

Title of thesis: An intervention programme for weight management in overweight and obese Malaysian adults

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under the supervision of

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Abstract

The prevalence of obesity and its co-morbidities in the Malaysian population has been increasing dramatically through the past decade, and has now become the leading preventable cause of morbidity and mortality in the Malaysian population. The main aim of this doctoral thesis was to develop a dietary intervention programme tailored according to an individual's food preference, cultural habit and genetic makeup (namely fat mass and obesity-associated gene (*FTO*) and beta 2-adrenergic receptor (*ADRB2*) gene). Further, this project aimed to manage body weight and reduce metabolic risk factors through, the modelled dietary intervention in overweight and obese Malaysian adults (Malaysian Malays, Chinese and Indians) (Chapter 6). Thereafter, this project investigated the individual and combined response of the gene variants (*FTO* and *ADRB2* genes) on the post-intervention (6-month period) differences in anthropometric and cardio-metabolic parameters (Chapters 7 & 8).

Baseline investigation of 178 multi-ethnic Malaysian adults revealed that Malaysian Indians had significantly higher BMI, WC, fat mass, body fat percent, fasting insulin, HOMA-IR and lower HDL cholesterol levels, compared to the Chinese and Malays (Chapter 4). Interestingly, dietary analysis revealed that Malaysian Indians consumed lower dietary cholesterol and higher fibre, compared to their Malaysian Chinese and Malays counterpart ($p<0.05$). With respect to the risk of obesity and gene variants of *FTO* and *ADRB2*, our results revealed contrasting differences (Chapter 5). In case of *FTO* gene variants (n=178), the risk alleles carriers of *FTO* rs9930501 (GG), rs9930506 (GG), and rs9932754 (CC) were associated with increased odds of obesity 3.03 (CI=1.23-7.49, p=0.016), 2.87 (CI=1.14-7.19, p=0.025) and 3.04 (CI=1.22-7.59, p=0.017) compared to AA, AA and TT genotypes, respectively, in the additive model. Moreover, the risk allele carriers of *FTO* rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly higher BMI, WC, WHR, fat mass, body fat percent, hsCRP levels and lower HDL cholesterol levels compared to the non-carriers (AA, AA and TT, respectively) ($p<0.05$). Interestingly, we found that Malaysian Indians expressed significantly higher frequency of the risk alleles of *FTO* rs9930501 (G), rs9930506 (G) and rs9932754 (C) when compared to Chinese and Malays ($p<0.001$).

With respect to *ADRB2* (n=178), our results revealed that *ADRB2* rs1042713 and rs1042714 were not associated with increased odds of obesity ($p>0.025$). However, we found that the risk allele carriers of *ADRB2* rs1042713 (GG) were associated with increased odds of insulin resistance, 4.43 (CI=1.31-15.0, p=0.016) compared to the non-carriers (AA), in the additive

model. Obese individuals carrying the G allele of rs1042713 had significantly higher total cholesterol and LDL cholesterol levels compared to the non-carriers (A), whereas obese individuals carrying the G allele of rs1042714 had significantly higher WHR compared to the non-carriers (C) ($p<0.025$). However, such differences were not observed in non-obese individuals ($p>0.025$). Furthermore, Malaysian Chinese had significantly lower frequency of the risk allele (G) of *ADRB2* rs1042714 compared to Malaysian Malays and Malaysian Indians combined (11.9% vs 28.7%) ($p=0.028$).

Gene-diet interaction analysis (n=126) revealed that individuals carrying the risk alleles of *FTO* rs9930501 (G), rs9930506 (G) and rs9932754 (C) showed significantly lower hsCRP levels with higher vitamin E intake (≥5.4mg/day of energy adjusted vitamin E intake) ($p=0.036$, $p=0.038$ and $p=0.036$, respectively) and higher percent energy from protein (≥14%/day) ($p=0.034$, $p=0.049$ and $p=0.034$, respectively). Individuals carrying the risk allele of *ADRB2* rs1042713 (G) had significantly lower fasting glucose levels with intake of relatively higher PUFA: SFA ratio (≥0.8/day) ($p=0.006$) and lower SFA intake (<7.3% of TE/day) ($p=0.011$). Moreover, the latter had significantly lower fasting insulin levels ($p=0.036$) and HOMA-IR ($p=0.026$) when consuming higher PUFA intake (≥6% of TE/day).

Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention was formulated and developed to reduce body weight and cardio-metabolic risk factors in Malaysian adults (Chapter 6). A 6-month RCT revealed that overweight and obese Malaysian adults following Hipcref diet (n=65) had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the generalised dietary instruction on weight loss based on Malaysian Dietary Guidelines (MDG) 2010 (n=63) ($p<0.05$). After 6 months of intervention, Hipcref diet group was found to consume significantly higher percent energy from protein, higher percent energy from PUFA, higher vitamin E (mg), higher fibre (g), lower total energy, lower percent energy from fat and lower percent energy from carbohydrate ($p<0.05$). We opine that the success of the Hipcref diet in achieving positive outcomes in overweight/obese Malaysian adults may be, due to the combined effect of the nutrient composition of the Hipcref diet.

Further, the effect of the interaction between gene variants (*FTO* and *ADRB2* genes) and dietary intervention on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters was assessed (Chapter 7). In the individual SNP analysis, general linear

regression analysis revealed that the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 were not associated with any post-intervention differences in dietary intake, anthropometric and cardio-metabolic parameters after 6 months of dietary intervention ($p>0.05$). However, significant effect of the interaction between *ADRB2* rs1042714 x dietary group was found on the post-intervention differences in fasting insulin (p interaction =0.012) and HOMA-IR (p interaction =0.021), in response to a 6-month Hipcref dietary intervention. Individuals carrying both the risk allele (CG+GG) and the non-risk allele (CC) of rs1042714 had significantly greater reduction in fasting insulin ($p<0.001$ and $p=0.001$, respectively) and HOMA-IR ($p<0.001$ and $p=0.006$, respectively) after a Hipcref diet compared to a control diet. With respect to dietary intake, individuals carrying the risk allele of rs1042713 (AG+GG) had significantly greater reduction in SFA intake ($p=0.006$) and MUFA intake ($p=0.019$) after a Hipcref diet. Further, we assessed the combined response of *FTO* and *ADRB2* gene variants on the above aspect by computing polygenic risk scores (PRS) (Chapter 8). General liner regression analysis revealed significant effect of the interaction between PRS x dietary group on the post-intervention difference in hsCRP levels (p interaction=0.048). Participants from the second and third tertiles of PRS had significantly greater reduction in hsCRP levels with Hipcref diet compared to the control diet (-2.5 ± 0.9mg/L vs -0.03 ± 0.6mg/L, $p=0.025$; and -2.4 ± 1.0mg/L vs 0.8 ± 1.0mg/L, $p=0.025$, respectively), after 6 months of intervention period. Such difference was not observed in the first tertile of PRS ($p>0.05$).

In conclusion, the findings from this project suggest that gene variants may affect individual's susceptibility to obesity-related phenotypes and it may interact with dietary intakes and modulate the effect of obesity-related phenotypes, in Malaysian adults. Individualised dietary intervention (Hipcref diet) tailored according to individual's food preferences, cultural habits and genetic makeup had positive impact on body composition and cardio-metabolic parameters compared to generalised weight loss advice based on Malaysian Dietary Guidelines (MDG) 2010. Personalisation seems to be an important factor when developing strategies for weight management, especially in a multi-ethnic Malaysian population. Furthermore, individuals with different gene variants responded differently to the dietary intervention programme with respect to body composition and cardio-metabolic parameters.

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List of publications, presentations and awards

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1. Tan, P. Y., Mitra, S. R., & Amini, F. (2019). Lifestyle interventions for weight control modified by genetic variation, a review of evidence. *Public Health Genomics,* 21(5-6), 169-185.

2. Mitra, S. R., & Tan, P. Y. (2019). Effect of an individualised high protein, calorie-restricted diet on anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults: A 6-month randomised controlled study. *British Journal of Nutrition,* 121(9), 1-36. http://doi.org/10.1017/S0007114519000345.

3. Mitra, S. R., Tan, P. Y., & Amini, F. (2019). Association of *ADRB2* rs1042713 with obesity and obesity-related phenotypes and its interaction with dietary fat in modulating glycaemic indices in Malaysian adults. *Journal of Nutrition and Metabolism,* Volume 2019, Article ID 8718795, 1-10. http://doi.org/10.1155/2019/8718795.

4. Mitra, S. R., Tan, P. Y., & Amini, F. (2018). Effect of *FTO* rs9930506 on obesity and interaction of the gene variants with dietary protein and vitamin E on C-reactive protein levels in multi-ethnic Malaysian adults. *Journal of Human Nutrition and Dietetics,* 31(6), 758–77.

Publications in proceedings:

1. Mitra, S. R., Tan, P. Y., & Amini, F. (2017). Obesity, insulin resistance and gene variation in multi-ethnic Malaysian adults. *Journal of Nutrition & Food Science 2017*, 7(Suppl 1), 61. https://doi.org/10.4172/2155-9600.C1.039.

2. Mitra, S. R., Tan, P. Y., & Amini, F. (2015). Dietary intake, blood lipid levels and insulin resistance in Malaysian adults. *Proceedings of the Nutrition Society*, 74(OCE5), E345. https://doi.org/10.1017/S0029665115003924.

Oral presentation in international conference:

1. "Association of *ADRB2* rs1042713 with obesity and obesity-related phenotypes and its interaction with dietary fat in modulating glycaemic indices in Malaysian adults" - 13th Asian Congress of Nutrition 2019, at Bali International Convention Centre, Bali, Indonesia, from 4th August to 7th August 2019. –co-author

2. "Dietary intake, blood lipid levels and insulin resistance in Malaysian adults", Nutrition Society UK Conference at Sutton Bonington Campus University of Nottingham, UK from 6th July to 9th July 2015. –co-author

Poster presentations in international conferences:

1. "Diet, biochemical markers and interaction with *FTO* gene polymorphisms in Malaysian adults", 10th Asia Pacific Conference on Clinical Nutrition at Adelaide, Australia from 26th November to 29th November 2017.

2. "Polymorphisms in *FTO*, *TP53* and *LEP* genes, dietary intake and metabolic biomarkers in obese Malaysian adults", European Society of Human Genetics: ESHG 2016 Conference at Barcelona, Spain from 21st May to 24th May 2016. –co-author

3. "Macronutrient intake and blood bio-chemical parameters in obese Malaysian adults", 12th Asian Congress of Nutrition 2015 at Pacifico Yokohama, Japan from 14th May to 18th May 2015.

Oral presentations in national conferences:

1. "*FTO* gene variants (rs9930501, rs9930506 and rs9932754) and post-intervention differences in anthropometric and cardio-metabolic parameters after a 6-month Hipcref diet intervention in overweight and obese Malaysian adults" - 34th Scientific Conference by Nutrition Society of Malaysia at Hotel Istana, Kuala Lumpur, from 3rd July to 4th July 2019.

2. "Improved anthropometric and metabolic profile in Malaysian adults – A 6-month dietary intervention with 30% calories from protein", 33rd Scientific Conference by Nutrition Society of Malaysia at Kuala Lumpur from 25th July to 26th July 2018. – co-author

3. "Obesity and polymorphisms in *FTO* gene in multi-ethnic Malaysian adults", 1st Southeast Asia Public Health Nutrition Conference 2017 at Hotel Istana, Kuala Lumpur from 14th May to 17th May 2017.

4. "Association of serum leptin levels and *LEP* gene variation with body composition and insulin resistance in obese Malaysian adults", 31st Annual Scientific Conference 2016 by Nutrition Society of Malaysia at Kuala Lumpur from 31st May to 1st June 2016.

Awards

1. Second runner up - Postgraduate student conference support poster competition, by the Faculty of Science, University of Nottingham Malaysia (UNM) on 15th September 2017, with the title of "Gene variations in *FTO* and interaction with obesogenic phenotypes and diet in multi-ethnic Malaysian adults".

2. University Postgraduate Prize Award 2018/19 – University of Nottingham Malaysia (UNM)

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List of abbreviations

Abbreviation	Meaning
ADRB2	Beta 2-adrenergic receptor
AHA	American Heart Association
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
BMR	Basal metabolic rate
CHO	Carbohydrate
CI	Confidence interval
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DSM-BIA	Direct segmental multi-frequency- bioelectrical impedance analysis method
EDTA	Ethylenediaminetetraacetic acid
FBG	Fasting blood glucose
FFAs	Free fatty acids
FFM	Fat free mass
FM	Fat mass
FTO	Fat mass and obesity-associated
GLM	General linear model
GWAS	Genome-wide association studies
HC	Hip circumference
HDL-C	High-density lipoprotein cholesterol
Hipcref	High-protein, calorie-restricted, high vitamin E and normal fibre
HOMA	Homeostatic model assessment
HOMA-IR	Homeostatic model assessment -insulin resistance
hsCRP	High-sensitivity C-reactive protein
HWE	Handy-Weinberg equilibrium
IL	Interleukin
IFNγ	Interferon gamma
LDL-C	Low-density lipoprotein cholesterol
MANS	Malaysian adult nutrition survey
MET	Metabolic equivalent
MDG	Malaysian Dietary Guidelines
MI	Myocardial infarction
MOH	Ministry of Health Malaysia
MREC	Medical Research and Ethics Committee
MS	Muscle mass
MUFA	Monounsaturated fatty acids
NCDs	Non-communicable diseases
NEFA	Non-esterified fatty acids
NHMS	National Health and Morbidity Survey
OR	Odds ratio
PBF	Percent body fat
PCR	Polymerase chain reaction
PGC1α	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PRO	Protein
PRS	Polygenic risk scores

PUFA	Polyunsaturated fatty acids
RDA	Recommended dietary allowance
RE	Restriction enzyme
RNI	Recommended nutrient intake
SBP	Systolic blood pressure
SE	Standard error of mean
SFA	Saturated fatty acids
SIRT1	NAD-dependent deacetylase sirtuin-1
SNPs	Single nucleotide polymorphisms
TC	Total cholesterol
TE	Total energy intake
TFA	Trans fatty acids
TG	Triglyceride
TNFα	Tumor necrosis factor-alpha
UCSI	University College Sedaya International
UNM	University of Nottingham Malaysia
Vit	Vitamin
VLDL	Very-low-density lipoprotein
vs	Versus
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip-ratio
χ^2	Chi square test

Chapter 1

Introduction

1.1 Background

The prevalence of obesity has reached epidemic proportions globally (Upadhyay et al., 2018). It is caused by a state of positive energy balance, when energy consumption exceeds energy expenditure and results in storage of excess body fat, to the extent that it may impair health (Spiegelman & Flier, 2001). Consumption of energy-dense food coupled with sedentary lifestyle, are the main contributors towards positive energy balance and the development of obesity and related complications nowadays. World Health Organization (WHO) 2016 reported that more than 1.9 billion adults, 18 years and above, were overweight, and of these over 650 million were obese, which translated to 39% of the adults aged 18 years and over were overweight, and 13% were obese (WHO, 2016). A research published in 'The Lancet' also reported that the current prevalence of overweight and obesity in Malaysian women and men is about 49% and 44%, respectively (Ng et al., 2014). Interestingly, disparities in the prevalence of obesity within the ethnic groups in Malaysian population had been reported earlier. According to NHMS 2015, the prevalence of obesity was highest in Malaysian Indian (35%), followed by Malays (31%) and Chinese (28.1%) (Institute for Public Health, Malaysia, 2015). Such differences may be due to the differences in dietary intakes, physical activity levels and genetic factors (Tan et al., 2011; Ghee, 2016).

Obesity has become a major public health problem in Malaysia due to its clinical implications in increasing the prevalence of comorbid conditions such as hypertension, hyperglycaemia, dyslipidaemia, insulin resistance and inflammation. These complications are known to be associated with increased risk of developing metabolic syndrome and chronic diseases such as cardiovascular diseases, hypertension, type 2 diabetes and some type of cancers (Guh et al., 2009). National Health and Morbidity Survey Malaysia 2011 reported that, about 15% and 35% of Malaysian adults above 18 were suffering from type 2 diabetes mellitus and hypercholesterolemia respectively (Institute for Publihc Health, Malaysia, 2011). Furthermore, Department of Statistics Malaysia reported that in 2017, obesity has becoming the leading preventable cause of morbidity and mortality in the Malaysian population (13.9% of death due to ischaemic heart disease) (Department of Statistics, Malaysia, 2018).

Obesity in Malaysia is now a challenge to the health care system as it has increased the economic burden of the government on the health care expenses in order to diagnose, treat and manage obesity (Tan et al., 2011). According to Tan et al., 2011, the treatment of obesity and its primary comorbidities are estimated to cost the Malaysian health care system approximately

US$162 million per year. Ironically, these non-communicable diseases are preventable and reversible through small changes in lifestyle that promote active lifestyle and practice adherence to balanced diet. This has been supported by previous findings which has shown that a reduction in the risk of developing these chronic diseases is associated with weight reduction especially reduction in abdominal fat (Kishida, Funahashi, & Shimomura, 2011).

Previous evidence has illustrated that a moderate weight loss of 5-10% in body weight (Brown et al., 2016; Wing & Phelan, 2005), particularly in visceral adipose tissue (Goodpaster et al., 1999; Kwon, Kim, & Kim, 2017; Pascale et al., 1992) had beneficial effects in insulin sensitivity, glycaemic control and cardiovascular disease risk. Therefore, an effective weight management programme is necessary to combat the high prevalence of obesity and obesity-related non-communicable diseases in the Malaysia population. Numerous studies have investigated interventions based on various dietary patterns by varying the dietary macronutrient composition (Goss et al., 2013; Sacks et al., 2009). Dietary composition plays an important role in determining the energy density of the diet, thus affecting the total caloric intake (Rolls et al., 1994; Rolls, Hetherington, & Burley, 1988). Low-fat diets are one of the most common dietary strategies to lose weight as fat is a high energy dense and poor satiating food. Fleming et al., 2002 reported that significant reduction in body weight, total cholesterol levels, LDL cholesterol levels, triglyceride levels, total cholesterol/HDL cholesterol was found in low-fat and moderate-fat diets. Only individuals following high-fat diets showed a worsening effect on cardio-metabolic parameters such as the levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, fibrinogen and etc., despite achieving statistically significant weight loss. However, conflicting results have been reported in other studies. A meta-analysis reported that low-carbohydrate diet (≤45% of energy from carbohydrates) is more favourable in improving body weight and blood lipid profile in obese individuals, by decreasing triglyceride levels and increasing HDL cholesterol levels compared to a low-fat diet (≤30% of energy from fat) (Hu et al., 2012). However, extremely low-carbohydrate diet is not recommended for sustained weight loss program due to the adverse effects such as breath odor, constipation, loss of appetite, kidney distress and organ failure (Bilsborough & Crowe, 2003).

In recent, high-protein diet has becoming a popular strategy for weight loss as it promotes satiety, reduces energy intake, induces weight loss and maintains lean muscle tissue (Astrup, Raben, & Geiker, 2015; Clifton, 2012). A high protein diet with 25-30% of total energy from protein showed greater reduction in weight and waist circumference and greater improvement in glycaemic control and cardio-metabolic profile in Asian Indians, Iranian and Australian

populations (Azadbakht et al., 2013; Farnsworth et al., 2003; Gulati et al., 2017). A meta-analysis of randomised controlled trials reported that by comparing with a standard protein, low-fat diet, low-carbohydrate, high-protein diets provided greater reduction in body weight, blood triglyceride levels and preservation of fat-free mass (Wycherley et al., 2012). Elevated protein diet was associated with increased satiety, thermogenesis and glycogenesis which may lead to greater weight loss and improvement in insulin sensitivity (Westerterp-Plantenga et al., 2006). A high-protein, very low-carbohydrate, very high-fat diet promoted by Atkins (60% of energy from fat, 35% of energy from protein and 5% of energy from carbohydrate) is perhaps the most popular high-protein diet in America for weight loss. However, healthcare professionals do not recommend long-term adherence to an Atkins-like, high-protein diet due to the high fat content of the diet (~60% energy), particularly the saturated fat (~25% of energy from SFA), which may eventually lead to elevations in total cholesterol and LDL cholesterol levels (Mahdi, 2006). Extremely low carbohydrate-high protein diets may have short-term beneficial effects on weight control, however concerns have also been expressed on long-term effects, notably with respect to cardiovascular outcomes. Long-term high protein or high meat intake in humans may lead to hyperuricemia (leading to joint pain and gout), hypercalcuria (leading to kidney stones, hypocalcaemia, and osteoporosis), renal malfunction, increased cancer risk, liver malfunction, and increased risk of coronary artery disease (Delimaris, 2013). Moreover, extremely low-carbohydrate diets may also lead to ketosis, nausea, dizziness, constipation, headache, fatigue, and smelly breath (Mahdi, 2006).

Diets high in protein (30% of energy from protein) but low in fat (<30% of energy from fat) and moderate in carbohydrate (40% of energy from carbohydrate) are as effective at promoting weight loss as Atkins diets, and reducing total cholesterol and LDL cholesterol as well as triglycerides (Baba et al., 1999; Johnston, Tjonn, & Swan, 2004; Parker et al., 2002). However, these diets have not received the same attention as the Atkins diet. Johnston et al., 2004 reported that energy-restricted, low-fat diets (<30% of energy from fat) of varying protein content (15% or 30% of energy from protein) were equally effective at promoting steady weight loss over 6 weeks of the intervention period, but diet satisfaction was greater in those consuming the high-protein, low-fat diet (Johnston, Tjonn, & Swan, 2004). The authors reported that blood lipid profile, insulin sensitivity, uricosuria, hypercalciuria, and glomerular filtration rates, were not adversely affected by the high-protein, low-fat diets. Farnsworth et al., 2003 also reported that a high-protein, moderate carbohydrate diet (HP diet: 27% of energy from protein, 44% of energy from carbohydrate, and 29% of energy from fat) had beneficial metabolic effects and

no adverse effects on markers of bone turnover or calcium excretion compared to a standard-protein diet (SP diet: 16% of energy from protein, 57% of energy from carbohydrate, and 27% of energy from fat). Therefore, high-protein diets that are low in fat and moderate in carbohydrate (30% of energy from protein, 40% of energy from carbohydrate and 30% of energy from fat) may represent an ideal diet plan for safe and healthy weight loss and deserve further investigation.

Dietary fibre is known to be a key component in healthy eating. A review of studies reported that higher dietary fibre intake was associated with increased satiety and decreased hunger (Howarth, Saltzman, & Roberts, 2001). Previous studies have reported a favourable metabolic profile with high fibre diet (Jung & Choi, 2017; Kabir et al., 2002). Large prospective cohort studies consistently reported significant associations between high dietary fibre intake (>25 g/d in women and >38 g/d in men) and reduced risk of developing type 2 diabetes (T2D) (20-30% of reduced risk) (Weickert & Pfeiffer, 2018). The proposed mechanisms for this relationship remain unclear, but the type of fibre may play a role. In the gut, certain soluble fibres which form a viscous gel matrix is believed to slow gastric emptying and lead to a greater feeling of fullness (Slavin & Green, 2007). Soluble fibres slow absorption of glucose further in the small intestine and lead to lower postprandial glycaemic and insulinemic responses (Yu et al., 2014). Therefore, high soluble fibres may contribute to the improvement in glycaemic control and insulin sensitivity. Moreover, in both the cross-sectional and longitudinal analyses, fibre intake was found to be protective against high inflammation levels (Griffith, et al., 2006). It was suggested that dietary fibre decreases lipid oxidation, which in turn associated with decrease in inflammation (King, 2005). Therefore, incorporating food sources that are high in fibre such as whole grains, vegetables, fruits, peas and nuts into our diets may help to improve metabolic health.

Over the years, strategies to combat obesity remained ineffective, particularly in long term weight loss programmes (Bombak, 2014). General recommendations based on dietary guidelines, e.g. recommended nutrient intake (RNI) or recommended dietary allowance (RDA), are often found to be ineffective and end in unsuccessful weight management programmes as there is no one-size-fits-all diet that is more effective than the others. A recent review by Koliaki et al., 2018 reported that an optimal diet to manage obesity should be individualised, safe, efficacious, nutritionally adequate, culturally sensitive, cost effective, adoptable and should ensure long term maintenance of weight loss. Setting a realistic weight loss goal and following a balanced dietary plan tailored according to the individual's need, food preference

and cultural habit are the key principles to facilitate sustainable weight loss in obese individuals and most importantly to reduce their overall cardio-metabolic risk. According to a current study, Malaysians of different ethnic background continue to practice traditional meal patterns, habitually (Abdullah, Teo, & Foo, 2016). Thus, individualised dietary intervention according to food preferences and cultural habits could have higher impact in establishing consumer's adherence to the intervention diet compared to a generalised dietary advice on weight loss, and hence end in successful and sustainable weight management in the Malaysian population.

It has been accepted that obesity is a multifactorial disease of the complex interaction between multiple gene variants, lifestyle and environmental factors (Marti et al., 2004). Epidemiological studies have reported that the changes in lifestyle during the last decade has promoted an increase in the consumption of energy dense foods and sedentary lifestyle, thus creating an 'obesogenic' environment which resulted in positive energy balance leading to weight gain in individuals of the population (Loos, 2009). However, not everyone living in the obesogenic environment is obese, suggesting that genetic components play an important role in contributing to the risk of developing obesity and obesity-related chronic diseases. Multiple genetic loci have been successfully determined by genome-wide association studies (GWAS) that increase the susceptibility to obesity and diabetes in a cumulative manner (Speliotes et al., 2010).

The first successfully determined candidate gene is the *FTO* (Fat-mass and obesity associated) gene (Loos & Bouchard, 2008). The strong association of *FTO* gene and obesity-related traits have been consistently observed in studies involving various populations worldwide (Dina et al., 2007; Peeters et al., 2008; Ri et al., 2015). Previous evidence reported that the AA genotype of *FTO* rs9930609 weighed 3kg more and had a 1.7 fold increased risk of obesity compared to individuals carrying TT genotype (Frayling et al., 2007). This association has been replicated in many European studies involving different ethnic groups across the globe (Dina et al., 2007; Peeters et al., 2008; Ri et al., 2015). Furthermore, a meta-analysis involving 18 published articles revealed significant association between *ADRB2* rs1042714 and susceptibility to obesity (Zhang, Wu, & Yu, 2014). These findings have suggested that gene variants play a critical role in weight management. Therefore, candidate genes that are involved in lipid metabolism, adipogenesis, energy expenditure, appetite control, insulin signaling and adipokine secretion should be largely investigated to understand the interplay between gene variants and lifestyle factors particularly dietary intake and physical activity in modulating obesity-related traits (Deram & Villares, 2009).

In randomised controlled trials, several weight loss strategies have reported reduction in weight and improvement in metabolic risk factors in obese individuals. However, the studies reported individual variability in response to the same intervention, suggesting that the differences in such responses are due to genetic factors (Bray, 2008; Loos & Rankinen, 2005). Individual genetic background may trigger phenotypes of obesity when an individual is exposed to obesogenic environment. Thus, we can see the emergence of precision nutrition tailored according to individual's genetic background, age, gender, physical activity level, food preferences and ethnic background as a new therapeutic approach, to optimise health so as to prevent disease (Gaboon, 2011). Therefore, it is important to investigate the role of gene-diet interactions in the development of obesity and obesity-related traits as this information may help to address sub-groups of individuals with individualised strategies. Despite an increased research interest in this area in the past few years, existing evidence is not strong enough to drive concrete policy recommendations for obesity prevention indicating a need for more gene-diet interaction studies in specific populations.

1.2 Problem statement

Obesity is a public health problem which has now reached epidemic proportions worldwide. Substantial evidence has proved that obesity has clinical implications in increasing the prevalence of comorbid conditions such as hypertension, hyperglycaemia, dyslipidaemia, insulin resistance and inflammation, which subsequently increase the risk of developing non-communicable diseases including cardiovascular diseases, type 2 diabetes and various cancers. It has been mentioned earlier that according to the Department of Statistic Malaysia 2017, obesity has become the leading preventable cause of morbidity and mortality in Malaysian population (Department of Statistics, Malaysia, 2018). Furthermore, it has been reported that Malaysia has the highest prevalence of metabolic syndrome in Asia (Mohamud et al., 2011). This has highly increased the economic burden of the Malaysia government on health care system. The related-health care costs are substantially high (Tan et al., 2011). Therefore, the recent challenge is to develop and establish an effective weight management strategy to combat the high prevalence of obesity and obesity-related chronic diseases in overweight and obese Malaysian population (Malaysian Chinese, Malays and Indians).

A public health approach to weight management strategies should be designed based on the target factors contributing to obesity in the population and should be multifaceted. To date, there are only a few weight management intervention studies which have been experimented in the Malaysian population using strategies such as dietary or physical activity-based programmes or behavior modification programmes (Soon et al., 2013; Kassim et al., 2017; Moy, Sallam, & Wong, 2006; Ramli et al., 2013). Most of the intervention programmes were conducted in community settings and government (public) sectors. However, the results were conflicting and unsatisfactory. Generalised advice on weight loss in multi-ethnic Malaysian population seem to be less effective in increasing individual's compliance to the intervention and hence end in unsuccessful weight management programme. Individualised dietary intervention according to individual's food preference and cultural habit could have higher efficacy in establishing individual's adherence to the intervention diet, and hence can be successful and sustainable weight management programmes. Therefore, investigating the differences in dietary patterns, physical activity levels, obesity-related phenotypes, and genetic factors between the ethnic groups is an important factor in designing an effective individualised dietary intervention to manage body weight.

Another important factor which could not be neglected in designing and establishing personalised weight management programmes would be individual's variability of genetic background (Loos & Rankinen, 2005). It has been mentioned earlier that obesity is a multifactorial disease which involves complex interactions between multiple gene variants and lifestyle factors particularly dietary intakes and physical activity levels. Genetic background may modulate the effect of weight management intervention in the post intervention changes of obesity-related phenotypes and result in individual variability in weight loss outcomes (Bray, 2008). Therefore, a better understanding of the interplay between lifestyle factors and genetic factors of obesity-related traits is important in developing useful diagnostic tools for obesity, and as well establishing an effective weight management programme. It is believed that early identification of these candidate gene variants and its interactions with dietary modulation may allow for the provision of good quality personalised recommendation to achieve effective weight loss and positive outcome in obesity related morbidities (Martínez & Milagro 2015).

Therefore, in this PhD thesis, an individualised Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention which tailored according to individual's food preference and cultural habit was developed for weight control and reduction in metabolic risk factors. To the best of our knowledge, this is the first study that aims to evaluate the effect of a 6-month individualised Hipcref dietary intervention in improving obesity-related anthropometric and blood cardio-metabolic parameters in multi-ethnic overweight and obese Malaysian adults, as well as to assess the gene-diet interactions on the post-intervention differences in the aforementioned parameters, in response to different dietary interventions.

1.3 Aims, objectives and research questions

1.3.1 Main aim:

The main aim of this doctoral thesis was to evaluate the efficacy of an intervention programme in managing body weight and reducing metabolic risk factors in overweight and obese Malaysian adults. This project recruited Malaysian adults from the three major Malaysian ethnic groups namely Malaysian Malays, Chinese and Indians to investigate the effect of a 6-month individualised dietary intervention on anthropometric and cardio-metabolic parameters. In addition to the above, this project investigates the modulatory effect of gene variants on the post-intervention differences in obesity-related anthropometric and cardio-metabolic parameters, after 6 months of dietary intervention. To achieve this, this thesis is organised into different sections with the following objectives.

1.3.2 Specific objectives and research questions:

The objectives of this doctoral thesis were framed to address the following issues:

1. To determine the differences in dietary intakes, physical activity levels, obesity-related anthropometric and blood biochemical parameters between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians),

   Research question 1: Are there any differences in dietary intake, physical activity level and obesity-related anthropometric and blood biochemical parameters between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians)?

2. To assess the differences in genotype distribution and allele frequencies of the gene variants between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians),

   Research question 2: Are there any differences in genotype distributions and allele frequencies of gene variants between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians)?

3. To investigate the association between gene variants and obesity and obesity-related traits,

Research question 3: How do gene variants affect the phenotypes of obesity-related traits of Malaysian adults?

4. To evaluate the interaction between gene variants and dietary factors in modulating the obesity-related anthropometric and biochemical parameters,
   Research question 4: How do gene variants interact with dietary factors and modulate the effect of obesity-related phenotypes in the Malaysian adults?

5. To investigate the effect of an individualised dietary intervention programme on anthropometric and cardio-metabolic parameters of the study participants,
   Research question 5: How does an individualised dietary intervention programme (calorie-restricted, high-protein diets with high vitamin E and normal fibre intake) benefit the overweight and obese Malaysian adults with respect to obesity-related anthropometric and blood biochemical parameters?

6. To assess the response of the variants of the same gene on the post-intervention differences in anthropometric and cardio-metabolic parameters after 6 months, if any, and,
   Research question 6: Do individuals with different variants of a single gene respond differently to the dietary intervention programme with respect to anthropometric and cardio-metabolic parameters?

7. To assess the combined response of multiple gene variants using polygenic risk scoring on the post-intervention differences in anthropometric and cardio-metabolic parameters after 6 months of dietary intervention.
   Research question 7: Do individuals with different variants of multiple genes respond differently to the dietary intervention programme with respect to anthropometric and cardio-metabolic parameters?

1.4 Ethical approval

This study was reviewed and approved by the Science and Engineering Research Ethics Committee, University of Nottingham Malaysia (UNM) (Appendices 1 & 2). This study was also registered under the Medical Research and Ethics Committee (MREC) of National Medical Research Registry, Ministry of Health Malaysia (MOH) (Appendix 3). Written informed consent was requested and obtained from each individual participant (Appendices 6 & 7). The study protocol was prepared in accordance with the ethical standards laid down by the Declaration of Helsinki, 1964 and follows the Good Clinical Practice Guidelines of the government of Malaysia (Third Edition Oct 2011© Ministry of Health Malaysia).

Chapter 2

Literature review

2.1 Obesity

2.1.1 Definition

Obesity is a state of positive energy balance, when energy consumption exceeds energy expenditure and results in the storage of excess body fat (Spiegelman & Flier, 2001). It can be defined as a condition of excess fat accumulation in adipose tissue, to the extent that it may impair health. For practical purposes, obesity is defined as excess body weight rather than excess body fat, as direct measurement of body fat is more difficult than measuring body weight. Body mass index (BMI), a simple index of weight-to-height is used as a population-level measure of obesity. It is calculated as an individual's weight in kilograms divided by the square of height in meters (kg/m^2), and is commonly used as a practical indicator to classify underweight, overweight and obese in adults. World Health Organization (WHO) has defined underweight as BMI<18.5kg/m^2, overweight as BMI 25kg/m^2-29.9kg/m^2 and obesity as BMI≥30kg/m^2 (WHO Expert Consultation, 2004). However, investigators have found that compared to Caucasians, Asian were more likely to be compromised with hyperglycaemia, hypertension, and hypertriglyceridemia at lower levels of BMI and waist circumference. Therefore, for Asian populations, World Health Organization (WHO) has redefined the cut off point for overweight and obesity, with BMI value ≥23 kg/m^2 and ≥27.5 kg/m^2, respectively (WHO Expert Consultation, 2004).

Numerous studies have investigated and compared various anthropometric indices including BMI, waist circumference (WC) and waist-to-hip ratio (WHR) in assessing obesity and predicting the obesity-related health risks (Czernichow et al., 2011). However, there is no agreement on which index should be applied universally for defining obesity. BMI cut offs are set based on the co-morbidities risk associated with BMI. However, the use of BMI does not distinguish the weight associated with muscle mass and the weight associated with fat mass. WC, a measure of abdominal fat or central fat accumulation has been suggested to be a better predictor for obesity-related cardiovascular risk factors and other forms of chronic diseases compared to BMI (Klein et al., 2007). Therefore, an assessment of abdominal fat/central fat accumulation is of great importance to define obesity. According to the WHO recommendations for Asian populations, WC of 85 cm and above in men and 80 cm and above in women are associated with increased risk of obesity-related comorbidities (Alberti, Zimmet, & Shaw, 2005). Whereas, a waist–hip ratio values of 0.90 and 0.80 for men and women, respectively indicated abdominal fat accumulation which is associated with increased risk of

cardio-metabolic risks in Asian populations (WHO Expert Consultation, 2008). However, the use of WHR has been recently questioned and challenged due to few reasons. The measurement of hip circumference is hard to perform and is less reliable. Moreover, WHR is not useful in practical risk management as the ratio could remain constant when the weight of individual increases or decreases.

2.1.2 Prevalence of obesity and its comorbidities in the Malaysian population

The prevalence of overweight and obesity has been increasing worldwide over the past three decades, with the number of overweight and obese individuals increased from 921 million in 1980 to 2.1 billion in 2013 (Ng et al., 2014). World Health Organization (WHO) has reported that in 2016, more than 1.9 billion adults, 18 years and older, were overweight, and of these over 650 million were obese, which translated to 39% of the adults aged 18 years and above were overweight, and 13% were obese (WHO, 2016). Obesity was once considered to only affect high-income or developed countries, but the prevalence of obesity has now increased tremendously in developing countries. As with other developing countries, the prevalence of obesity in Malaysia has reached epidemic proportions and has now become the leading cause of morbidity and mortality of the country.

National Health and Morbidity Survey (NHMS) 2015 reported that 5.6 million Malaysian adults aged 18 and above were overweight and 3.3 million were obese (Institute for Public Health, Malaysia, 2015). Based on previous NHMS carried out in 2006, 2011 and 2015, an increasing trend of the prevalence of overweight and obesity was observed among the Malaysian adults aged 18 years and older: 29.1% and 14.5% in 2006 (Noor Safiza et al., 2008), 29.4% and 15.1% in 2011 (Institute for Public Health, Malaysia, 2011), 30.0% and 17.7% in 2015 (Institute for Public Health, Malaysia, 2015), respectively. Women had higher prevalence of obesity (33.6%) compared to the men (27.8%), according to NHMS 2015 (Institute for Public Health, Malaysia, 2015).

Numerous studies have reported that excess body weight is associated with increased risk of developing chronic diseases such as cardiovascular diseases, hypertension, type 2 diabetes and some type of cancers (Guh et al., 2009). This was reflected in the high prevalence of obesity-related comorbidities in the Malaysian population. National Health and Morbidity Survey (NHMS) 2015, reported that about 2.5 million and 9.6 million of Malaysian adults above 18 were suffering from type 2 diabetes mellitus and hypercholesterolemia, respectively. Moreover, it has been consistently reported that obesity as the risk factor for metabolic syndrome

(Monteiro & Azevedo, 2010). Metabolic syndrome refers to a cluster of conditions including central obesity, hypertension, hyperglycaemia, hyperinsulinemia, glucose intolerance, and dyslipidaemia (including hypertriglyceridemia, elevated non-esterified fatty acids (NEFAs) and decreased HDL cholesterol) (Mottillo et al., 2010). Malaysia was reported as the country with the highest prevalence of metabolic syndrome in Asia (Mohamud et al., 2011).

2.1.3 Disparities in the prevalence of obesity and its co-morbidities between the three ethnic groups (Malaysian Chinese, Malays and Indians)

Malaysia is a rapidly developing country with multi-ethnic heterogeneous population consisting of Bumiputera (69.1%), Chinese (23%), Indians (6.9%) and other mixed ethnic groups (1%) (Department of Statistics, Malaysia, 2018). Disparities in the prevalence of obesity and metabolic syndrome between the three ethnic groups (Malaysian Malays, Chinese and Indians) had been described earlier. According to NHMS 2015, the prevalence of obesity was the highest in Malaysian Indian (35%), followed by Malays (31%) and Chinese (28.1%) (Institute for Public Health, Malaysia, 2015). A large scale population-based survey in the Malaysian population conducted by Rampal et al, 2012, also reported that ethnic Indians had the highest prevalence of metabolic syndrome, followed by ethnic Malays and ethnic Chinese. The authors also reported that higher prevalence of metabolic syndrome was found in Malaysian women (30.1%) compared to men (24.8%) (Rampal et al., 2012). Malaysian Indians were more likely to exhibit central obesity, elevated fasting glucose and low HDL cholesterol levels compared to other ethnic groups (Tan, Dunn, & Yen, 2011). These findings suggest that obesity and metabolic syndrome disproportionately affect Malaysian Indians.

Previous evidence suggested that such differences between the ethnic groups may be due to the differences in cultural and lifestyle practices. Tan et al., 2011 reported that compared to Malaysian Malays and Chinese, Indians were less likely to engage in physical activity and were less likely to consume fruits and vegetables. This may have contributed to the high prevalence of obesity and metabolic risk in the Malaysian Indians. Moreover, genetic factors may have further contributed to the disparities in the prevalence of obesity and its complications between the ethnic groups. Previous studies have reported that higher prevalence of the abdominal obesity, diabetes, hyperinsulinemia, and dyslipidaemia was found in migrant Asian Indians compared to British Caucasians and Europeans, residing in London, United Kingdom (McKeigue et al., 1988; McKeigue et al., 1992). This finding suggests that ethnic Indians may be predisposed to several features of the metabolic syndrome. Therefore, understanding the

diffrences in genotype distributions, dietary intakes and obesity-related anthropometric and cardio-metabolic parameters between the three ethnic groups (Malaysian Chinese, Malays and Indians) is essential for the prevention and treatment of obesity in the Malaysian population.

2.2 Obesity and obesity-related cardio-metabolic parameters

2.2.1 Obesity and inflammation

Inflammation is a physiological response of our body to harmful stimuli, which can be physical, chemical or biological, to restore homeostasis (Monteiro & Azevedo, 2010). The normal acute inflammatory response involves the delivery of plasma components and leucocytes to the site of insult. This process is initiated by tissue-resident macrophages and mast cells, leading to a production of different types of inflammatory mediators (chemokines, cytokines, vasoactive amines, eicosanoids and etc.) (Rock & Kono, 2008). If successful, the harmful agent will be eliminated and the resolution of inflammation will be restored. Tissue leucocytes undergo apoptosis after their action, and are phagocytised by the macrophages and left the inflamed site by lymphatic drainage (Fox et al., 2010). The clearance of apoptotic neutrophils switches the lipid mediators from pro-inflammatory to anti-inflammatory. This process is essential for the resolution of inflammation and the restoration of tissue homeostasis.

However, an excessive response or a persisted inflammatory process may cause a state of chronic inflammation, and this can lead to deleterious health effects and increase the risk of developing cardiovascular diseases (Willerson, 2004). Earlier evidence reported that obesity, which is a feature of metabolic syndrome, was associated with chronic inflammation in obese individuals (Monteiro & Azevedo, 2010). Adipose tissue, besides being a storage of energy reserves in the form of triglycerides, it has important function as an endocrine organ, producing a variety of molecules called adipocytokines such as interleukin-1 (IL-1), IL-6, IL-8, interferon gamma (IFNγ), tumor necrosis factor-alpha (TNFα), leptin and resistin (Castro, Macedo-de la Concha, & Pantoja-Meléndez, 2017).

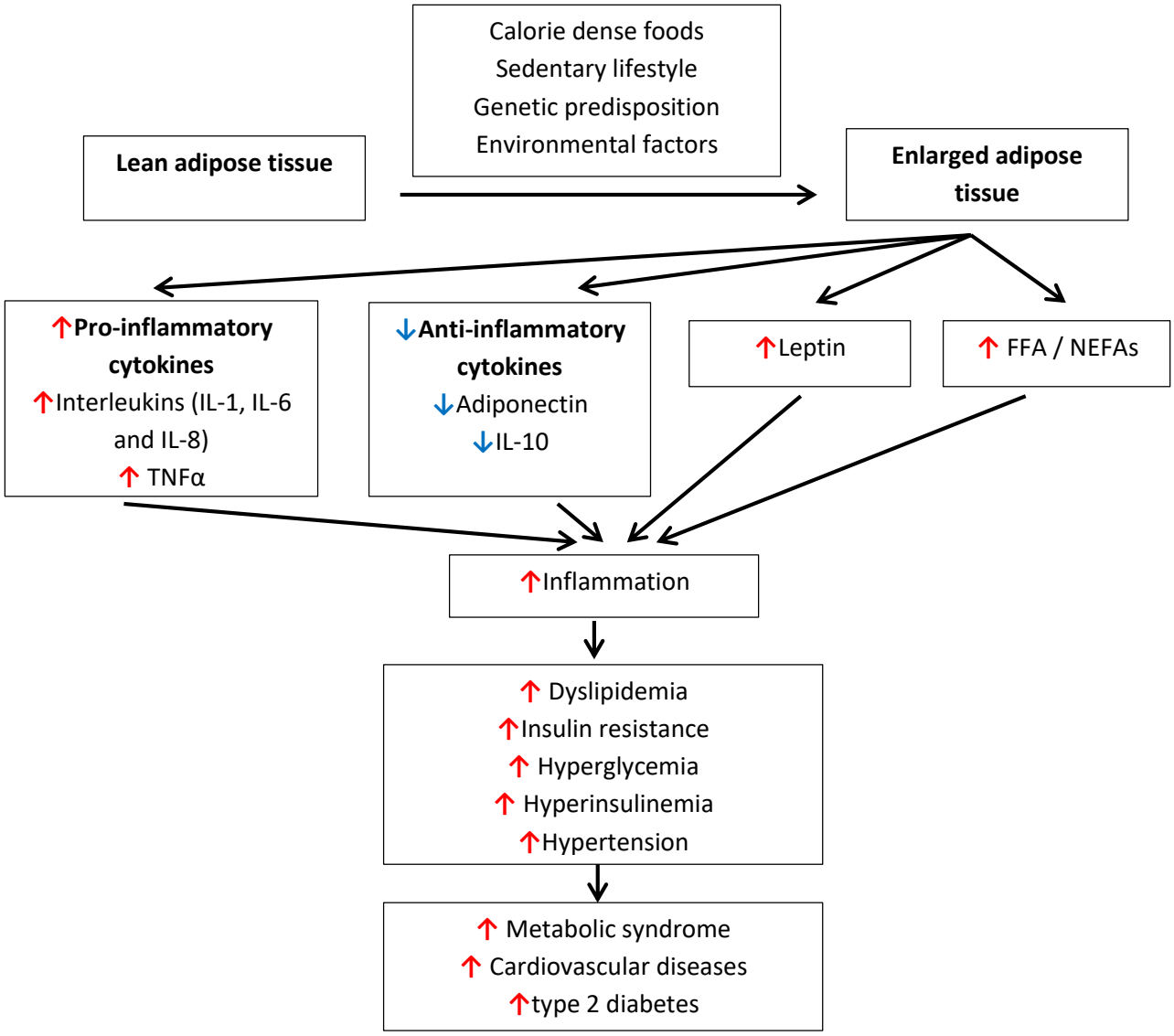


Figure 2.1: Secretion of inflammatory adipokines in obese state. In an obese state, excess adipose tissue leads to increased release of free fatty acids (FFAs), leptin and dysregulated secretion of adipokines, which resulted in metabolic comorbidities such as dyslipidemia, insulin resistance, hyperglycemia, hyperinsulinemia and hypertension. Obesity induces a phenotypic switch in adipose tissue from anti-inflammatory to pro-inflammatory macrophages. The production of pro-inflammatory adipokines such as interleukins (IL-1, IL-6 and IL-8), TNFα and CRP are increased in obese state, and the production of adipokines with anti-inflammatory properties, such as adiponectin, IL-10 and SFRP5 are reduced in obese state. Red arrow indicates increase in obesity-induced metabolic changes (pointing upward) and blue arrow indicates decrease in obesity-induced metabolic changes (pointing downward). FFAs, free fatty acids; NEFA, non-esterified fatty acids; IL, interleukin; TNF-α, tumor necrosis factor-α.

In the obese state, excess adipose tissue leads to increased release of free fatty acids (FFAs) and dysregulated secretion of adipokines which triggers a series of inflammation-related pathophysiological processes (Van Gaal, Mertens, & De Block, 2006) (Figure 2.1). Obesity induces a phenotypic switch in adipose tissue from anti-inflammatory to pro-inflammatory

macrophages. The activation of innate immune system in adipose tissue promotes an increase in the production and release of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, TNFα and C-reactive protein (CRP) and a reduction in the anti-inflammatory cytokines such as adiponectin and IL-10. This has contributed to the development of metabolic comorbidities including hypertension, hyperglycemia, hyperinsulinemia, dyslipidemia and insulin resistance, which eventually increase the risk of cardiovascular diseases and type 2 diabetes.

Previous studies implicated chronic low grade inflammation in the interplay between obesity and metabolic complications including atherosclerosis, cardiovascular diseases, diabetes and hypertension. Numerous studies have reported significant positive association between inflammatory markers, mainly high sensitivity C-reactive protein (hsCRP) levels with obesity and features of metabolic syndrome, including adiposity, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and low HDL cholesterol (Rodríguez-Hernández et al., 2013). hsCRP is a systemic inflammatory marker and is now widely known to be an independent risk factor for future cardiovascular events (Devaraj, Singh, & Jialal, 2009). The accumulation of free fatty acids in obesity activates pro-inflammatory serine kinase cascades, such as IkB kinase and c-Jun N-terminal kinase, which in turn promotes adipose tissue to release IL-6. The release of IL-6 triggers hepatocytes to synthesise and secrete hsCRP, indicating a state of inflammation. Previous studies have reported that weight loss was associated with a decline in hsCRP levels (Selvin, Paynter, & Erlinger, 2007). A systematic review reported that reduction of hsCRP levels may be due to the consequence of weight loss following a moderate reduction in calorie intake (Selvin, Paynter, & Erlinger, 2007). A reduction in the excess body fat stores can significantly reduce the inflammatory state (Petelin et al., 2014). Therefore, weight loss is essential to reduce inflammation and the risk of cardiovascular disease in overweight and obese individuals.

2.2.2 Obesity and hypertension

Hypertension is a chronic medical condition when the blood pressure persistently at or >140/90 mmHg, but not at the normal level which is defined as 100–140mmHg and 60–90 mmHg for systolic and diastolic blood pressure, respectively (Pickering et al., 2005). National Health and Morbidity Survey (NHMS) 2015, reported that one in three (33%) which translated to about 6.1 million of Malaysians were suffering from hypertension (Institute for Public Health, Malaysia, 2015). Hypertension is an important risk factor for myocardial infarction, stroke, kidney failure and premature death (Lai et al., 2014). Previous studies have mentioned that

elevated high blood pressure was highly associated with obesity, and the prevalence of hypertension substantially increased with increasing BMI (Tesfaye et al., 2007). Although the relationship between obesity and hypertension is well established, but the mechanism by which obesity directly causes hypertension is still under investigation. The pathogenesis of obesity-related hypertension described earlier included i) the activation of the sympathetic nervous system (SNS), ii) activation of the renin-angiotensin system (RAS), iii) hyperinsulinemia, iv) structural changes in the kidney, and v) elaboration of adipokines (hormones produced in fat itself) such as leptin (Esler et al., 2006).

The activation of the renin-angiotensin system (RAS) increases the production of circulating renin, angiotensinogen, and angiotensin II. This has led to elevated blood pressure by inducing systematic vasoconstriction, increasing renal tubular reabsorption of sodium and stimulating the synthesis of the sodium-retaining hormone aldosterone. Similarly, obesity is associated with hyperinsulinemia. The increase in insulin can in some circumstances enhances tubular reabsorption of sodium, and hence increases arterial pressure. Structural changes in the kidney secondary to obesity, may also important, as the fat deposit around kidney together with abdominal pressure secondary to obesity, has been proposed as an additional cause of renal sodium reabsorption disorder (Serra et al., 2008). Leptin, a hormone produced in fat promotes satiety by activating sympathetic nervous system to enhance thermogenesis, can cause hypertension as well (Bravo et al., 2006). A meta-analysis of 25 randomised controlled trials (RCTs) provided evidence that weight loss was significant associated with reduction in blood pressure (Neter et al., 2003). Therefore, weight loss is an important factor for the prevention and treatment of hypertension.

2.2.3 Obesity and dyslipidaemia

After the consumption of a diet containing fat, triglyceride (TG) is lipolysed into free fatty acids (FFAs) and 2-monoacylglycerols (MAG) and both are taken up by the enterocytes via passive diffusion (Hussain, 2014). Cholesterol is taken up by the enterocytes via the specific cholesterol transporter Niemann-Pick C1 Like 1 protein (NPC1L1). Once at the enterocytes, cholesterol is transformed to cholesterol ester, whereas FFAs and MAG assembled to TG again. Cholesterol ester and TG are then packed together with phospholipids and apo-lipoprotein (apo) B48 to form chylomicrons. Liver synthesises TG-rich-lipoprotein, known as very low-density lipoprotein (VLDL), which is increased post-prandially when the TG and FFAs reached liver (Lewis, 1997). Chylomicrons and VLDL deliver FFA to heart, skeletal muscle and adipose

tissue for energy expenditure and storage. Adequate lipolysis of TG-rich lipoprotein (e.g. chylomicrons and VLDL) is necessary to release FFA into the circulation. Lipoprotein lipase (LPL) is the primary enzyme for the TG lipolysis (Goldberg, Eckel, & Abumrad, 2009). The amount of the FFA released into the circulation is depended on the activity of LPL, which is stimulated by insulin (Sadur & Eckel, 1982). Therefore, postprandial increase in the insulin is very important in regulating the fuel storage, as increase in insulin inhibits the activity of LPL, thus reducing the lipolysis of TG. Chylomicrons and VLDL will shrink during the lipolysis to form chylomicron remnants and small dense LDL. Chylomicron remnants will be taken up by liver via apo E, hepatic lipase, LDL receptor or LDL receptor-related protein, while dense LDL will be taken up by liver via LDL receptor (Cooper, 1997).

Intestine and liver also play an important role in the reverse cholesterol transport by the synthesis of HDL particles (Rothblat & Phillips, 2010). HDL promotes the uptake of excess cholesterol from the peripheral tissue and delivers them back to the liver. Enterocytes and hepatocytes synthesise apo A1, which is a structural protein to HDL. Apo A1 enters the bloodstream and arrives at the peripheral tissue such as heart. In the circulation, Apo A-1 interacts with a complex molecule known as ATP-Binding Cassette, Sub-Family A, Member 1 (ABCA1), expressed on the surface of macrophages in the arterial wall to remove excess cholesterol from the macrophages and to form nascent HDL-c particles (pre-β HDL) (Lee & Parks, 2005). Subsequently, the nascent HDL cholesterol interacts with scavenger receptor class B member 1 (SR-B1) and ATP-binding cassette, sub-family G, member 1 (ABCG1), to incorporate more cholesterol to form a mature molecule of HDL cholesterol (α-HDL) (Tall, 1994). The cholesterol within the HDL is converted to cholesteryl esters by the lecithin-cholesterol acyltransferase (LCAT). In the circulation, cholesterol ester in HDL is transferred to other lipoproteins (e.g. LDL and VLDL) in exchanged for TG. In the liver, hepatic lipase hydrolyses HDL-associated TG inducing the formation of smaller HDL cholesterol which contributes again to the reverse cholesterol transport. Therefore, lipid metabolism is a dynamic process and is highly depended on numerous factors such as postprandial state, TG-rich lipoprotein concentration, HDL level, insulin level and adipose tissue formation (Klop, Elte, & Cabezas, 2013).

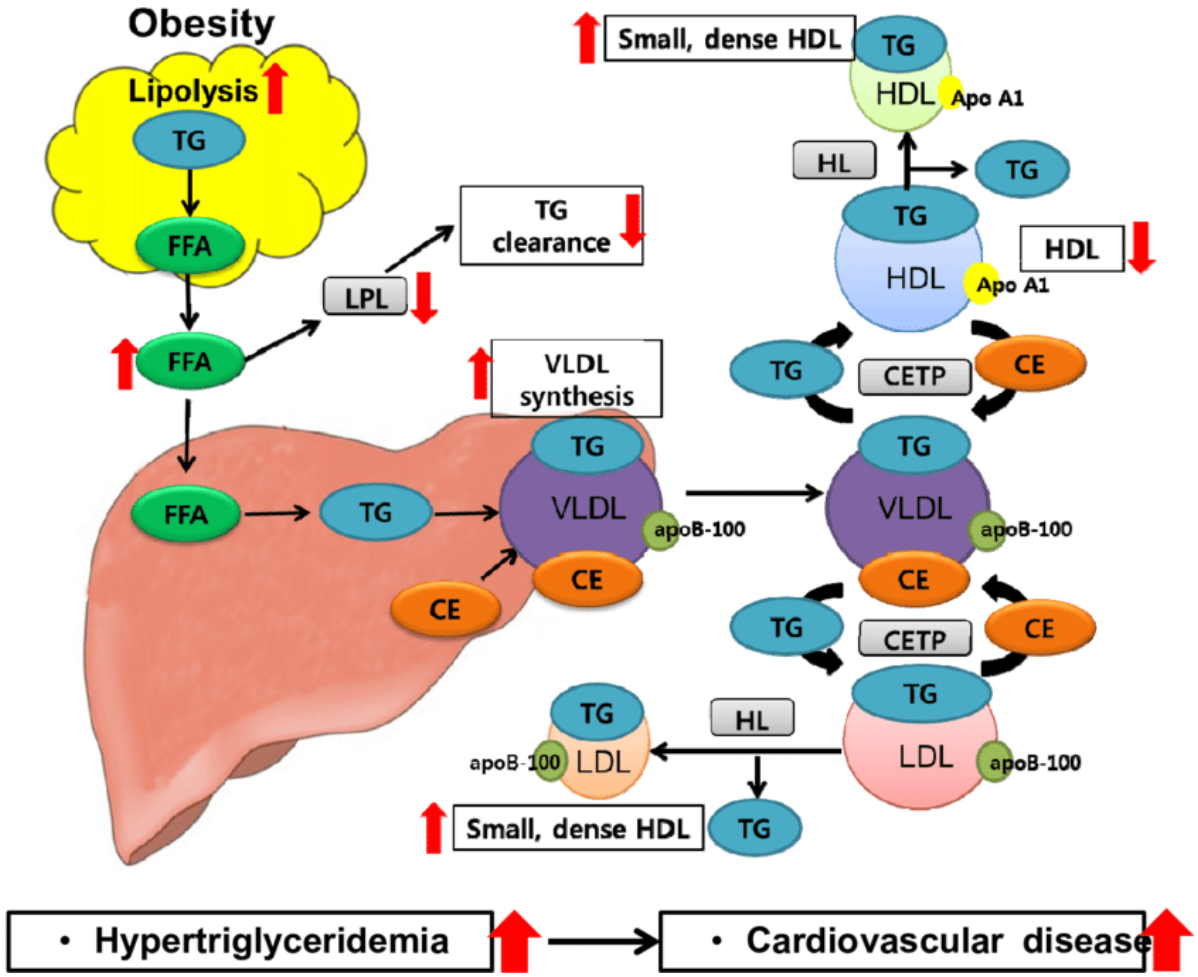


Figure 2.2: The hallmark of dyslipidaemia in obesity. The hallmark of dyslipidaemia in obesity is characterised by hypertriglyceridemia which is due to the increase influx of free fatty acid (FFA) to the liver, cause hepatic accumulation of triglycerides (TG). This leads to an increase in the synthesis of TG-rich lipoprotein, very low density lipoprotein (VLDL), which impairs the lipolysis of chylomicrons due to competition of lipoprotein lipase (LPL) with increased remnant TG being transported to the liver. The inhibition of lipoprotein lipase in adipose tissue and skeletal muscle, further promotes hypertriglyceridemia. Hypertriglyceridemia further induce and increase the exchange of TG from VLDL with cholesterol esters from HDL and LDL, producing TG rich HDL and LDL. The TG-rich LDL is hydrolysed by hepatic lipase (HL) and ultimately leads to the formation of small, dense LDL particles Moreover, lipolysis of this TG-rich HDL by hepatic lipase (HL) will reduce the affinity of apo A1, which leads to dissociation of apo A1from HDL. The decreased HDL concentration and formation of small, dense LDL particles are linked to a higher risk of cardiovascular disease. Red arrow represents increased (when pointing upward) or decreased (when pointing downward) responses to obesity. CE, cholesteryl esters; CETP, cholesteryl ester transport protein; FFA, free fatty acids; HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; LPL, lipoprotein lipase; TG, triglyceride; VLDL, very-low-density lipoprotein. Source of figure: (Jung & Choi, 2014)

The hallmark of dyslipidaemia in obesity is characterised by elevated circulating free fatty acids, triglyceride levels and LDL cholesterol with increase small dense LDL, and reduction in HDL cholesterol levels (Figure 2.2) (Klop et al., 2013). Obesity will increases circulating free fatty acids (FFAs) due to i) expanded fat mass (intracellular lipolysis of adipose tissue); ii) increased lipolysis of TG-rich lipoprotein from the circulation iii) inhibited anti-lipolytic effect

of insulin and iv) reduced FFA clearance (Boden, 2008). Increase influx of free fatty acids (FFAs) in liver will then leads to hepatic de novo lipogenesis and accumulation of triglycerides which subsequently increases synthesis of TG-rich lipoprotein, very low density lipoprotein (VLDL) (Jung & Choi, 2014). Increase in the VLDL hampers the lipolysis of chylomicrons due to the competition at the level of LPL, which contributes to hypertriglyceridemia. Lipolysis is further impaired by obesity due to the reduction in the mRNA expression of LPL in adipose tissue (Clemente-Postigo et al., 2011) and reduced LPL activity in skeletal muscle (Pollare, Vessby, & Lithell, 1991), which also promoting hypertriglyceridemia.

Hypertriglyceridemia further triggers the exchange of triglycerides for cholesterol esters between triglyceride-rich lipoprotein (VLDL, immediate-density lipoprotein) and lipoproteins which are relatively richer in cholesterol esters (HDL and LDL). This eventually leads to a decrease in HDL-cholesterol concentration and increase in small, dense LDL cholesterol (Jung & Choi, 2014). The increase in TG-rich LDL is hydrolysed by hepatic lipase, which ultimately leads to the formation of small, dense LDL particles. Moreover, lipolysis of this TG-rich HDL by hepatic lipase will reduce the affinity of apo A1, which leads to dissociation of apo A1 from HDL (Chatterjee & Sparks, 2011). This may impair reverse cholesterol transport. The decrease in the HDL concentration and the formation of small, dense LDL particles are linked to a higher risk of cardiovascular disease (Ivanova et al., 2017).

2.2.4 Obesity and insulin resistance

The association of obesity and type 2 diabetes has been well recognised and the major basis for this link is the ability of obesity to induce insulin resistance (Hardy, Czech, & Corvera, 2012). Insulin resistance is the fundamental aspect for the pathology of type 2 diabetes and was associated with other co-morbidities hypertension, dyslipidaemia, metabolic syndrome and atherosclerosis. It is a condition where the somatic cells fail to respond to insulin or they do not use it effectively (Qatanani & Lazar, 2007). This may lead to hyperglycaemia and hyperinsulinemia as the pancreatic β-cell produce more insulin to cope with the elevated blood glucose levels (Reaven, 2004).

As mentioned earlier, adipose tissue is one of the insulin-responsive tissue, which insulin stimulates the storage of TG in adipose tissue through multiple mechanisms such as i) inducing the differentiation of pre-adipocytes to adipocytes, ii) increasing the uptake of glucose and fatty acids derived from circulating lipoproteins and lipogenesis in mature adipocytes, and iii)

inhibiting lipolysis (Jung & Choi, 2014). Insulin signalling is initiated when insulin binds to the receptors on the cell membranes and activates the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway (Hemmings, 2012). Phosphorylation of insulin receptor substrate (IRS) proteins will triggers the activation of PI3K and conversion by its catalytic domain of phosphatidylinositol (3,4)-bisphosphate (PIP2) lipids to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Membrane-bound PIP3 recruits and activates PDK-1, which phosphorylates and activates AKT/PKB. Ultimately, these signalling events lead to the translocation of glucose transporter 4 to the plasma membrane, and increase the adipocyte glucose uptake (Boucher, Kleinridders, & Ronald Kahn, 2014). In the adipose tissue, insulin also exert an anti-lipolytic effect, whereby the activation of PI3K stimulates phosphodiesterase-3 to hydrolyse adenosine 3',5'-cyclic monophosphates, which in turn limits the release of fatty acids from adipocytes.

Previous evidence suggested that excess free fatty acids (FFAs) and various adipokines released from adipose tissue have been involved in abnormal insulin signalling. Enlarged adipose tissues reduce the release of adiponectin which then reduce mitochondrial fatty acid oxidation at multiple tissues, and causes increase in intracellular FA content (Boden, 2011). Limited ability to adjust fat oxidation to match with fat intake resulted in positive fat balance and weight gain in long term (Weyer et al., 2000). Increase in adipose tissue increases leptin levels which is supposed to enhance fatty acid oxidation. However, due to leptin resistance, it further promotes cytosolic FA build up. As a result, accumulation of excess intracellular fatty acid causes the development of insulin resistance. The consequences of the accumulation of intracellular fatty acids content included i) reduction of fatty acid oxidation and glucose uptake in skeletal muscle and ii) decrease in hepatic glycogen synthesis and increase hepatic gluconeogenesis which lead to hyperglycaemia. Increase in plasma glucose levels stimulates the release of insulin in pancreas, which eventually leads to hyperinsulinemia. Obesity-induced insulin resistance is also associated with increased secretion of cytokines and other bioactive substances from adipose tissue as well as the number of adipose macrophages. The accumulation of M1 macrophages in adipose tissue will result in increased secretion of various pro-inflammatory chemokines and cytokines such as monocyte chemotactic protein (MCP-1), tumour necrosis factor (TNFα), interleukin (IL)-1, IL-6 and IL-8 that may interfere with insulin signalling and enhance adipocytes insulin resistance through induction of proteins that bind to the insulin receptors. Moreover, the production of insulin sensitizing adipokines with anti-inflammatory properties such as adiponectin is reduced in the obese state.

Concurrently, insulin resistance has featured with atherogenic lipid profiles such as elevated total cholesterol, serum triglyceride, LDL cholesterol and a decline in HDL cholesterol (Savage, Petersen, & Shulman, 2007). Hypertriglyceridemia involves in the early step of insulin action pathway, but and alternatively, insulin resistance may also cause hypertriglyceridemia (Al-mahmood, Afrin, & Hoque, 2014). Hypertriglyceridemia increases intrahepatic lipid accumulation which subsequently leads to stimulation of β-oxidation and production of reactive oxygen species (ROS). ROS increases the gene transcription and synthesis of cytokines such as TNF and IL-6. This leads to the activation of JNK pathway which phosphorylates IRS-1 serine (Insulin receptor substrate) that inhibits phosphorylation of IRS-1 at the tyrosine residue. Eventually, it diminishes insulin signaling and increases the risk of developing insulin resistance. On the other hands, insulin plays a role in mediating the secretion of VLDL. Hypersecretion of VLDL leads to hypertriglyceridemia that consequently lowers the circulating HDL cholesterol and increases the small dense LDL cholesterol which are the hallmark of dyslipidaemia in obesity (Sparks, Sparks, & Adeli, 2012). The ability of insulin to inhibit VLDL secretion may be diminished in obese individuals with insulin resistance, causing VLDL hypersecretion and hypertriglyceridemia (Choi & Ginsberg, 2011).

2.2.5 Obesity and leptin

Leptin, produced by adipose tissue, is the best known hormone marker for obesity. It is an anorexigenic peptide hormone which associated with insulin resistance and metabolic syndrome (Gupta et al., 2010). It plays a critical role in regulating body weight by decreasing the food intake during energy imbalance and increasing energy expenditure (Halaas et al., 1997). It regulates body weight by signalling the hunger and satiety centre in the brain as an indication of satiety, thus inhibiting the release of orexigenic peptide hormones and subsequently reduce food intake. Although leptin reduces appetite as a circulating signal, however obese individuals generally exhibit higher level of circulating leptin concentration than normal individuals due to higher percentage of body fat. This is supported by previous studies which have proven that serum leptin level is positively correlated with body mass index (BMI) and body fat percentage in obese population (Al Maskari & Alnaqdy, 2006). The overproduction of leptin and over activation of leptin receptor in obese individuals will activate negative feedback loops that block the leptin signalling, and this may be one of the factors that contribute to the development of leptin resistance (Myers et al., 2010). Thus, despite the high circulating leptin or the existence of functional receptors, human brain fails to control and

modulate body weight due to leptin resistance. The diminished expected anorexigenic effect of leptin will lead to hyperphagia and obesity. A study reported that significant weight loss of 23% from baseline, with a low caloric diet resulted in significant improvement and restoration of circulating leptin level in obese individuals (Rolland, Hession, & Broom, 2011).

2.3 Lifestyle factors contribution to obesity

2.3.1 Dietary factors

Consumption of energy-dense food coupled with sedentary lifestyle, are the main contributors for the development of obesity and its complications nowadays. The 'Westernization' of diet had led to an increase in the consumption of fast foods, high energy-dense foods, sugar, animal fats and refined carbohydrate (Soon, 2014). Malaysian Adult Nutrition Survey (MANS) 2014 reported that there was an increase in the consumption of 'processed foods' added with salt and condiments compared to the MANS 2003 (Institute of Public Health, Malaysia, 2014). This unhealthy dietary practice and lack of physical activity are the major contributors for the rising epidemic of obesity and associated chronic diseases in the Malaysia population.

A number of scientific studies have suggested that varying the dietary macronutrient composition of the intervention diet can vary the magnitude of reduction of the individual's weight. Dietary composition plays an important role in determining the energy density of the diet, thus affecting the total caloric intake of the diet (Rolls et al., 1994; Rolls, Hetherington, & Burley, 1988). At the cellular level, this will lead to change in appetite regulation, thermogenesis and metabolism (Abete et al., 2010). However, the effectiveness and possible advantages of different macronutrient ratio intake on weight loss have not been established. Some studies showed that low-carbohydrate, high-protein diets resulted in greater weight loss compared to a conventional carbohydrate diet (Piatti et al., 1994), but some trials reported that high-carbohydrate, low-fat vegetarian diet resulted in greater weight loss compared to a conventional high-carbohydrate, low-fat diet (Barnard et al., 2009). Thus, there is no one-size-fits-all dietary regime that is more effective than others for weight loss.

A meta-analysis reported that low-carbohydrate diet (≤45% of energy from carbohydrates) is more favourable in improving blood lipid profile in obese individuals, by decreasing triglyceride levels and increasing HDL cholesterol levels compared to a low-fat diet (≤30% of energy from fat) (Hu et al., 2012). While low-fat diets are more pronounced in reducing LDL cholesterol and total cholesterol levels compared to low-carbohydrate diets. Some studies

reported that severely obese individuals with diabetes or the metabolic syndrome lost more weight after six months of carbohydrate-restricted diet compared to a calorie- and fat-restricted diet, with a relative improvement in insulin sensitivity and triglyceride levels (Samaha et al., 2003; Yancy et al., 2004). However, extremely low carbohydrate diet is not recommended for sustained weight loss program. A 6-week intervention study reported that ketogenic low carbohydrate diet (5% calories from carbohydrate) and non-ketogenic low carbohydrate diet (40% calories from carbohydrate) resulted in the same reduction in body weight, but ketogenic diet is associated with side effects such as emotional effects and adverse metabolic responses (Johnston et al., 2006). Besides, it may lead to breath odor, constipation, loss of appetite, kidney distress and organ failure (Bilsborough & Crowe, 2003).

Sacks et al., 2009 reported that reduction in calories resulted in clinically beneficial regardless of the macronutrient intake. In this study, participants were assigned into one of the four group diets which the energy derived from fat, protein and carbohydrate were, 20%, 15% and 65%; 20%, 25% and 55%; 40%, 15% and 45%; 40%, 25% and 35%. All the diets showed improvement in lipid profile, insulin level and reduction from the initial weight (Goetz-Perry, 2009). This finding was supported by other clinical trial which investigated the effect of energy restricted diet on weight loss (Luscombe et al., 2003). They found significant weight loss after a 12-week of energy-restricted diet (6.5MJ/day) and 4-week of energy-balanced diet (8.2MJ/day) on non-diabetic obese volunteers with hyperinsulinemia, regardless of the macronutrient composition of the diet. These findings suggest that caloric restriction, rather than macronutrient composition may play a role in determining weight loss. Therefore, the initial approach to weight loss should start with reduction in total energy consumption.

2.3.2 Physical activity

In the past decade, Malaysia has been experiencing an accelerated phase of industrialisation and urbanisation. This has brought about change in dietary pattern coupled with reduced physical activity in the Malaysia population (Noor, 2002). According to the findings from the Malaysian Adult Nutrition Survey (MANS), only 16% of the Malaysian men and 10% of the Malaysian women were considered to be physically active (Poh et al., 2010). Sedentary lifestyle with low physical activity level is one of the most critical determinants that increase the risk of developing obesity (Shields & Tremblay, 2008). As we mentioned previously, to promote weight loss, it is necessary to create energy deficit or negative energy balance. Physical activity is known to be the only variable component of energy expenditure which can

be manipulated to modify body weight (Jakicic & Otto, 2005). Therefore, increase physical activity is a common strategy to increase energy expenditure and reduce body weight in weight management programmes.

Exercise not only can promote the loss of body fat mass, but also can prevent the weight loss-induced loss of lean muscle mass and increase basal metabolic rate, and hence promote total energy expenditure (Stiegler & Cunliffe, 2006). All these outcomes are unable to be achieved by dieting alone. American College of Sports Medicine and American Heart Association has recommended healthy adults to perform ≥150 min/week of moderate-intensity aerobic physical activity to maintain health (Haskell et al., 2007). However, Institute of Medicine (IOM) suggested that at least 60 min/ day (420min/week) of moderate-intensity physical activity is necessary to prevent transition to overweight or obesity (Lee et al., 2010). Moreover, previous studies suggested that exercise may be able to trigger self-initiate change to adapt active lifestyle and healthy eating pattern, which may result in sustainable weight maintenance (Atlantis et al., 2004). Therefore, high level of physical activity can be the key behaviour to maintain healthy eating habit and to achieve sustainable weight loss outcomes.

2.3.3 Smoking

Smoking is also an important contributing factor for obesity. In short term, nicotine increases energy expenditure and may reduce appetite which subsequently leads to weight loss (Hofstetter et al., 1986). This may explain the association of smoking with lower body weight and body mass index compared to the non-smokers, and the association of smoking cessation with weight gain (Filozof, Fernández Pinilla, & Fernández-Cruz, 2004) (Chiolero et al., 2008). Given that the aforementioned metabolic effect of smoking on body weight, it is expected that greater number of cigarettes smoked should be associated with lesser body weight. However, heavy smokers were found to have greater body weight compared to the light smokers or non-smokers, particularly in men (Chiolero et al., 2007). The mechanisms underlying the association of heavy smokers and greater body weight were poorly understood. One explanation could be that heavy smokers are more likely to adopt risk behaviours favourable to weight gain. Chiolero et al., 2006 reported that strong clustering risk behaviours such as low physical activity, low intakes of fruit and vegetables, and high alcohol intake were found to be correlated with the level of cigarettes smoked. In addition, smoking was associated with the central fat accumulation, impaired glucose tolerance and insulin resistance, and these factors

subsequently increase the risk of developing cardiovascular diseases and type 2 diabetes (Chiolero et al., 2008).

Although majority of the smokers gained weight after they quit smoking, however smoking cessation was associated with substantial health benefits and was found to improve insulin sensitivity, despite of the weight gain (Filozof et al., 2004). The authors reported that a dietary intervention (intermittent very-low-calorie diet) plus nicotine gum was introduced to a group of women who failed to quit smoking due to weight gain. The study participants had successfully quit smoking and prevented the post-cessation weight gain at the end of the intervention (Filozof et al., 2004). Therefore, the combination of smoking cessation and calorie restriction dietary intervention may be effective in inducing weight loss and improving metabolic health in the smokers.

2.3.4 Alcohol consumption

Alcohol is an important source of energy. Despite the given fact that 1 gram of alcohol provides 7.1 kcal (29 kJ), and studies showed that increase in total energy with alcohol consumption can certainly promote positive energy balance and may ultimately lead to weight gain (Yeomans, 2010), it is still controversial whether increase in alcohol consumption increases the risk of obesity and weight gain. Evidence from several metabolic studies reported that alcohol consumption is associated with suppression of lipid oxidation, and could lead to positive fat balance in the long term due to the substitution of ethanol for fatty acids as the major hepatic fuel (Baraona & Lieber, 1979). The degree of fat accumulation depends on the supply of dietary fat, and the accumulated excess fats in liver may result in moderate hyperlipidaemia (Baraona & Lieber, 1979). Moreover, alcohol consumption before or during meals may stimulate food intake. This may due to the enhancement in the short-term rewarding effects of foods. Although this finding may suggest that alcohol is a risk factor for obesity, however epidemiological data suggests that moderate alcohol intake may be protective against obesity, particularly in women.

Obesity is a multi-factorial disease and it is difficult to truly assess the clear cause-and-effect association between alcohol intake and weight gain based on the current mixed and conflicting available evidence. A systematic review reported that the findings from large cross-sectional studies including prospective and cohort studies were inconsistent and contradictory, and the overall results do not conclusively confirm a significant positive association between alcohol consumption and weight gain (Sayon-Orea, Martinez-Gonzalez & Bes-Rastrollo, 2011). However, positive association was found in higher levels of alcohol suggesting that such effect

on obesity and weight gain was commonly in heavy drinkers compared to light drinkers. Recent prospective studies also reported that light-to-moderate alcohol intake was not associated with adiposity gain, whereas heavy drinking was more consistently related to obesity and weight gain (Traversy & Chaput, 2015). Therefore, moderation in alcohol consumption is still an important dietary recommendation, together with a healthy eating habit and lifestyle to prevent obesity and weight gain.

2.4 Obesity and weight loss

Obesity and obesity related non-communicable diseases are known to be preventable through small changes in lifestyle that promote active lifestyle and healthy eating. Previous evidence reported that a moderate weight loss of 5-10% in body weight (Brown et al., 2016; Wing & Phelan, 2005), particularly in visceral adipose tissue (Goodpaster et al., 1999; Kwon, Kim, & Kim, 2017; Pascale et al., 1992) had beneficial effects in insulin sensitivity, glycaemic control and cardiovascular disease risk. Thus, reducing body weight in overweight and obese individuals is important in improving the health status of the Malaysian population. However, over the years, strategies to combat obesity remained ineffective, particularly in long term weight loss programmes (Bombak, 2014). Past studies have investigated interventions based on various dietary patterns by varying the dietary macronutrient composition (Goss et al., 2013; Sacks et al., 2009). Other strategies that have been used to facilitate weight management include reduction in energy density, portion control through meal replacement, and improved diet quality (Davis et al., 2010; Fung et al., 2015; Heymsfield et al., 2003; Rolls et al., 2005). A key strategy for weight management that can be applied across dietary patterns is to reduce energy density of the meals. Clinical trials reported that reducing energy density is effective for weight loss and weight loss maintenance (Ello-Martin et al., 2007; Ledikwe et al., 2007).

2.4.1 Weight management programmes conducted in the Malaysian population

To date, there are only a few weight management intervention studies which have been experimented in the Malaysian population using different strategies such as dietary and physical activity based programmes or behavior modification programmes (Table 2.1). Most of the intervention programmes were conducted in community settings and government (public) sectors. However, the results were conflicting and unsatisfactory. A 12-week weight loss intervention study using the combination of exercise and 2-hours of nutritional education per month reported no improvement in dietary intake and changes in steps/physical activity in 28 employees of University Putra Malaysia (UPM) with abdominal obesity (Soon et al., 2013).

Although no significant improvement was found in body weight, BMI and blood lipids profile in the intervention group, but fasting blood glucose was significantly reduced compared to the control group. The authors concluded that behavior modification programmes through changes in dietary intake and physical activity level may be effective in reducing fasting blood glucose levels in individuals with abdominal obesity.

A study reported that CORFIS (Community-based Cardiovascular Risk Factors Intervention Strategies) resulted in significant improvement in blood pressure and reduction in body weight and waist circumference in patients with hypertension. Dietician counselling was provided to the study participants to promote healthy lifestyle behaviors such as regular exercise by walking >10,000 steps/day, increase fruits and vegetables consumption and reduce salt intake (Karupaiah et al., 2015). However, this study did not evaluate the health outcomes on obesity-related blood metabolic markers such as blood lipids levels and glycaemic indices. Therefore, the beneficial effects of CORFIS on metabolic health in the Malaysian adults were unable to be evaluated. A recent weight loss intervention, My Body is Fit and Fabulous at Home (MyBFF@Home), which recruited overweight and obese housewives (mainly Malays, 88.6%) with low social-economic status, reported only a mean weight loss of 1.13kg (±2.70SD) was observed in the intervention group after 6 months of the intervention period (Kassim et al., 2017). The intervention provided individual and group counselling on behavior modification such as reduction of a minimum of 500 kcal/day (through diet and physical activity), and approximately 45 to 60 minutes per day of moderate physical activity (target PA level>1.70). However, this study did not evaluate the outcomes on obesity-related blood metabolic markers.

A 2-year follow-up worksite health promotion programme was conducted in 186 Malay-Muslim male security guards (Moy, Sallam, & Wong, 2006). This programme provided one-to-one counselling (twice a year) and group teaching (3-4 times per year) of dietary advice based on the National Cholesterol Education Programme (NCEP) Step I diet (<30% of total energy from fat, <10% of energy from saturated fat, and <300mg cholesterol per day) and the American Heart Association (AHA) guide to improve cardiovascular health at the community level. The study reported significant reduction in total cholesterol levels (mean reduction of 0.21mmol/L) and significant increase in HDL cholesterol levels (mean increase of 0.21mmol/L) in the intervention group compared to baseline value. However, no improvement in BMI was found in the intervention group. A 10-week of lifestyle intervention programme reported a mean weight loss of 5.76 kg and significant reduction in total cholesterol, LDL cholesterol, triglyceride and fasting blood glucose levels in 41 overweight and obese individuals recruited

from an organisation-wide workplace wellness initiative (Ang et al., 2017). However, the sustainability of the weight loss outcomes was questionable as the duration of the intervention was short. A 6-month worksite health programme (n=28) which provided two weekly sessions of unsupervised exercise and monthly dietary/health education sessions reported significant reduction in percent body fat (a reduction of 0.9%) compared to the baseline (Ramli et al., 2013). However, no significant improvement was found in body weight. Therefore, it is essential to develop and establish an effective weight management strategy to combat the high prevalence of obesity and obesity-related chronic diseases in overweight and obese Malaysian population.

Table 2.1: Weight management programmes conducted in the Malaysian population

Author, Year	Population characteristics (sample size, location and age range)	Intervention	Duration and study design	Short description of the intervention programmes	Outcome measures	Results
Soon et al., 2013	56 employees from Universiti Putra Malaysia (UPM) with abdominal obesity (WC≥90 cm for men and ≥80cm for women); aged 22-55 years	Lifestyle modification (diet and exercise)	12 weeks of RCT	Participants in the intervention group were required to walk at least 10,000 steps per day and to reduce daily calorie intake, intakes of saturated fat, trans-fat and cholesterol, increase fibre intake, and minimise salt intake.	Body weight, BMI, WC, HC, WHR, SBP, DBP, TC, LDL-C, HDL-C, TG, FBG, calorie intake and steps per day	Significant increase in HC and reduction in FBG was found in the intervention group compared to the control group.
Moy, Sallam, & Wong, 2006	186 Malay males security guard recruited from a public university in Kuala Lumpur; Mean age of 46 (±SD 7) years and 48 (±SD 5) years in the intervention and control groups, respectively; Mean BMI of 26 (SD±3) kg/m^2	Lifestyle modification (diet and exercise)	2 years of quasi-experimental study	Intervention group received one-to-one counselling (at least twice yearly) and group teaching (3–4 times per year) on nutrition, physical activity and smoking cessation. Dietary advice was based on the National Cholesterol Education Programme (NCEP) Step I diet and the American Heart Association Guide for improving cardiovascular health at the community level.	Body weight, BMI, WC, HP, SBP, DBP, TC, LDL-C, HDL-C, TG, FBG, dietary practices, physical activity, smoking	The intervention group had significant reduction in TC levels compared to the control group. Reduction in the amount of cigarettes smoked was found in the intervention group as well.
Ramli et al., 2013	28 overweight and obese public sector office workers from the Ministry of Higher Education in Putrajaya, Malaysia; BMI ≥$25kg/m^2$; Aged 18-60 years	Lifestyle modification (diet and exercise)	6 months of pre-post quasi-experimental study	A 6-month obesity health programme, which consisted of two weekly unsupervised exercise sessions and monthly dietary/health education sessions.	Body weight, PBF, $V0_{2max}$, IPAQ score, EBBS score, sit and reach distance, push up repetition and partial curl up repetition	Participants showed significant reduction in PBF, V02max, partial curl up repetition and sit and reach distance, but no effect was found on body mass, self-perceived level of physical activity and behaviour toward exercise.

Kassim et al., 2017	328 overweight and obese low socio-economic status housewives in Klang Valley; BMI 25-39.9kg/m^2; Aged 18-59 years	Lifestyle modification (diet and exercise)	6 months of pre-post quasi-experimental study	My Body is Fit and Fabulous at home (MyBFF@home) study intervention consisted of a healthy diet, physical activity, and self-monitoring behaviour by trained health professionals (dietitians, nutritionists and physiotherapists). Participants were advised to reduce calorie intake and engage in moderate physical activities such as brisk walking, stairs climbing, and housework up to 45 minutes per day.	Body weight, BMI, SBP, and DBP	No significant difference was found between the intervention and control groups for all parameters.
Karupaiah et al., 2015	209 patients with hypertension; Mean BMI of 29.3 (SD±5.2) kg/m^2; Mean age of 49 (SD±9) years	Lifestyle modification (diet and exercise)	6 months of pre-post quasi-experimental study	CORFIS (Community-Based Cardiovascular Risk Factors Intervention Strategies) provided monthly dietician counselling with 7 individual appointments over 6 months. A pedometer was provided to monitor daily number of footsteps. Participants were categorised into i) nonadherent/N-A (Precontemplation, Contemplation, and Preparation), ii) newly adherent/NA (Action) and iii) totally adherent/TA (Maintenance) groups based on their self-reported compliancy with advocated healthful behaviours.	Body weight, BMI, WC, SBP, DBP, dietary sodium intake, pedometer steps, total MET activity and fruits and vegetables intake	Reduction in body weight and WC were largest in NA, followed by TA and N-A groups. Patients reporting a shift to positive stages of change behaviours had significant reduction in SBP, DBP, weight, and WC.
Ang et al., 2017	41 overweight and obese employees with BMI >23kg/m^2; Mean age of 34 (SD±7) years	Lifestyle modification (diet and exercise)	10 weeks of pre-post quasi-experimental study	Participants attended a 3-full day educational group classes held at the premises of their workplace over a 10-week period. The participants met as a group with the preventive care specialist, health educators and dietitians once a month.	Body weight, BMI, WC, PBF, SBP, DBP, TC, LDL-C, HDL-C, TG, FBG, self-body image and health quality of life	Mean weight loss of 5.76 kg or 7% of initial weight ($p < 0.001$) was found at week 10 of the intervention. Significant reduction was found in blood lipid levels and FBG. Self-body image and health quality of life were significantly improved.

BMI, body mass index; EBBS, exercise benefits and barrier; DBP, diastolic blood pressure; FBG, fasting blood glucose; HC, hip circumference; HDL-C, high-density-lipoprotein cholesterol; IPAQ, international physical activity questionnaire; LDL-C, low-density-lipoprotein cholesterol; MET, metabolic equivalent; PBF, percent body fat; RCT, randomised controlled trial; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VO_2 max, maximal oxygen uptake; WC, waist circumference; WHR, waist-hip ratio.

2.4.2 Recommended dietary interventions

2.4.2.1 Intermittent fasting or calorie-restriction?

In recent, intermittent fasting has becoming a popular strategy for weight loss. Various forms of intermittent fasting, such as complete alternate-day, 5:2 diets (severe energy restriction for 2 non-consecutive days a week and ad libitum eating the other 5 days), time-restricted feeding (eating for only 8 hours then fasting for the other 16 hours of the day), alternate-day fasting and religious fasting have been described earlier (Patterson et al., 2015). Promising effects on fat mass and insulin sensitivity have been reported when fasting duration is routinely extended beyond sixteen consecutive hours (Templeman et al., 2019). At the cellular level, intermittent fasting may increase resistance against oxidative stress, decrease inflammation, and promote longevity. However, scientific evidence for the health benefits of intermittent fasting in humans is often derived from animal studies, based on observational data on religious fasting, or from experimental studies with modest sample size and short duration. To date, the existing research is not sufficient to suggest intermittent fasting as a standard practice in healthcare setting. It is unknown which form of intermittent fasting is more effective and who will be benefitting the most from intermittent fasting in long-term. A long episode of fasting may lead to binge eating or large portions of unhealthy foods at the end of the fast. Intermittent fasting may only be benefitted to those individuals who are able to avoid overeating following fasting periods. More human clinical trials are needed to assess the long-term effect of intermittent fasting on behavioural or physiological changes, weight loss and metabolic health.

A recent review study has reported that, in clinical trials, caloric restriction and intermittent fasting resulted in similar degrees of weight loss and improvement in insulin sensitivity (Stockman et al., 2018). Calorie restriction is a dietary regime which reduces the total calorie intake but maintains proper nutrition. It is the only intervention that consistently found to be associated with decrease in the biological rate of aging and increase both average and maximal lifespan (Redman & Ravussin, 2011). Given that calorie restriction is a proven method for weight loss, for those who are interested in weight loss, calorie restriction may be easier to adopt compared to intermittent fasting, and it is as effective as intermittent fasting. Previous evidence suggested that, for overweight and obese individuals with a BMI of 27-31kg/m^2, a decrease of 300-500kcal per day was recommended to bring about a reduction of 0.25-0.5kg per week in body weight (Warrell, Cox, & Firth, 2005). Therefore, in our dietary intervention

study, a reduction of 300-500 kcal/day of total energy intake (depending on the individual's baseline energy intake and basal metabolic rate) was introduced in the formulated diet charts.

2.4.2.2 Low fat diets

To date, various calorie restriction dietary regimens have been proposed as conducive to weight control through various mechanisms (Raynor & Champagne, 2016). Low-fat diets are also one of the most common dietary strategies to lose weight as fat is a high energy dense and poor satiating food. Obese individuals tend to consume high energy dense foods compared to lean people because high energy dense food is generally more palatable. This may have caused passive overconsumption of total calorie intake, which subsequently lead to positive energy balance and weight gain (Halton & Hu, 2004; Slavin & Green, 2007). A review from 28 studies reported that a reduction of 10% in the percentage of dietary fat from total energy intake resulted in weight loss with 16g/d, but the effect of reducing dietary fat intake alone without reduction in total energy intake showed a little potential in weight loss (Bray & Popkin, 1998). Besides, other studies have reported that obese individuals showed a blunted lipid response following a plasma cholesterol lowering diet. It is suggested that diets low in saturated fat and cholesterol are less effective in improving lipid profile in obese individuals compared to lean individuals due to the excess adipose tissue (Flock, Green, & Kris-Etherton, 2011). Multiple mechanisms such as increased inflammation, diminished LDL receptor activity, abnormality in the enzyme activity involved in lipid metabolism and insulin resistance due to excess adipose tissue have altered lipid response to a plasma cholesterol lowering diet in obese individuals. In obese individuals, macronutrient composition of the diets has little effect on the degree of weight loss over the short term unless nutrient composition influences caloric intake (Hill, Drougas, & Peters, 1993).The beneficial effects on blood lipids and insulin resistance after a dietary intervention may due to weight reduction, not the changes in macronutrient composition (Westerterp-Plantenga et al., 2009). Hypocaloric diets (reduction of 500kcal from daily energy requirement) which bring about weight loss seem to have beneficial effects in improving lipid profile in obese individuals. Thus, bringing about weight loss and normalizing adipose tissue first is an important goal to improve lipid profile in obese individuals (Jansen et al., 1998).

2.4.2.3 High-protein diets with low or moderate carbohydrate intake

The most popular among the weight loss diets emphasise reduction of carbohydrate intake, and hence encouraging high protein intake, as high fat diets are generally avoided due to higher energy density. A high-protein diet with 25-30% of total energy from protein showed greater

reduction in weight and waist circumference and greater improvement in glycaemic control and cardio-metabolic parameters in various populations (Azadbakht et al., 2013; Farnsworth et al., 2003; Gulati et al., 2017). A meta-analysis of randomised controlled trials reported that by comparing with a standard protein, low-fat diet, high-protein, low fat diets provided greater reduction in body weight, blood triglyceride levels and preservation of fat-free mass (Wycherley et al., 2012). It is potentially effective in promoting satiety, reducing energy intake, inducing weight loss and maintaining lean muscle tissue (Astrup et al., 2015; Clifton, 2012). Elevated protein diet was associated with increased satiety, thermogenesis and glycogenesis which may lead to greater weight loss and improvement in insulin sensitivity (Westerterp-Plantenga et al., 2006).

Atkin diet, a high-protein, very low-carbohydrate, high-fat diet promoted by Atkins (60% of energy from fat, 35% of energy from protein and 5% of energy from carbohydrate) is perhaps the most popular high-protein diet in America for weight loss. However, healthcare professionals do not recommend long-term adherence to an Atkins-like, high-protein diet due to the high fat content of the diet (~60% energy), particularly the saturated fat (~25% of energy from SFA) and extremely low carbohydrate intake, which may eventually lead to elevations in total cholesterol and LDL cholesterol levels. Weight loss is initially high after Atkin diets and this may due to the fluid loss related to reduced carbohydrate intake, calorie reduction, and ketosis-induced appetite suppression (Weigle et al., 2005). These diets may have short-term beneficial effects on weight control, but concerns have also been expressed on long-term effects, notably with respect to cardiovascular outcomes. Long-term high protein or high meat intake in humans may lead to hyperuricemia (leading to joint pain and gout), hypercalcuria (leading to kidney stones, hypocalcaemia, and osteoporosis), renal malfunction, increased cancer risk, liver malfunction, and increased risk of coronary artery disease (Delimaris, 2013). Extra protein which is not used efficiently by the body and may impose a metabolic burden on the bones, kidneys, and liver. Extreme low-carbohydrate diets may also lead to ketosis, nausea, dizziness, constipation, headache, fatigue, and smelly breath.

Diets high in protein (30% of energy from protein) but low in fat (<30% of energy from fat) and moderate in carbohydrate (40% of energy from carbohydrate) are as effective at promoting weight loss as Atkins diets, and reduce total cholesterol and LDL cholesterol as well as triglycerides (Baba et al., 1999; Johnston et al., 2004; Parker et al., 2002). However, these diets have not received the same attention as the Atkins diet. Johnston et al., 2004 reported that energy-restricted, low-fat diets of varying protein content (15% or 30% of energy from protein)

were equally effective at promoting steady weight loss over 6 weeks of the intervention period, but diet satisfaction was greater in those consuming the high-protein, low-fat diet. The authors reported that the biomarkers of health and disease, including blood lipid profile, insulin sensitivity, uricosuria, hypercalciuria, and glomerular filtration rates, were not adversely affected by the high-protein, low-fat diet. Farnsworth et al., 2003 also reported that high-protein, moderate-carbohydrate diets which replace carbohydrate with protein from meat, poultry, and dairy foods (HP diet: 27% of energy from protein, 44% of energy from carbohydrate, and 29% of energy from fat) has beneficial metabolic effects and no adverse effects on markers of bone turnover or calcium excretion compared to a standard-protein diet (SP diet: 16% of energy from protein, 57% of energy from carbohydrate, and 27% of energy from fat). Therefore, high-protein diets that are low in fat and moderate in carbohydrate may represent an appealing diet plan for healthful and safe weight loss and deserve further investigation. In our dietary intervention study, we introduced a high-protein diet that is moderate in carbohydrate and fat intakes (30% total energy from protein, 40% total energy from carbohydrate and <30% of total energy from fat), to manage body weight and reduce metabolic risk factors for non-communicable diseases, in overweight and obese Malaysian adults.

2.4.2.4 Low-carbohydrate, high-protein diets and risk cardiovascular disease

A prospective cohort study reported that low carbohydrate-high protein diets, used on a regular basis and without consideration of the nature of carbohydrates or the source of proteins, are significantly associated with increased risk of cardiovascular disease in Swedish women, after adjusting for confounding variables such as total energy, saturated and unsaturated fat intake (Lagiou et al., 2012). The authors reported that a one tenth decrease in carbohydrate intake (20g) or increase in protein intake (5g) or a 2-unit increase in the 20-unit low carbohydrate-high protein score were statistically associated with a 5% increase in the incidence of cardiovascular disease overall. No heterogeneity existed in the association of any of these scores with the five studied cardiovascular outcomes, ischaemic heart disease, ischaemic stroke, haemorrhagic stroke, subarachnoid haemorrhage, and peripheral arterial disease. This can be explained by the food sources such as vegetables, fruits, cereals, and legumes, which are the core components of healthy dietary patterns, are also important sources of carbohydrates. Thus, reduced intake of these food groups is likely to have adverse effects on cardiovascular health (McCullough et al., 2002). Moreover, a prospective cohort and meta-analysis reported that both high (>70%) and low percentages (<40%) of carbohydrate diets were associated with increased mortality, with minimal risk observed at 50–55% carbohydrate intake (Seidelmann et al., 2018).

With respect to protein sources, previous evidence reported that the associations of low-carbohydrate, high-protein diets, with cardiovascular outcomes were significantly different between individuals whose protein intake was mainly of animal origin and those whose protein intake was mainly of plant origin (Lagiou et al., 2012). A statistically significant positive association was found between low-carbohydrate diets of mainly animal origin and cardiovascular outcomes, but a statistically significant inverse association was found between low-carbohydrate diets of mainly vegetable origin. Moreover, a positive association between intake of red meat and risk of ischaemic heart disease among women was reported from the Nurses' Health Study (Bernstein et al., 2010). Similar findings were found in patients with type 2 diabetes. Campmans-Kuijpers et al., 2015 reported that substitution of 10 gram dietary carbohydrate with plant protein was not significantly associated with weight gain, and was significantly associated with lower all-cause mortality risk, in patients with type 2 diabetes. However, no association was found with CVD mortality risk with total or animal protein.

These findings suggest low-carbohydrate dietary patterns containing animal-derived protein and fat intakes, from sources such as lamb, beef, pork, and chicken, were associated with higher mortality. On the other hand, those that favoured plant-derived protein and fat intakes, from sources such as vegetables, nuts, peanut butter, and whole-grain breads, were associated with lower mortality, suggesting that the source of food notably modifies the association between carbohydrate intake and mortality. Therefore, it should not be precluded that dietary regimens low in refined carbohydrates and high in plant proteins or unsaturated lipids could have no adverse effects on cardiovascular health. Future research is needed to assess whether dietary guidelines should not actively promote substitution of carbohydrates by total protein, or to focus on substitution of carbohydrates with plant protein.

2.4.2.5 High fibre intake

A review of studies reported that higher dietary fibre intake was associated with increased satiety and decreased hunger (Howarth, Saltzman, & Roberts, 2001). Previous studies have reported a favourable metabolic profile with high fibre diet (Jung & Choi, 2017; Kabir et al., 2002). Large prospective cohort studies consistently reported significant associations between high dietary fibre intake (>25 g/d in women and >38 g/d in men) and reduced risk of developing type 2 diabetes (T2D) (20-30% of reduced risk) (Weickert & Pfeiffer, 2018). The proposed mechanisms for this relationship remain unclear, but the type of fibre may play a role. In the gut, certain soluble fibres which form a viscous gel matrix is believed to slow gastric emptying

and lead to a greater feeling of fullness (Slavin & Green, 2007). Soluble fibres slow absorption of glucose further in the small intestine and lead to lower postprandial glycaemic and insulinemic responses (Yu et al., 2014). Therefore, high soluble fibres may contribute to the improvement in glycaemic control and insulin sensitivity. Moreover, in both the cross-sectional and longitudinal analyses, fibre intake was found to be protective against high inflammation levels (Griffith, et al., 2006). It was suggested that dietary fibre decreases lipid oxidation, which in turn associated with decrease in inflammation (King, 2005). Therefore, incorporating food sources that are high in fibre such as whole grains, vegetables, fruits, peas and nuts into weight loss diets may help to improve individual's metabolic health. In our dietary intervention study, a daily consumption of ≥25g of fibre was introduced into the formulated diet charts for better metabolic health, as per the recommendation of Malaysian Dietary Guidelines (MDG) 2010 (meaning at least 3 servings of vegetables per day) (National Coordinating Committee on Food and Nutrition, 2010).

2.4.3 Individualised weight management programme

In response to the high prevalence of obesity in the Malaysian population, various efforts and strategies have been implemented in the past decade to address this issue. These include new national nutrition policy and strategies, updated dietary guidelines and healthy lifestyle campaigns in Malaysia (Mohamad Nor et al., 2018). General recommendations based on dietary guidelines, e.g. recommended nutrient intake (RNI) or recommended dietary allowance (RDA), are often ineffective and end in unsuccessful weight management programmes, as there is no one-size-fits-all diet that should be recommended universally. Malaysia Dietary Guidelines (MDG), published by the Ministry of Health Malaysia, aims to promote healthy dietary and active lifestyle to the Malaysian population for the prevention of nutrition-related disorders (Tee, 2011). The first MDG was published in 1999 and was revised and updated in 2010. The updated MDG 2010 comprises 14 key messages and 55 recommendations that are considered to be most relevant to promoting healthy eating and active living in Malaysian population. However, a study conducted by Norimah et al., 2010 reported that 63% of the Malaysians were not aware of the existence of the MDG. Moreover, there was an ethnicity difference in the understanding of the 5 key health messages of MDG, with Malaysian Chinese had better mean score of the understanding of the five messages in the MDG compared to the Malays and Indians (Norimah et al., 2010). Therefore, a weight management programme which tailored according to individual's food preference and cultural habit may be effective in

increasing individual's dietary adherence and hence improve the weight loss outcomes in different ethnic groups in Malaysian population.

A recent review by Koliaki et al., 2018 reported that an optimal diet to treat obesity should be safe, efficacious, nutritionally adequate, culturally sensitive, cost effective, adoptable and should ensure long term maintenance of weight loss. Setting a realistic weight loss goal and following a balanced dietary plan tailored according to individual's need, food preference, cultural habit, and medical condition, are the key principles to facilitate sustainable weight loss in obese individuals and most importantly to reduce their overall cardio-metabolic risk. Ethnic diversity in Malaysia is unique (Bumiputera – 69.1%, Chinese – 23%, Indians – 6.9% and others – 1%) (Department of Statistics, Malaysia, 2018). According to a current study, Malaysians of different ethnic background continue to practice traditional meal patterns, habitually (Abdullah et al., 2016). Individualised dietary intervention according to cultural preferences could have higher efficacy in establishing consumer's adherence to the intervention diet and hence can be successful and sustainable weight management in the Malaysian population. Therefore, it is essential to identify an effective food-based dietary strategy which is cost effective, culturally sensitive and practical under real life situations for long term weight control for multi-ethnic overweight and obese Malaysian population.

2.5 Genetic contribution to obesity

Consumption of energy-dense food coupled with sedentary lifestyle, are the main contributors for the development of obesity and its complications nowadays (Racette, Deusinger, & Deusinger, 2017). However, not all individuals living in the same 'obesogenic environment' is obese. The variations in the individual's response to the 'obesogenic' environment suggesting that genetic factors may play an important role in modulating body weight regulation (Loos & Rankinen, 2005). Recent study of US national cohort reported that the magnitude of the association between obesity and genetic risk was stronger in more recent birth cohorts compared to earlier years of birth cohort, indicating that such genetic predisposition to obesity may exert greater effect in more recent obesogenic environment (Walter et al., 2016). Genetic predisposition to obesity may have interacted with such an obesogenic environment in determining the obesity epidemic. Gene-environment interaction may explain why some individuals are more prone to weight gain than others in a similar environment (Hetherington & Cecil, 2009).

Emerging evidence from gene-diet interactions from observational studies and from randomised controlled trials have supported the fact that the epidemic of obesity and its co-morbidities are the consequences of the complex interactions between multiple gene variants, lifestyle and environment factors (Hu, 2011; Ordovas & Shen, 2008). Obesity is commonly classified into subgroups depending on suspected etiology: monogenic obesity (extremely severe obesity in the absence of developmental delays) and polygenic or common form of obesity, (which affects the general population and may have associated health risks, such as increased risk of CVD) (Mutch & Clément, 2006). In monogenic form of obesity, obesity is caused by a mutation in a single gene which is rare and highly inheritable (Muñoz Yáñez, García Vargas, & Pérez-Morales, 2017). In common form of obesity (polygenic obesity), polymorphisms do not necessarily lead to disease. However, the susceptibility to obesity increases if associated with other genetic (gene-gene interaction) or environmental factors (gene-environment interaction) (Muñoz Yáñez et al., 2017).

2.5.1 Monogenic obesity

Monogenic obesity is defined as rare and severe early-onset of obesity with abnormal feeding behaviour and endocrine disorders which caused by a single mutation that occurs de novo or segregates in the family (Huvenne et al., 2016). This is mainly due to autosomal recessive mutations in genes of the leptin-melanocortin pathway which play a key role in the hypothalamic control of food intake (Farooqi & O'Rahilly, 2005). The impact of genetics in monogenic form of obesity is high and only little dependent on the environmental factors. Several different monogenic forms of obesity have been described, of which the first, caused by the mutations in the leptin gene (*LEP*) was identified in 1997 (Montague et al., 1997). Later on, a successful profound weight loss in leptin deficient patients with morbid obesity upon administration of recombinant leptin was reported by Licinio et al., 2004. This finding proved that a pharmacology therapy for weight management can be highly effective if a clear molecular basis of human obesity was identified.

To date, there are about 176 human obesity cases due to single-gene mutations in 11 different genes that result in an autosomal form of obesity (Rankinen et al., 2006). Apart from leptin, several obesity-causing genes have been identified mostly within the leptin-proopiomelanocortin (*POMC*) melanocortin pathway, such as leptin receptor (*LEPR*), *POMC*, prohormone convertase 1 (*PC1*) and melanocortin receptor 4 (*MC4R*). However, despite providing valuable insights into biological pathways and mechanisms leading to excessive

weight gain, the development of such diseases as a result of mutation in a single gene (monogenic forms of obesity) can occur, but it is rare and affects only a small fraction of population. The most common monogenic form of obesity is *MC4R* gene mutations with a prevalence of at least 0.15% in normal weight population, 1.7% among obese adults (Stutzmann et al., 2008) and 0.5%-6% among obese children (Farooqi et al., 2003; Miraglia del Giudice et al., 2002). Therefore, recent large scale molecular genetic studies have substantiated that the genetic predisposition to obesity is in many cases due to the combined effect of polygenic variants, a result from the interaction between multiple genes (polygenic) and environmental factors. An individual's susceptibility to complex diseases is underpinned by the unique combination of SNPs distributed throughout their genome (Loos, 2009). Thus, identification of genetic variations involved in common forms of obesity applicable to general population proved to be an arduous task.

2.5.2 Polygenic or common obesity

Polygenic or common form of obesity arises when an individual's genetic makeup is susceptible to an environment that promotes energy consumption over energy expenditure (Mutch & Clément, 2006). Unlike monogenic obesity, multiple genes and chromosomal regions contribute to defining the common obese phenotype, with each variant of many different genes making a small difference in effect (Sørensen & Echwald, 2001). Studies of polygenic obesity are based on the analysis of single nucleotide polymorphisms (SNPs) within or near a candidate gene. Single nucleotide polymorphism (SNP) refers to a single base pair alteration that is common in the population (Marth et al., 1999). Large scale molecular studies have substantiated that individual's susceptibility to obesity is in many cases due to the combined effect of polygenic variants of candidate genes, meaning a result of the interaction of multiple genes, instead of the individual effect of a single gene variant (Loos, 2009). Over the past few years, the number of the studies reporting the associations between gene variants and obesity phenotypes have been increasing. Multiple genetic loci have been successfully determined by genome-wide association studies (GWAS) that increase the susceptibility to obesity and diabetes in a cumulative manner (Speliotes et al., 2010). Candidate genes that involved in lipid metabolism, adipogenesis, energy expenditure, appetite control, insulin signaling and adipokine secretion were largely investigated as they play a critical role in weight management (Deram & Villares, 2009).

According to the human obesity gene map in 2005, it has identified 426 positive associations between 127 genes which have direct and indirectly effect on the onset of obesity in general population (Rankinen et al., 2006). A promising observation is that 22 genes were each supported by at least five positive studies. Among them, those showing replications in 10 studies and more included *PPARG* (30 studies), *ADRB3 (*29), *ADRB2* (20), *LEPR* (16), *GNB3* (14), *UCP3* (12), *ADIPOQ* (11), *LEP* (11), *UCP2* (11), *HTR2C* (10), *NR3C1* (10), and *UCP1* (10). However, in contrast to genetically identical mice whose environments can be rigorously controlled, the genetic and environmental diversity in humans has caused data replication to be challenging. The results were conflicting that cast doubt on potentially interesting candidate genes. Overall, these associations are relatively small and the results are not very consistent.

2.5.2.1 Evidence on observational studies

a) *FTO* gene variants

Genome-wide association studies (GWAS) have identified multiple genetic loci containing gene variants that affect body weight and the first successfully determined candidate gene is the *FTO* (Fat-mass and obesity associated) gene (Loos & Bouchard, 2008). *FTO* gene is positioned at chromosome region 16q12.211, encoding a Fe (II)- and 2-oxoglutarate (OG) dependent nucleic acid demethylase (Gerken et al., 2007). It was first discovered in a genome-wide study identifying type 2 diabetes-susceptibility genes in Europeans, with 1,924 cases and 2,938 controls (Frayling et al., 2007). The study reported that a cluster of single nucleotide polymorphisms (SNPs) in *FTO* gene was significant associated with increased risk of type 2 diabetes. However, such associations abolished after adjusting for BMI, suggesting that *FTO* gene variants predisposed to type 2 diabetes through an effect on BMI. A follow-up analyses revealed that individuals carrying the AA genotype of *FTO* rs9930609 weighed 3kg more and had a 1.7 fold increased risk of obesity compared to individuals carrying TT genotype (Frayling et al., 2007). Other studies have shown that a cluster of SNPs in the first intron of the *FTO* gene correlates with an increase in body mass index (BMI) in both children and adults, regardless of gender (Berulava & Horsthemke, 2010; Volckmar et al., 2016). The strong association of *FTO* gene and obesity-related traits have been consistently observed in studies involving various populations worldwide (Dina et al., 2007; Peeters et al., 2008; Ri et al., 2015). This association has been replicated in many European studies involving different ethnic groups across the globe (Dina et al., 2007; Peeters et al., 2008; Ri et al., 2015). However, other studies

analysing association of *FTO* gene variants and obesity (defined by BMI) in the Asian population have reported controversial results (Li et al., 2008).

A Singaporean study reported positive association between 9 *FTO* single nucleotide polymorphisms (SNPs) and obesity in the ethnic Chinese and Malays (Tan et al., 2008). The authors concluded that the risk alleles of the 9 *FTO* SNPs were associated with obesity in the same direction as reported by previous European studies. It is not only associated with increased risk of obesity but also with the risk of developing type 2 diabetes, atherogenic lipid profile and myocardial infarction (Doney et al., 2009). Rees et al., 2011 also reported a strong association between *FTO* gene variants with BMI and waist circumference in the South Asian population. However, two Malaysian studies which involved a total of 911 multi-ethnic Malaysians indicated no such association was found between *FTO* rs9939609 and rs17817288 with obesity-related anthropometric measurements (Apalasamy et al., 2012; Chey, Fan, & Say, 2013). Studies on Caucasian population have found significant association between *FTO* rs9930506 and obesity (Sentinelli et al., 2012; Wrzosek et al., 2016). To date, there is no data reported on *FTO* rs9930506 and its association with obesity and obesity-related metabolic parameters in the Malaysian population.

A recent study by Kalantari et al., 2018 reported that a complete linkage disequilibrium (LD) was found between the three neighbouring SNPs of intron 1 region of the *FTO* gene (rs9930501, rs9930506 and rs9932754). This haplotype in the first intron of the *FTO* gene (rs9930501, rs9930506 and rs9932754) with complete linkage disequilibrium (LD), had a strong association with obesity indices (e.g. BMI and body fat percent) in Iranian male adolescents. The authors suggested that the effect of *FTO* gene (rs9930501, rs9930506 and rs9932754) on obesity-related anthropometric variables was exerted through haplotypes (and not single SNPs). This finding was supported by an earlier study which reported that the haplotype in the first intron of the *FTO* gene (rs9930501, rs9930506 and rs9932754) was highly associated with increased odds of obesity (in BMI) and body fat percent in adolescent boys even after the adjustment for age, total energy intake and physical activity (Kalantari et al., 2018). The authors suggested that *FTO* gene polymorphisms (rs9930501, rs9930506 and rs9932754) may exert their effects on obesity indices as a haplotype, and not as single SNPs. To the best of our knowledge, the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 has not been investigated in the Malaysian population.

The function of the FTO protein is still largely elusive. It is known that *FTO* is a DNA/RNA demethylase which influences splicing, stability and export of mRNA (Lesbirel & Wilson, 2018). Initial observation suggested that it plays an important role in regulating energy homeostasis by signalling the hunger and satiety centre in brain, but it is unknown whether it influences eating behaviour or energy expenditure (Scuteri et al., 2007). The mechanism of action of *FTO* in mediating satiety and hunger signals is yet to be elucidated. Animal studies provided evidence that *FTO* is expressed in the arcuate nucleus of the hypothalamus and is associated with appetite and energy intake. A study conducted by Karra et al., 2013, showed that *FTO* gene variants were associated with obesity and obesity-prone behaviour due to higher level of circulating 'hunger hormone', ghrelin that eventually increased food intake and a preference for energy-dense foods.

Recently, a new mechanism was proposed, suggesting variants in the *FTO* gene exert effect on the genes located nearby and determining obesity (Smemo et al., 2014). It is assumed that SNPs located within the first intron may affect obesity by altering *FTO* expression directly, or, these SNPs may also remotely alter the expression of other downstream genes such as genes involved in body mass regulation by changes in epigenetic mechanisms including methylation of other genes (Almén et al., 2012; Zhou, Hambly, & McLachlan, 2017). The presence of *FTO* variants increased adipocyte-specific expression of IRX3 and IRX5 during early adipocyte differentiation, causing a reduction in mitochondrial thermogenesis and increased lipid storage (Claussnitzer et al., 2015). To support this mode of action, knockdown of *Irx3* and *Irx5* in adipocytes in mice resulted in weight reduction and increased energy dissipation without any changes in appetite and physical activity. Thus, as a transcriptional coactivator, *FTO* may play a significant role in the transcriptional regulation of adipogenesis and may be involved in the regulation of fat mass development and maintenance.

b) *ADRB2* gene variants

Obesity is caused by positive energy balance when energy consumption exceeds energy expenditure and resulted in excess body fat storage (Spiegelman & Flier, 2001). Energy expenditure is an important component in energy balance system and hence affects the regulation of body weight. The components of total energy expenditure are i) obligatory energy expenditure required to perform cellular and organ functions; ii) physical activity and iii) adaptive thermogenesis induced by diet or cold exposure (Lowell & Bachman, 2003). At the cellular level, biochemical processes involved in energy expenditure that happen in the

mitochondria hold a key to the modulation of adaptive thermogenesis in order to expend excess energy and prevent obesity. Therefore, identification of target tissue and intracellular mechanisms mediating adaptive thermogenesis is an area of intense interest to combat obesity.

Many pieces of evidence support the view that brown adipose tissue is an important site for beta-adrenergic receptor mediated adaptive thermogenesis, as well as dissipating caloric excess in response to feeding with a high caloric diet in humans (Vosselman et al., 2013). The evidence for this is as follows: i) cold exposure and diet increase sympathetic nerve activity, ii) exogenous administration of neurotransmitters (norepinephrine and epinephrine) stimulate energy expenditure, and iii) the thermogenic target tissue, brown adipose tissue is heavily innervated by sympathetic nerves. Beta 2-adrenergic receptor (*ADRB2*) gene coding for beta-2 adrenergic receptor has received significant attention in this respect. Beta 2-adrenergic receptor (*ADRB2*) is a member of a family of three G-protein-coupled receptors (Rosenbaum, Rasmussen, & Kobilka, 2009). It is a major lipolytic receptor in human white adipose tissue, associated with lipid mobilisation (Arner & Hoffstedt, 1999). Adrenergic system regulates energy balance through both thermogenesis and lipid mobilisation in the fat stores. It stimulates lipid mobilisation through lipolysis in adipocytes which then regulates body fat accumulation and account for energy expenditure (Taylor, 2007). In addition to lipolysis, they also mediate vaso and bronchial dilation (Dias, Gowdak, & Pereira, 2011).

A strong association between obesity and a mutation in codon 27 of the beta-2 adrenoceptor gene replacing glutamine to glutamic acid (rs1042714 or Gln27Glu) has been reported earlier (Large et al., 1997). Another gene variant located at codon 16 substituting arginine for glycine (rs1042713 or Arg16Gly) has been reported by Green et al., 1995. Single nucleotide polymorphisms (SNPs) in *ADRB2* gene such as rs1042713 and rs1042714 cause alterations in the structural conformation of the receptor which eventually affect the function of β-adrenergic receptors (Green et al., 1994). This may influence the binding of catecholamines to the beta 2 adrenoceptors and hence alters lipolysis. Masuo et al., 2005 reported that insulin resistant subjects had higher frequencies of the G allele of rs1042713 in Japanese population. Total body fat mass and blood pressure levels were also higher in non-obese and non-hypertensive men with G allele in the study population. The authors speculated that insulin resistance could, in part be determined by the genetic variant of the beta-2 adrenoceptor gene and that polymorphism and higher plasma adrenaline could increase insulin resistance, adiposity and high blood pressure in their subjects. Thus, the G allele could lead to heightened sympathetic nerve activity, insulin resistance and higher blood pressure and adiposity in non-obese and non-

hypertensive individuals. Other studies have adequately reported that insulin resistance was strongly associated with heightened sympathetic nerve activity (Masuo et al., 1997). In other words, adrenergic receptor defects lead to sympathetic nervous system over activity that may play a role in the development of insulin resistance, hypertension and obesity (Masuo & Lambert, 2011).

A study from Swedish population have reported that *ADRB2* rs1042713 was significantly associated with elevated central body fat accumulation, systolic blood pressure, serum leptin and triglyceride levels, but not with obesity (Rosmond et al., 2000). A meta-analysis involving 18 published articles revealed that there was an association between rs1042714 and susceptibility to obesity, but no association was found in rs1042713 (Zhang, Wu, & Yu, 2014). Earlier studies in Saudi population reported significant association between the *ADRB2* rs1042713 and rs1042714 polymorphisms and the development of obesity, insulin resistance and dyslipidemia (Daghestani et al., 2010; Daghestani et al., 2012). However, findings from populations such as Japanese and Korean, revealed, population specific outcomes with respect to *ADRB2* and associated SNPs (Hayakawa et al., 2000; Kim et al., 2002). To date, there is no data reported on *ADRB2* rs1042713 and rs1042714 gene polymorphisms in the Malaysian population.

c) *TP53* gene variants

The tumour protein p53 encoded by *TP53* gene, is known as a critical tumour suppressor in human cancer, which is activated after DNA damage (Levine & Oren, 2009). p53 inhibits tumour development by regulating the expression of genes associated with programmed cell death (apoptosis), cell cycle arrest, and senescence. These processes are important for the protection against cancer, and polymorphisms in *TP53* gene which led to a loss of wild-type p53 activity have been widely associated with various types of cancer (Muller & Vousden, 2014). *TP53* gene, in addition of being a tumour suppresor, it also plays an important role in metabolism. The most common *TP53* polymorphism occurs at codon 72 of p53 (rs1042522), which characterised by C>G change and resulted in the changes of the ancestral arginine into a proline (Dumont et al., 2003).

A study by Minamino et al., 2009 reported that p53 expression in adipose tissue was found to be associated with insulin resistance and subsequently with age-related cardiovascular disorders. Accumulation of oxidative stress in the adipose tissue of mice with type 2 diabetes-like disease was observed in excessive calorie intake. This led to senescence-like changes, such

as increased activity of senescence-associated beta-galactosidase, increased expression of p53 and increased production of pro-inflammatory cytokines. Inhibition of p53 activity in adipose tissue markedly ameliorated these senescence-like changes, decreased the expression of pro-inflammatory cytokines and improved insulin resistance in mice with type 2 diabetes-like disease. Conversely, upregulation of p53 in adipose tissue may cause an inflammatory response that led to insulin resistance. Therefore, recent research has been focused on the new role of TP53 beyond cancer progression, such as obesity, insulin resistance, metabolic syndrome, adipose tissue inflammation and age-related cardiovascular risk factors (Minamino et al., 2009). An association was also found between CC genotype of *TP53* rs1042522 and changes in blood pressure and waist circumference over time (Reiling et al., 2012).

2.6 Gene-diet interactions on obesity and obesity-related traits

Several weight loss strategies reported reduction in weight and improvement in metabolic risk factors in obese individuals, however, each individual has shown huge variability in response to the same intervention, suggesting that the differences in such responses moderately are due to genetic factors (Bray, 2008; Loos & Rankinen, 2005). Predicting obesity risk from genetic background is complicated by its interaction between genetic variants and dietary factors (Perusse & Bouchard, 2000). Gene-lifestyle interaction may reflect the causal mechanism of the interaction between gene variants and dietary nutrients and its contribution to the causation of a disease or condition. This interaction may account for greater part of genetic risk seen across genotypes. Therefore, it is important to understand the interplay between genetic and lifestyle factors in the development of obesity and obesity-related traits.

Gene-environment interaction is defined as "a different effect of an environmental exposure on disease risk in persons with different genotypes," or "a different effect of a genotype on disease risk in persons with different environmental exposures" (Ottman, 1996). Genetic predisposition to obesity may have interacted with such environments in determining the obesity epidemic. This has been proven in recent randomised controlled trials (RCTs), which reported that individual genetic profile was found to modify the effect of dietary interventions on weight loss and post intervention changes in obesity-related anthropometric and metabolic risk parameters (Qi, 2014). This finding supported that individual genetic background may have affected the success of the dietary interventions in the prevention and treatment of obesity and obesity-related non-communicable diseases. The degree to which genetic variants, interact with the intake of specific dietary nutrients, can contribute to the risk of obesity and chronic

diseases is yet to be explored. Therefore, there is no one diet that should be recommended universally to reduce body weight and improve metabolic health. Personalised nutrition which tailored nutritional advice or recommendations based on individual's genetic makeup should be introduced to optimise individual's response to particular interventions (Deram & Villares, 2009).

2.6.1 Evidence of gene-diet interactions on intervention studies

a) *FTO* gene variants

FTO gene was found to be the most investigated gene for gene-lifestyle interactions in randomised controlled trials (RCTs). Between 2009 and 2015, there were a total of six studies investigating the interactions between *FTO* rs9939609, rs1558902 and rs8050136 SNPs with various weight loss strategies such as hypocaloric diet with different macronutrient composition or exercise intervention. Matsuo et al., 2012 reported that no significant association was found between rs9939609 (T>A) and weight loss outcomes after a 14-week calorie-restricted dietary intervention in obese Japanese women. In a population of obese Spanish women, Labayen et al., 2015 also reported no significant association between rs9939609 alleles (T>A) and the changes in body weight and body composition after 14 weeks of calorie-restricted dietary intervention. Individuals with the risk allele (AA) of *FTO* rs9939609 had similar improvement in body weight, anthropometric parameters and blood biomarkers compared to the non-carriers (TT).

A 2-year POUNDS LOST trial was conducted to compare the effects of an energy-restricted diet (an energy deficit of 750kcal/day) with different compositions of fat, protein and carbohydrate. Improvements in weight management and insulin sensitivity was observed in both gene variants (risk allele carriers (AA) and non-carriers (TT) of *FTO* rs9939609) in an American population, and such differences were not statistically significant (Zheng et al., 2015). However, a 10-week randomised controlled trial, NUGENOB, conducted by Grau et al 2009, reported that TT genotype carriers of the same gene polymorphism (*FTO* rs9939609) had greater reduction in HOMA-IR and HOMA-β with 10-week low fat diet compared to high fat diet in the European population (K Grau et al., 2009). The beneficial effect of low-fat diet on insulin sensitivity was not found in the risk allele carriers (AA) (K Grau et al., 2009). It is to be noted that female carriers of TT genotype of rs9939609, were in higher numbers in the NUGENOB study (75%) compared to the POUNDS LOST trial (62%). Moreover, NUGENOB participants were significantly younger (age ranged 20-50 years) compared to the POUNDS

LOST participants, 50.1y (±9.9 SD) (age ranged 20-70 years). The differences observed between the two studies (POUNDS LOST and NUGENOB), maybe explained by the differences in age and gender. Further, a study conducted by Zhang et al., 2012, comparing the effect of 4 hypocaloric-diets with different macronutrient composition, reported that the risk allele carriers (A) of rs1558902 showed higher reduction in BMI and body adiposity on high protein diet compared to non-carriers (T) in overweight and obese American adults (Zhang et al., 2012). Overall, dietary intervention does not affect individuals with *FTO* minor and major alleles differentially.

Evidence from previous observational studies suggest that physical activity can attenuate the effect of genetic mutations of *FTO* rs9939609 on obesity and body fat accumulation (Andreasen et al., 2008; Sonestedt et al., 2009). This finding was replicated in a recent observational study in a Nigerian population. The study reported that the carriers of the risk allele (A) of *FTO* rs9939609 had higher odds of being overweight/obese compared to non-carriers (T), but this association was attenuated by high physical activity of the participants (Oyeyemi et al., 2017). Further, a lifestyle intervention (through lower energy intake and increased physical activity) showed better improvement in C-reactive protein and fasting plasma glucose in Brazilians at high cardio-metabolic risk who carried the risk allele (A allele) of *FTO* rs9939609 (Curti et al., 2013). Similar positive results were reported in gene variant rs8050136. Individuals carrying the risk allele (A) demonstrated greater reduction in weight compared to the non-carriers (C allele) after a moderate-intensity exercise intervention in Caucasian postmenopausal women (Mitchell et al., 2010). Therefore, individuals with *FTO* gene variants who are genetically predisposed to obesity may benefit from increased physical activity.

A study conducted by Karra et al., 2013, reported that *FTO* gene variants were associated with obesity and obesity-prone behaviour due to higher level of circulating 'hunger hormone', ghrelin that eventually increased food intake and a preference for energy-dense foods. It is known that exercise can suppress appetite by reducing the circulating ghrelin levels in an intensity dependent way. Thus, exercise followed by reduced energy intake can eventually improve body composition (Hazell et al., 2016). Thus, individuals with *FTO* gene variants who are genetically predisposed to obesity may benefit from increased physical activity. It must be emphasised that although physical activity modifies the effects of *FTO,* the latter has no influence on energy expenditure. This has been adequately proved by a study by Berentzen et

al., 2008, where the authors report no effect of *FTO* on resting energy expenditure, glucose induced thermogenesis, cardiorespiratory fitness and leisure time physical activity.

b) *ADRB2* gene variants

ADRB2 gene coding for beta 2 adrenergic receptor, has recently received significant attention in gene-diet interactions studies. These receptors are part of the adrenergic system, which stimulate lipid mobilisation in adipose tissue through the action of catecholamines (Kurokawa et al., 2008). A 2-year DIETFITS intervention study reported no significant gene-diet interaction between the two alleles of *ADRB2* rs1042714 (C>G), in weight loss, in response to two hypocaloric diets (low carbohydrate diet vs low fat diet) in overweight and obese Americans (Gardner et al., 2018). In an obese Brazilian population, polymorphisms in *ADRB2* rs1042714 (C>G), *ADRB2* rs1042713 (G>A) and *ADRB3* rs4994 (C>G), did not affect the two allele carriers differently, to a 9-week lifestyle modification with a combination of dietary and exercise intervention (Saliba et al., 2014). Mutation in the aforementioned genes did not lead to greater or lesser weight loss in response to caloric restriction. Another study with calorie restriction as dietary intervention conducted by Ruiz et al., 2011, reported that both genotypes of *ADRB2* rs1042713 (G>A) showed similar reduction in body weight, however, obese Spanish women carrying risk allele (G) of *ADRB2* rs1042714 had greater reduction in body weight compared to the non- carriers (CC), after 3 months of energy-restricted diet. This finding suggests that there may be a gender-specific genetic association between rs1042714 and intervention employed on the changes in body weight.

2.7 Mendelian randomisation (MR) studies

Many of the significant gene-diet associations or interactions observed in the observational studies were failed to replicate in RCTs. This may be explained by residual confounding, reverse causation and incorrect causal inference in the observational studies (Sattar & Preiss, 2017). Mendelian randomisation (MR) method makes use of genetic data to examine the causal relationship between the risk factors and disease outcomes in observational data (Sekula et al., 2016). It is known as the non-experimental alternative to RCTs as both of them have shared several common features. In RCTs, participants are randomly assigned to intervention or control groups to balance confounders and examine the causal inference. However, in MR, alleles are randomly allocated at conception (Davies, Holmes, & Davey Smith, 2018). The fundamental difference between MR studies and RCTs is that, in RCTs, the intervention applied to the treatment group is usually the intervention that is proposed in clinical practice,

whereas, in MR studies, the 'intervention' leading to differences in disease outcomes between the groups is the presence of a genetic variant (Burgess et al., 2012) (Figure 2.3).

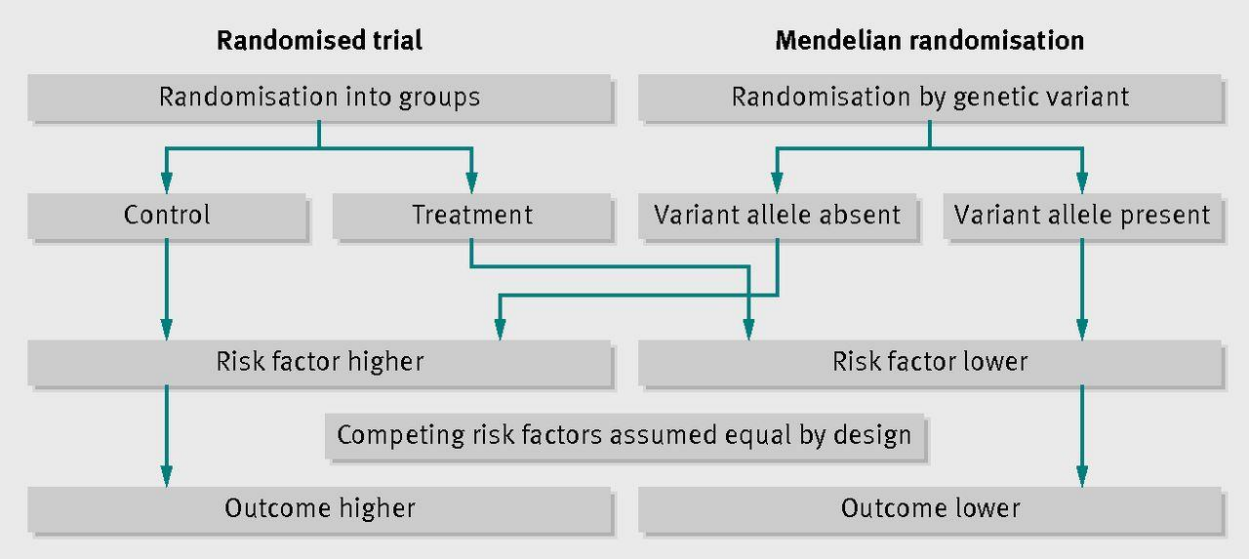


Figure 2.3: Comparison of RCTs and MR studies. Photo sources: Burgess et al., 2012.

MR studies are increasingly being used because it can overcome a major limitation of evidence from observational studies, which is the unmeasured confounding. Moreover, compared to RCTs, MR studies have relatively lower cost. However, the ability of the genetic variant in MR studies and the proposed intervention in RCTs, to provide the same specific estimate on the risk factors is questionable as there few limitations existed which may affect the validity of MR studies (Plotnikov & Guggenheim, 2019). These reasons included the association of the variant with one or more other unrelated risk factors (pleiotropy), the association between the variant and other neighbouring genetic variants that are inherited together (linkage disequilibrium), the presence of genetic differences on disease outcomes between the subgroups in the population under investigation (population stratification) and developmental compensation due to the adaptation to a genetically determined phenotype (canalisation) (Burgess et al., 2012). Therefore, mendelian randomisation approach may be qualitatively informative for the selection of potential treatments in a clinical intervention, but the genetically derived estimate may not be corresponding to the magnitude of the effect in practice. It could be misleading to generalise the magnitude of the Mendelian randomisation estimate to the effect of a potential treatment or intervention on the risk factors practically.

2.8 Future perspectives on personalised nutrition

Gene variants not only contributing to the development of obesity risk, but also modulating the effect of dietary nutrients and physical activity intervention on weight loss outcomes. Therefore, inter-individual variances at the genetic level cannot be ignored when developing strategies for weight management in overweight and obese population as there is no one-size-fits-all diet that can suit all individuals (Ferguson et al., 2016). Genetic background may affect the success of the interventions in the prevention and treatment of obesity and obesity-related non communicable diseases. Inter-individual variances at the genetic level cannot be ignored when developing strategies for weight management in overweight or obese population (Loos & Rankinen, 2005). Personalised nutrition tailored according to individual's genetic background, age, gender, physical activity level, cultural habits and food preferences was proposed as a new therapeutic approach to optimise health and to prevent obesity-related chronic disease compared to general recommendations (Gaboon, 2011). Early identification of the candidate gene variants that have potential to influence dietary nutrients may allow for the provision of good quality personalised recommendation to achieve effective weight loss and successful maintenance of weight (Martínez & Milagro, 2015).

However, there are several limitations in the studies reported so far, including small sample size, short duration for the intervention, and measurement of different outcomes. Most of the intervention studies focus on the effect of variants of single candidate gene on weight loss. Large scale molecular genetic studies have substantiated that the genetic predisposition to obesity is in many cases due to the combined net effect of polygenic variants, a result from the action of multiple genes (polygenic) and environmental factors. An individual's susceptibility to complex diseases is underpinned by the unique combination of multiple SNPs distributed throughout their genome (Loos, 2009). However, there was little evidence about the interactions between polygenic risk scores (PRS) and dietary intervention on the post intervention changes in obesity-related traits, in order to provide personalised nutrition based on genotype. Therefore, further evidence from large scale studies are necessary to assess the effect of multiple candidate genes to compute a gene score that could be used as a model in intervention programme. Future studies should focus on evaluating the interaction between polygenic risk scores (PRS) and the changes in body composition and metabolic parameters in response to various weight loss strategies.

At present, technology targeting personalisation of health care may be a better way to fight the epidemic of obesity and obesity-related non-communicable diseases. However, for the latter to materialise we need better understanding of gene variants and corresponding biochemical phenotypes. With the current data available, it is still premature to introduce or apply the use of genetic testing, prior to weight loss intervention trials in the community. There has been some scepticism regarding to the use of personalised nutrition according to individual's genetic makeup in preventing or managing obesity as the available data on genes and association with genes may not be sufficient to be translated into clinical recommendations and public health initiatives (Guasch-Ferré, Dashti, & Merino, 2018). Gene-lifestyle interaction studies on the same candidate gene in different populations have reported information which is challenging to interpret. Thus, it is difficult to arrive at a particular model for a strategy on weight management at this point in time. Moreover, due to the high cost involved in such diagnostics, only a handful of individuals will be able to afford such tests. At present, healthy lifestyle with a balanced diet and regular physical activity will benefit individuals who carry the risk alleles of the obesity-related candidate genes. This message should be the main stay of the recommendations and guidelines published by the nutrition societies across the world.

Chapter 3

Methodology

3.1 Study design

In this PhD thesis, different study designs such as cross-sectional studies, case-control studies and randomised controlled trial (RCT) were conducted to test different objectives and were reported in different chapters (Chapters 4-8). The detailed information on the sample sizes and the study designs for the respective objectives are summarised in Table 3.1.

Table 3.1: Detailed information on the sample sizes and the study designs in each chapter

Sample size	Study design	Objectives	Chapter
178 Malaysian adults	Cross-sectional study	**O1:** To determine the differences in dietary intakes, physical activity levels and obesity-related anthropometric and blood biochemical parameters in adult Malaysians of the three different ethnic groups (Malays, Chinese and Indians).	4
		O2: To assess the differences in genotype distribution and allele frequencies of the gene variants between the three different ethnic groups (Chinese, Malays and Indians), and **O4:** To assess the effect of gene variants on obesity-related phenotypes and its interaction with dietary nutrients in modulating the obesity-related phenotypes in the Malaysian adults.	5
	Case-control study	**O3:** To investigate the association between gene variants and the odds of obesity and insulin resistance in the Malaysian adults.	5
126 overweight and obese Malaysian adults	RCT	**O5 & 6:** To assess the effect of Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet on anthropometric and cardio-metabolic parameters, compared to a control diet, in overweight and obese Malaysian adults.	6
		O7: To assess the effect of the variants of the two genes (*FTO* and *ADRB2*) on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to a 6-month intervention diet or control diet.	7
		O8: To assess the combined effect of the variants of the two genes (*FTO* and *ADRB2*) on the post-intervention differences in anthropometric and cardio-metabolic parameters, by computing polygenic risk scores (PRS), in response to a 6-month intervention diet or control diet.	8

3.2 Study participants

Participants were recruited at random through advertisements and flyers distributed at UNM campus, supermarkets and schools in the vicinity of UNM (Beranang, Jalan Broga and Bandar

Kajang). Six waves of participant recruitment had staggered start dates between June 2015 and June 2018 (3 years). Each wave of the participant recruitment consisted two stages of screening and participant selection, for two different studies, i) cross-sectional/case-control studies (Chapters 4 & 5) and, ii) RCT (Chapters 6-8). The participant recruitment for both the i) cross-sectional/case-control studies and ii) RCT was conducted concurrently over the 4-year period of the study. The flow chart of the screening and participant selection for the cross-sectional/case-control studies and RCT is summarised in Figure 3.1.

a) First stage of the screening and participant selection- cross-sectional/case-control studies

During the first stage of screening and participant selection, individuals were initially informed that a 'free body composition measurement and blood analysis', was provided by UNM to Malaysian adults, with the following inclusion and exclusion criteria:

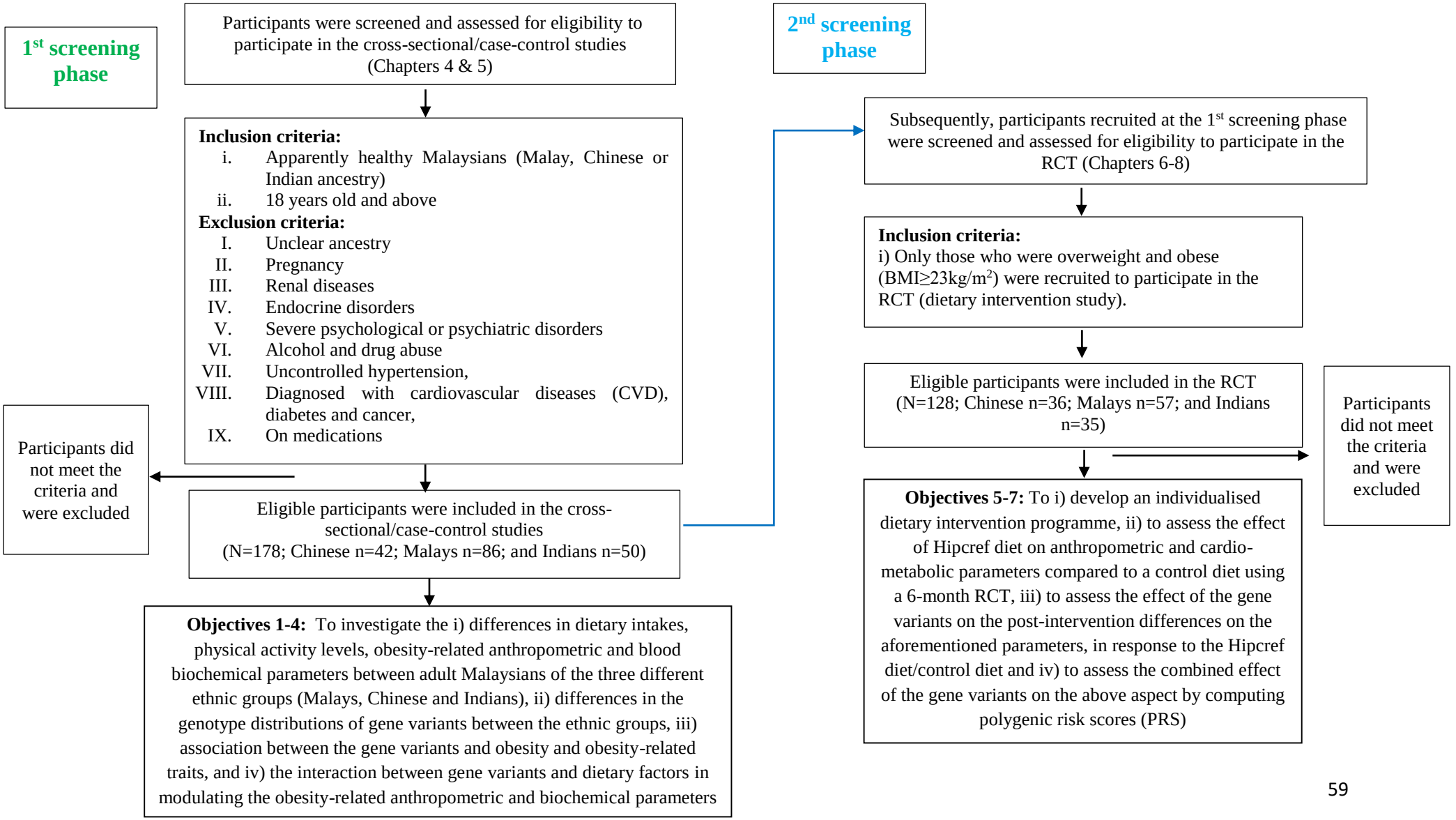
Inclusion Criteria:

i. Apparently healthy Malaysians of Malay, Chinese or Indian ancestry
ii. 18 years old and above

Exclusion Criteria:

I. Unclear ancestry
II. Pregnancy
III. Renal diseases and endocrine disorders such as hypothyroidism
IV. Severe psychological or psychiatric disorders
V. Alcohol and drug abuse
VI. Uncontrolled hypertension (>160/100 mm Hg),
VII. Diagnosed with cardiovascular diseases (CVD), diabetes and cancer,
VIII. On medication such as cholesterol lowering and hypoglycaemic drugs, antidepressants, antipsychotics, steroids, chemotherapeutics.

Figure 3.1: The flow chart of the screening and participant selection for the cross-sectional/case-control studies (1st screening phase) and RCT (2nd screening phase) in each batch of the participant recruitment



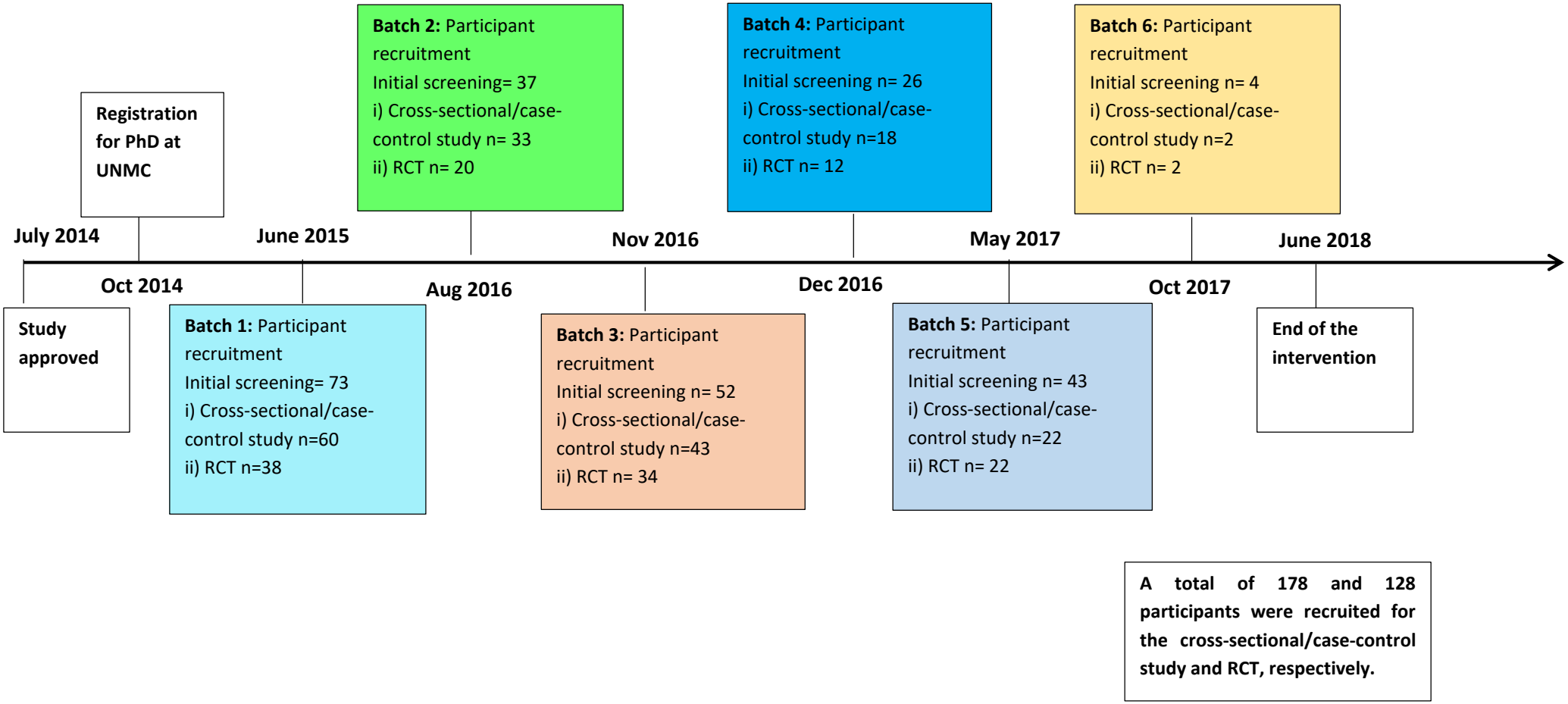
Interested participants completed a general characteristics questionnaire (Appendix 8) and a health and lifestyle questionnaire which included questions on ethnic background, past diseases, family history of past diseases, substance abuse (Appendix 9) and physical activity level. Structured questionnaire was used to record race and ethnicity and the questions were directed to trace back three generations to confirm ancestry. Smoking status and alcohol consumption were reported as i) never, ii) former and iii) current. Participants who did not meet the inclusion and exclusion criteria will not be considered into this study. Each participant was briefed about the programme and written informed consent was requested and obtained from each participant (Appendices 4-7). Participants were free to withdraw at any point in time during the programme without giving prior notice. No monetary rewards or payments were associated with this program.

b) <u>Second stage of screening and participant selection- RCT</u>

Subsequently, participants who have been recruited at the 1st stage of screening (for cross-sectional/case-control studies) were invited to participate in a 6-month, parallel-arm, RCT (dietary intervention programme). However, only overweight and obese Malaysian adults with a BMI≥23kg/m^2 were eligible to participate in the RCT. Participants who were non-overweight and non-obese (BMI<23kg/m^2) were excluded for RCT.

After 6 batches of participant recruitment, a total of 178 and 128 participants were successfully recruited for the cross-sectional/case-control studies and RCT, respectively. The timeline and the number of participants recruited at each batch are illustrated at Figure 3.2.

Figure 3.2: Timeline for the participant recruitment for both the cross-sectional/case-control studies and RCT: six batches of recruitment



3.3 Measurement of obesity-related anthropometric parameters

All individuals were subjected to light clothing for all intents and purposes.

3.3.1 Height

Height of individuals (barefoot) was measured with a Seca 213 portable stadiometer (Seca GmbH & Co. KG, German). Participants were requested to stand on the centre of the base, with his or her heel touching the bottom of the stadiometer upright and his or her back, buttock and calf muscle touching the height rule upright.

3.3.2 Measurement of body composition

Body weight and body composition including muscle mass (kg), fat mass (kg), fat free mass (kg) and percent body fat (%) of the individuals were evaluated using a body composition analyser with direct segmental multi-frequency (20 kHz and 100 kHz) bioelectrical impedance analysis method (DSM-BIA) InBody 230 (InBody Co., Ltd., Seoul, Korea). Bioelectrical impedance analysis (BIA) is a non-invasive, portable, cost-effective and commonly used body composition assessment in large epidemiology studies. BIA produces the estimates of total body water (TBW), fat-free mass (FFM), and fat mass by measuring the resistance to an electric current, with least resistance to fat-free mass due to its high water content compared to fat mass (Duren et al., 2008).

3.3.3 Body mass index (BMI)

Body mass index (BMI) was arrived at by using the formula, weight in kilograms divided by the square of height in meter (kg/m^2). For Asian population, World Health Organization has redefined the cut off point for normal, overweight and obesity, with BMI value 18-23kg/m^2 as normal, 23-27.5 kg/m^2 as overweight and ≥27.5 kg/m^2 as obesity (WHO Expert Consultation, 2004).

3.3.4 Measurement of waist and hip circumference

Waist circumference was measured at a level midway between lower rib margin and top of the hip bone with a constant tension tape wrapped around the body horizontally and hip circumference was measured around the widest portion of the buttocks (WHO Expert Consultation, 2008). Waist–hip ratio was calculated using the ratio of the circumference of the waist to the hips.

3.3.5 Measurement of blood pressure

Consumption of food, alcohol, caffeine, nicotine, medication, and exercise are not allowed within 30 minutes before blood pressure measurement as these are the factors that cause significant deviation in blood pressure. Participants were directed to sit in a chair crossed legged. Talking was strictly prohibited during the measurement as it could increase the blood pressure. Systolic and diastolic blood pressure was measured using a digital blood pressure monitor (HEM 402C, Omron Healthcare), following the recommendation from American Heart Association (AHA) (Pickering et al., 2005).

3.4 Assessment of dietary intake

Both 24-hour dietary recall and food-frequency questionnaire (FFQ), administered by a single interviewer, were the two dietary assessment techniques used in this research study. Initially, a 24-hour diet recall survey was conducted to capture personal food preferences, likes-dislikes, and the habitual macro and micronutrient intake of the participant (n=60; batch 1 of the participant recruitment). These food items were incorporated into a validated FFQ (Loy et al., 2011). The same interviewer then administered this revised FFQ to every participant to estimate their energy, macro and micronutrient intake per day as accurately as possible The details of both the techniques are briefly described below:

3.4.1 24-hour dietary recall

24 hour recalls (2 recalls, one week day and one week-end day) were conducted on 128 participants to capture and include food items habitually consumed on weekdays and weekends (Willett, 2012). 24-hour dietary recall is a subjective measure using open-ended survey to collect the detailed information about the food consumed over the previous 24 hours. It was conducted by an in-depth interview (by the researcher) and required 30-45mins to collect a single day recall. Detailed information on the meal time, venue, food preparation methods, the brand name of the commercial products consumed and ingredients used in mixed dishes were collected and documented as well. The portion sizes of the food consumed were estimated using pictures of standard household measures, such as bowls, cups, glasses, teaspoons and tablespoons to assist in quantifying the amount of food consumed (Suzana, 2009). The strengths of the 24-hour dietary recall are that, detailed information on dietary intake is acquired and relatively small respondent burden is imposed on the participants (Shim, Oh, & Kim, 2014). This technique obtained sufficient information on the habitual intake of the

participants by acquiring information on foods consumed on week days and on weekend days. Food items listed in the validated food frequency questionnaire (FFQ) (Loy et al., 2011) were then modified to include items popular to all three ethnic groups based on the 24 hour recalls (n=60). This effectively tailored the listed food items in the above FFQ to suit the participants of the current study.

3.4.2 Food-frequency questionnaire (FFQ)

Diet survey using a food-frequency questionnaire is a technique based on the principle of taking diet history retrospectively for a given period of time. In an interviewer administered diet survey using a FFQ, the respondents are asked to recall the frequency and the portion size of the foods and beverages consumed over a specific period of time (Shim et al., 2014). This method assesses and quantifies the individual's usual dietary intake in a relatively simple manner and it imposes minimal burden on the respondents as it is administered by the interviewer.

In the current study, an existing validated FFQ developed by Loy et al., 2011 was modified. Food items listed in the validated food frequency questionnaire were modified to include items popular to the current study participants (Malaysian Malays, Chinese an Indians) based on the 24 hour recalls as described above. This FFQ consisted of 156 food items which were listed under 12 categories (grains/cereals, meat and meat products, fish and seafood, egg and eggs products, legumes, milk and milk products, vegetables, fruits, drinks, confectionaries, bread spread and flavoring). Detailed information related to the brands, methods of cooking, supplementation of vitamins and minerals and oil consumption were collected and documented to avoid under-reporting. For each item, participants indicated how frequently they consumed it in a week (never, once a week, 2–4 times a week, 5–6 times a week, once per day, 2–3 times per day, 4–5 times per day); and the number of standard portions consumed per sitting. Photographs of standard portion sizes of the food items were provided during the interview to assist the researcher to quantify the amount of food consumed (Suzana, 2009). Total daily intake of macro and micronutrients was calculated by multiplying the energy and nutrient content of one portion of each food item by the number of portions consumed per sitting, times a factor representing the frequency of consumption for the past week, divided by 7 to give per day consumption. This was finally summed across all food items to give an estimate of the total daily intake of energy, macronutrients and micronutrients per participant.

3.4.3 Assessment of energy and nutrient intake

The per day consumption data obtained from 24-hour dietary call and FFQ were entered into an energy and nutrient assessment software, Dietplan7 (Forestfield Software Ltd., UK) to compute energy, macro and micro nutrient intakes per day. The Dietplan7 database is pre-installed with the full set of UK food tables - all the foods and nutrients from the 7th Edition of McCance and Widdowson's 'The Composition of foods' plus the revised 'Composition of Foods Integrated Data Set'. Nutrient data is available from a number of other sources such as Irish Food Composition Database, Australia/New Zealand NUTTAB 2010, U.S. Department of Agriculture SR 27 2014, USDA Isoflavone data and USDA Flavonoid data. All foods items not found in the Dietplan7 database were keyed in from the 'Nutrient Composition of Malaysian Foods 1997' by Tee, 1997 or information was collected from the 'nutrition facts' of the commercial products or by matching similar food items from data gathered from local 7-11 and other similar food outlets. The intakes of macronutrients were expressed as a percentage of total energy intake, while micronutrients were energy adjusted to unit/total energy intake(kcal)x1000kcal.

3.4.4 Under and over-reporting

To estimate under-reporting in the study population, a cut-off of total energy intake/basal metabolic rate (EI: BMR) <1.2 was used to indicate under-reporting (Black et al., 1996; Black et al., 1991) (Caan et al., 2004). EI: BMR ≥2.4 was used to identify over-reporting (Johansson et al., 1998). In the current study, none of the participants were found to be under or over-report. Therefore, all the participants were included in the analysis. Basal metabolic rate (BMR) for adult Malaysians was computed from the equations based on age, gender and body weight (kg) (Ismail et al., 1998) (Table 3.2):

Table 3.2: BMR predictive equations for adult Malaysians

Age group	Formula
Male	
18-30	0.0550 (Weight in kg) + 2.480
30-60	0.0432 (Weight in kg) + 3.112
Female	
18-30	0.0535 (Weight in kg) + 1.994
30-60	0.0539 (Weight in kg) + 2.147

3.5 Assessment of physical activity level

Physical activity data were collected through an interviewer-administered, 7-days activity record sheet. Detailed information on the activities performed in a week was documented. The information such as (1) the type and intensity of the physical activity (e.g., sitting, laying, jogging, brisk walking, running etc.) and (2) the time and duration (mins) of each activity performed, in a day for a week time were collected. According to the Compendium of Physical activity by Ainsworth et al 2000, a list of physical activities was reported with its corresponding metabolic equivalent (MET) values (Ainsworth et al., 2000). The type of the reported physical activity was then categorised into light, moderate and vigorous intensity physical activity which was defined as <3 METs, 3-6 METs and >6 METs, respectively according to Ainsworth et al 2000 (Ainsworth et al., 2000) . The total time (mins) spent on sedentary, moderate and vigorous intensity physical activity in a week was computed for each participant. According to the Ministry of Health Malaysia, individuals who accumulate at least 150 minutes/week of moderate intensity physical activity or 60 minutes/week of vigorous intensity physical activity are considered to be physically active, else they are considered to be physically inactive (National Corrdinating Committee on Food and Nutrition, 2010). Accordingly, participants of the current study were then categorised into two groups; physically active and physically inactive for further analysis.

3.6 Blood collection and biochemical analysis

3.6.1 Blood samples collection

Fasting blood samples were collected from each participant early in the morning between 7am to 10am following an overnight fasting, meaning no food was allowed except water for 10-12 hours before the blood collection. To collect the blood samples, participants were requested either i) to visit an appointed diagnostic centre by their own during their convenience time ii) or a designated venue (e.g. schools) on a given time and date where a phlebotomist from a diagnostic centre was assigned to. However, a minimum of 15 participants per trip was requested by the diagnostic centre. Therefore, it was necessary to consult and to get agreement of best meeting dates for everyone.

12ml of fasting venous blood sample was drawn from an antecubital vein into vacutainer tubes (Becton Dickinson, Oxford, United Kingdom) by an experienced phlebotomist. 1ml of fasting blood was drawn into vacutainer tubes containing fluoride oxalate for plasma glucose analysis,

6ml of fasting blood was drawn into vacutainer tube with clot activator and gel for serum separation for serum lipids (including total cholesterol, triglyceride and HDL-cholesterol), insulin, leptin and hsCRP analysis and 5ml of whole blood was drawn into vacutainer tubes containing EDTA for genetic analysis.

3.6.2 Blood biochemical analysis

The blood biochemical parameters were analysed following the standard laboratory procedures by professional technicians from the diagnostic centre (outsourced): (1) fasting plasma glucose level, (2) serum lipid profile including total cholesterol, triglyceride and HDL-cholesterol levels (3) fasting serum insulin level and (4) high-sensitivity C-reactive protein (hsCRP) level. Leptin level was analysed by an in-vitro enzyme-linked immunosorbent assay, RayBio® Human Leptin ELISA Kit with the antibody raised to highly purified recombinant human leptin. This analysis was carried out at UCSI laboratory. The details on blood sample handling, pre-processing and protocol for each blood biochemical analysis are reported in Table 3.3.

Insulin sensitivity was assessed by homeostatic model assessment (HOMA). This model has been proved to be a reliable tool for the assessment of insulin resistance (Bonora, 2000). HOMA is an estimate of the insulin sensitivity and β-cell function from fasting insulin and glucose concentrations. The steady-state of basal blood glucose and insulin concentrations reflects the balance between the hepatic glucose efflux and insulin secretion, which is determined by a feedback loop between the liver and β-cells (Matthews et al., 1985). According to homeostatic model assessment (HOMA), insulin resistance (HOMA-IR) was calculated as the product of fasting plasma glucose (mmol/L) and fasting serum insulin (uU/ml) divided by 22.5 (Wallace, Levy, & Matthews, 2004).

Low-density lipoprotein (LDL) cholesterol was calculated from the values of total cholesterol, HDL cholesterol, and triglyceride using Friedewald formula: LDL-cholesterol = Total cholesterol – [(Triglyceride/5) + HDL-cholesterol] (Friedewald, Levy, & Fredrickson, 1972). Total cholesterol per HDL-cholesterol ratio was calculated by dividing total cholesterol by HDL-cholesterol levels. This equation was first introduced to the clinical setting in 1972 due to the unavailability of ultracentrifugation in most of the laboratories for direct LDL measurement and this procedure is relatively costly and time-consuming (Friedewald, Levy, & Fredrickson, 1972). Mora et al., 2009 reported that the association between LDL cholesterol levels and CVD using Friedewald equation and direct LDL measurement was highly identical when using fasting blood samples. Choi et al 2016 also reported that Friedewald equation had

the highest accuracy and correlation in determining LDL cholesterol levels with direct LDL measuring compared to other formulas such as Chen, Vujovic, Hattori, de Cordova, and Anandaraja (Choi et al., 2016).

Table 3.3: The blood sample handling, pre-processing and protocol for each blood biochemical analysis

Biochemical analysis	Amount of fasting blood needed and the type of vacutainer tube used	Pre-processing of blood before analysis	Method	Instrumentation
Glucose analysis (outsourced)	 1ml of fasting blood was drawn into Grey top Vacutainer tubes containing fluoride oxalate (Vacutainer 367692; Becton Dickinson)	The tube was inversed several times to ensure a proper mixing of the additive with the blood. Plasma was then separated by centrifugation 2000x g for 15 minutes at 4 °C	**<u>Hexokinase method</u>** Glucose was phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP) (Duxbury, 2004). Glucose-6-phosphate dehydrogenase (G-6-PD) specifically oxidised G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide phosphate (NADP) to nicotinamide adenine dinucleotide phosphate reduced (NADPH). One micromole of NADPH was produced for each micromole of glucose consumed. The NADPH produced absorbs light at 340 nm and was detected spectrophotometrically.	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).
Serum lipids profile, insulin, hsCRP analysis (outsourced) **Leptin analysis (by own)**	 Total 6ml of fasting blood was drawn into Gold top Vacutainer tubes with clot activator and gel for serum separation (Vacutainer 368430; Becton Dickinson)	The tube was left to clot immediately at room temperature for 30 minutes, centrifuged at 1500x g at 4°C for 15 minutes. The supernatant (serum) was then aliquoted into 1.5ml labelled Eppendorf tubes and stored at -20°C until analysis	**Total cholesterol:** **<u>Enzymatic-colorimetric methods</u>** Cholesterol was hydrolysed into free cholesterol and fatty acids by cholesterol esterase (CHE) (H. S. Huang, Kuan, & Guilbault, 1975). The cholesterol was oxidised into cholest-4-ene-3-one and hydrogen peroxide (H_2O_2) in the reaction catalysed by cholesterol oxidase (CHOD). The hydrogen peroxide combined with Hydroxybenzoic Acid (HBA) and 4-aminoantipyrine to form a chromophore (Quinone mine dye) which was quantitated at 500 nm.	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).
			HDL-cholesterol: **<u>Enzymatic-direct determination method</u>** The determination of HDL cholesterol with homogeneous procedure is based on a novel methodology – the elimination method. The elimination method consists of two specific steps. In the first step, chylomicron, VLDL and LDL fractions were eliminated under specific conditions so cholesterol was derived only from HDL (Warnick, Nauck, & Rifai, 2001). These fractions were oxidised to cholestenone and hydrogen peroxide, which subsequently degraded by catalase. In the second step, after various enzymatic reactions and in the presence of specific surfactants,	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).

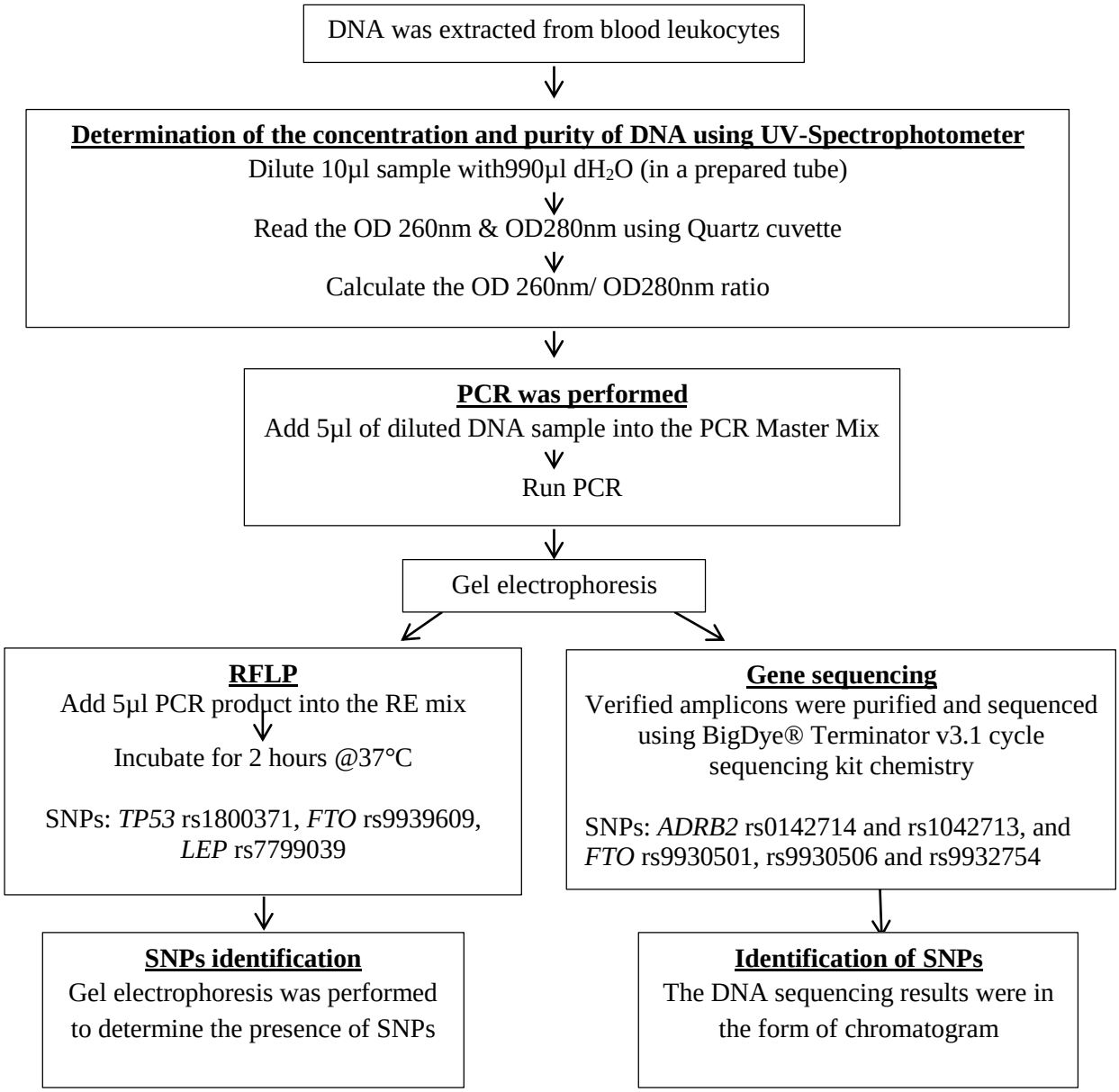
			remaining HDL cholesterol was measured specifically as a color formation (Quinone pigment), which intensity was proportional to the concentration of HDL cholesterol contained in sample.	
			Triglyceride: **<u>Enzymatic- colorimetric method</u>** Triglycerides were hydrolysed by lipoprotein lipase (LPL) to glycerol and fatty acids; glycerol was phosphorylated to glycerol-3-phosphate in presence of glycerol kinase (GK) and adenosine 5-triphosphate (ATP) and then converted by glycerol-3-phosphate oxidase (GPO) into dihydroxyacetone phosphate and hydrogen peroxide (Fossati & Prencipe, 1982). In the presence of peroxidase (POD), the hydrogen peroxide oxidised the chromogen 4-aminoantipyrine and 4-chlorophenol (4-CP) to form a red compound which color intensity was proportional to the concentration of triglyceride present in the sample.	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).
			Serum insulin analysis: **<u>Chemi-luminescent micro-particle immunoassay (CMIA)</u>** It is a one-step immunoassay to determine the presence of insulin in human serum using Chemiluminescent Micro particle Immunoassay (CMIA) technology (Cinquanta, Fontana, & Bizzaro, 2017). Insulin present in the samples bind to the anti-insulin coated micro particles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions were added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship existed between the amount of C-peptide in the sample and the RLUs detected by the optical system.	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).
			Serum hsCRP analysis: **<u>Immunoturbidimetric method</u>** The hsCRP assay is based on a latex enhanced immunoturbidi- metric assay (Denham et al., 2007). When an antigen-antibody reaction occurs between hsCRP in a sample and anti-hsCRP which has been sensitised to latex particles, agglutination results. This agglutination is detected as an absorbance change (570nm), with the magnitude of the change being proportional to the quantity of hsCRP in the sample. The actual	Architect ci8200 system (Abbott Diagnostics, Abbott Park, IL, USA).

			concentration is then determined by the interpolation from a calibration curve prepared from calibrators of known concentration.	
			Serum leptin analysis: **<u>Enzyme-linked immunosorbent assay (ELISA) method</u>** Antibody specific to human leptin was coated on the 96 wells (Aydin, 2015). Standards and samples are added into the wells and Leptin present is bound to the wells by the immobilised antibody. The wells are washed and biotinylated antihuman Leptin antibody is added. Unbound biotinylated antibody, HRP conjugated streptavidin is washed off by wash buffer. The intensity of the color develops in proportion to the amount of human leptin and was measured at 450nm.	RayBio® Human Leptin ELISA Kit

3.7 Genetic analysis

Both restriction fragment length polymorphism (RFLP) and direct gene sequencing methods were employed to explore the variations in DNA sequences, and to identify the presence of the gene polymorphisms. A schematic diagram (flow chart) of the lab experiment for genotyping is demonstrated in Figure 3.3. The detailed information on each procedure is described next as well.

Figure 3.3: Flow chart of the lab experiment for genotyping



3.7.1 DNA extraction

5ml of whole blood was drawn from antecubital vein into vacutainer tubes containing EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ). Genomic DNA was isolated from blood leukocytes using MasterPure DNA Purification kit according to manufacturer's instructions. DNA samples were then stored at -30 °C until use.

3.7.2 DNA purity and concentration

Determination of DNA purity and concentration is essential for downstream applications, such as polymerase chain reaction (PCR) (Boesenberg-Smith, Pessarakli, & Wolk, 2012). The purity and concentration of DNA samples can be determined by measuring the absorbance of ultraviolet light (in optical density/OD) using Eppendorf BioPhotometer® D30 (Eppendorf AG, Hamburg, Germany). A260/280 was used to measure DNA purity in the presence of nucleic acid. Pure DNA should have an A260/280 ratio of 1.8 or above, readings below 1.8 may indicate significant amounts of protein contamination. For pure DNA, the most common method to determine the concentration is by reading the absorbance at 260nm where an OD (optical density) in a 1cm path length corresponds to 50 µg/ml double stranded DNA solution (1 OD_{260} unit = 50 µg/ml). The DNA concentration was calculated using the formula below (Barbas et al., 2007):

$$DNA\ Concentration\ (\mu g/ml) = OD_{260} \times dilution\ factor \times 50 \mu g/ml$$

The total DNA yield (µg) was obtained by multiplying the DNA concentration by the sample volume:

$$DNA\ yield\ (\mu g) = DNA\ concentration\ (\mu g/ml) \times total\ sample\ volume\ (ml)$$

3.7.3 Polymerase chain reaction (PCR)

Targeted regions of DNA were amplified using PCR method (Jane Gurr, 1991). Optimisation of PCR reaction condition is very important as failure of PCR will lead to many non-specific DNA products of varying sizes that appear as a smear of bands on agarose gels, or no product form at all. Therefore, optimisation was employed by varying the PCR conditions (e.g. the time and temperature needed for annealing) to obtain a better PCR outcomes (e.g. a clean and clear band). The PCR protocols and optimal conditions for each gene polymorphism are summarised in Table 3.4. The amplicons obtained from PCR were verified using electrophoresis on 2% agarose gel and visualised under ultraviolet illumination after staining by ethidium bromide.

Table 3.4: PCR protocol for each gene polymorphism

Thermal cycle	**Step 1**	**Step 2: 35 cycles of denaturation, annealing and extension**			**Step 3**
Gene polymorphisms	**Initial denaturing (°C, time)**	**Denaturation (°C, time)**	**Annealing (°C, time)**	**Extension (°C, time)**	**Final extension (°C, time)**
***FTO* rs9939609**	95ºC for 5 min	94ºC for 45	57ºC for 60s	72ºC for 45 sec	72ºC for 5 min
***FTO* rs9930501, rs9930506 and rs9932754**	95ºC for 5 min	94ºC for 45	53ºC for 30s	72ºC for 45 sec	72ºC for 5 min
***ADRB2* rs1042713 and rs1042714**	95ºC for 5 min	94ºC for 45	64ºC for 40s	72ºC for 45 sec	72ºC for 5 min
***TP53* rs1800371**	95ºC for 5 min	94ºC for 45	53.1ºC for 60s	72ºC for 45 sec	72ºC for 5 min
***LEP* rs7799039**	95ºC for 5 min	94ºC for 45	55ºC for 60s	72ºC for 45 sec	72ºC for 5 min

The main components to perform PCR include (i) a DNA template which contains the target sequence, (ii) PCR oligonucleotide primers, which are complemented to the 3' ends of each strand of DNA to serve as a starting point for DNA amplification, (iii) deoxynucleotide triphosphates (dNTPs) such as adenine (A) thymine (T), guanine (G) and cytosine (C) are required to provide the building block for DNA replication, (iv) a heat-stable DNA polymerase, called *Taq* polymerase which is isolated from heat-tolerant bacterium, *Thermus aquaticus* to elongate the DNA strand by adding nucleotides to the sequence, and (v) a buffer solution containing magnesium chloride ($MgCl_2$) to stabilise the activity of DNA polymerase (Ho, Hunt, Horton, Pullen, & Pease, 1989). All the reagents were kept on ice throughout the experiment to avoid degradation of DNA samples and reagents. The reaction mix was prepared and PCR was performed following the standard procedure of the manufacturer's instruction in Table 3.5.

Table 3.5: Reaction components for PCR

Components	**Volume**	**Final Concentration**
ddH_20	14.6 µl	
*DNA sample	5 µl	1ng–1µg
**100 µM Primer_F	0.2 µl	***0.08 µM (0.05 µM-1 µM)
**100 µM Primer_R	0.2 µl	***0.08 µM (0.05 µM-1 µM)
[a]5x Red Master Mix	5 µl	1X
Total	25 µl	

[a]5x Red Master Mix is a premixed solution which containing *Taq* polymerase, dNTPs, MgCl2 and reaction buffer.

*DNA is diluted by adding 10µl of DNA sample into 990 µl ddH_20 (with dilution factor, 100)

** 10 µl of 100 µM primers is added into 99 µl ddH_20. Then adding 0.2 µl of diluted primer to 25µl solution will give a final concentration of 0.08 µM.

***Calculation for final concentration of primers:

$V_1M_1 = V_2M_2$

(0.2 µl)(10 µM) = (25 µl) M_2

M_2 = 0.08 µM

3.7.3.1 Primers for polymerase chain reaction (PCR)

A PCR primer is a short, artificial, single DNA strand which generally about 18-25 bases. It serves as a starting point for DNA amplification. The primers anneal to the targeted sequence of the single strand DNAs and allow the elongation of nucleotides to the existing strand of DNAs by DNA polymerases. Generally, primer concentration between 0.1 µM and 0.5 µM is optimal for PCR. Higher primer concentration may promote mispriming which leads to the accumulation of primer-dimers and non-specific products (Jane Gurr, 1991). These primer-dimers and non-specific products may subsequently compete with the desired products, primers and dNTPs, and results in lower yield of desired products. The primers used in PCR and the expected outcomes for each gene polymorphism investigated in this doctoral thesis are reported in Table 3.6. The primers were obtained from previously published studies except for *TP53* rs1800371 which was self-designed by using the primer designing program, Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The details of the specific primers pair (such as base length, optimal melting temperature and etc.) can be retrieved.

Table 3.6: The primers used in PCR and the expected outcomes for each gene polymorphism

Gene	SNPs	Location	Nucleotide substitution	Primer sequence	Expected size of PCR product (bp)	References
ADRB2	rs1042714 and rs1042713	Chromosome 5	C→G A→G	Forward: 5'-CCGCCGTGGGTCCGCC -3' Reverse: 5'-CCATGACCAGATCAGCAC -3'	310	Macho-Azcarate et al., 2002
FTO	rs9930501, rs9930506 and rs9932754	Chromosome 16	A→G A→G T→C	Forward: 5'-TGATGAGAATGTAAGAAG GGAGA -3' Reverse: 5'-TCATTTGACAGATGGACT TTTCA -3'	237	Gemma et al., 2009
TP53	rs1800371	Chromosome 17	C→G	Forward: 5'-TTCACCCATCTACAGTCC C-3' Reverse: 5'-CCAGACGGAAACCGTAGC -3'	253	Self-designed
FTO	rs9939609	Chromosome 16	T→ A	Forward: 5'-AACTGGCTCTTGAATGAA ATAGGATTCAGA -3' Reverse: 5'-AGAGTAACAGAGACTAT CCAAGTGCAGTAC -3'	182	Bracho-Riquelme et al., 2011
LEP	rs7799039	Chromosome 7	G →A	Forward: 5'-TTTCCTGTAATTTTCCCGT GAG -3' Reverse: 5'-AAAGCAAAGACAGGCAT AAAAA -3'	242	Bracho-Riquelme et al., 2011

ADRB2, beta-2 adrenergic receptor; *FTO*, fat and obesity-associated; *TP53*, tumor protein p53; *LEP*, leptin; SNPs, single nucleotide polymorphisms.

Below is the list of the characteristics that were considered when selecting a primer (Lorenz, 2012):

1. The length of primer should be around 15-30bp
2. Optimal G-C content should range between 40-60%
3. The final optimal melting temperatures (Tm) for both primers should not differ more than 5 °C.
4. Avoid di-nucleotide repeats (e.g. TATATATA) and single base runs (e.g. TTTT) as it will result in the formation of hairpin loop structure or causing slipping along the primed segment of DNA.
5. The first and last 20-30 bases of DNA sequencing are usually unresolved and small, thus it was recommended to design the primer at least 50bp upstream of the sequence of interest.

Once the primers have been selected, it can be tested using Sequence Manipulation Suite (Source: http://www.bioinformatics.org/sms2/pcr_products.html). It is a visual digest of PCR products by inserting the DNA sequences of interested (in FASTA format) (Appendix 10) and the selected primers to predict the outcomes of the PCR products.

3.7.4 Gel electrophoresis

Gel electrophoresis was performed i) to visualise and reveal the presence or absence of the amplified PCR and RE products and ii) to determine the size of the bands produced which were then compared with the predicted result. A 2% agarose gel was obtained by mixing 0.8g of agarose powder with 40ml of electrophoresis buffer (Tris-borate-EDTA (TBE)) and 1µl of ethidium bromide (EtBr) was added into the gel. Ethidium bromide is a fluorescent dye and upon binding of the molecule to the DNA and illumination with a UV light source, hence the DNA banding pattern can be visualised and identified A 100bp DNA ladder was loaded together with the PCR and RE products as a molecular size marker.

After running the PCR products on agarose gel, DNA fragments of 182bp containing *FTO* rs9939609 were observed (Figure 3.4); DNA fragments of 242bp containing *LEP* rs7799309 were observed (Figure 3.5); DNA fragments of 253bp containing *TP53* rs1800371 were observed (Figure 3.6); DNA fragments of 237bp containing *FTO* rs9930501, rs9930506 and rs9932754 were observed (Figure 3.7) (Appendix 11); DNA fragments of 310bp containing *ADRB2* rs1042713 and rs1042714 were observed (Figure 3.8) (Appendix 12).

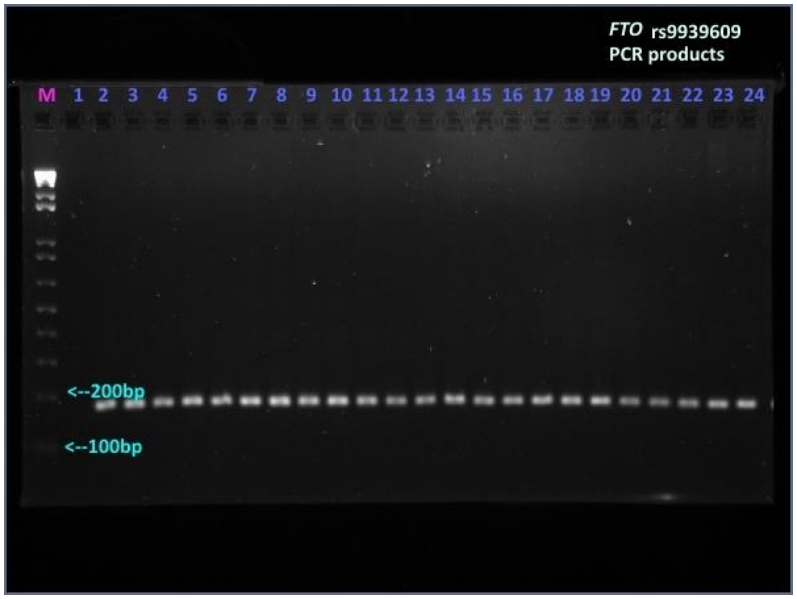


Figure 3.4: Visualisation of amplified PCR products containing *FTO* rs9939609 (182bp).
Lane M: 100bp DNA marker
Lane 1: Negative control (no DNA samples)
Lane 2-24: DNA samples from participants

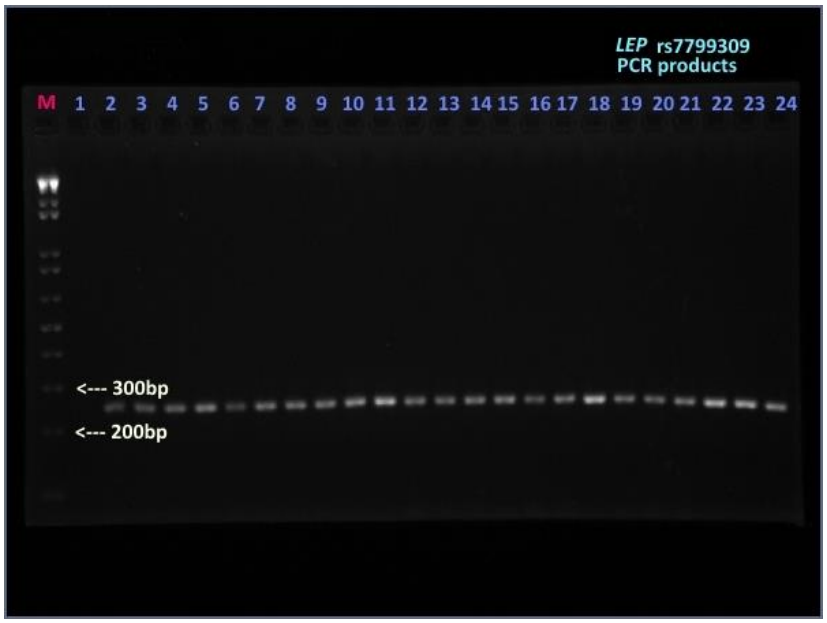


Figure 3.5: Visualisation of amplified PCR products containing *LEP* rs7799309 (242bp).
Lane M: 100bp DNA marker
Lane 1: Negative control (no DNA samples)
Lane 2-24: DNA samples from participants

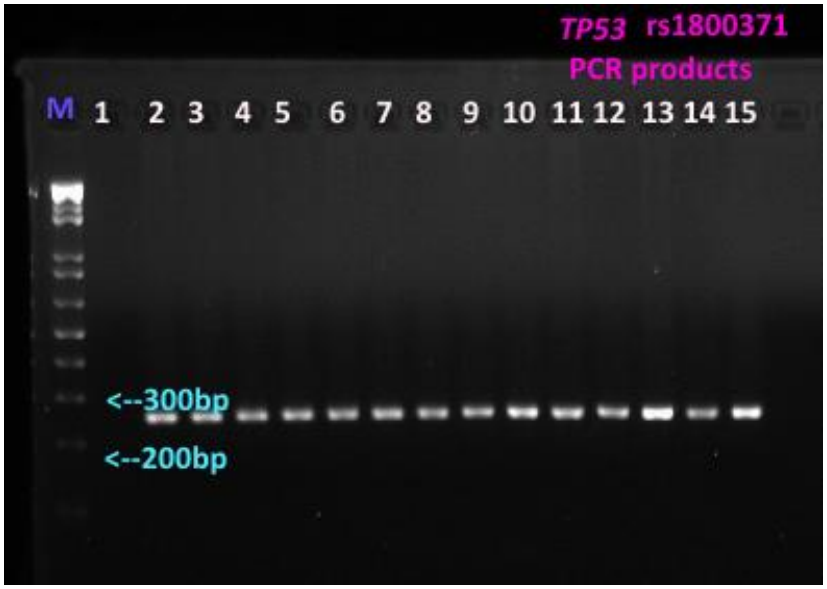


Figure 3.6: Visualisation of amplified PCR products containing *TP53* rs1800371 (253bp).
Lane M: 100bp DNA marker
Lane 1: Negative control (no DNA samples)
Lane 2-15: DNA samples from participants

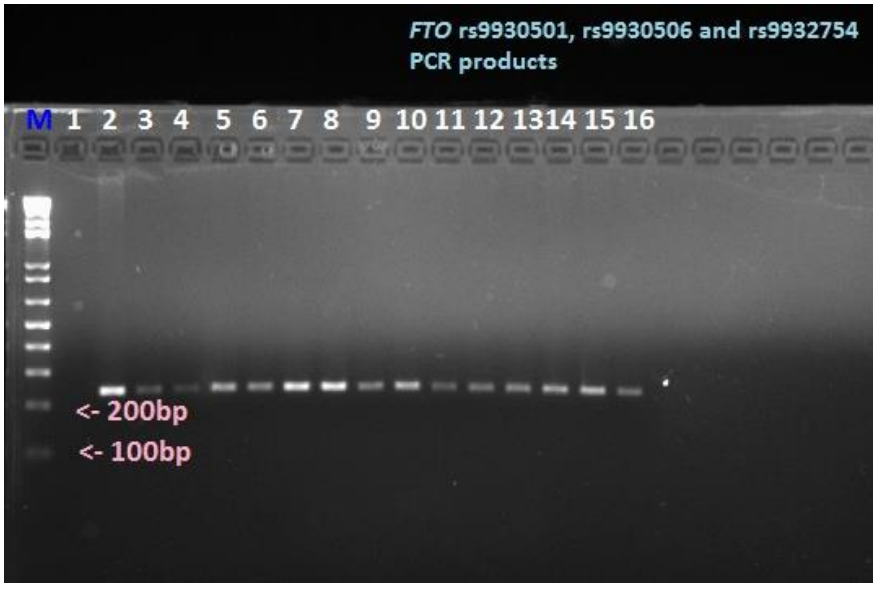


Figure 3.7: Visualisation of amplified PCR products containing *FTO* rs9930501, rs9930506 and rs9932754 (237bp)
Lane M: 100bp DNA marker
Lane 1: Negative control (no DNA samples)
Lane 2-16: DNA samples from participants

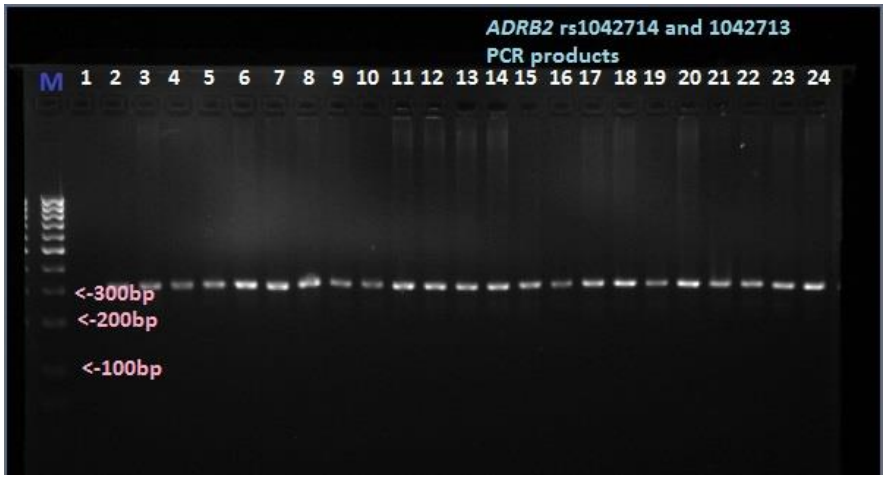


Figure 3.8: Visualisation of amplified PCR products containing *ADRB2* rs1042713 and rs1042714 (310bp).
Lane M: 100bp DNA marker
Lane 1: Negative control (no DNA samples)
Lane 2-24: DNA samples from participants

3.7.5 Genotyping using RFLP or gene sequencing methods

The SNP-containing PCR amplicons (*FTO* rs9930609, rs9930501, rs9930506 and rs9932754, *ADRB2* rs1042714 and rs1042713, *TP53* rs1800371 and *LEP* rs7799309) were then genotyped using either restriction fragment length polymorphism (RFLP) method or direct gene sequencing method. RFLP method was initially performed to identify the presence of gene polymorphisms (*TP53* rs1800371, *FTO* rs9939609 and *LEP* rs7799039) in this doctoral thesis. However, this genotyping method only allows detection of a single base change at a specific restriction site. Therefore, under much consideration, direct gene sequencing method was then preferred to be used to identify the subsequent SNPs (*ADRB2* rs1042713 and rs1042714 and *FTO* rs9930501, rs9930506 and rs9932754) due to its ability to discover more than one nucleotide variations.

3.7.5.1 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a conventional method which allows detection of single nucleotide polymorphism (SNP) after the genomic sequence was amplified by PCR (Ota et al., 2007). It is a process where DNA molecules are digested into smaller pieces with a special enzyme called restriction endonucleases or restriction enzyme (RE). DNA restriction enzymes recognise specific sequences in DNA and catalyse endonucleolytic cleavages, yielding fragments of different lengths (Botstein et al., 1980). Gel electrophoresis of the RE products reveals a specific banding profile which allows easy identification for the

presence or absence of the SNP. Smaller DNA fragments are generated when the restriction site is present whereas the DNA fragments lack of restriction site resulted in an undigested PCR product. The restriction enzymes required in identifying the presence of *FTO* rs9939609 and *LEP* rs7799039 gene polymorphisms were obtained from previously published studies (Table 3.6). However, the restriction enzyme for *TP53* rs1800371 was self-designed using program Restriction mapper on http://www.restrictionmapper.org/. A virtual digest of the restriction enzyme (RE) on the PCR amplicons was performed by using RestrictionMapper (Source: http://www.restrictionmapper.org/) to ensure the restriction digestion outcomes. The restriction enzyme (RE) and the expected outcomes of restriction digestion (product sizes of the bands) for each gene polymorphisms are reported in Table 3.7.

Table 3.7: Restriction enzymes (REs) and the expected outcomes of RFLP for each gene polymorphism

Gene	SNPs	RE	Recognition site	Expected size of RE products (bp)	References
TP53	rs1800371	NciI	CC^SGG GGS^CC	G/G = 253bp G/C = 253, 189 and 58bp C/C= 195 and 58bp	Self-designed
FTO	rs9939609	ScaI	AGT^ACT TCA^TGA	T/T= 182bp T/A = 182, 154 and 28bp A/A = 154 and 28bp	Bracho-Riquelme et al., 2011
LEP	rs7799039	HhaI	GCG^C C^GCG	A/A = 242bp G/A= 242, 181 and 61bp G/G = 181 and 61bp	Bracho-Riquelme et al 2011

RE, restriction endonucleases or restriction enzyme; RFLP, restriction fragment length polymorphisms; SNPs, single nucleotide polymorphisms.

The products of restriction digestion for each polymorphism were run on the gel electrophoresis to visualise the profile of the bands (Figures 3.9-3.11). To identify the presence of *FTO* rs9939609, 182bp of PCR product was digested with ScaI restriction endonuclease which recognises the restriction site of AGT^ACT. Thus, individuals carrying TT genotype of *FTO* rs9939609 produced only one band (182bp), individuals carrying AA genotype produced two bands (154bp and 28bp), and individuals carrying both T and A alleles of *FTO* rs9939609 produced three bands (182bp, 154bp and 28bp) (Figure 3.9). With respect to *LEP* rs7799039, 242bp of PCR product was digested with HhaI restriction endonuclease which recognises the restriction site of GCG^C. Thus, individuals carrying AA genotype produced one band (242bp), individuals carrying GG genotype produced two bands (181bp and 61bp), and individuals carrying both A and G allele of *LEP* rs7799039 produced three bands (242np, 181bp and 61bp) (Figure 3.10). To identify *TP53* rs1800371 gene polymorphism, 253bp of PCR product was digested with NciI restriction endonuclease which recognises the restriction site of CC^SGG

(Figure 3.11). Thus, individuals carrying GG genotype of *TP53* rs1800371 produced only one band (253bp), individuals carrying CC genotype produced two bands (195bp and 58bp) and individuals carrying both G and C alleles of *TP53* rs1800371 produced three bands (253bp, 195bp and 58bp) when performing gel electrophoresis.

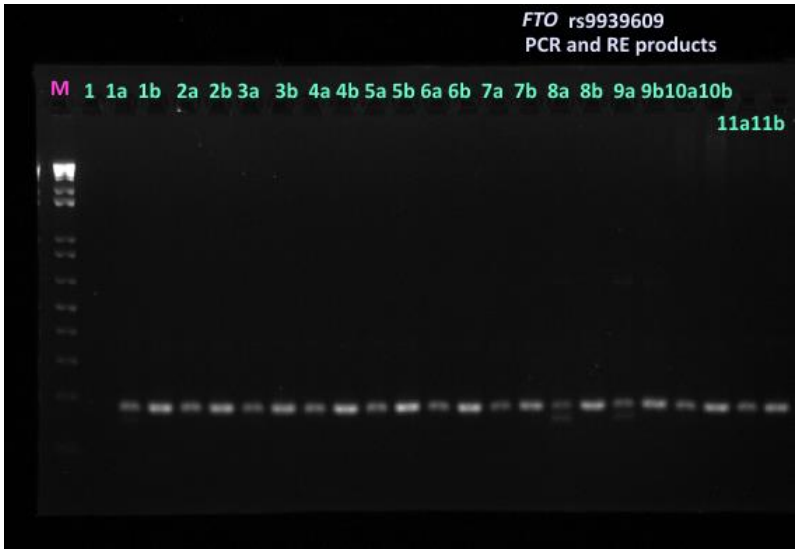


Figure 3.9: Restriction digested of 182 base pair amplified product of *FTO* gene using ScaI. Lane M: 100bp DNA Marker; Lane 1: Negative control; Alphabet a and b represent the RE and PCR products from respective DNA sample; Lane 1a, 8a and 9a: Heterozygous TA allele having normal bands 182bp, 154bp and 28bp fragments; Lane 2a-7a and 10a: DNA remained uncut showing homozygote TT allele with 182bp fragment.

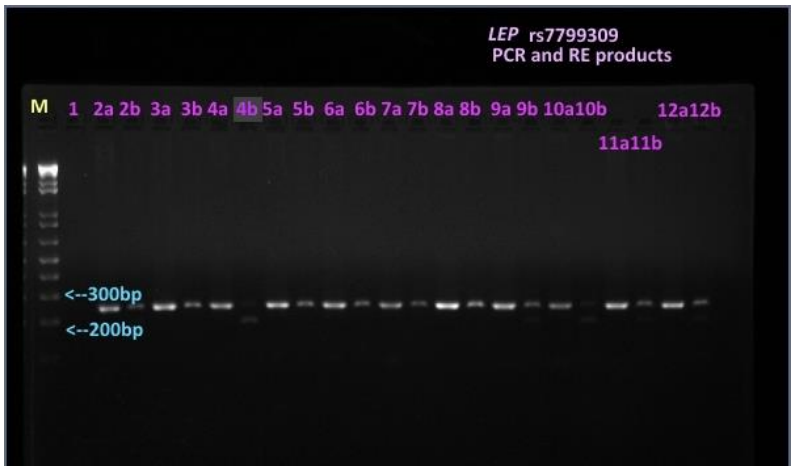


Figure 3.10: Restriction digested of 242 base pair amplified product of *LEP* gene using HhaI. Lane M: 100bp DNA Marker; Lane 1: Negative control; Alphabet a and b represent the RE and PCR products from respective DNA sample; Lane 9b, 10b, 11b and 12b: Heterozygotes GA allele having normal bands of 242bp, 181bp and 61bp fragments; Lane 2b, 3b, 5b, 6b, 7b and 8b: DNA remained uncut showing homozygote mutant AA allele with 242 bp fragment; Lane 4b: Homozygote wild type GG allele showing normal bands of 181bp and 61bp fragments as the cutting site of HhaI is at G allele.

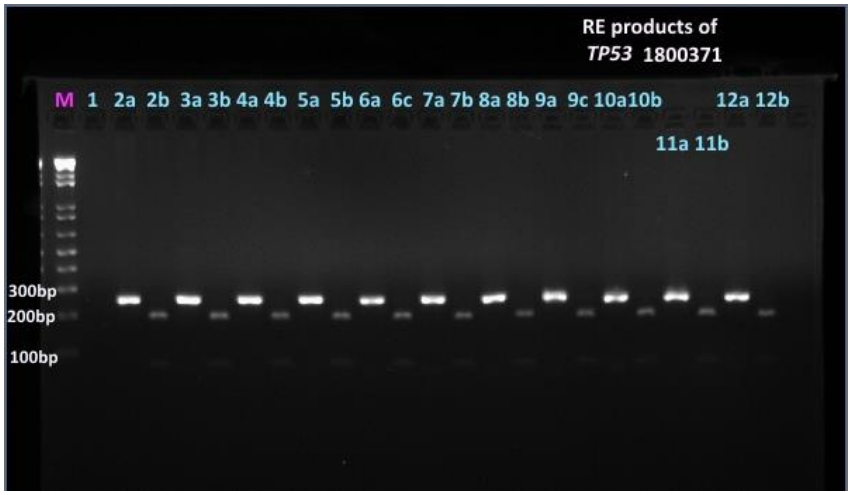


Figure 3.11: Restriction digested of 253 base pair amplified product of *TP53* gene using NciI. Lane M: DNA Marker; Lane 1: Negative control; Alphabet a and b represent the RE and PCR products from respective DNA sample; All lanes with alphabet a: Homozygote CC genotype is showing bands of 195bp and 58bp fragments as the cutting site of NciI is at C allele.

3.7.5.2 Direct gene sequencing method

The verified PCR amplicons were purified and sequenced using dideoxy approach (Sanger's method) with BigDye® Terminator v3.1 cycle sequencing kit chemistry performed by First Base Sequencing Company. Sanger's method is also known as dideoxy chain termination method which is similar with DNA replication. The single-stranded DNA molecules are annealed to oligonucleotide primers and elongated using a mixture of deoxynucleotide triphosphates (dNTPs such as dATP, dCTP, dTTP and dGTP) to provide the needs for adenine (A), cytosine (C), thymine (T), and guanine (G) nucleotides to generate new double-stranded DNA molecules (Shendure et al., 2011). In addition, a small quantity of chain-terminating dideoxynucleotide triphosphates (ddNTPs) (ddATP, ddGTP, ddCTP, ddTTP) is included as well. ddNTPs are similar to dNTPs except that they contain a hydrogen group on the 3' carbon instead of a hydroxyl group (OH), which prevent the elongation of further nucleotides once they integrated into the sequence. At that point, DNA chains are terminated and different lengths of strands are produced. Each strand ended with a specific dideoxynucleotide, which is associated with a fluorescent marker. Green indicated Adenine (A), red indicated thymine (T), black indicated guanine (G) and blue indicated cytosine (C). Different colours of dyes fluoresce at different wavelengths. Therefore, these fluorescence-labelled ddNTPs can be detected after electrophoresis and a sequence in the form of a chromatogram can be generated. Chromatogram is a diagram of different colored peaks that correspond to different nucleotides in the sequence. FinchTV, a chromatogram viewing program was used to view the chromatogram of the sequenced DNA.

3.7.5.2.1 Interpretation of DNA chromatogram

The signal intensities were presented in a graph with four differently colored-peaks, with each colour corresponding to a different DNA base (Figures 3.12 & 3.13). The heterozygosity of the single nucleotide polymorphism appeared as double peak with different colors and homozygosity of the single nucleotide polymorphism appeared as single peak. Green indicated Adenine (A), red indicated thymine (T), black indicated guanine (G) and blue indicated cytosine (C). The DNA sequences obtained from chromatogram were compared with the reference sequences provided by NCBI database (Human RefSeqGene Sequences) using Nucleotide Blast.

a) *FTO* rs9930501, rs9930506 and rs9932754

Double peak with different colors indicated heterozygosity of *FTO* rs9930501, rs9930506 and rs9932754 (Figure 3.12a). Double peak of green and black colors represents heterozygote AG genotype of rs9930501 and rs9930506; while double peak of red and blue colors represents heterozygote TC genotype of rs9932754. Single peak indicated homozygosity of *FTO* rs9930501, rs9930506 and rs9932754 (Figures 3.12b & 3.12c). A single green colored-peak represents homozygote AA genotype of rs9930501 and rs9930506, and single red colored-peak represent homozygote TT genotype of rs9932754 (Figure 3.12b). A single black colored-peak represents homozygotes GG genotypes of rs9930501 and rs9930506, and single blue colored-peak represents homozygote CC genotype of rs9932754 (Figure 3.12c).

Figure 3.12a)

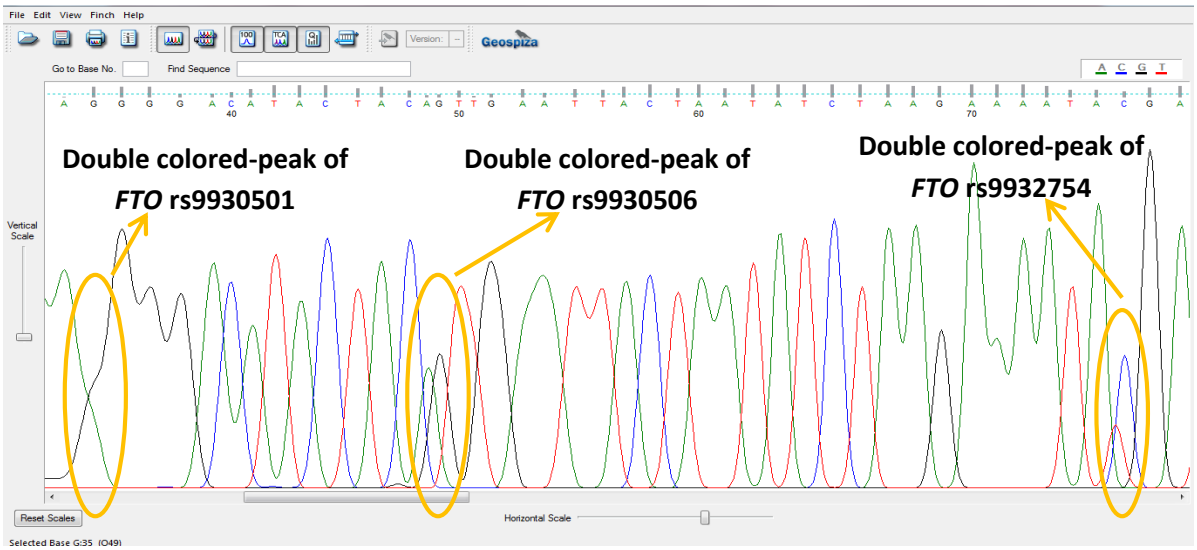


Figure 3.12 (a-c): DNA sequences which included the polymorphisms in *FTO* rs9930501, rs9930506 and rs9932754 in the form of chromatogram.

Figure 3.12b)

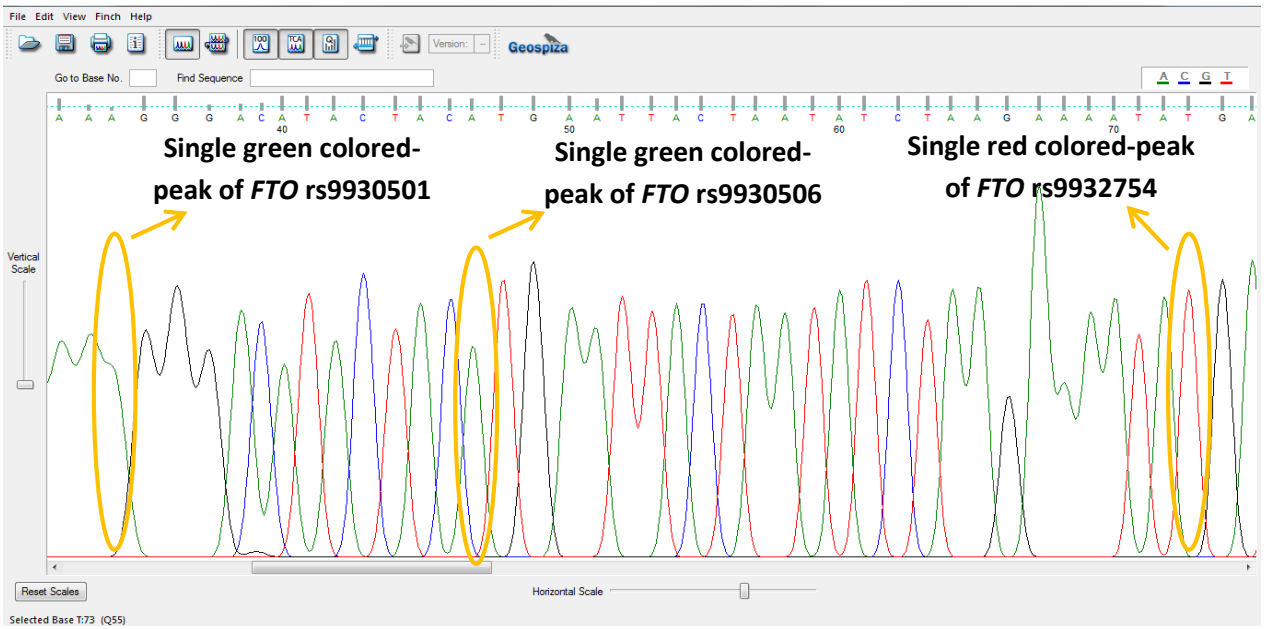


Figure 3.12c)

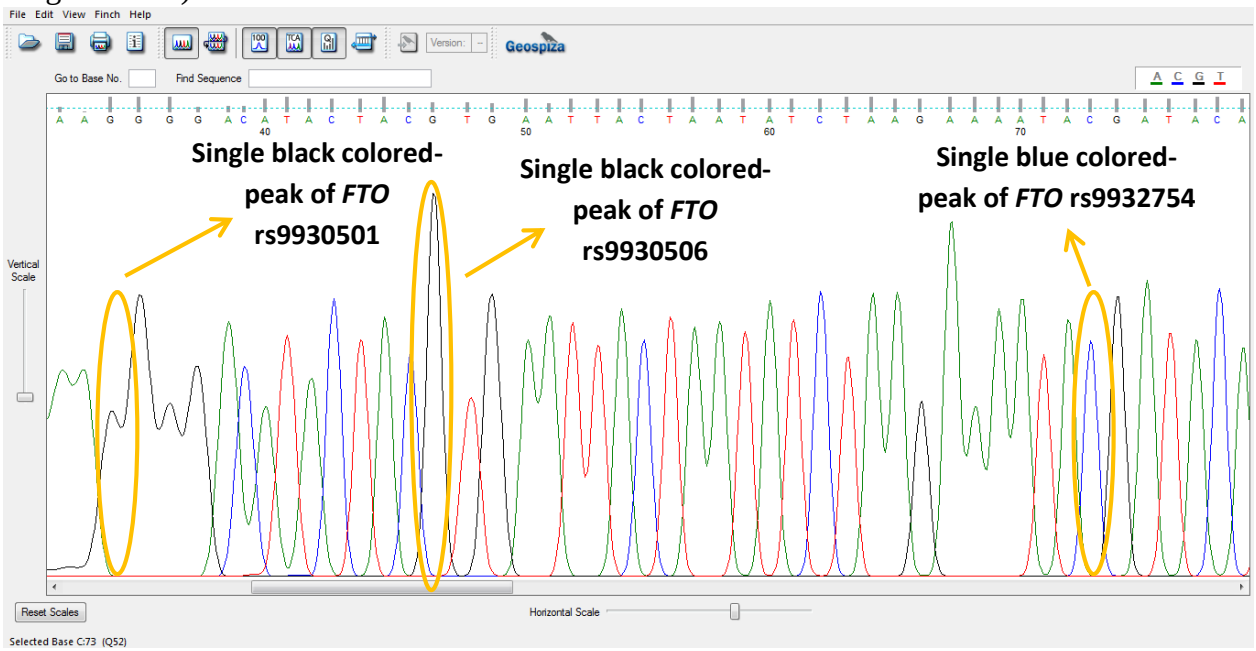


Figure 3.12 (a-c): DNA sequences which included the polymorphisms in *FTO* rs9930501, rs9930506 and rs9932754 in the form of chromatogram.

b) *ADRB2* rs1042714 and rs1042713

Double peak with different colors indicated heterozygosity of *ADRB2* rs1042714 and rs1042713 (Figure 3.13a). Double peak of black and blue colors represents heterozygote of GC genotype of rs1042714 (the complement base was CG genotype) and double peak with red and blue colors represents heterozygote of TC genotype of rs1042713 (the complement base was AG genotype). Single peak indicated homozygosity of *ADRB2* rs1042714 and rs1042713 (Figures 3.13b & 3.13c). A single black colored-peak of rs1042714 and single red colored-peak of rs1042713 represent homozygotes GG and TT genotypes (with the complement base of CC and AA genotypes), respectively (Figure 3.13b); A single blue colored-peak of rs1042714 and rs1042713 represents homozygote CC genotype (with the complement base of GG genotype) (Figure 3.13c).

Figure 3.13a)

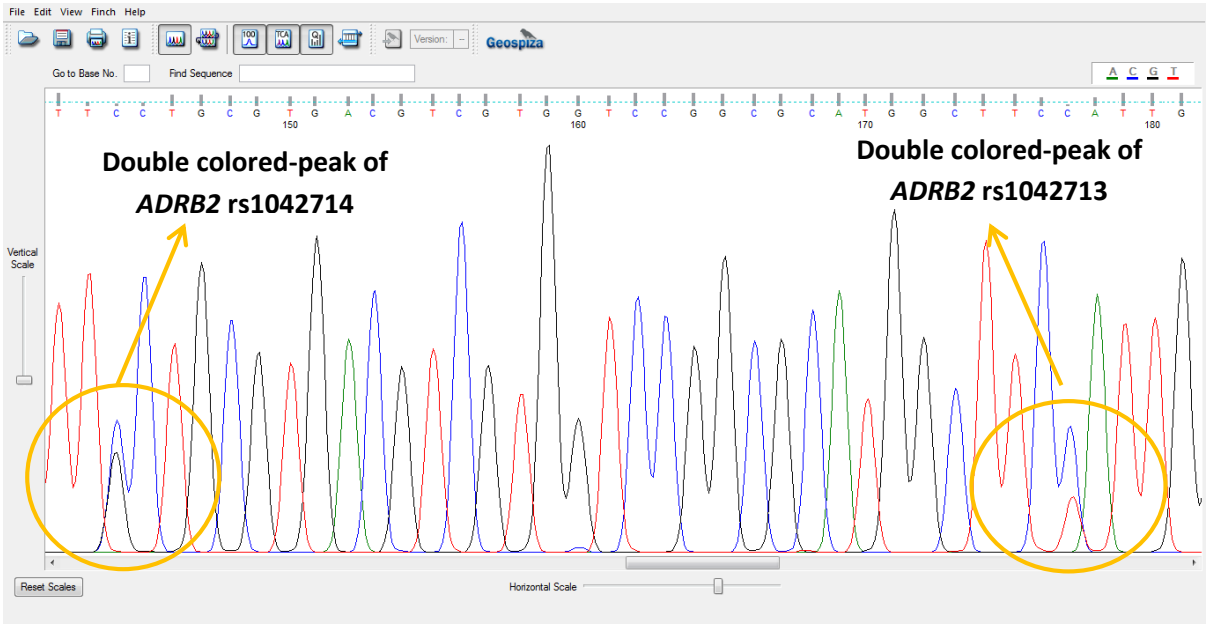


Figure 3.13 (a-c): DNA sequences which included the polymorphisms in *ADRB2* rs1042713 and rs1042714 in the form of chromatogram.

Figure 3.13b)

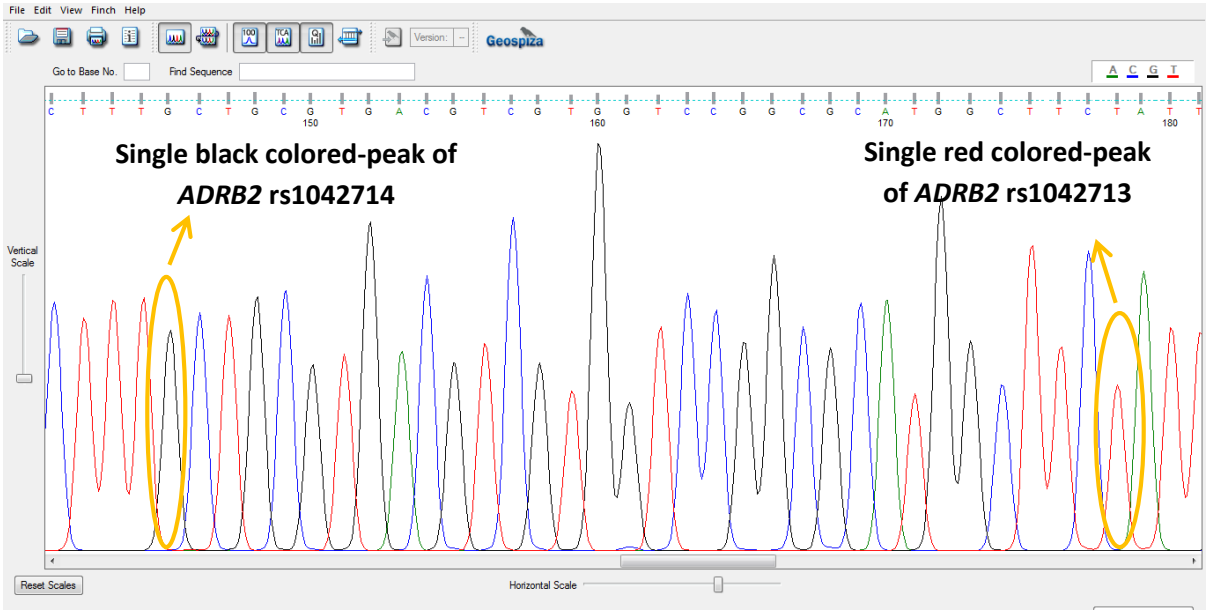


Figure 3.13c)

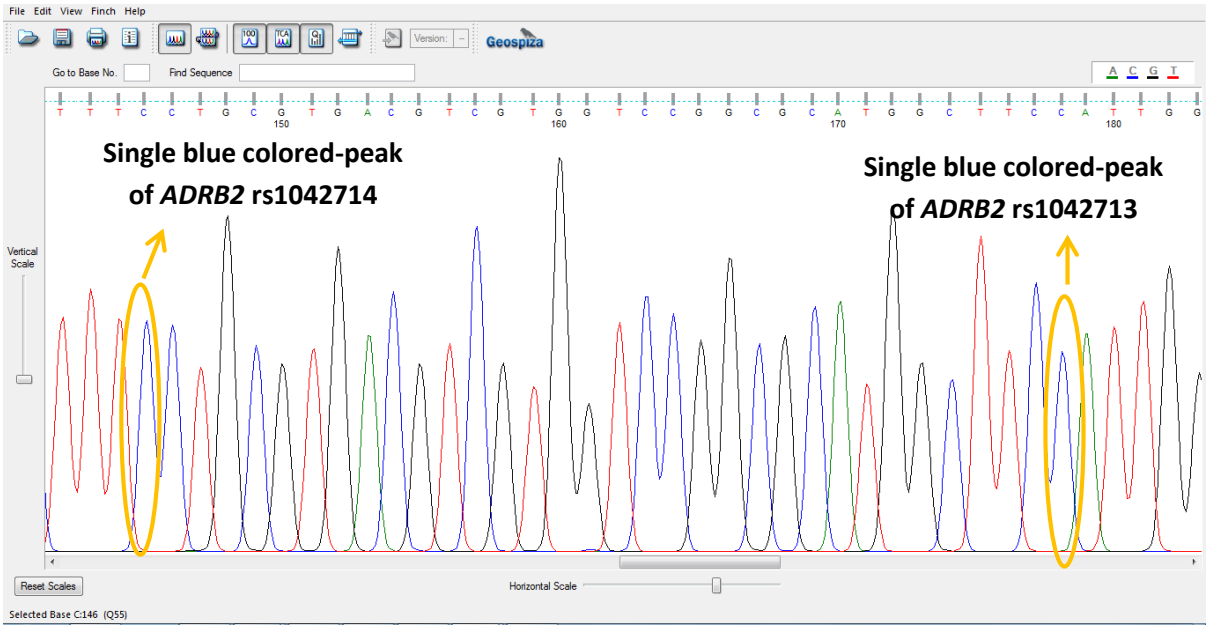


Figure 3.13 (a-c): DNA sequences which included the polymorphisms in *ADRB2* rs1042713 and rs1042714 in the form of chromatogram.

3.7.6 Obesity-associated gene polymorphisms investigated in this doctoral thesis

After a pilot study (n=40), it was decided that *TP53* rs1800371, *FTO* rs9930609 and *LEP* rs7799309 gene polymorphisms to be excluded from this doctoral thesis. *TP53* rs1800371 was excluded as all the study participants were carrying the C allele of *TP53* rs1800371, limiting the study for comparative analysis; *LEP* rs7799309 was excluded as *LEP* rs7799309 was not successfully genotyped, the observed bands of PCR products from gel electrophoresis did not match with the expected results (185bp and 58bp); *FTO* rs9930609 was excluded as this SNP had been previously reported in Malaysian adults by Chey et al., 2013, and no association was found between this SNP and risk of obesity. Therefore, *FTO* rs9930501, rs9930506 and rs9932754 and *ADRB2* rs1042713 and rs1042714 gene polymorphisms (not reported in the Malaysian population) were investigated in this doctoral thesis.

Chapter 4

Dietary intakes and obesity-related traits in multi-ethnic Malaysian adults (Chinese, Malays and Indians)

4.1 Introduction

The prevalence of obesity and its complications are increasing over the past decade. National Health and Morbidity Survey (NHMS) 2015 reported that 5.6 million Malaysian adults aged 18 and above were overweight and 3.3 million were obese (Institute for Public Health, Malaysia, 2015). Numerous studies have reported that excess body weight is associated with increased risk of developing chronic diseases such as cardiovascular diseases, hypertension, type 2 diabetes and some type of cancers (Guh et al., 2009). This was reflected in the high prevalence of obesity-related comorbidities in the Malaysian population. National Health and Morbidity Survey (NHMS) 2015, reported that about 2.5 million and 9.6 million of Malaysian adults aged 18 and above were suffering from type 2 diabetes mellitus and hypercholesterolemia, respectively (Institute for Public Health, Malaysia, 2015). Long term practice of unhealthy eating habit and inactive lifestyle are the major contributors for the rising epidemic of obesity and associated chronic diseases in the Malaysia population. Malaysian Adult Nutrition Survey (MANS) 2014 reported that there was an increase in the consumption of 'processed foods' added with salt and condiments compared to the MANS 2003 (Institute for Public Health, Malaysia, 2015). The rapid economic growth of the country had led to an increase in the consumption of fast foods, high energy-dense foods, sugar, animal fats and refined carbohydrate.

Malaysia is a multi-ethnic and multi-cultural country with a population of 29.1 million according to Current Population Estimate, Malaysia 2017-2018 reported by Department of Statistics Malaysia (Department of Statistics, Malaysia, 2018). The three major ethnic groups in Malaysian population are Bumiputera, Chinese and Indians which accounted for 69.1%, 23% and 6.9%, of the total population, respectively (Department of Statistics, Malaysia, 2018). Ethnic diversity in Malaysia is unique and each of the ethnic group retains their own cultures, languages and religions. Interestingly, disparities in the prevalence of obesity and metabolic syndrome within the ethnic groups had been described earlier. Previous evidence indicated that obesity and its complications disproportionately affect Indians in Malaysian population. According to NHMS 2015, the prevalence of obesity was highest in Malaysian Indian (35%), followed by Malays (31%) and Chinese (28.1%) (Institute for Public Health, Malaysia, 2015). A large scale population-based survey in Malaysian population conducted by Rampal et al., 2012, also reported that ethnic Indians had the highest prevalence of metabolic syndrome, followed by ethnic Malays and ethnic Chinese.

Malaysians of different ethnic background continue to practice their own traditional meal patterns, habitually (Abdullah et al., 2016). Therefore, individualised dietary intervention according to individual's food preference, cultural habit and genetic makeup could be more effective in establishing the individual's adherence to the intervention diet and promote successful and sustainable weight management in the Malaysian population. Understanding the differences in dietary intakes and obesity-related anthropometric and metabolic parameters between the adult Malaysians of the three different ethnic groups is essential for the prevention and treatment of obesity in the Malaysian population.

4.2 Objective

The aim of this chapter is to assess the differences in dietary intake and obesity-related phenotypes between the three major ethnic groups in our study participants (Malaysian Malays, Chinese and Indians).

4.3 Methodology

4.3.1 Study design

This is a cross-sectional study conducted from 2015-2018, which aimed to assess the differences in dietary intakes and obesity-related phenotypes between the three ethnic groups (Malaysian Malays, Chinese and Indians).

4.3.2 Participant selection

A total of 178 Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above were recruited at random through advertisements and flyers, distributed at UNM campus, supermarkets and schools in the vicinity of UNM (Beranang, Jalan Broga and Bandar Kajang). Interested participants completed a health and lifestyle questionnaire which included questions on ethnic background, past diseases, family history of past diseases, physical activity level and substance abuse. Smoking status and alcohol consumption were reported as i) never, ii) former and iii) current. Individuals diagnosed with cardiovascular diseases (CVD), diabetes, cancer, hypertension, renal diseases, endocrine disorders such as hypothyroidism were excluded. Individuals on medications for cholesterolemia, hypoglycemia and psychiatric disorders were excluded. Pregnant and lactating women were also excluded from the study. Interested individuals, attended initial screening to determine whether or not the participant met the inclusion and exclusion criteria. In total 79 obese and 99 non-obese Malaysian adults were

recruited. Each participant was briefed about the programme and written informed consent was requested and obtained from each participant (Appendices 4-7).

4.3.3 Measurement of anthropometric parameters

Detailed information on the measurement of anthropometric parameters was reported in an earlier chapter (Chapter 3). Height of the individual (barefoot) was measured with a standard height rod. Body composition including weight, fat mass (kg), skeletal muscle mass (kg), fat free mass (kg) and percent body fat (%) were measured using a body composition analyzer DSM-BIA Inbody 230 (InBody Co., Ltd., South Korea). Body mass index (BMI) was arrived at by using the formula, weight in kilograms divided by the square of height in meters (kg/m^2). Obesity was defined by BMI $\geq$27.5kg/m^2 (WHO Expert Consultation, 2004). Waist circumference was measured at a level midway between lower rib margin and top of the hip bone with a constant tension tape wrapped around the body horizontally and hip circumference was measured around the widest portion of the buttocks (WHO Expert Consultation, 2008). Waist–hip ratio was calculated using the ratio of the circumference of the waist to the hips. Systolic and diastolic blood pressure were measured using a digital blood pressure monitor (HEM 402C, Omron Healthcare).

4.3.4 Assessment of dietary intake

Detailed information on the assessment of dietary intake has been described in an earlier chapter (Chapter 3). The intake of macronutrients including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and trans fatty acid (TFA) were expressed as a percentage of total energy intake (TE) consumed. The intakes of micronutrients were energy adjusted to unit/total energy intake(kcal)x1000kcal.

4.3.5 Assessment of physical activity level

Physical activity data were collected through an interviewer administered structured questionnaire. Detailed information on the assessment of physical activity level has been described in an earlier chapter (Chapter 3). According to the Ministry of Health Malaysia, individuals who accumulate at least 150 minutes/week of moderate intensity physical activity or 60 minutes/week of vigorous intensity physical activity are considered as physically active, else they are considered as physically inactive (National Coordinating Committee on Food and Nutrition, 2010). Accordingly, participants of the current study were then categorised into two groups; physically active and physically inactive for statistical analysis.

4.3.6 Blood collection and biochemical analysis

Detailed information on the blood collection and biochemical analysis has been described in an earlier chapter (Chapter 3). Fasting blood samples were collected from each participant early in the morning between 7am to 10am following an overnight fast, at baseline and at month 6 of the intervention period. Fasting venous blood was drawn by an experienced phlebotomist from the antecubital vein into vacutainer tubes containing fluoride oxalate for plasma glucose analysis and vacutainer tube with clot activator and gel for serum separation (Becton Dickinson, Oxford, United Kingdom) for serum lipids (including total cholesterol, triglyceride and HDL-cholesterol), serum insulin and hsCRP analysis. All the aforementioned biochemical analysis was assessed using Abbott Architect CI8200 Automatic System according to manufacturer's instructions. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as the product of fasting plasma glucose (mmol/L) and fasting serum insulin (uU/ml) divided by 22.5 according to homeostatic model assessment (HOMA) (Wallace, Levy, & Matthews, 2004). Low-density lipoprotein (LDL) cholesterol was calculated from values of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride using the Friedewald formula: LDL cholesterol = Total cholesterol – [(Triglyceride/5) + HDL cholesterol] (Friedewald, Levy, & Fredrickson, 1972).

4.3.7 Power and sample size calculation

Sample size was computed using the formula $n = [(r+1)/r]*[(SD^2 (Z_\beta + Z_{1-\alpha/2})^2)]/d^2$, according to (Charan & Biswas, 2013). r is the ratio of the number of participants in different groups, 1 for equal number in all the three ethnic groups. To detect a 10-unit difference (d) in body weight, with a population standard deviation (SD) of 11.2 (Ambak et al., 2018), and with a significance level of 95% ($Z_{1-\alpha/2}$= 1.96) and power of 80% (Z_β = 0.84), the required number of participants per ethic group computed was 35. However, we decided to recruit as many participants as possible to secure a good sample size in case attrition rate went up later. Therefore, a total of 178 participants (Chinese n=42; Malays =86; Indians n=50) were recruited for the current study.

4.3.8 Statistical analysis

Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). Data were expressed as mean ± standard error (SE) or number (percentage). Log transformation was performed to transform non-normally distributed data into normally distributed data. Differences in means between ethnic groups and obesogenic phenotypes and dietary components were assessed by using one way ANCOVA with Tukey post hoc test. Variance inflation factor (VIF) was taken as a measure for testing multicollinearity among related parameters (e.g. BMI, WC, body fat, percent body fat). Stepwise regression was performed to select the model that had the highest R-squared value. In all linear regression analysis assessing correlations, BMI was used as a covariate to adjust for the above instead of all other related variables (e.g. WC, body fat, percent body fat) (pg 114 & 131). Adjustment for covariates such as age, gender, ethnicity, physical activity status, smoking status, alcohol consumption, BMI and total energy intake were applied where appropriate. Comparisons of blood pressure and blood biochemical parameters with respective diagnostic cut offs in the three different ethnic groups were assessed using one sample t test. Same analysis was performed to compare the actual dietary nutrients intake with respective diagnostic cut offs according to Recommended Nutrient Intake (RNI) for Malaysians (2017) (National Coordinating Committee of Food and Nutrition, 2017) in different ethnic groups. A statistical probability level of $p<0.05$ (two-sided) was considered significant.

4.4 Results

4.4.1 General characteristics: Differences between the three ethnic groups (Malaysian Malays, Chinese, and Indians)

In total, 178 Malaysian adults were recruited for anthropometric measurement {Chinese n=42 (Female=37, Male=5); Malays n=86 (Female=78, Male=8); Indians n=50 (Female=39, Male=11)}. For biochemical and dietary analysis, 126 participants were available {Chinese n=22 (Female=19, Male=3); Malays n=57 (Female=51, Male=6); Indians n=47 (Female=36, Male=11)}. All analysis was done on the pooled data. The general characteristics of the study participants are reported according to their ethnic groups in Table 4.1. No significant difference was found in the distribution of gender (p=0.106), physical activity status (p=0.825), alcohol consumption status (p=0.457) and smoking status (p=0.754) between the ethnic gorups. However, Malays had significantly lower age (p=0.010) compared to the Indians and lower height (p=0.023) compared to the Chinese.

Table 4.1: General characteristics: Differences between the three ethnic groups (Malaysian Malays, Chinese, and Indians)

Variables	Overall (n=178)	Chinese (n=42)	Malay (n=86)	Indian (n=50)	p value
Age (year)	41.7 ± 0.9	42.3 ± 1.9	**39.0 ± 1.2a**	**45.7 ± 1.6b**	**0.010[1]***
Height (cm)	157.8 ± 0.5	**159.6 ± 1.0a**	**156.7 ± 0.7b**	158.4 ± 1.1	**0.023[1]***
Gender					
Female	154	37 (88.1%)	78 (90.7%)	39 (78%)	0.106[2]
Male	24	5 (11.9%)	8 (9.3%)	11 (22%)	
Physical activity status					
Physically inactive	171	41 (97.6%)	82 (95.3%)	48 (96%)	0.825[2]
Physically active	7	1 (2.4%)	4 (4.7%)	2 (4%)	
Smoking status					
Never	175	42 (100%)	84 (97.6%)	49 (98%)	0.754[2]
Former	1	0	1 (1.2%)	0	
Current	2	0	1 (1.2%)	1 (2%)	
Alcohol consumption status					
Never	176	42 (100%)	85 (98.8%)	49 (98%)	0.457[2]
Former	1	0	0	1 (2%)	
Current	1	0	1 (1.2%)	0	

[1] Data were expressed in mean (±SE) and p value was based on ANCOVA with Tukey post hoc test, after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption.
[2] Data were expressed in total number (percentage) and p value was based on chi square test.
*p<0.05 was considered as significant.
a,b - Figures not sharing a common letter are significantly different at p<0.05.

4.4.2 Differences in anthropometric parameters and blood pressure between the three ethnic groups (Malaysian Malays, Chinese, and Indians)

In comparison to Malays and Chinese, Indians had significantly higher WC (p<0.001), WHR (p<0.001), body fat percent (p=0.001), even after adjusting for covariates such as age, gender, physical activity status, smoking status, alcohol consumption (Table 4.2). Indians had signficantly higher BMI (p=0.028) and fat mass (p=0.014) compared to the Chinese.

4.4.3 Differences in blood biochemical parameters between the three ethnic groups (Malaysian Malays, Chinese, and Indians)

In comparison to Malays and Chinese, Indians had significantly higher fasting insulin (p<0.001), HOMA-IR (p<0.001) and lower HDL cholesterol levels (p<0.001), even after adjusting for covariates such as age, gender, physical activity status, smoking status, alcohol consumption, BMI, WC, fat mass and body fat percent (Table 4.2). Indians had also signficantly higher total cholesterol per HDL cholesterol (p=0.031) compared to the Malays.

Table 4.2: Differences in the mean values (±SE) of anthropometric and blood biochemical between the three ethnic groups in Malaysian adults (Malaysian Malays, Chinese and Indians)

Variables	Overall (n=178)	Chinese (n=42)	Malay (n=86)	Indian (n=50)	p value
[1]Weight (kg)	67.4 ± 1.3	64.6 ± 2.6	66.2 ± 1.7	72.0 ± 2.6	0.173
[1]BMI (kg/m^2)	27.0 ± 0.4	**25.2 ± 0.8a**	26.9 ± 0.6	**28.6 ± 0.8b**	**0.028***
[1]Waist circumference (cm)	82.0 ± 1.6	**72.5 ± 3.2a**	**79.6 ± 2.3b**	**94.2 ± 2.5c**	**<0.001***
[1]Waist hip ratio	0.89 ± 0.01	**0.88 ± 0.01b**	**0.87 ± 0.01b**	**0.93 ± 0.01a**	**<0.001***
[1]Muscle mass (kg)	22.4 ± 0.4	22.8 ± 1.0	21.8 ± 0.5	23.1 ± 0.9	0.63
[1]Fat mass (kg)	26.6 ± 0.8	**23.8 ± 1.7a**	26.1 ± 1.1	**29.8 ± 1.7b**	**0.014***
[1]Fat-free mass (kg)	40.9 ± 0.6	40.7 ± 1.2	40.2 ± 0.8	42.2 ± 1.4	0.903
[1]Body fat percent (%)	38.3 ± 0.6	**35.6 ± 1.2b**	**38.3 ± 0.9b**	**40.8 ± 1.2a**	**0.001***
[1]Systolic blood pressure (mmHg)	122.6 ± 1.3	124.0 ± 3.4	122.2 ± 1.7	122.5 ± 2.5	0.377
[1]Diastolic blood pressure (mmHg)	80.8 ± 0.9	80.5 ± 2.4	80.9 ± 1.3	80.9 ± 1.6	0.73
[1]Pulse rate	76.5 ± 1.0	73.8 ± 1.8	76.4 ± 1.5	77.7 ± 1.7	0.323
Blood biochemical markers	**Overall (n=126)**	**Chinese (n=22)**	**Malay (n=57)**	**Indian (n=47)**	**p value**
[2]Fasting serum leptin (ng/ml)	8.9 ± 0.8	8.7 ± 1.6	8.8 ± 1.2	9.3 ± 1.6	0.167
[2]Fasting glucose (mmol/L)	5.2 ± 0.2	4.7 ± 0.1	5.1 ± 0.1	5.7 ± 0.4	0.197
[2]Fasting insulin (uU/mL)	10.0 ± 0.9	**6.9 ± 1.1b**	**7.7 ± 0.7b**	**14.0 ± 2.0a**	**<0.001***
[2]HOMA-IR	2.5 ± 0.3	**1.4 ± 0.2b**	**1.8 ± 0.2b**	**3.8 ± 0.7a**	**<0.001***
[2]Total cholesterol (mmol/L)	5.5 ± 0.1	5.6 ± 0.2	5.6 ± 0.1	5.4 ± 0.2	0.47
[2]Triglyceride (mmol/L)	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.5 ± 0.2	0.815
[2]HDL cholesterol (mmol/L)	1.5 ± 0.03	**1.7 ± 0.1b**	**1.6 ± 0.04b**	**1.3 ± 0.04a**	**<0.001***
[2]LDL cholesterol (mmol/L)	3.4 ± 0.1	3.3 ± 0.2	3.4 ± 0.1	3.3 ± 0.2	0.815
[2]Total cholesterol/HDL cholesterol	3.8 ± 0.9	3.5 ± 0.3	**3.6 ± 0.1b**	**4.1 ± 0.2a**	**0.031***
[2]hsCRP (mg/L)	5.7 ± 0.7	2.7 ± 0.9	5.0 ± 0.9	7.7 ± 1.4	0.091

One way ANCOVA with Tukey post hoc test was performed to determine the differences in anthropometric and biochemical parameters, between the three ethnic groups, after adjusting for covariates in different models [1]age, gender, physical activity status, smoking status and alcohol consumption; and [2] model[1] + BMI.

*p<0.05 was considered as significant.

a,b - Figures not sharing a common letter are significantly different at p<0.05.

SE, standard error of mean; HOMA-IR, Homeostatic model assessment -insulin resistance; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.

Note: Serum leptin level was only available in 35 (9 Chinese, 14 Malays and 12 Indians) participants.

4.4.4 Differences in dietary parameters between the three ethnic groups (Malaysian Malays, Chinese, and Indians)

With respect to dietary intake, Malaysian Malays had significantly higher total energy intake compared to the Chinese and Indians even after adjusting for covariates, age, gender, physical activity status, alcohol consumption, smoking status and BMI (p<0.001) (Table 4.3). Indians consumed significantly lower dietary cholesterol (p=0.001) and higher percent energy from carbohydrate (p=0.009) compared to the Chinese, and consumed significantly higher dietary

fibre (p=0.005) compared to the Malays. Malaysian Chinese consumed significantly higher percent energy from protein (p<0.001) compared to the Malays and Indians.

Table 4.3: Differences in the mean values (±SE) of dietary parameters between the three ethnic groups in Malaysian adults (Malaysian Malays, Chinese and Indians)

Dietary nutrients intake	Overall (n=126)	Chinese (n=22)	Malays (n=57)	Indians (n=47)	p value
Total energy intake (kcal)	2007.0 ± 30.8	**1856.2 ± 47.1b**	**2125.0 ± 51.9a**	**1929.0 ± 42.4b**	**<0.001***
Actual total carbohydrate intake (g)	253.9 ± 5.7	**220.9 ± 9.5a**	262.6 ± 10.1	**259.1 ± 7.9b**	**0.011***
Actual total protein intake (g)	73.8 ± 2.0	**83.3 ± 4.6a**	**77.6 ± 2.8b**	**64.3 ± 3.1b**	**<0.001***
Actual total dietary fat intake (g)	82.9 ± 1.9	75.2 ± 2.2	90.6 ± 3.1	76.8 ± 2.9	0.285
Percent energy from carbohydrate (%)	47.1 ± 0.7	**44.4 ± 1.2a**	45.5 ± 1.2	**50.3 ± 1.1b**	**0.009***
Percent energy from protein (%)	14.6 ± 0.4	**17.8 ± 0.8a**	**14.7 ± 0.5b**	**13.1 ± 0.5b**	**<0.001***
Percent energy from dietary fat (%)	36.8 ± 0.6	36.1 ± 1.3	38.3 ± 1.0	35.2 ± 1.0	0.253
Saturated fatty acids (%TE)	7.8 ± 0.3	7.3 ± 0.6	8.7 ± 0.5	6.9 ± 0.5	0.108
Monounsaturated fatty acids (%TE)	11.2 ± 0.4	12.3 ± 1.0	11.4 ± 0.6	10.4 ± 0.6	0.088
Polyunsaturated fatty acids (%TE)	6.4 ± 0.3	7.1 ± 0.7	5.8 ± 0.4	6.9 ± 0.6	0.254
Trans fatty acids (%TE)	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.02	0.350
Dietary cholesterol (%TE)	106.0 ± 7.3	**165.8 ± 19.1a**	113.5 ± 10.5	**69.0 ± 9.1b**	**0.001***
Total sugar (%TE)	7.3 ± 0.4	7.1 ± 0.9	6.9 ± 0.5	7.9 ± 0.7	0.812
Glucose (%TE)	1.2 ± 0.1	1.3 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	0.933
Fructose (%TE)	1.2 ± 0.1	1.5 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	0.508
Sucrose (%TE)	4.1 ± 0.3	3.5 ± 0.5	3.6 ± 0.4	4.8 ± 0.5	0.492
Energy-adjusted fibre (g)	5.8 ± 0.3	6.6 ± 0.6	**4.4 ± 0.3a**	**7.2 ± 0.6b**	**0.005***
Energy-adjusted Vitamin C (mg)	31.3 ± 3.7	44.7 ± 13.8	27.8 ± 4.1	29.4 ± 5.1	0.315
Energy-adjusted Vitamin D (µg)	1.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	1.7 ± 0.3	0.380
Energy-adjusted Vitamin E (mg)	5.8 ± 0.3	5.8 ± 0.6	5.8 ± 0.4	5.9 ± 0.6	0.822

One way ANCOVA with Tukey post hoc test was performed to determine the differences in dietary parameters between the three ethnic groups, after adjusting for covariates age, gender, physical activity status, smoking status and alcohol consumption, BMI and total energy intake.

*p<0.05 was considered as significant.

a,b - Figures not sharing a common letter are significantly different at p<0.05.

SE, standard error of mean.

4.4.5 Comparison of blood biochemical parameters with respective diagnostic cut offs between the three different ethnic groups

With respect to blood lipid levels, our results revealed that HDL cholesterol levels in all the three ethnic groups (Malaysian Chinese, Malays and Indians) were positively, significantly higher than the diagnostic cut off, indicating lesser metabolic risk (1mmol/L) (p<0.05). However, LDL

cholesterol levels were significantly higher than the diagnostic cut off, indicating higher CVD risk (2.6mmol/L) in the Malaysian Chinese, Malays and Indians (3.3±0.2mmol/L, p=0.002; 3.4±0.1mmol/L, p<0.001; and 3.3±0.2mmol/L, p<0.001, respectively) (Figure 4.1). Moreover, Malaysian Malays had significantly higher total cholesterol levels (5.6±0.1mmol/L, p=0.002) than the diagnostic cut off, indicating higher CVD risk (5.2mmol/L).

Malaysian Chinese and Malays had significantly lower fasting glucose (p<0.001 and p<0.001, respectively), insulin levels (p=0.004 and p<0.001, respectively) and triglyceride levels (p=0.017 and p<0.001, respectively) compared to the diagnostic cut off, indicating lesser metabolic risk (5.6mmol/L, 10.6uU/mL and 1.7mmol/L, respectively). However, Malaysian Indians had higher fasting glucose and insulin levels compared to the diagnostic cut offs, although these differences did not reach statistical significance. HOMA-IR (3.8±0.7, p=0.002) and hsCRP levels (7.7±1.4mg/L, p=0.002) in the Malaysian Indians were significantly above the diagnostic cut offs (1.7 and 3mg/L, respectively) indicating insulin resistance and inflammation.

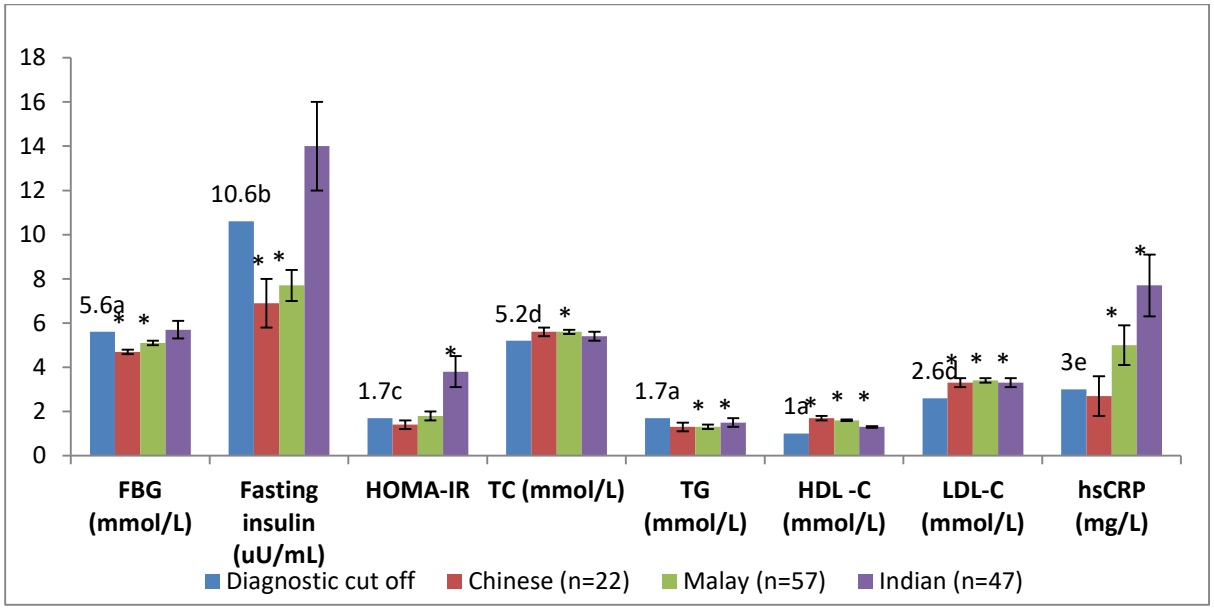


Figure 4.1: Comparisons of blood biochemical parameters with respective diagnostic cut offs, in the three different ethnic groups (mean ± SE)

One sample t test was performed to compare the differences between the actual blood biochemical parameters with respective diagnostic cut offs in the three different ethnic groups.

*p<0.05 was considered significant different compared to the respective diagnostic cut off.

a Alberti, Zimmet, & Shaw, 2006

b Lee, et al., 2006

c Yamada, Moriyama, & Takahashi, 2012

d National Cholesterol Education Program 2002

e Pearson, et al., 2003

FBG, fasting blood glucose; HOMA-IR, Homeostatic model assessment -insulin resistance; TC, total cholesterol, HDL, High-density lipoprotein; LDL, Low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.

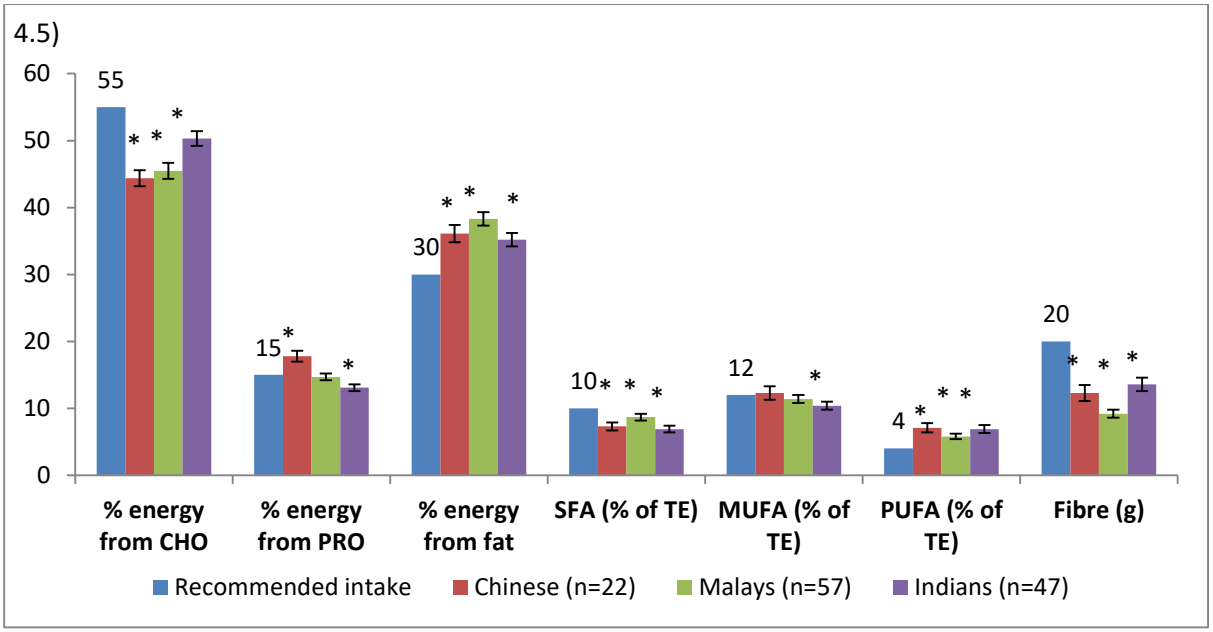


Figure 4.2: Comparison of actual dietary intake with Recommended Nutrient Intake (RNI) for Malaysians (2017): differences between the three ethnic groups (mean ± SE).
One sample t test was performed to assess the differences between the actual dietary intake and the recommended intake.
Recommended intake was based on the Recommended Nutrient Intake (RNI), for Malaysians (2017) (National Coordinating Committee on Food and Nutrition, 2017).
*p<0.05 was considered as significant different compared to the recommended intake.
TE, total energy; CHO, carbohydrate; PRO, protein; SFA, saturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid.

4.4.6 Comparison of actual dietary intake with Recommended Nutrient Intake (RNI) for Malaysians (2017): differences between the three ethnic groups

According to Recommended Nutrient Intake (RNI) for Malaysians (2017), percent energy from carbohydrate was significantly lower than the recommended intake, 55% per day (acceptable range from 50-65% per day) in Malaysian Chinese ($p<0.001$), Malays ($p<0.001$) and Indians ($p<0.001$) (Figure 4.2). However, percent energy from fat was significantly higher than the recommended intake, 30% per day (acceptable range from 20-30% per day) in Malaysian Chinese ($p<0.0010$), Malays ($p<0.001$) and Indians ($p<0.001$). Percent energy from protein in Chinese ($p=0.001$) was significantly higher than the recommended intake 15%, and was significantly lower in Indians ($p=0.001$), but both intakes were still within the acceptable range (10-20% of total energy intake per day). Saturated fatty acids (SFA) intake in all the three ethnic groups were significantly below the limit (should be <10% of total energy intake per day) ($p<0.05$). Polyunsaturated fatty acids (PUFA) intake in all the three ethnic groups were significantly higher than the lower limit (4% of total energy intake per day) and were within the acceptable range from 4-8% of total energy intake per day. However, monounsaturated fatty acid (MUFA) intake in Indians was significantly below the recommended value (12% of total energy per day) ($p=0.010$). Fibre intake in all the three ethnic groups, Chinese ($p<0.001$), Malays

($p<0.001$) and Indians ($p<0.001$) were significantly below the recommended intake, 20g per day (acceptable range from 20-30g per day).

4.5 Discussion

4.5.1 Differences in obesity-related phenotypes between the three ethnic groups

Malaysia is a multi-ethnic country where Malaysians with different ethnic background continue to practice their own cultural and traditional meal patterns. Therefore, understanding the differences in dietary intakes and obesity-related phenotypes between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians) is important in establishing individualised dietary interventions, to manage body weight in the Malaysian population. In our study, our results revealed that Malaysian Indians had significantly higher obesity-related anthropometric parameters (e.g. BMI, WC, fat mass, body fat percent), fasting insulin, HOMA-IR and lower HDL cholesterol levels compared to the Malays and Chinese.

When compared to the respective diagnostic cut offs, we found that HOMA-IR and hsCRP levels in Indians were significantly above the diagnostic cut offs (1.7 and 3.0mg/L), indicating insulin resistance and inflammation. Such findings were not observed in Malays and Chinese. Moreover, Malays and Chinese had significantly lower fasting glucose, insulin and triglyceride levels compared to the diagnostic cut offs (5.6mmol/L, 10.6uU/mL and 1.7mmol/L, respectively), indicating lesser metabolic risk. However, Indians showed higher fasting glucose and insulin levels compared to the diagnostic cut offs, although such differences did not reach statistical significance. These findings suggest that Indians were compromised with higher obesity-related anthropometric parameters and had the least favourable metabolic profile in term of glycaemic indices, insulin sensitivity and inflammation compared to the Chinese and Malays.

Our findings were in line with the previous evidence from Tan et al., 2011, which reported that the prevalence of metabolic syndrome was higher in Malaysian Indians compared to the Malays and Chinese. They were more likely to exhibit central obesity, elevated fasting glucose levels and lower HDL cholesterol levels compared to the Malays and Chinese (Tan, Dunn, & Yen, 2011). Similar findings were found in Rampal et al., 2012 which reported that ethnic Indians had the highest prevalence of metabolic syndrome, followed by ethnic Malays and ethnic Chinese. This suggests that metabolic syndrome may disproportionately affect Indians in the Malaysian population. This evidence emphasises the need for individualised dietary recommendations tailored to account for the traditional diet and customs of the different ethnic populations living

in Malaysia to improve phenotypic risk factors of obesity-related chronic diseases in the Malaysian population.

4.5.2 Hyperlipidaemia in our study participants

With respect to blood lipid levels, HDL cholesterol levels in all the three ethnic groups (Malaysian Chinese, Malays and Indians) were positively, significantly higher than the diagnostic cut off (1mmol/L), and triglyceride levels were lower than the diagnostic cut off (1.7mmol/L), indicating lesser metabolic risk. However, LDL cholesterol levels in all the three ethnic groups were significantly higher than the diagnostic cut off, indicating dyslipidaemia and higher CVD risk (2.6mmol/L). The total cholesterol levels in all the three ethnic groups were slightly above the diagnostic cut off (5.2mmol/L), although such difference did not reach statistical significance in Malaysian Chinese and Indians. These findings suggest that regardless of ethnicity, Malaysian adults in general, were compromised with higher total cholesterol and LDL cholesterol levels. High total cholesterol levels in the Malaysian population had been described earlier. National Health and Morbidity Survey (NHMS) 2015, reported that about 9.6 million, which was equivalent to 47.7% of Malaysian adults above 18 were suffering from hypercholesterolemia (Institute for Public Health, Malaysia, 2015). Previous evidence indicated that hyperlipidaemia is one of the main modifiable risk factors for cardiovascular disease (CVD) (Nelson, 2013). Therefore, immediate action should be taken to prevent and to treat hyperlipidaemia in the Malaysian population.

4.5.3 Serum leptin levels in our study participants

In the current study, our results revealed no significant difference in the serum leptin levels between the three ethnic groups in Malaysian adults. In an earlier pilot study of 35 participants (9 Chinese, 14 Malays and 12 Indians) (Appendix 13), our results revealed that serum leptin level was positively correlated with BMI ($r=0.583$, $p=0.001$), waist circumference ($r=0.453$, $p=0.040$), fat mass ($r=0.600$, $p=0.001$), fat percentage ($r=0.559$, $p=0.001$), fasting insulin ($r=0.385$, $p=0.014$) and HOMA-IR ($r=0.391$, $p=0.013$). These findings suggest that increase in BMI, WC, fat mass, body fat percent, fasting insulin and HOMA-IR was significantly correlated with increase in serum leptin levels. Further, stepwise multiple linear regression revealed that percent body fat and fasting insulin were the independent variables associated positively with serum leptin levels. With a 1-point increase in percent body fat, serum leptin increased by 0.4ng/ml. With a 1-point increase in fasting insulin, serum leptin increased by 0.33 ng/ml. These findings were accepted and presented at the 31st Annual Scientific Conference 2016 (Appendix

14). However, due to the limited resources the serum leptin levels were not assessed in all the study participants (n=178). The small sample size (n=35) may limit this experiment for comparative analysis and the power to detect association. Therefore, it was decided that serum leptin analysis would be excluded from this doctoral thesis.

4.5.4 Macronutrient intake: Differences between the three ethnic groups

With respect to dietary intake, the total energy intake of our study participants was close to the Malaysian recommendation (1660-1900kcal/per day, sedentary activity, female; 1920-2190kcal/per day, sedentary activity, male (National Coordinating Committee of Food and Nutrition, 2017)). Using EI/BMR <1.2 and ≥2.4, we did not identify any under or over reporter in our participants (Chapter 3). Therefore, all participants were included for analysis. Our results revealed that Malaysian Malays consumed significantly higher total energy intake compared to the Chinese and Indians, after adjusting for covariates incuding BMI.

When compared to the Recommended Nutrient Intake (RNI) for Malaysians (2017), Malaysian adults in all the three ethnic groups had failed to meet the recommendation for dietary carbohydrate intake (55% of total energy intake per day; acceptable range from 50-65% of total energy intake per day). In addition, we found that Malaysian adults generally, regardless of ethnicity, exceeded the recommendation for total dietary fat intake (<30% of total energy intake per day; acceptable range from 20-30% of total energy intake per day), given that the intakes of SFA (<10% of total energy intake per day) and PUFA (acceptable range from 4-8% of total energy intake per day) in all the three ethnic groups were within the favourable range. However, we found that Malaysian Indians consumed significantly lower dietary cholesterol intake compared to the Malays and Chinese.

Previous evidence reported that reduction or modification of dietary fat intake in individuals may result in the reduction of future cardiovascular events (Hooper, 2002). Replacing SFA with PUFA had consistently showed reduction in total cholesterol and LDL cholesterol levels (DiNicolantonio & O'Keefe, 2018). A systematic review and meta-analysis of randomised controlled trials suggested that higher consumption of PUFA, in place with SFA, can reduce the cardiovascular diseases risk (Mozaffarian, Micha, & Wallace, 2010). A large prospective cohort study had also reported that replacing SFA with low glycemic index (GI) carbohydrate from whole fruits, vegetables, pulses and grains reduced the risk of myocardial infarction (MI), whereas replacing SFA with carbohydrates with high-GI was associated with a higher risk of MI (Jakobsen et al., 2010). Therefore, it is important to reduce the total dietary fat intake in the

Malaysian population, preferably replacing SFA with PUFA or low-GI carbohydrates to lower down their blood lipid levels and to reduce the risk of developing dyslipidaemia and CVD in future.

Moreover, we found that Malaysian Chinese had significantly higher consumption of dietary protein (18% of total energy intake per day) compared to Malays (15% of total energy intake per day) and Indians (13% of total energy intake per day). When compared to the RNI for Malaysians (2017), percent energy from protein in Malaysian Chinese was significantly higher than the recommended intake 15%, whereas percent energy from protein in Malaysian Indians was significantly lower. This finding was similar with the previous evidence which reported that the consumption of poultry, meat and dairy products were relatively low in Malays and Indians estate workers resided at Johor and Kedah. The average of percent energy from protein in Malays and Indians were 14% and 12%, respectively (Chee et al., 1996). Therefore, it is important to increase the consumption of high protein food sources, especially in Malaysian Indians to improve their diet quality.

4.5.5 Low fibre intake in our study participants

In addition, we found that Malaysian Indians consumed higher fibre intake compared to the Malaysian Malays and Chinese. However, when compared to the RNI for Malaysians (2017), the fibre intakes in all the three ethnic groups were significantly below the recommended intake, 20g per day (acceptable range from 20-30g/day). A review reported that high consumption of soluble dietary fibre reduces postprandial glucose responses as well as decreases inflammatory marker hsCRP and blood lipid levels (e.g. total cholesterol and LDL cholesterol levels) (Weickert & Pfeiffer, 2008). These effects may be due to the viscous property of soluble which fibre slows down the process of gastric emptying and macronutrient absorption in the gut. Large prospective cohort studies reported that diets high in insoluble cereal dietary fibre and whole grains may improve insulin sensitivity and eventually reduce the risk of diabetes (De Munter et al., 2007). Therefore, it is important to increase the consumption of both dietary soluble and insoluble fibre intake (>20g of total dietary fibre intake per day) such as whole grains, cereals, fruits and vegetables in the Malaysian population to improve their metabolic health.

4.5.6 Factors affecting obesity-related phenotypes

In our study participants, we observed that Indians were compromised with negative obesity-related anthropometric parameters (e.g. BMI, WC, fat mass, body fat percent), and had the least

favourable metabolic profile in terms of glycaemic indices, insulin sensitivity and inflammatory markers compared to the Chinese and Malays, even though they had lower dietary cholesterol intake, protein intake and higher fibre intake compared to the Malays and Chinese. Tan et al., 2011 reported that obesity risk in the Malaysian population are highly affected by sociodemographic determinants (such as ethnicity, gender, education level) and health–lifestyle factors (such as family history of serious illnesses and smoking status). Moreover, other studies have reported that differences in the prevalence of obesity and its complications may be due to the consequence of the complex interactions between multiple gene variants and lifestyle factors (Heianza & Qi, 2017; Marti et al., 2004). Therefore, further studies are needed to include a wide range of determinants such as genetic, socioeconomic, sociodemographic and health-and-lifestyle related factors to assess the potential determinants underlying the ethnic disparities in obesity and obesity-related metabolic risk factors in the Malaysian population. A better understanding on the significance and the magnitude of these factors in contributing to the ethnic disparities in obesity-related phenotypes, can provide policy makers with the fundamental information needed to design the interventions which ultimately reduce the health disparities between the ethnic groups in the Malaysian population.

4.5.7 Limitations

There are few limitations exist in this study. Our participants were predominantly middle-aged women with sedentary lifestyle, recruited from the supermarkets and schools in the vicinity of UNM (Beranang, Jalan Broga and Bandar Kajang). This study sample is not representative of the Malaysian population and the findings of current experiments may not be generalisable to the Malaysian population as a whole. Therefore, the results are reported as the associations found in the participants of the current study. It is inferred that similar investigations in other geographical locations in Malaysia, including higher male participation is necessary for extrapolation of the results obtained from this study.

Moreover, detailed information on other unmeasured or unknown confounding factors such as individual's mental health (e.g. stress, sleep problems, worry, depression and anxiety), sociodemographic (e.g. location of residence, marital status) and socioeconomic indicators (e.g. educational attainment, social class, employment status, monthly household income and housing tenure) were not assessed. These are the important factors that may contribute to the differences in dietary intake, anthropometric and cardio-metabolic parameters between the three ethnic

groups. Therefore, the associations observed in our study may be due to the aforementioned confounding factors.

Furthermore, our revised FFQ was not validated. However, the original FFQ is a validated FFQ (Loy et al., 2011). Initially, a 24-hour diet recall survey was conducted to capture personal food preferences, likes-dislikes, and the habitual macro and micronutrient intake of the participant. These food items were incorporated into a validated FFQ (Loy et al., 2011). The same interviewer then administered this revised FFQ to every participant to estimate their energy, macro and micronutrient intake per day as accurately as possible. Similarly, our physical activity questionnaire was not validated. Hence the results may not reflect the real individual's dietary intake and physical activity status, and may have caused bias in the current findings. This may have contributed to the extremely low number of participants who were considered as physically active in our study participants.

4.6 Summary

In this chapter, we aimed to assess the differences in dietary intakes and obesity-related phenotypes between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians). Our results revealed that our participants in general, regardless of ethnicity, were compromised with higher total cholesterol and LDL cholesterol levels. Besides, the latter had significantly higher total dietary fat intake and lower carbohydrate intake and fibre intake compared to the RNI for Malaysians (2017). Therefore, it is important to increase the consumption of dietary vegetables and to reduce the total dietary fat intake in our study participants, preferably replacing SFA with PUFA or low-GI carbohydrates to reduce the risk of hyperlipidaemia and CVD in future. It is interesting to note that Indians had significantly higher obesity-related anthropometric parameters (BMI, WC, fat mass, body fat percent) and had the least favourable metabolic profile in term of glycaemic indices, insulin sensitivity and inflammation, compared to the Chinese and Malays, even they consumed lower dietary cholesterol intake and higher dietary fibre intake compared to Malays and Chinese. However, our study sample is not representative of the Malaysian population, and hence the findings from the current study may not be generalisable to the whole Malaysian population. It is undeniable that the associations observed in this study may be due to the unmeasured or unknown confounding factors. Large scale studies are required to replicate the findings of this study in the Malaysian population from other geographical locations.

Chapter 5

Effect of gene variants on obesity and obesity-related phenotypes and its interaction with dietary nutrients in modulating obesity-related phenotypes in Malaysian adults

5.1 Introduction

Obesity is a major public health problem which has clinical implications in increasing the prevalence of comorbid conditions such as hypertension, hyperglycaemia, dyslipidaemia, insulin resistance and inflammation (Finer, 2015). These complications often increase the risk of developing other non-communicable diseases (NCDs) such as cardiovascular disease, stroke, dyslipidaemia and type 2 diabetes. Elevated circulating levels of hsCRP and triglyceride and reduced levels of HDL cholesterol are believed to contribute to increased risk of cardiovascular diseases (CVD) and myocardial infarction (MI) (Bilhorn et al., 2012; Fonseca & Izar, 2016; Mahalle et al., 2014; Shrivastava et al., 2015). According to National Health and Morbidity Survey (NHMS) 2015, it was reported that about 5.6 million Malaysian adults aged 18 and above were overweight and 3.3 million were obese (Institute for Public Health, Malaysia, 2015). Moreover, there was about 2.5 million and 9.6 million of Malaysian adults above 18 were suffering from type 2 diabetes mellitus and hypercholesterolemia, respectively.

Obesity is a state of positive energy balance, when energy consumption exceeds energy expenditure and results in the storage of excess body fat (Spiegelman & Flier, 2001). The excess fat accumulation in adipose tissue may impair health. Consumption of energy-dense food coupled with sedentary lifestyle, are the main contributors for the development of obesity and its complications nowadays (Racette, Deusinger, & Deusinger, 2017). However, not all individuals living in the same 'obesogenic environment' is obese. The variation in the individual's response to the 'obesogenic' environment suggesting that genetic factors may play an important role in modulating body weight regulation (Loos & Rankinen, 2005). Gene-environment interaction may explain why some individuals are more prone to weight gain than others in a similar environment (Hetherington & Cecil, 2009).

Over the past few years, the number of the studies reporting associations between gene variants and obesity phenotypes has been increasing. Multiple genetic loci have been determined by genome-wide association studies (GWAS) that increase body index mass (BMI) and the susceptibility to obesity in a cumulative manner (Speliotes et al., 2010). Genes that involved in lipid metabolism, adipogenesis, energy expenditure, appetite control, insulin signaling and adipokine secretion have been largely investigated as they play a critical role in weight management (Deram & Villares, 2009). According to the human obesity gene map in 2005, 426 findings of positive associations from 127 genes have been identified to have direct and indirectly effect on the onset of obesity in general population (Rankinen et al., 2006). A

promising observation is that 22 genes were each replicated in at least five positive studies. Among them, those showing replications in 10 studies and more included *PPARG* (30 studies), *ADRB3 (*29), *ADRB2* (20), *LEPR* (16), *GNB3* (14), *UCP3* (12), *ADIPOQ* (11), *LEP* (11), *UCP2* (11), *HTR2C* (10), *NR3C1* (10), and *UCP1* (10). Therefore, understanding the molecular pathophysiology of obesity is essential for its prevention and treatment.

In Chapter 4, our results revealed that Malaysian Indians had significantly higher obesity-related anthropometric parameters (BMI, WC, fat mass, body fat percent) and had the least favourable metabolic profile in term of glycaemic indices, insulin sensitivity and inflammation, compared to the Malaysian Chinese and Malays, even they consumed lower dietary cholesterol intake and higher dietary fibre intake compared to the Chinese and Malays. Such differences indicate that obesity and its complications are most likely the results of the complex interactions between multiple gene variants, dietary intake and physical activity level (Heianza & Qi, 2017; Marti et al., 2004). This complex interaction may eventually affect energy balance and body weight regulation. Therefore, it is important to understand the genetic effect on the development of obesity and its interactions with dietary nutrients in modulating obesity-related phenotypes, in the Malaysian population.

5.2 Objectives

The aim of this chapter is to investigate the i) differences in genotype distributions between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians), ii) association between gene variants and obesity and obesity-related phenotypes, and iii) the interaction between gene variants and dietary nutrients in modulating obesity-related phenotypes, in Malaysian adults.

Chapter 5-Section I: *FTO* gene

5I.1 Introduction

Genome-wide association studies (GWAS) have identified multiple genetic loci containing gene variants that affect body weight and the first successfully determined candidate gene is the *FTO* (Fat-mass and obesity associated) gene (Loos & Bouchard, 2008). *FTO* gene is positioned at chromosome region 16q12.211, encoding a Fe (II)- and 2-oxoglutarate (OG) dependent nucleic acid demethylase (Gerken et al., 2007). It was first discovered in a genome-wide study identifying type 2 diabetes-susceptibility genes in Europeans, with 1,924 cases and 2,938 controls (Frayling et al., 2007). The study reported that a cluster of single nucleotide polymorphisms (SNPs) in *FTO* gene was significant associated with increased risk of type 2 diabetes. However, such associations abolished after adjusting for BMI, suggesting that *FTO* gene variants predisposed to type 2 diabetes through an effect on BMI. A follow-up analyses revealed that individuals carrying the AA genotype of *FTO* rs9930609 weighed 3kg more and had a 1.7 fold increased risk of obesity compared to individuals carrying TT genotype (Frayling et al., 2007).

The strong association of *FTO* gene and obesity related phenotypes have been consistently observed in studies involving various populations worldwide (Dina et al., 2007; Peeters et al., 2008; Ri et al., 2015). Studies have shown that a cluster of SNPs in the first intron of the *FTO* gene correlates with an increase in body mass index (BMI) in both children and adults, regardless of gender (Berulava & Horsthemke, 2010; Volckmar et al., 2016). The function of the FTO protein is still largely elusive. It is known that *FTO* is a DNA/RNA demethylase which influences splicing, stability and export of mRNA (Lesbirel & Wilson, 2018). Initial observation suggested that it plays an important role in regulating energy homeostasis by signalling the hunger and satiety centre in brain, but it is unknown whether it influences eating behaviour or energy expenditure (Scuteri et al., 2007). Recently, a new mechanism was proposed, suggesting variants in the *FTO* gene exert effect on the genes located nearby and determining obesity (Smemo et al., 2014). It is assumed that SNPs located within the first intron may affect obesity by altering *FTO* expression directly, or, these SNPs may also remotely alter the expression of other downstream genes such as genes involved in body mass regulation by changes in epigenetic mechanisms including methylation of other genes (Almén et al., 2012; Zhou et al., 2017).

A Singaporean study reported positive association between 9 *FTO* single nucleotide polymorphisms (SNPs) and obesity in ethnic Chinese and Malays (Tan et al., 2008). The authors concluded that the risk alleles of the 9 *FTO* SNPs were associated with obesity in the same direction as reported by previous European studies. It is not only associated with increased risk of obesity but also with the risk of developing type 2 diabetes, atherogenic lipid profile and myocardial infarction (Doney et al., 2009). However, two Malaysian studies which involved a total of 911 multi-ethnic Malaysians found no association between *FTO* rs9939609 and rs17817288 with obesity related anthropometric measurements (Apalasamy et al., 2012; Chey et al., 2013). Studies on Caucasian population have found significant association between *FTO* rs9930506 and obesity (Sentinelli et al., 2012; Wrzosek et al., 2016).

In recent, Kalantari et al., 2018 reported that a complete linkage disequilibrium (LD) was found between the three neighbouring SNPs of intron 1 region of the *FTO* gene (rs9930501, rs9930506 and rs9932754). This haplotype in the first intron of the *FTO* gene (rs9930501, rs9930506 and rs9932754) with complete linkage disequilibrium (LD), had a strong association with obesity indices (e.g. BMI and body fat percent) in Iranian male adolescents. To the best of our knowledge, the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 has not been investigated in the Malaysian population. Further, relatively little is known with respect to the interaction between dietary nutrients and *FTO* SNPs on obesity and obesity related metabolic phenotypes in Asian populations.

5I.2 Objectives

The aim of this section is to investigate i) the differences in genotype distributions of *FTO* gene variants (rs9930501, rs9930506 and rs9932754) between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians), ii) the association between the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and the odds of obesity, iii) the association between the *FTO* gene variants and obesity-related phenotypes, and iv) the interaction between the *FTO* gene variants and dietary nutrients on obesity-related phenotypes, in the Malaysian adults.

5I.3 Methodology

5I.3.1 Study design

This cross-sectional study was conducted from 2015-2018 on 178 multi-ethnic Malaysian adults (Malaysian Malays, Malaysian Chinese and Malaysian Indians) aged between 18-74 years.

5I.3.2 Participant selection

Detailed information on the participant selection has been previously described in Chapter 4. Briefly, a total of 178 Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above were recruited at random through advertisements and flyers, distributed at UNMC campus, supermarkets and community centres in the vicinity of UNMC. Structured questionnaire was used to record information on race and ethnicity, and the questions were directed to trace back three generations to confirm ancestry. Participants completed a health and lifestyle questionnaire which included questions on past diseases, family history of past diseases, physical activity level and substance abuse. Smoking status and alcohol consumption were reported as i) never, ii) former and iii) current. Individuals diagnosed with cardiovascular diseases (CVD), diabetes, cancer, hypertension, renal diseases, endocrine disorders such as hypothyroidism were excluded. Individuals on medications for cholesterolemia, hypoglycemia and psychiatric disorders were excluded. Pregnant and lactating women were also excluded from the study. Interested individuals, attended initial screening to determine whether or not the participant met the inclusion and exclusion criteria. In total 79 obese and 99 non-obese Malaysian adults were recruited.

5I.3.3 Assessment of anthropometric, dietary, blood biochemical parameters, substance abuse and physical activity level

Detailed information on the assessment of anthropometric parameters, dietary nutrients analysis, blood biochemical parameters, substance abuse and physical activity levels have been previously described in Chapter 4.

5I.3.4 DNA extraction and genotyping assay

5ml of whole blood was drawn from an antecubital vein into vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) containing EDTA. Genomic DNA was extracted from blood leukocytes using MasterPure DNA Purification kit according to manufacturer's

instructions. DNA samples were then stored at -20 °C until use. A DNA fragment of 237bp containing the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 was amplified by using polymerase chain reaction (PCR) with specific primers (Forward primer: 5'-TGATGAGAATGTAAGAAGGGAGA-3' and reverse primer: 5'-TCATTTGACAGATGGACTTTTCA-3') derived from an earlier study (Gemma et al., 2009). PCR was performed using 5µl of genomic DNA (~1ng/µl), 0.2 µM forward primer and reverse primer with 5µl of Taq 5X Master Mix. Thermal cycling was performed as follows: initial denaturing at 95ºC for 5 min; 35 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC for 30s and extension at 72ºC for 1 min, and then a final extension at 72ºC for 10 min. The amplicons were verified by using electrophoresis on 2% agarose gel and visualised under ultraviolet illumination after staining by ethidium bromide (Appendix 11). The verified amplicons were then sequenced by using the BigDye® Terminator v3.1 cycle sequencing kit chemistry.

5I.3.5 Power and sample size calculation

We performed power calculation using software- QUANTO, Version 1.2.4 to find the minimum detectable effect for a given sample size. This calculation takes into account the type 1 error rate of 0.05, and the population prevalence of obesity (BMI≥27.5 kg/m2) of 27.7%, as reported by Ahmad et al., 2016. Given that the minor allele frequency (G) of *FTO* rs9930506 in our study, was 0.37, with 79 obese and 99 non-obese participants, we had 50.0% power to detect an effect of 2.87 (odds ratio) (additive model). With regard to the gene-diet interaction, given that the mean of hsCRP levels in our population was 5.72mg/L, with a SD of 6.82, environmental effect (differences of hCRP levels between high and low vitamin E intake) of -2.75 (4.42mg/L-7.17mg/L), genetic effect {differences of hsCRP levels between the carriers of the risk allele (G) of *FTO* rs9930506 and the non-carriers (A)} of 3.43 (6.93mg/L-3.50mg/L) and interaction effect of 2.30 (7.87mg/L-5.57mg/L), a power of 13% for the gene-diet interaction was computed.

5I.3.6 Statistical analysis

Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). All parametric results were expressed as mean ± standard error (SE) for each group. Log transformation was performed to transform non-normally distributed data into normally distributed data. Allele frequency was estimated by gene counting and Chi-square test was used to assess deviation from Hardy-Weinberg

equilibrium (HWE) (Namipashaki, Razaghi-Moghadam, & Ansari-Pour, 2015). One way ANCOVA and Chi square test were performed to assess the differences in the baseline continous variables and categorical variables, respectively, between the *FTO* genotypes. Variance inflation factor (VIF) was taken as a measure for testing multicollinearity among related parameters (e.g. BMI, WC, body fat, percent body fat). Stepwise regression was performed to select the model that had the highest R-squared value. In all linear regression analysis assessing correlations, BMI was used as a covariate to adjust for the above instead of all other related variables (e.g. WC, body fat, percent body fat). Adjustment for covariates such as age, gender, ethnicity, physical activity status, smoking status, alcohol consumption, BMI and total energy intake were applied where appropriate.

To study the effect of *FTO* gene variants on obesity, data were dichotomised into obese and non-obese groups (obesity was defined by BMI $\geq$27.5 kg/m^2) (WHO Expert Consultation, 2004). Linear regression was performed to determine the model that best fits the association of *FTO* gene variants and obesity. Odds ratio (OR) with 95% confidence interval (95% CI) was estimated for each genotype by logistic regression to determine the odds of obesity associated with *FTO* gene variants, after adjusting for covariates age, gender, physical activity status, smoking status and alcohol consumption. The common homozygotes of *FTO* rs9930501 (AA), rs9930506 (AA) and rs9932754 (TT) were used as the reference group in the additive model.

In total, 27 macro and micronutrients were analysed to investigate differences in dietary nutrient intake between ethnic groups (Chapter 4). We found that ethnicity was associated with dietary carbohydrate, protein, cholesterol and fibre intakes only. Further, we found that *FTO* gene variants were associated with vitamin E and fibre intake regardless of ethnicity (Table 5I.3). In a similar analysis, we found significant association between *FTO* gene variants and HDL cholesterol and hsCRP levels only (Table 5I.3). Thereafter, we investigated interaction between presence of risk allele, macronutrients, vitamin E and fibre, on hsCRP and HDL cholesterol levels using multivariate general linear model (GLM). Dietary data were dichotomised into two groups based on the median value of the nutrients for analysis purposes. Adjustment for coviariates age, gender, physical activity status, smoking status, alcohol consumption, BMI, WC, fat mass and total energy intake were applied. A statistical probability level of $p<0.05$ (two-sided) was considered significant. Significance was found only in the interaction between *FTO* gene variants and dietary protein and vitamin E on hsCRP levels (Figures 5I.1 & 5I.2). No signifcance was found in dietary cholesterol intake, fibre intake and HDL cholesterol levels, therefore the results were not reported.

5I.4 Result

In total, 178 Malaysian adults were recruited for anthropometric measurement and genetic analysis {Chinese n=42 (Female=37, Male=5); Malays n=86 (Female=78, Male=8); Indians n=50 (Female=39, Male=11)}. For biochemical and dietary analysis, 126 participants were available {Chinese n=22 (Female=19, Male=3); Malays n=57 (Female=51, Male=6); Indians n=47 (Female=36, Male=11)}. All analysis was done on the pooled data.

5I.4.1 Allele frequencies and genotype distributions of *FTO* gene variants

The allele frequencies for minor allele (G) of *FTO* rs9930501 and rs9930506 were 0.371 and 0.365 respectively, and the allele frequency for minor allele (C) of *FTO* rs9932754 was 0.371, which did not deviate from Hardy-Weinberg equilibrium as tested by Chi square test (χ^2=3.15, χ^2= 1.90 and χ^2= 2.12, repectively) (Table 5I.1). Due to the similar findings between the three *FTO* SNPs, rs9930501, rs9930506 and rs9932754, which were in complete linkage disequilibrium (LD), we reported only rs9930506 as the tag SNP for the following analyses.

Table 5I.1: Allele frequencies and genotype distributions of *FTO* gene variants

SNPs	rs9930501			rs9930506			rs9932754		
	Genotype	Overall n=178	$\chi2$	Genotype	Overall n=178	$\chi2$	Genotype	Overall n=178	$\chi2$
Additive model	**AA**	76 (42.7%)	3.15	**AA**	76 (42.7%)	1.90	**TT**	75 (42.1%)	2.12
	AG	72 (40.4%)		**AG**	74 (41.6%)		**TC**	74 (41.6%)	
	GG	30 (16.9%)		**GG**	28 (15.7%)		**CC**	29 (16.3%)	
Dominant model	**AA**	76 (42.7%)		**AA**	76 (42.7%)		**TT**	75 (42.1%)	
	AG + GG	102 (57.3%)		**AG + GG**	102 (57.3%)		**TC + CC**	103 (57.9%)	
Recessive model	**AA + AG**	148 (83.1%)		**AA + AG**	150 (84.3%)		**TT + TC**	149 (83.7%)	
	GG	30 (16.9%)		**GG**	28 (15.7%)		**CC**	29 (16.3%)	
Allele frequency	**A**	224 (62.9%)		**A**	226 (63.5%)		**T**	224 (62.9%)	
	G	132 (37.1%)		**G**	130 (36.5%)		**C**	132 (37.1%)	

Note: The top most gene variant denotes the wild variant of the SNP in each category, and the last gene variant denotes the mutant variant.
The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with $\chi2<3.841$ considered as no deviation from Hardy Weinberg Equilibrium.
$p<0.05$ was considered significant.

5I.4.2 Association between *FTO* rs9930506 and obesity

Linear regression was performed to determine the model that best fits the association of *FTO* gene variants and obesity. Based on the linear regression analysis, our results revealed that the additive model (r^2=0.037, p=0.009) showed the best fit to BMI, compared to recessive model (r^2=0.027, p=0.028) and dominant model (r^2=0.025, p=0.033). Therefore, we decided to report

only the additive model for statistical analysis (Table 5I.2). Logistic regression was performed to examine the independent effect of *FTO* rs9930506 on the odds of obesity (obesity was defined as BMI≥27.5kg/m^2). In the additive model, rare homozygotes of rs9930506 (GG) was associated with increased odds of obesity, 2.65 (CI=1.09-6.45, unadjusted p=0.032), compared to the common homozygotes (AA) (Table 5I.2). After adjusting for covariates, age, gender, physical activity status, alcohol consumption and smoking status, the odds of obesity in rare homozygotes of rs9930506 (GG) was increased to 2.87 (CI=1.14-67.19, adjusted p=0.025).

Table 5I.2: Genotype distribution and allele frequency of *FTO* rs9930506 in obese and non-obese groups (in additive model)

SNPs	Genotype	Overall n=178	χ2	Obese BMI≥27.5 n=79	Non-obese BMI<27.5 n=99	Unadjusted OR (95%CI)	Unadjusted p value	Adjusted OR (95%CI)[a]	p value[a]
rs9930506	**AA**	76 (42.7%)	1.90	28 (35.5%)	48 (48.5%)	1	-	1	-
	AG	74 (41.6%)		34 (43.0%)	40 (40.4%)	1.46 (0.76-2.80)	0.258	1.54 (0.77-3.07)	0.219
	GG	28 (15.7%)		17 (21.5%)	11 (11.1%)	2.65 (1.09-6.45)	**0.032***	2.87 (1.14-7.19)	**0.025***

The agreement of genotype frequency with Hardy Weinberg Equilibrium was tested by using chi Square test, with χ2 < 3.841 considered as no deviation from Hardy Weinberg Equilibrium.
Logistic regression was conducted to determine the risk of obesity associated with *FTO* rs9930506. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each genotype. The common homozygote (AA) was used as the reference group in the additive model.
[a] adjusted for age, gender, physical activity status, smoking status and alcohol consumption.
*p<0.05 was considered significant.

5I.4.3 General characteristics: Differences between the *FTO* genotypes

Further, we investigated the differences in general characteristics between the individuals carrying the risk allele (G) of *FTO* rs9930506 compared to the non-carriers (AA) (Table 5I.3a). The ages between the two genotype groups of *FTO* rs9930506 (AA vs AG+GG) were 41.1±1.3y and 42.1±1.2y, respectively, which did not differ significantly (p=0.705). The gender distribution was not significantly different between the two genotype groups (p=0.150). Similarly, no significant difference was found between the two genotype groups in physical activity (p=0.441), smoking (p=0.497) and alcohol consumption (p=0.352), respectively. No significant difference was found in body weight (p=0.076) and height (p=0.501) between the two genotype groups.

5I.4.4 Differences in *FTO* genotype distributions between Malaysian Indians versus Malaysian Chinese and Malaysian Malays

There was significant difference in genotype distribution of *FTO* rs9930506 between ethnic groups (p<0.001) (Table 5I.3a). On further analysis, ethnicity of 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Indians vs Malaysian Chinese and Malays) for further analysis. In the dominant model, Malaysian Indians had significantly higher frequency of the risk allele (G) of rs9930506 compared to Malaysian Chinese and Malaysian Malays combined (78% vs 49.2%, p<0.001) (Table 5I.3b).

Table 5I.3a: General characteristics: Differences between the *FTO* genotypes

SNPs	**rs9930506 (dominant model)**		
Genotype	**AA (n=76)**	**AG+GG (n=102)**	**p value**
Age (years)	41.1 ± 1.3	42.1 ± 1.2	0.705 [a]
Gender			
Female (n)	69 (90.8%)	85 (83.3%)	0.150 [b]
Male (n)	7 (9.2%)	17 (16.7%)	
Ethnicity			
Malays	39 (51.3%)	47 (46.1%)	**<0.001 [b*]**
Chinese	26 (34.2%)	16 (15.7%)	
Indians	11 (14.5%)	39 (38.2%)	
Physical activity status			
Physically inactive	74 (97.4%)	97 (95.1%)	0.441 [b]
Physically active	2 (2.6%)	5 (4.9%)	
Smoking status			
Never	74 (97.4%)	101 (99%)	0.497 [b]
Former	1 (1.3%)	0	
Current	1 (1.3%)	1 (1%)	
Alcohol consumption status			
Never	75 (98.7%)	101 (99%)	0.352 [b]
Former	0	1 (1%)	
Current	1 (1.3%)	0	
Weight (kg)	64.2 ± 1.5	69.9 ± 1.9	0.076 [a]
Height (cm)	157.6 ± 0.8	158.0 ± 0.7	0.501 [a]

Data were expressed as mean (±SE) or number (percentage).
[a] p value based on ANCOVA after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption.
[b] p value based on Chi square test.
*p<0.05 was considered as significant.
Note: 'Physically active' was defined as accumulation of at least 150 minutes/week of moderate intensity activity (3-6 METs) or 60 minutes/week of vigorous physical activity (>6 METs).

Table 5I.3b: Differences in *FTO* genotype distribution between Malaysian Indians versus Malaysian Chinese and Malaysian Malays

***FTO* SNPs**	**rs9930506 (dominant model)**		
Ethnicity	**AA (n=76)**	**AG+GG (n=102)**	**p value**
Indian (n=50)	11 (22.0%)	39 (78.0%)	**<0.001***
Chinese & Malay (n=128)	65 (50.8%)	63 (49.2%)	

Ethnicity with 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Indians versus Malaysian Chinese and Malaysian Malays) for further analysis. Data were expressed in total number and percentages. The difference in genotype frequency between ethnic groups was assessed by Chi square test.
*p<0.05 was considered significant.

5I.4.5 Differences in the mean values of anthropometric, biochemical and dietary parameters between the *FTO* genotypes

Our results reveaeled that the individuals carrying the the risk allele (G) of rs9930506 had significantly higher BMI (p=0.036), WC (p=0.032), WHR (p=0.005), fat mass (p=0.038), body fat percent (p=0.039), hsCRP levels (p=0.018) and lower HDL cholesterol levels (p=0.011) compared to the non-carriers (AA), after adjusting for covariates such as age, gender, physical activity status, smoking status and alcohol consumption, BMI, WC, body fat mass and body fat percent (Table 5I.4). With respect to dietary nutrient intake, no significant difference was found between *FTO* rs9930506 and macronutrient intake (p>0.05). However, the carriers of the risk allele (G) of rs9930506 had significantly lower intake of vitamin E (p=0.015) and higher fibre intake (p=0.016) compared to the non-carriers (AA), even after adjusting for covariates age, gender, physical activity status, alcohol consumption status, smoking status, BMI, WC, fat mass, body fat percent and total energy intake.

Table 5I.4: Differences in the mean values (± SE) of dietary, anthropometric and biochemical parameters between the *FTO* genotypes

SNPs	**rs9930506 (dominant model)**		
Genotype	**AA (n=76)**	**AG+GG (n=102)**	**p value**
[1]Weight (kg)	64.2 ± 1.5	69.9 ± 1.9	0.076
[1]BMI (c)	**25.9 ± 0.6**	**27.8 ± 0.6**	**0.036***
[1]WC (cm)	**77.5 ± 2.3**	**85.5 ± 2.1**	**0.032***
[1]WHR	**0.87 ± 0.01**	**0.91 ± 0.01**	**0.005***
[1]Muscle Mass (kg)	22.0 ± 0.6	22.7 ± 0.5	0.942
[1]Fat Mass (kg)	**24.5 ± 1.0**	**28.2 ± 1.2**	**0.038***
[1]Fat-free Mass (kg)	39.8 ± 0.8	41.7 ± 0.9	0.519
[1]% Body Fat	**37.2 ± 0.9**	**39.2 ± 0.8**	**0.039***
[1]Systolic Blood pressure (mmHg)	120.2 ± 2.2	124.1 ± 1.7	0.197
[1]Diastolic Blood pressure (mmHg)	78.9 ± 1.4	82.0 ± 1.2	0.152
[1]Pulse rate	76.7 ± 1.8	76.3 ± 1.2	0.957
Blood biochemical parameters	**AA (n=48)**	**AG+GG (n=78)**	**p value**
[2]Fasting Glucose (mmol/L)	4.94 ± 0.2	5.40 ± 0.3	0.270
[2]Fasting Insulin (uU/mL)	8.75 ± 1.2	10.77 ± 1.2	0.329
[2]HOMA-IR	1.92 ± 0.3	2.82 ± 0.4	0.239
[2]Total cholesterol (mmol/L)	5.60 ± 0.2	5.47 ± 0.1	0.391
[2]Triglyceride (mmol/L)	1.33 ± 0.1	1.41 ± 0.1	0.851
[2]HDL Cholesterol (mmol/L)	**1.65 ± 0.1**	**1.45 ± 0.04**	**0.011***
[2]LDL cholesterol (mmol/L)	3.38 ± 0.1	3.35 ± 0.1	0.784
[2]Total cholesterol/HDL cholesterol	3.57 ± 0.2	3.86 ± 0.1	0.234
[2]hsCRP (mg/L)	**3.50 ± 0.6**	**6.93 ± 1.0**	**0.018***
Dietary nutrients intake	**AA (n=48)**	**AG+GG (n=78)**	**p value**

[2]Total energy intake (kcal)	1993.4 ± 41.8	2014.4 ± 42.3	0.576
[3]Total carbohydrate intake (g)	249.3 ± 8.9	256.8 ± 7.5	0.781
[3]Total protein intake (g)	74.0 ± 2.7	73.7 ± 2.7	0.483
[3]Total dietary fat intake (g)	81.8 ± 2.9	83.6 ± 2.5	0.839
[3]Percentage energy from carbohydrate (%)	46.3 ± 1.2	47.6 ± 0.9	0.356
[3]Percentage energy from protein (%)	15.0 ± 0.6	14.4 ± 0.4	0.474
[3]Percentage energy from dietary fat (%)	37.0 ± 1.0	36.6 ± 0.8	0.740
[3]Saturated fat (%TE)	7.7 ± 0.5	7.8 ± 0.5	0.924
[3]Monounsaturated fat (%TE)	11.0 ± 0.6	11.3 ± 0.5	0.861
[3]Polyunsaturated fat (%TE)	6.5 ± 0.5	6.4 ± 0.4	0.579
[3]Trans fat (%TE)	0.1 ± 0.02	0.1 ± 0.03	0.212
[3]Dietary cholesterol (%TE)	127.3 ± 12.9	93.0 ± 8.5	0.113
[3]Total sugar (%TE)	7.2 ± 0.6	7.3 ± 0.5	0.999
[3]Glucose (%TE)	1.0 ± 0.2	1.4 ± 0.2	0.247
[3]Fructose (%TE)	1.1 ± 0.2	1.3 ± 0.2	0.389
[3]Sucrose (%TE)	4.2 ± 0.4	4.0 ± 0.3	0.760
[3]Fibre (g)	**5.1 ± 0.4**	**6.3 ± 0.4**	**0.016***
[3]vitamin E (mg)	**6.6 ± 0.5**	**5.3 ± 0.3**	**0.015***

One way ANCOVA was performed to determine the differences in the mean values of dietary, anthropometric and biochemical parameters between the *FTO* genotypes, after adjusting for covariates in different models [1]age, gender, physical activity status, smoking status and alcohol consumption; [2] model [1]+ BMI and; [3] model[2] + total energy intake.
*p<0.05 was considered significant.

5I.4.6 Gene-diet interaction on obesity-related traits

To study the effect of the interaction between percent energy from dietary protein and vitamin E intake with *FTO* rs9930506 on hsCRP levels, data were then dichotomised into two groups based on the median value of the two nutrients The groups were i) low percent energy from dietary protein (<14%/day) and high percent energy from dietary protein (≥14%/day) and ii) low vitamin E intake (<5.4mg/day of energy adjusted vitamin E intake) and high vitamin E intake (≥5.4mg/day of energy adjusted vitamin E intake). The effect of gene-diet interaction on biochemical parameters was evaluated by general linear model (GLM) after adjusting for age, gender, physical activity status, smoking status, alcohol consumption, BMI, WC, fat massand total energy intake. A statistical probability level of p<0.05 (two-sided) was considered significant. The above analysis revealed differences between the 2 groups (Figure 5I.1 and Figure 5I.2). Individuals carrying the risk allele (G) *FTO* rs9930506 had significantly lower hsCRP levels (6.35±1.3mg/L vs 7.46±1.6mg/L, p=0.007; Figure 5I.1) when consuming high percent energy from dietary protein (≥14%/day), compared to low percent energy from dietary protein, even after adjusting for covariates (age, gender, physical activity status, smoking status, alcohol consumption, BMI, WC, fat mass and total energy intake). Similarly, we found significantly lower hsCRP levels (5.57±1.2mg/L vs 7.87±1.6mg/L, p=0.035; Figure

5I.2) in the individuals carrying the risk allele (G) of *FTO* rs9930506 when consuming high vitamin E compared to low vitamin E after adjusting for the same covariates.

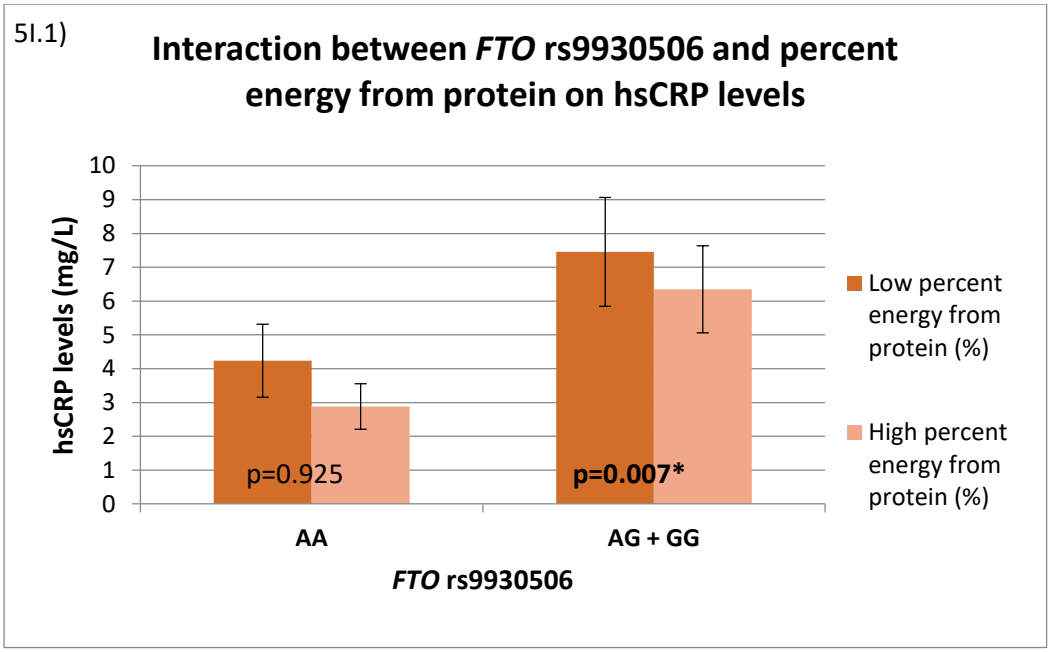


Figure5I.1: The effect of the interaction between *FTO* rs9903506 and percent energy from dietary protein on hsCRP levels. Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, physical activity status, smoking status, alcohol consumption, total energy intake and BMI. Percent energy from dietary protein was dichotomised into 2 groups by using the median value of 14%/day (for all participants), for analysis.
*p<0.05 was considered significant.

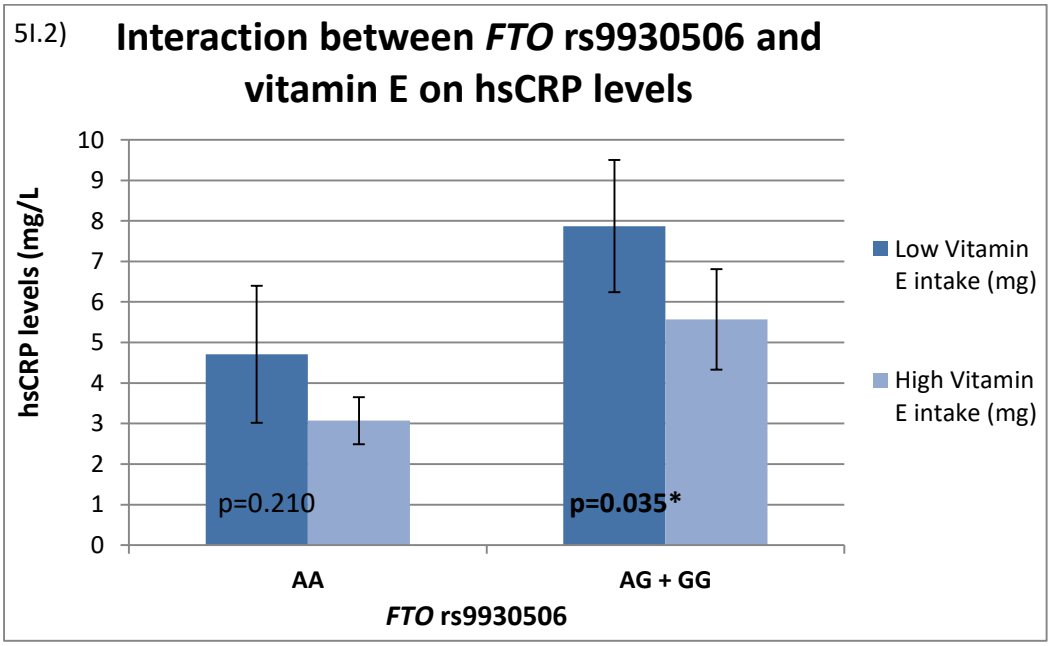


Figure 5I.2: The effect of the interaction between *FTO* rs9930506 and vitamin E intake on hsCRP levels. Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, physical activity status, smoking status, alcohol consumption, BMI and total energy intake. Energy adjusted vitamin E intake was dichotomised into 2 groups by using the median value of 5.4mg/day (for all participants), for analysis.
*p<0.05 was considered significant.

5I.5 Discussion

Single nucleotide polymorphisms in *FTO* gene was the first reported gene variant, to be associated with obesity in the European population; with AA genotype of rs9930609 having a 1.7 fold increased risk of obesity compared to TT genotype (Frayling et al., 2007). Similar finding was also reported in a Pakistani population (Rees et al., 2011). However, other studies analysing association of *FTO* gene variants and obesity (defined by BMI) in Asian population have reported conflicting information (Li et al., 2008). Rees et al., 2011 reported a strong association between *FTO* gene variants with BMI and waist circumference in South Asian population. However, two Malaysian studies which involved a total of 911 multi-ethnic Malaysians indicated no such association between *FTO* rs9939609 and rs17817288 with obesity related anthropometric measurements (Apalasamy et al., 2012; Chey et al., 2013). Other polymorphisms such as rs9930506 have also been observed to be associated with increased risk of obesity. Previous studies have reported that the GG genotype of rs9930506 polymorphism was associated with obesity in the Polish, Japanese and Italian populations (Hotta et al., 2008; Sentinelli et al., 2012; Wrzosek et al., 2016), however this association was not observed in the Chinese Han population (Li et al., 2008). To date, there is no data reported on the haplotype of *FTO* rs9930501, rs9930506 and rs9943754 and its association with metabolic parameters or its association with habitual diet, in the Malaysian population.

5I.5.1 Complete linkage disequilibrium of *FTO* rs9930501, rs9930506 and rs9932754

The association between *FTO* rs9930506 and obesity has been studied frequently. However, the other two adjacent SNPs (rs9930501 and rs9932754) have been less explored for their potential impact on obesity-related anthropometric measurements. The linkage disequilibrium (LD) between rs9930506, rs9930501 and rs9932754 has been mentioned in genome-wide association studies (Kalantari, Keshavarz Mohammadi, et al., 2018; Kalantari, Mohammadi, et al., 2018). Recent findings from Kalantari et al., 2018 also reported that a complete linkage disequilibrium (LD) in a haplotype block comprised of 3 SNPs, rs9930501 (risk allele G), rs9930506 (risk allele G) and rs9932754 (risk allele C) located at the intron 1 region of the *FTO* gene was identified in Iranian male adolescents. The authors suggested that the *FTO* gene variants may play their roles as a haplotype (and not single SNPs). Given the close proximity of these variants, it can be also considered as a new hypothesis that these variants exert their effects on expression of the other genes through a sequence of intron 1 region.

5I.5.2 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and association with diet

Our data does not reveal any interaction between macronutrients and *FTO* genotypes, but the risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly lower vitamin E and higher fibre intake compared to the non-carriers (AA, AA and TT, respectively). Given that the food choices are affected by environment, traditions, meal skipping, behaviour and stress, it is not surprising that there is no association between gene variants and energy and macronutrient intake. However, Steemburgo et al., 2013, in their study reported that carriers of the AA genotype of the *FTO* rs9939609, had increased fat and decreased fibre consumption, independent of BMI. It should be noted though that the participants of their study were diabetic while the participants of the current study were healthy individuals with normal blood glucose levels (<5.6mmol/L).

5I.5.3 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and association with anthropometric and biochemical parameters

We found that the risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly higher BMI, WC, WHR, fat mass, body fat percent, hsCRP levels and lower HDL cholesterol levels compared to the non-carriers (AA, AA and TT, respectively). We report that the risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) expressed higher hsCRP levels, even after adjusting for BMI and body fatness. High sensitivity C-reactive protein (hsCRP) is known to be the best systemic marker for inflammation. It is also an independent predictor of future cardiovascular events (Devaraj et al., 2009). Apparently healthy individuals with higher concentration of hsCRP had a higher risk of developing CVD and type 2 diabetes (Kolb & Mandrup-Poulsen, 2005; Pearson et al., 2003). Fisher et al., 2009 reported that the risk allele (A allele) of *FTO* rs9939609 contributed to higher levels of plasma hsCRP in their population, independent of its effect on BMI and fatness. It is suggested that *FTO* gene variations may not only contribute to the increase in the size of fat mass, but may as well enhance the inflammatory state of adipose tissue stores thus increasing systemic inflammation.

We found that the risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) also presented lower HDL cholesterol levels even after adjusting for BMI and body fatness (Table 5I.3) (Appendix 15). This result replicates the previous findings from the Polish and Italian populations, which report that risk allele of *FTO* rs9930506 was associated with lower HDL-C levels (Franczak et al., 2017; Sentinelli et al., 2012). Elevated HDL cholesterol and reduced hsCRP levels are known to be protective against risk of cardiovascular diseases (Bilhorn et al.,

2012; Ou et al., 2009). The presence of risk alleles of *FTO* gene in our population predisposes susceptible individuals to CVD events in future.

5I.5.4 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and insulin resistance

In our study, no significant association was found between the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and insulin resistance (HOMA-IR) (Table 5I.3). Sentinelli et al., 2012 and Wrzosek et al., 2016 reported on *FTO* rs9930506 and rs9939609 and found no association with the latter gene variants and insulin resistance in their Polish and Italian populations respectively. However, Doney et al., 2009 reported significant association of *FTO* rs9939609 with increased fat mass, increased risk of type 2 diabetes, increase in atherogenic lipid profile and increased risk of myocardial infarction with carriers of the risk allele in their Scottish population. It is interesting to note that the average age (±SD) of the Polish population was 33.9 ± 12.7 years and the median age of Italian populations was 46 years; whereas the average age (±SD) of the Scottish population was 63.7±11.7 years. Our study population had an average age (±SE) of 41.7 ± 0.9 years. We conclude that advanced age in the Scottish population may have precipitated the comorbidities in their population in the group with the risk allele.

5I.5.5 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754, ethnicity and association with obesity

In our study, we found significant differences in the *FTO* genotype distribution between the ethnic groups. Malaysian Indians had significantly higher frequency of risk alleles of rs9930501 (G), rs9930506 (G) and rs9932754 (C) compared to the Malaysian Chinese and Malays (78% vs 49.2%; 80% vs 48.4%; and 80% vs 49.2%, respectively) (Table 5I.1b) (Appendix 16). A previous study reported a similar finding that the risk allele of rs9939609 (A allele) was less common in the Chinese population than in the European population (12.6 vs. 45%, respectively) (Chang et al., 2008). Again, Li et al., 2008 reported that the minor allele frequencies of *FTO* rs8050136, rs9939609, and rs9930506 were significantly lower in the Chinese Han population compared to the European population. Our study adds to the existing evidence that differences in gene variants in *FTO* does exist in various ethnic groups.

In our study, we found that rare homozygotes of *FTO* rs9930506 (GG), rs9930501 (GG) and rs9932754 (CC) had increased odds of obesity, 2.87 (CI=1.14-7.19, p=0.025), 3.03 (CI=1.23-7.49, p=0.016) and 3.04 (CI=1.22-7.59, p=0.017) compared to the non-carriers (AA, AA and

TT, respectively), in the additive model, after adjusting for age, gender, physical activity status, alcohol consumption and smoking status (Table 4) (Appendix 17). Previous studies on Polish (n=442) and Italian (n=752) populations reported that the G allele of *FTO* rs9930506 was associated with increased odds of obesity (Scuteri et al., 2007; Sentinelli et al., 2012; Wrzosek et al., 2016). Our small sample size (n=178) may have limited our investigation with respect to ethnicity, gene variation and association with obesity.

5I.5.6 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754, and interaction with biochemical parameters and diet

Interestingly, the gene-diet interaction analysis of our data suggest that dietary vitamin E intake and percent energy from protein may modulate the effect of the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 on serum hsCRP levels, respectively. Individuals carrying the risk alleles of rs9930501 (G), rs9930506 (G) and rs9932754 (C) showed significantly lower hsCRP levels with high vitamin E intake (Figure 5I.2) (Appendix 18). It is common knowledge that vitamin E (a micronutrient with anti-oxidant and immune modulating properties) can reduce oxidative stress and influence inflammatory processes (Singh & Jialal, 2004). A significant inverse association of vitamin E and hsCRP has been demonstrated in some studies (Devaraj et al., 2007; Scheurig et al., 2008). A previous study has reported that consumption of vitamin E and omega-3 fatty acid can increase the expression of SIRT1 and PGC1α genes, suppressing pro-inflammatory genes in coronary artery disease patients (Saboori et al., 2015).

In our study, both carriers and non-carriers of the risk alleles had met the Malaysian dietary recommendations for vitamin E intake (7.5mg/day for women and 10mg/day for men) (National Coordinating Committee of Food and Nutrition, 2017). However, vitamin E intake was significantly lower in individuals carrying the risk alleles of *FTO* SNPs compared to the non-carriers (unadjusted value of vitamin E; 10.6±0.7mg vs 13.5±1.2mg; data not presented in table). We opine that the interaction of gene variants on vitamin E intake in our study participants has influenced their serum hsCRP levels. Increased intake of vitamin E for metabolically unhealthy individuals should be considered as a key dietary recommendation in weight management programmes.

We found that individuals carrying the risk alleles of rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly lower hsCRP levels with high percentage energy from protein (≥14% of TE) compared to low percentage energy from protein (Figure 5I.1) (Appendix 19). This suggests that *FTO* gene variants may interact with dietary protein intake and modulate

hsCRP levels. A randomised weight loss intervention trial has reported that the carriers of the risk allele of *FTO* gene exhibited greater reduction in food cravings with a calorie restricted and high- protein diet (Huang et al., 2014). Moreover, a high protein diet led to weight loss and improvement in body composition in the individuals carrying the risk allele of the *FTO* gene (Zhang et al., 2012). Our Chinese participants were consuming significantly higher percent energy from protein (17.8±0.8% of TE, p=0.001; Chapter 4) and Indians were consuming significantly lower percent energy from protein (13.1±0.5% of TE, p=0.001; Chapter 4) compared to Malaysian recommendation (15% of TE/per day) (data not presented in table). This finding along with evidence from the current study that Indians expressed phenotypes which predispose them to risk of non-communicable diseases is key to personalised recommendations in weight management programmes.

5I.5.7 Haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and physical activity status

Multiple studies have reported that *FTO* SNPs have no impact on basal metabolic rate (Cecil et al., 2008). There is some evidence that physical activity level can affect BMI positively in the individuals with risk alleles of *FTO* (Andreasen et al., 2008). A meta-analysis of 218,166 adults reported that the association between *FTO* rs9939609 and the odds of obesity was attenuated by 30% in physically active adults, suggesting that individuals who were genetically predisposed to obesity can benefit from increased physical activity (Kilpeläinen et al., 2011). However, other studies were not able to replicate this finding (Lee et al., 2010). Gustavsson et al., 2014 found that the minor allele of *FTO* gene was associated with higher proportion of subjects with overweight and obesity. They also reported that there were no association of *FTO* genotypes with blood lipids and physical activity levels (Gustavsson et al., 2014). In our study, 96% of the study participants were categorised as physically inactive and only a small proportion of them (3-5%) were physically active. In all our analysis, physical activity status was adjusted for where appropriate.

Emerging evidence points to the fact that human genotypes may influence dietary modulation and affect weight loss and weight maintenance (Heianza & Qi, 2017). General recommendations based on dietary guidelines, e.g. recommended nutrient intake (RNI) or recommended dietary allowance (RDA), are often ineffective and end in unsuccessful weight management programmes. Inter-individual variances at the genetic level cannot be ignored when developing strategies for weight management in an overweight/obese population as there is no one-size-fit-all diet that can suit all individuals. Precision nutrition or personalised dietary

regime tailored to individual's genetic and ethnic background should be considered for successful weight loss strategies.

5I.6 Summary

This chapter aimed to evaluate i) the differences in genotype distributions of *FTO* gene variants (rs9930501, rs9930506 and rs9932754) between the ethnic groups (Malaysian Malays, Chinese and Indians), ii) the association between the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 and the odds of obesity, iii) the association between the *FTO* SNPs and obesity-related phenotypes, and iv) the interaction between the *FTO* SNPs and dietary nutrients on phenotypic traits, in Malaysian adults. 79 obese and 99 non-obese Malaysian adults were recruited. Our results revealed that Malaysian Indians expressed the highest risk allele frequencies of rs9930501 (G), rs9930506 (G) and rs9932754 (C) compared to the Malays and Chinese. Individuals carrying the homozygous mutant of *FTO* rs9930501 (GG), rs9930506 (GG), and rs9932754 (CC) were associated with increased odds of obesity compared to AA, AA and TT genotypes, respectively, in the additive model. The risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly higher BMI, WC, WHR, fat mass, body fat percent, hsCRP levels and lower HDL cholesterol levels compared to the non-carriers (AA, AA and TT genotypes, respectively). The risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) consumed significantly lower vitamin E and higher fibre intake compared to the non-carriers (AA, AA and TT, respectively). Gene-diet interaction analysis revealed that risk alleles carriers of rs9930501 (G), rs9930506 (G) and rs9932754 (C) had lower hsCRP levels with higher energy from protein (≥14%/day) and higher Vitamin E (≥5.4mg/day). These findings suggest that the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 may interact with dietary protein and Vitamin E and modulate hsCRP levels.

Chapter 5-Section II: *ADRB2* gene

5II.1 Introduction

Obesity and related chronic diseases have become the leading cause of morbidity and mortality worldwide. Consumption of energy-dense food coupled with sedentary lifestyle, are the main contributors to the development of obesity and related complications (Spiegelman & Flier, 2001). Moreover, multiple genetic loci determined by genome-wide association studies (GWAS) have been found to be associated with increased susceptibility to obesity, diabetes and dyslipidemia (Speliotes et al., 2010). In the recent years, attention has been focused on the adrenergic receptor system for its role in the stimulation of thermogenesis and in activating lipid mobilisation from the fat stores. With respect to this, the beta-2 adrenoceptor gene is a candidate gene since it is the dominating lipolytic receptor in the human white adipose tissue (Arner & Hoffstedt, 1999). It stimulates lipid mobilisation through lipolysis in adipocytes, regulates body fat accumulation and energy expenditure (Taylor, 2007).

A strong association between obesity and a miss-sense mutation in codon 27 of the beta-2 adrenoceptor gene replacing glutamine to glutamic acid (Gln27Glu or rs1042714) has been reported (Large et al., 1997). Another gene variant located at codon 16 substituting arginine for glycine (Arg16Gly or rs1042713) has been reported by Green et al., 1995. Masuo et al., 2005 reported that insulin resistant subjects had higher frequencies of the G allele of rs1042713. Total body fat mass and blood pressure levels were also higher in non-obese and non-hypertensive men with G allele in the Japanese population. The authors speculated that insulin resistance could, in part be determined by the genetic variant of the beta-2 adrenoceptor gene and that polymorphism and higher plasma adrenaline could increase insulin resistance, adiposity and high blood pressure in their subjects. Thus, the G allele could lead to heightened sympathetic nerve activity, insulin resistance and higher blood pressure and adiposity in non-obese and non-hypertensive individuals. Other studies have adequately reported that insulin resistance was strongly associated with heightened sympathetic nerve activity (Masuo et al., 1997). In other words, adrenergic receptor defects lead to sympathetic nervous system over activity that may play a role in the development of insulin resistance, hypertension and obesity (Masuo & Lambert, 2011).

A study on the Swedish population reported that *ADRB2* rs1042713 was significantly associated with elevated central body fat, systolic blood pressure, serum leptin and triglyceride

levels, but not with obesity (Rosmond et al., 2000). Studies from Saudi Arabia reported significant association between *ADRB2* rs1042713 and rs1042714 polymorphisms and the development of insulin resistance, dyslipidemia, overweight, and obesity (Daghestani et al., 2010; Daghestani et al., 2012). However, findings from Asian populations (Japanese and Korean) reported negative association between obesity and *ADRB2* gene polymorphisms. Moreover, these studies did not investigate interaction of gene variants with dietary nutrients (Hayakawa et al., 2000; Kim et al., 2002).

Individual or population differences in the development of obesity related metabolic diseases may result not only from genetic variation, but may also be the modulatory effect of dietary nutrients on gene and gene variants (Heianza & Qi, 2017). In the Malaysian population, relatively little is known with respect to the interaction between dietary nutrients and *ADRB2* gene variations on obesity, insulin resistance and glucose homeostasis. It is believed that early identification of the candidate gene variants and their interaction with diet may allow for the provision of good quality personalised dietary recommendation to achieve effective weight loss and reduction in metabolic risk factors (Fenech et al., 2011). Although a couple of intervention studies have reported lipid outcomes associated with *ADRB2* rs1042713, there is no such study done in Malaysian population. Since there has been conflicting results with respect to *ADRB2* rs1042713 between Asian, Caucasian and Arabic populations, the current study on the Malaysian population is valuable and will shed light and add to the existing evidence on gene variants and phenotypic outcomes and influence of diet on the latter. To the best of our knowledge, this is the first study in Malaysian adults, that investigated the interaction between *ADRB2* rs1042713 and rs1042714 and dietary nutrients on obesity-related metabolic traits.

5II.2 Objectives

The aim of this section is to investigate i) the differences in genotype distributions of *ADRB2* gene variants (rs1042713 and rs1042714) between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians), ii) the association of *ADRB2* rs1042713 and rs1042714 with obesity and insulin resistance; iii) the association between *ADRB2* rs1042713 and rs1042714 and obesity-related phenotypes in obese and non-obese individuals; and iv) the interaction between *ADRB2* rs1042713 and rs1042714 and dietary nutrients on obesity-related phenotypes, in the Malaysian adults.

5II.3 Methodology

5II.3.1 Study design

This cross-sectional study was conducted from 2015-2018 on 178 multi-ethnic Malaysian adults (Malaysian Malays, Malaysian Chinese and Malaysian Indians) aged between 18-74 years.

5II.3.2 Participant selection

Detailed information on the participant selection has been described in an earlier chapter (Chapter 4). Briefly, a total of 178 Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above were recruited at random through advertisements and flyers, distributed at UNMC campus, supermarkets and community centres in the vicinity of UNMC. Structured questionnaire was used to record information on race and ethnicity, and the questions were directed to trace back three generations to confirm ancestry. Participants completed a health and lifestyle questionnaire which included questions on past diseases, family history of past diseases, physical activity level and substance abuse. Smoking status and alcohol consumption were reported as i) never, ii) former and iii) current. Individuals diagnosed with cardiovascular diseases (CVD), diabetes, cancer, hypertension, renal diseases, endocrine disorders such as hypothyroidism were excluded. Individuals on medications for cholesterolemia, hypoglycemia and psychiatric disorders were excluded. Pregnant and lactating women were also excluded from the study. Interested individuals, attended initial screening to determine whether or not the participant met the inclusion and exclusion criteria. In total 79 obese and 99 non-obese Malaysian adults were recruited.

5II.3.3 Assessment on anthropometric, dietary, blood biochemical parameters, substance abuse and physical activity level

Detailed information on the assessment of anthropometric parameters, dietary nutrients analysis, blood biochemical parameters, substance abuse and physical activity levels have been described in an earlier chapter (Chapter 4).

5II.3.4 DNA extraction and genotyping assay

5ml of whole blood was drawn from an antecubital vein into vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ) containing EDTA. The genomic DNA was extracted from leukocytes using MasterPure DNA Purification kit (Lucigen Corporation Middleton, WI, USA) according to manufacturer's instructions. DNA samples were stored at -20 °C until use. A DNA fragment of 310bp containing rs1042713 and rs1042714 polymorphisms was amplified by using polymerase chain reaction (PCR) for the identification of *ADRB2* rs1042713 gene polymorphism with specific primers (Forward primer: 5'-CCGCCGTGGGTCCGCC-3' and reverse primer: 5'-CCATGACCAGATCAGCAC-3') derived from an earlier study (Macho-Azcarate et al., 2002). PCR was performed using 5µl of genomic DNA (~1ng/µl), 0.2 µM forward primer and reverse primer with 5µl of Taq 5X Master Mix. Thermal cycling was performed as follows: initial denaturing at 95ºC for 5 min; 35 cycles of denaturation at 94ºC for 45 s, annealing at 64ºC for 40s and extension at 72ºC for 45 sec, and then a final extension at 72ºC for 5 min. The amplicons were verified by using electrophoresis on 2% agarose gel and visualised under ultraviolet illumination after staining by ethidium bromide (Appendix 12). The verified amplicons were then sequenced by using BigDye® Terminator v3.1 cycle sequencing kit chemistry.

5II.3.5 Power and sample size calculation

We performed power calculation using software QUANTO, Version 1.2.4 to find the minimum detectable effect for a given sample size. This calculation takes into account the type 1 error rate of 0.05, and the population prevalence of insulin resistance (using a cut off of HOMA-IR≥1.7) of 45%, as reported in the present study. Given that the minor allele frequency (A) of *ADRB2* rs1042713 in our study, was 0.49, with 57 insulin resistant and 69 non-insulin resistant participants, we had 68.2% power to detect an effect of 2.83 (odds ratio) (dominant model). With regard to the gene-diet interaction, given that the mean of fasting glucose levels in our population was 5.2mmol/L, with a SD of 2.0, environmental effect (differences of fasting glucose levels between high and low PUFA:SFA ratio) of -0.4 (5.0mmol/L-5.4mmol/L), genetic effect {differences of fasting glucose levels between the carriers of G allele of *ADRB2* rs1042713 and the non-carriers (A)} of 0.3 (5.4mmol/L-5.1mmol/L) and interaction effect of -1.1 (4.83mmol/L-5.93mmol/L), a power of 61% for the gene-diet interaction was computed.

5II.3.6 Statistical analysis

Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). All parametric results were expressed as mean ± standard error (SE) for each group. Log transformation was performed to transform non-normally distributed data into normally distributed data. Independent t test and Chi square test were performed to assess the differences between the two genotype groups (AA vs AG+GG) on baseline continous variables and categorical variables, respectively. Allele frequency was estimated by gene counting and Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE) (Namipashaki et al., 2015). To study the effect of *ADRB2* gene variants on obesity, data were dichotomised into obese and non-obese groups (obesity was defined by BMI ≥27.5 kg/m^2) (WHO Expert Consultation, 2004). Odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each genotype by logistic regression to determine the odds of obesity associated with gene variants, after adjusting for covariates age, gender, physical activity status, smoking status and alcohol consumption. The AA genotype of *ADRB2* rs1042713 and CC genotype of rs1042714 were used as the reference group in both additive and dominant models. Same analysis was performed to study the odds of *ADRB2* gene variants on insulin resistance. Data were dichotomised into i) non-insulin resistant and insulin resistant groups (using a cut off of HOMA-IR≥1.7; Yamada, Moriyama, & Takahashi, 2012). Linear regression was performed to determine the model that best fits the association of *ADRB2* gene variants and insulin resistance.

Differences in the mean values of anthropometric, blood biochemical and dietary parameters between the gene variants, in obese and non-obese groups were assessed by using one-way analysis of covariance (ANCOVA). Variance inflation factor (VIF) was taken as a measure for testing multicollinearity among related parameters (e.g. BMI, WC, body fat, percent body fat). Stepwise regression was performed to select the model that had the highest R-squared value. In all linear regression analysis assessing correlations, BMI was used as a covariate to adjust for the above instead of all other related variables (e.g. WC, body fat, percent body fat). Adjustment for covariates such as age, gender, ethnicity, physical activity status, smoking status, alcohol consumption, BMI and total energy intake were applied where appropriate. Bonferroni corection was applied to account for the multiple comparisons and a Bonferroni corrected p value of <0.025 ($\alpha = 0.05/2$) was considered significant.

The intake of macronutrients (energy-adjusted) was dichotomised into two groups based on the median intake of the population. A multivariate general linear model (GLM) was used to investigate the effect of the interaction between dietary macronutrients and *ADRB2* gene variants on obesity related metabolic traits, after adjusting for potential confounders (age, gender, physical activity status, smoking status, alcohol consumption, BMI, and total energy intake). A statistical probability level of $p<0.05$ (two-sided) was considered significant.

5II.4 Result

5II.4.1 Allele frequencies and genotype distributions of *ADRB2* gene variants

G allele and C allele was the most frequent variant for rs1042713 and rs1042714 respectively, in our study population. The allele frequency for minor allele of rs1042714 (G allele) and rs1042713 (A allele) were 0.13 and 0.49, respectively, which did not deviate from Hardy-Weinberg equilibrium as tested by Chi square test (χ^2=0.42 and χ^2= 0.36, repectively (χ^2<3.841)) (Table 5II.1). In case of rs1042714, due to the lower frequency of homozygous mutant GG genotype (n=2) in the additive model, we combined the homozygous mutant GG genotype with the heterozygous CG genotype in a dominant model for all statistical analysis.

Table 5II.1: Allele frequencies and genotype distributions of *ADRB2* gene variants

SNPs	**rs1042713**			**rs1042714**		
	Genotype	**Overall n=178**	**X2 Hardy-Weinberg**	**Genotype**	**Overall n=178**	**X2 Hardy-Weinberg**
Additive model	**AA**	44 (24.7%)	0.36	**CC**	134 (75.3%)	0.42
	AG	86 (48.3%)		**CG**	42 (23.6%)	
	GG	48 (27.0%)		**GG**	2 (1.1%)	
Dominant model	**AA**	44 (24.7%)		**CC**	134 (75.3%)	
	AG+ GG	134 (75.3%)		**CG + GG**	44 (24.7%)	
Recessive model	**AA + AG**	130 (73.0%)		**CC + CG**	176 (98.9%)	
	GG	48 (27.0%)		**GG**	2 (1.1%)	
Allele frequency	**A**	174 (48.9%)		**C**	310 (87.1%)	
	G	182 (51.1%)		**G**	46 (12.9%)	

Note: The top most gene variant denotes the wild variant of the SNP in each category, and the last gene variant denotes the mutant variant.
The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with $\chi2<3.841$ considered as no deviation from Hardy Weinberg Equilibrium.
$p<0.05$ was considered significant.

5II.4.2 Association between *ADRB2* gene variants and insulin resistance and obesity

Linear regression was performed to determine the model that best fits the association of *FTO* gene variants and insulin resistance (HOMA-IR), Based on the linear regression analysis, our results revealed that additive model (r^2=0.030, p=0.049) showed the best fit to insulin resistance, compared to the dominant model (r^2=0.029, p=0.055) and recessive model (r^2=0.012, p=0.214).

Therefore, we decided to report only the additive model for rs1042714 for statistical analysis. With respect to rs1042714, our results revealed that dominant model (r^2=0.041, p=0.023) showed the best fit to insulin resistance (HOMA-IR), compared to the additive model (r^2=0.031, p=0.048) and recessive model (r^2=0.007, p=0.366). Therefore, we decided to report only the dominant model for rs1042714 for statistical analysis.

Logistic regression was performed to examine the independent effect of *ADRB2* rs1042714 and rs1042713 polymorpshims on the odds of insulin resistance and obesity. Our results revealed significant association between *ADRB2* rs1042713 and insulin resistance (using a cut off of HOMA-IR≥1.7) (Table 5II.2). The carriers of GG genotype of rs1042713 had increased odds of insulin resistance, compared to AA genotype in the additive model, 4.43 (CI=1.31-15.0, adjusted p=0.016), even after adjusting for covariates age, gender, BMI, physical activity status, smoking status and alcohol consumption. However, we found no significan association between rs1042714 and the odds of insulin resistance, 2.76 (CI=1.10-6.83, p=0.030), in the dominant model, after adjusting for covariates age, gender, BMI, physical activity status, smoking status and alcohol consumption.

With respect to the odds of obesity, we found no significant association between *ADRB2* gene variants (rs1042714 and rs1042713) and obesity (obesity as defined by BMI ≥27.5 kg/m^2), after adjusting for covariates such as age, gender, physical activity status, smoking status, alcohol consumption and BMI (Table 5II.3).

Table 5II.2: Genotype distribution and allele frequency of *ADRB2* gene variants (rs1042714 and rs1042713) in insulin resistant and non-insulin resistant groups

SNPs	Genotype	Overall n=126	X^2 Hardy-Weinberg	HOMA<1.7 n=69	HOMA≥1.7 n=57	Unadjusted OR (95%CI)	p value	Adjusted OR (95%CI)[a]	p value[a]
rs1042713	**AA**	30 (23.8%)	0.20	21 (30.5%)	9 (15.8%)	1	-	1	-
	AG	63 (50.0%)		33 (47.8%)	30 (52.6%)	2.12 (0.84-5.35)	0.111	2.31 (0.81-6.61)	0.118
	GG	33 (26.2%)		15 (21.7%)	18 (31.6%)	2.80 (1.0-7.91)	0.052	**4.43 (1.31-15.0)**	**0.016***
rs1042714	**CC**	94 (74.6%)		57 (82.6%)	37 (64.9%)	1	-	1	-
	CG + GG	32 (25.4%)		12 (17.4%)	20 (35.1%)	2.57 (1.12-5.87)	0.025	2.76 (1.10-6.83)	0.030

Odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each genotype by logistic regression to determine the risk of insulin resistance associated with *ADRB2* gene variants. The homozygous wildtype was used as the reference group in both additive and dominant models.
Non-insulin resistant and insulin resistant groups was dichotomised using a cut off of HOMA-IR≥1.7 (Yamada, Moriyama, & Takahashi, 2012).

*p<0.025 was considered significant after applying for Bonferroni correction
[a] adjusted for age, BMI, gender, physical activity status, smoking status and alcohol consumption.

Table 5II.3: Genotype distribution and allele frequency of *ADRB2* gene variants (rs1042714 and rs1042713) in obese and non-obese groups

SNPs	Genotype	Overall n=178	X^2 Hardy-Weinberg	Obese BMI≥27.5 n=79	Non-obese BMI<27.5 n=99	Unadjusted OR (95%CI)	p value	Adjusted OR (95%CI)[a]	p value[a]
rs1042713	**AA**	44 (24.7%)	0.36	19 (24.0%)	25 (25.2%)	1	-	1	-
	AG	86 (48.3%)		41 (51.9%)	45 (45.5%)	1.20 (0.58-2.49)	0.627	1.26 (0.59-2.71)	0.548
	GG	48 (27.0%)		19 (24.1%)	29 (29.3%)	0.86 (0.38-1.98)	0.726	0.94 (0.40-2.23)	0.884
rs1042714	**CC**	134 (75.3%)		60 (75.9%)	74 (74.7%)	1	-	1	-
	CG + GG	44 (24.7%)		19 (24.1%)	25 (25.3%)	0.94 (0.47-1.986	0.853	1.00 (0.48-2.06)	0.995

Note: The top most gene variant denotes the wild variant of the SNP in each category, and the last gene variant denotes the mutant variant.
The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with χ^2 < 3.841 considered as no deviation from Hardy Weinberg Equilibrium.
Logistic regression was conducted to determine the risk of obesity associated with *ADRB2* gene variants. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each genotype. The homozygous wildtype was used as the reference group in both additive and dominant models.
p<0.025 was considered significant after applying for Bonferroni correction
[a] adjusted for age, gender, physical activity status, smoking status and alcohol consumption.

5II.4.3 General characteristics: Differences between the *ADRB2* genotypes

General characteristics between the *ADRB2* genotypes are reported in the Table 5II.4. The gender distribution was not significantly different between the genotype groups of *ADRB2* rs1042713 (AA vs AG+GG) and rs1042714 (CC vs CG+GG) (p=0.587 and p=0.635, respectively). Similarly, no significant difference was found between the genotype groups of *ADRB2* rs1042713 (AA vs AG+GG) and rs1042714 (CC vs CG+GG) in physical activity status (p=0.256 and p=0.810), smoking status (p=0.606 and p=0.157) and alcohol consumption status (p=0.717 and p=0.717), respectively. The ages between genotype groups of *ADRB2* rs1042713 (AA vs AG+GG) were 43.4±1.8y and 41.1±1.0y, respectively and the ages between the genotype groups of rs1042714 (CC vs CG+GG) were 42.3±1.0y and 39.7±1.8y, respectively, which did not differ significantly (p=0.284 and p=0.162, respectively). No significant difference was found in body weight and height between the two genotype groups of *ADRB2* rs1042713 and rs1042714 (p>0.05). Due to higher number of female participants, all data analysis was adjusted for by gender.

No significant difference in the genotype distribution of *ADRB2* rs1042713 between the ethnic groups (p=0.556). However, there was significant difference in genotype distribution of *ADRB2* rs1042714 between the ethnic groups (p<0.001) (Table 5II.4). On further analysis

(Table 5II.5), ethnicity of 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Chinese vs Malaysian Malays and Indians). In the dominant model, Malaysian Chinese had significantly lower frequency of the risk allele (G) of rs1042714 compared to the Malaysian Malays and Indians combined (11.9% vs 28.7%, p=0.028).

Table 5II.4: General characteristics: Differences between the *ADRB2* gene variants

	rs1042713 (dominant model)			rs1042714 (dominant model)		
Genotype	**AA (n=44)**	**AG + GG (n=134)**	**p value**	**CC (n=134)**	**CG+GG (n=44)**	**p value**
Age (years)	43.4 ± 1.8	41.1 ± 1.0	0.284 [a]	42.3 ± 1.0	39.7 ± 1.8	0.162 [a]
Gender						
Female (n)	37 (84.1%)	117 (87.3%)	0.587 [a]	113 (74.7%)	39 (25.3%)	0.635 [b]
Male (n)	7 (15.9%)	17 (12.7%)		19 (79.2%)	5 (20.8%)	
Ethnicity						
Malays	20 (23.3%)	66 (46.7%)	0.556 [b]	64 (74.4%)	22 (25.6%)	**0.048 [b]***
Chinese	13 (31%)	29 (69%)		37 (88.1%)	5 (11.9%)	
Indians	11 (22%)	39 (78%)		33 (66.0%)	17 (34.0%)	
Physical activity status			0.256 [b]			0.810 [b]
Physically active	41 (24%)	130 (76%)		129 (75.4%)	42 (24.6%)	
Physically inactive	3 (42.9%)	4 (57.1%)		5 (71.4%)	2 (28.6%)	
Smoking status						
Never	44 (25%)	132 (75%)	0.606 [b]	132 (75.4%)	43 (24.6%)	0.157 [b]
Former	0	1 (100%)		0	1 (100%)	
Current	0	2 (100%)		2 (100%)	0	
Alcohol consumption status						
Never	44 (25.1%)	131 (74.9%)	0.717 [b]	132 (75%)	44 (25%)	0.717 [b]
Former	0	1 (100%)		1 (100%)	0	
Current	0	1 (100%)		1 (100%)	0	
Weight (kg)	65.7 ± 1.1	68.1 ± 1.6	0.199 [a]	67.7 ± 1.5	66.8 ± 2.1	0.928 [a]
Height (cm)	157.9 ± 1.1	157.8 ± 0.6	0.808 [a]	158.0 ± 0.6	157.2 ± 1.1	0.495 [a]

Data were expressed as mean (±SE) or number (percentage).

[a] p value based on ANCOVA after adjusting for age, gender, physical activity, smoking and alcohol consumption.

[b] p value based on Chi square test.

*p<0.05 was considered as significant.

Physically active' was defined as accumulation of at least 150 minutes/week of moderate intensity activity (3-6 METs) or 60 0minutes/week of vigorous physical activity (>6 METs) as defined by the Ministry of Health Malaysia.

Table 5II.5: Differences in *ADRB2* genotype distributions between Malaysian Chinese versus Malaysian Malays and Malaysian Indians

***ADRB2* SNP**	**rs1042714 (dominant model)**		
Ethnicity	**CC**	**CG+GG**	**p value**
Chinese (n=50)	37 (88.1%)	5 (11.9%)	**0.028***
Malay & Indian (n=128)	97 (71.3%)	62(28.7%)	

Ethnicity with 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Chinese versus Malaysian Malays and Malaysian Indians) for further analysis. Data were expressed in total number and percentages. The difference in genotype frequency between the ethnic groups was assessed by Chi square test.

*p<0.05 was considered significant.

5II.4.4 Differences in the mean values of anthropometric, biochemical and dietary parameters between the *ADRB2* genotypes in obese and non-obese groups

Further, we aimed to investigate the differences in the age, anthropometric, biochemical and dietary parameters of the study participants between the individuals carrying the risk allele of *ADRB2* gene variants compared to the non-risk allele carriers in obese and non-obese groups (Table 5II.6). In obese participants, we found that the risk allele carriers (G allele) of rs1042713 (AG+GG) had significantly higher total cholesterol (p=0.023) and LDL cholesterol levels (p=0.008), compared to the non-carriers (AA), even after adjusting for covariates age, gender, BMI, physical activity status, smoking status and alcohol consumption. Interestingly, such differences in blood biochemical parameters within genotypes were not observed in the non-obese group. With respect to rs1042714, obese individuals carrying the risk allele (G allele) (CG+GG) had significantly higher WHR (p=0.006) compared to the non-carriers (CC), even after adjusting for covariates age, gender, physical activity status, smoking status and alcohol consumption. No significance association was found between *ADRB2* rs1042714 and dietary parameters.

Table 5II.6: Differences in the mean values (± SE) of anthropometric, biochemical and dietary parameters between the *ADRB2* genotypes in obese and non-obese groups

SNP	rs1042713 (Dominant model)						rs1042714 (Dominant model)					
	Obese			Non-obese			Obese			Non-obese		
Genotype	**AA (n=19)**	**AG + GG (n=60)**	**p value**	**AA (n=25)**	**AG + GG (n=74)**	**p value**	**CC (n=60)**	**CG+GG (n=19)**	**p value**	**CC (n=74)**	**CG+GG (n=25)**	**p value**
[1]**Age (years)**	47.8 ± 2.0	42.8 ± 1.5	0.038	40.0 ± 2.7	39.7 ± 1.3	0.958	44.3 ± 1.5	43.0 ± 2.5	0.379	40.7 ± 1.4	37.2 ± 2.4	0.113
[1]**Weight (kg)**	76.0 ± 2.0	81.2 ± 2.3	0.097	57.9 ± 1.9	57.4 ± 1.0	0.957	80.5 ± 2.4	78.0 ± 1.9	0.994	57.3 ± 0.9	58.2 ± 2.2	0.748
[1]**Height (cm)**	157.7 ± 1.7	157.7 ± 1.0	0.688	158.1± 1.5	157.9 ± 0.8	0.871	158.3 ± 1.0	155.8 ± 1.4	0.556	157.8± 0.7	158.3 ± 1.5	0.680
[1]**BMI (kg/m2)**	30.5 ± 0.5	32.4 ± 0.6	0.086	23.0 ± 0.6	23.0 ± 0.3	0.936	31.9 ± 0.6	32.2 ± 0.8	0.735	23.0 ± 0.3	23.2 ± 0.7	0.549
[1]**WC (cm)**	97.6 ± 2.2	99.4 ± 1.6	0.173	68.6 ± 3.7	68.6 ± 2.0	0.899	98.2 ± 1.6	101.5 ± 1.8	0.031	67.8± 2.0	70.8 ± 4.1	0.307
[1]**WHR**	0.90 ± 0.01	0.93 ± 0.01	0.117	0.87 ± 0.01	0.87 ± 0.01	0.728	**0.91 ± 0.01**	**0.95 ± 0.01**	**0.006***	0.87 ± 0.01	0.88 ± 0.01	0.206
[1]**Muscle mass (kg)**	24.0 ± 0.9	24.4 ± 0.7	0.525	20.9 ± 1.0	20.9 ± 0.6	0.941	24.7 ± 0.7	23.3 ± 0.8	0.742	21.0 ± 0.6	20.5 ± 0.9	0.367
[1]**Fat mass (kg)**	32.3 ± 1.2	36.6 ± 1.4	0.065	19.2 ± 1.0	19.5 ± 0.6	0.887	35.6 ± 1.5	35.6 ± 1.4	0.751	19.2 ± 0.6	20.2 ± 1.2	0.303
[1]**Fat-free Mass (kg)**	43.8 ± 1.6	44.6 ± 1.2	0.475	38.6 ± 1.6	37.9 ± 0.7	0.678	45.0 ± 1.2	42.5 ± 1.3	0.645	38.1 ± 0.7	38.1 ± 1.5	0.513
[1]**Percent body Fat (%)**	42.5 ± 1.2	44.8 ± 0.7	0.110	33.3 ± 1.3	33.8 ± 0.8	0.683	43.8 ± 0.7	45.5 ± 1.2	0.503	33.4 ± 0.8	34.2 ± 1.4	0.227
[1]**Systolic BP (mmHg)**	124.0 ± 3.6	124.0 ± 2.0	0.712	122.6 ± 5.0	119.6± 2.3	0.587	123.6 ± 2.0	125.3 ± 3.6	0.433	119.3 ± 2.6	122.9 ± 3.2	0.741
[1]**Diastolic BP (mmHg)**	81.0 ± 2.3	81.5 ± 1.3	0.535	81.9 ± 2.7	79.2 ± 1.8	0.588	81.4 ± 1.3	81.2 ± 2.6	0.769	78.8 ± 1.7	82.5 ± 3.2	0.616
[1]**Pulse rate (bpm)**	75.7 ± 2.5	76.1 ± 1.3	0.948	75.1 ± 4.5	77.9 ± 2.0	0.556	74.8 ± 1.3	79.8 ± 2.0	0.081	78.0 ± 2.3	75.2 ± 3.1	0.602
Blood biochemical parameters	**AA (n=19)**	**AG + GG (n=60)**	**p value**	**AA (n=11)**	**AG + GG (n=36)**	**p value**	**CC (n=60)**	**CG+GG (n=19)**	**p value**	**CC (n=34)**	**CG+GG (n=13)**	**p value**
[2]**Fasting glucose (mmol/L)**	5.1 ± 0.2	5.3 ± 0.3	0.575	5.0 ± 0.3	5.2 ± 0.3	0.622	5.0 ± 0.1	6.2 ± 1.0	0.027	5.2 ± 0.3	5.1 ± 0.2	0.766
[2]**Fasting insulin (uU/mL)**	7.74 ± 1.6	11.2 ± 1.3	0.191	11.7 ± 3.7	7.5 ± 1.4	0.286	9.6 ± 0.9	14.9 ± 3.4	0.307	9.5 ± 1.9	6.0 ± 0.9	0.137
[2]**HOMA-IR**	1.9 ± 0.5	2.8 ± 0.3	0.165	2.9 ± 1.0	2.2 ± 0.7	0.402	2.2 ± 0.3	3.6 ± 0.8	0.115	2.7 ± 0.8	1.4 ± 0.2	0.164
[2]**Total cholesterol (mmol/L)**	**5.0 ± 0.2**	**5.7 ± 0.1**	**0.023***	5.4 ± 0.3	5.6 ± 0.2	0.909	5.5 ± 0.1	5.7 ± 0.3	0.378	5.5 ± 0.2	5.7 ± 0.4	0.326
[2]**Triglyceride (mmol/L)**	1.2 ± 0.2	1.4 ± 0.1	0.086	1.7 ± 0.4	1.4 ± 0.1	0.879	1.3 ± 0.1	1.5 ± 0.2	0.188	1.4 ± 0.2	1.6 ± 0.3	0.954
[2]**HDL cholesterol (mmol/L)**	1.6 ± 0.1	1.5 ± 0.1	0.752	1.5 ± 0.1	1.6 ± 0.1	0.589	1.5 ± 0.1	1.4 ± 0.1	0.210	1.6 ± 0.1	1.5 ± 0.1	0.995
[2]**LDL cholesterol (mmol/L)**	**2.9 ± 0.2**	**3.6 ± 0.1**	**0.008***	3.3 ± 0.4	3.3 ± 0.2	0.619	3.3 ± 0.1	3.6 ± 0.2	0.247	3.3 ± 0.2	3.3 ± 0.4	0.545
[2]**Total cholesterol/HDL cholesterol**	3.4 ± 0.2	3.9 ± 0.1	0.066	3.7 ± 0.3	3.7 ± 0.2	0.476	3.7 ± 0.1	4.1 ± 0.3	0.058	3.6 ± 0.1	4.1 ± 0.4	0.298
[2]**hsCRP (mg/L)**	5.2 ± 1.5	8.8 ± 1.4	0.042	2.2 ± 0.5	2.9 ± 0.5	0.546	7.2 ± 1.2	8.8 ± 2.3	0.297	2.6 ± 0.5	3.0 ± 0.8	0.789
Dietary parameters	**AA (n=19)**	**AG + GG (n=60)**	**p value**	**AA (n=11)**	**AG + GG (n=36)**	**p value**	**CC (n=60)**	**CG+GG (n=19)**	**p value**	**CC (n=34)**	**CG+GG (n=13)**	**p value**
[3]**Total energy intake (kcal)**	2145.5 ± 108.0	2065.6 ± 41.7	0.766	1897.5 ± 53.6	1867.5 ± 47.4	0.836	2078.0 ± 48.6	2106.6 ± 73.8	0.432	1857.4 ± 41.7	1919.3 ± 86.6	0.734
[3]**Actual total carbohydrate intake (g)**	272.3 ± 20.7	259.4 ± 7.2	0.979	237.8 ± 13.1	234.0 ± 11.0	0.612	262.6 ± 9.0	262.0 ± 11.6	0.734	237.7 ± 10.4	244.2 ± 17.1	0.946
[3]**Actual total protein intake (g)**	75.1 ± 5.3	77.1 ± 3.1	0.983	72.8 ± 5.3	70.0 ± 3.2	0.272	78.0 ± 3.2	72.3 ± 4.4	0.129	70.0 ± 3.0	66.7 ± 6.1	0.184
[3]**Actual total dietary fat intake (g)**	90.2 ± 5.5	84.7 ± 3.1	0.772	77.1 ± 4.1	77.7 ± 2.6	0.818	84.3 ± 2.9	91.6 ± 6.4	0.409	75.4 ± 2.6	83.0 ± 3.9	0.178

Dietary parameters	AA (n=19)	AG + GG (n=60)	p value	AA (n=11)	AG + GG (n=36)	p value	CC (n=60)	CG+GG (n=19)	p value	CC (n=34)	CG+GG (n=13)	p value
[3]Percentage energy from carbohydrate (%)	46.9 ± 1.8	46.9 ± 1.2	0.989	46.8 ± 2.1	47.7 ± 1.3	0.513	47.4 ± 1.1	45.3 ± 2.3	0.668	47.8 ± 1.3	46.8 ± 2.1	0.543
[3]Percentage energy from protein (%)	13.8 ± 0.6	14.9 ± 0.5	0.760	15.5 ± 1.2	14.4 ± 0.7	0.178	14.9 ± 0.5	13.7 ± 0.8	0.117	15.0 ± 0.7	13.8 ± 1.3	0.225
[3]Percentage energy from dietary fat (%)	37.9 ± 1.9	36.4 ± 1.0	0.697	36.4 ± 1.5	36.8 ± 1.1	0.972	36.3 ± 1.0	38.4 ± 1.8	0.467	36.1 ± 1.1	38.4 ± 1.3	0.143
[3]SFA (%TE)	7.0 ± 1.0	7.6 ± 0.5	0.528	7.0 ± 0.8	8.7 ± 0.6	0.311	7.5 ± 0.5	7.3 ± 0.9	0.384	8.4 ± 0.6	8.0 ± 0.9	0.944
[3]MUFA (%TE)	11.8 ± 1.1	10.3 ± 0.6	0.293	11.8 ± 1.1	12.1 ± 0.6	0.921	11.1 ± 0.6	9.5 ± 1.0	0.098	11.8 ± 0.6	12.5 ± 1.1	0.421
[3]PUFA (%TE)	8.0 ± 0.8	5.6 ± 0.4	0.033	6.5 ± 1.1	7.0 ± 0.6	0.524	6.4 ± 0.4	5.5 ± 1.0	0.147	6.5 ± 0.5	8.0 ± 1.2	0.189
[3]Trans fat (%TE)	0.1 ± 0.01	0.1 ±0.04	0.104	0.1 ± 0.03	0.1 ± 0.02	0.488	0.1 ± 0.03	0.1 ± 0.02	0.067	0.1 ± 0.02	0.1 ± 0.03	0.164
[3]Dietary cholesterol (%TE)	88.8 ± 14.0	108.3 ± 10.5	0.977	111.4 ± 22.9	109.7 ± 16.1	0.459	106.9 ± 10.0	93.3 ± 17.3	0.065	116.6 ± 14.3	93.1 ± 31.1	0.227
[3]Fibre (g)	6.1 ± 0.6	5.6 ± 0.5	0.310	6.8 ± 1.1	5.8 ± 0.5	0.589	5.9 ± 0.4	5.1 ± 1.0	0.106	6.4 ± 0.6	5.0 ± 0.5	0.122
[3]Vitamin E (mg)	7.2 ± 0.7	5.1 ± 0.4	0.045	6.3 ± 0.9	6.1 ± 0.5	0.934	5.6 ± 0.4	5.7 ± 1.1	0.318	6.2 ± 0.5	6.0 ± 1.0	0.678

One-way analysis of covariance was performed to determine the differences in the mean values of anthropometric, biochemical and dietary parameters between the gene variants, in obese and non-obese participants, after adjusting for covariates in different model [1]age, gender, physical activity status, smoking status and alcohol consumption; [2] model [1]+ BMI and; [3]model[2]+ total energy intake.

*p<0.025 was considered significant after applying for Bonferroni correction.

HOMA-IR, Homeostatic model assessment -insulin resistance; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; SFA, Saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid; % TE, percentage of total energy intake.

5II.4.5 Differences between blood biochemical parameters and respective diagnostic cut offs in obese individuals carrying *ADRB2* rs1042713 G allele

In obese individuals carrying the G alleles of *ADRB2* rs1042713 and rs0142714, HOMA-IR was significantly higher than the diagnostic cut off, indicating insulin resistance (1.7) (Table 5II.7). Although HDL cholesterol levels were positively, significantly higher than the diagnostic cut off (1mmol/L) and triglyceride levels were significantly lower than the diagnostic cut off (1.7mmol) in obese individuals carrying the G alleles of *ADRB2* rs1042713 and rs0142714, however LDL cholesterol and hsCRP levels were significantly higher than the respective cut offs, indicating dyslipidaemia and inflammation. Moreover, total cholesterol levels in obese individuals carrying the G alleles of *ADRB2* rs1042713 were significantly higher than the diagnostic cut off (5.2mmol/L), indicating dyslipidaemia.

Table 5II.7: Differences between blood biochemical parameters and respective diagnostic cut offs in obese individuals carrying G allele of *ADRB2* rs1042713 and rs1042714

Blood biochemical parameters	Diagnostic cut off	rs1042713 (Dominant model)		rs1042714 (Dominant model)	
		Mean (±SE)	p value	Mean (±SE)	p value
Fasting glucose (mmol/L)	5.6 (Alberti, Zimmet, & Shaw, 2006)	5.3 ± 0.3	0.327	6.2 ± 1.0	0.574
Fasting insulin (uU/mL)	10.6 (Lee et al., 2006)	11.2 ± 1.3	0.330	14.9 ± 3.4	0.231
HOMA-IR	1.7 (Yamada, Moriyama, & Takahashi, 2012)	**2.8 ± 0.3**	**0.003***	3.6 ± 0.8	**0.021***
Total cholesterol (mmol/L)	5.2 (National Cholesterol Education Program, 2002)	**5.7 ± 0.1**	**0.001***	5.7 ± 0.3	0.108
Triglyceride (mmol/L)	1.7 (Alberti, Zimmet, & Shaw, 2006)	**1.4 ± 0.1**	**<0.001***	1.5 ± 0.2	**0.210**
HDL cholesterol (mmol/L)	1.0 (Alberti, Zimmet, & Shaw, 2006)	**1.5 ± 0.1**	**<0.001***	1.4 ± 0.1	**<0.001**
LDL cholesterol (mmol/L)	2.6 (National Cholesterol Education Program, 2002)	**3.6 ± 0.1**	**<0.001***	3.6 ± 0.2	**0.001**
hsCRP (mg/L)	3 (Pearson et al., 2003)	8.8 ± 1.4	**<0.001***	8.8 ± 2.3	**<0.001***

One sample t test was performed to assess the difference between blood biochemical parameters and respective diagnostic cut offs

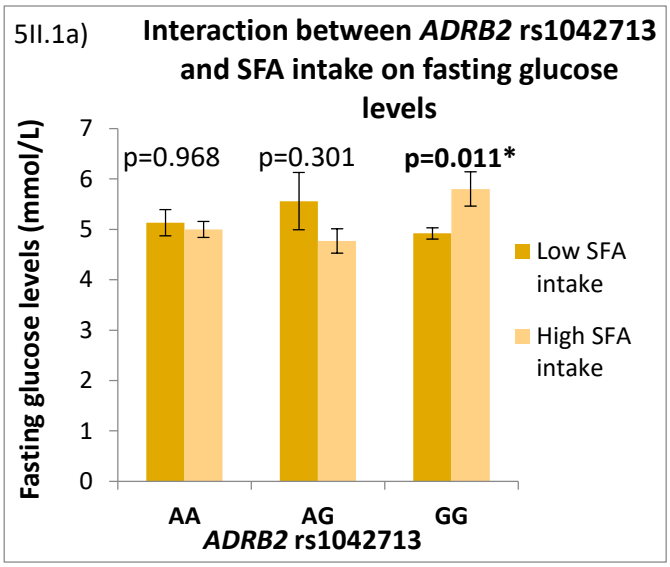
*p<0.05 was considered significant.

HOMA-IR, Homeostatic model assessment -insulin resistance; HDL, High-density lipoprotein; LDL, Low-density lipoprotein.

5II.4.6 Interaction between dietary fat intake and *ADRB2* rs1042713 on fasting glucose levels, insulin levels and HOMA-IR

Multivariate general linear model was performed to investigate the effect of *ADRB2* gene variants and dietary macronutrients on phenotypic variations. No significant interaction was found between rs1042713 and dietary nutrients on obesity-related phenotypes in dominant model, therefore, the results are reported in additive model. In our study, our results revealed that irrespective of obesity, the carriers of GG genotype of rs1042713 had significantly lower fasting glucose levels with low SFA intake (<7.3% of TE/day) (FBG: 4.92±0.1mmol/L vs 5.80±0.3mmol/L, p=0.011) (Figure 5II.1a), and high PUFA:SFA ratio (≥0.8/day) (FBG: 4.83±0.1 mmol/L vs 5.93±0.4 mmol/L, p=0.006) (Figure 5II.1b), even after adjusting for covariates age, gender, BMI, physical activity status, smoking status, alcohol consumption and total energy intake. No significant interaction was found between *ADRB2* rs1042714 and dietary intakes on obesity-related traits in Malaysian adults.

Moreover, the carriers of GG genotype of rs1042713 with high PUFA intake (≥6% of TE/day) had significantly lower HOMA-IR (1.5±0.3 vs 3.0±0.7, p=0.026) (Figure 5II.2a) and fasting insulin levels (6.8±1.6uU/mL vs 11.4±2.1uU/mL, p=0.036) (Figure 5II.2b) compared to low intake. No significant interaction was found between rs1042714 and dietary nutrients on obesity-related phenotypes. Therefore, the results are not reported.



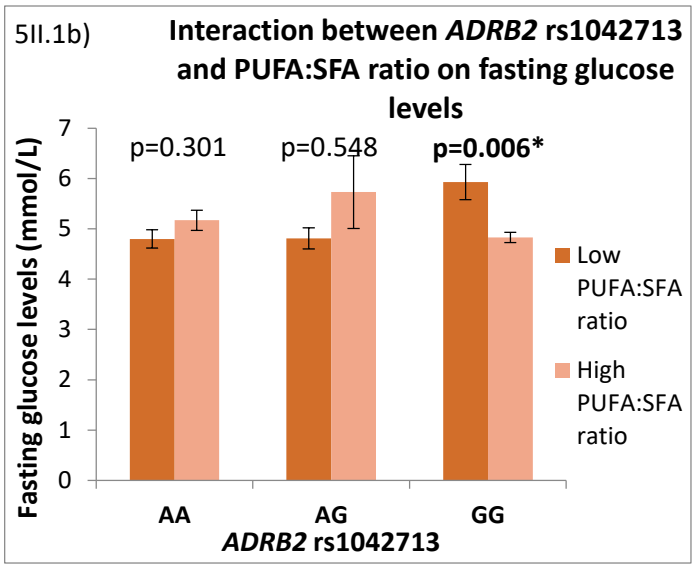


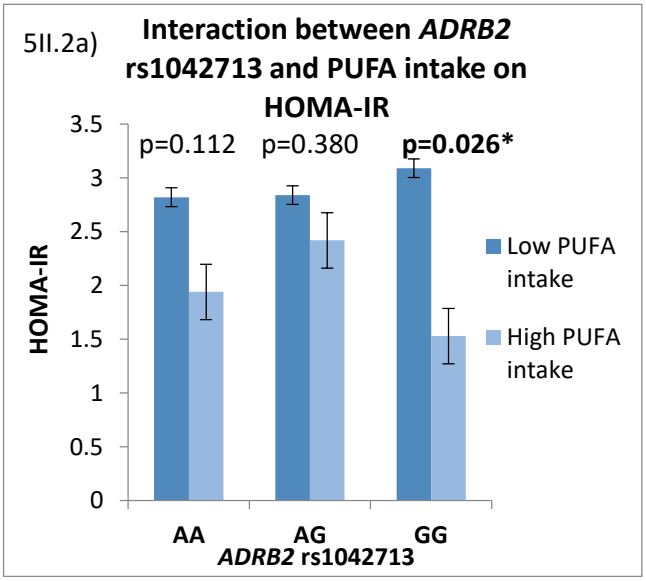
Figure 5II.1: The effect of the interaction between *ADRB2* rs1042713 and (5II.1a) SFA intake and (5II.1b) PUFA: SFA ratio on fasting glucose levels.

SFA and PUFA: SFA ratio were dichotomised into two groups by using the median value of 7.3% of TE/day and 0.8/day (for all participants), respectively, for analysis.

Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, BMI, physical activity status, smoking status, alcohol consumption and total energy intake.

*p<0.05 was considered statistically significant.

SFA, Saturated fatty acid; PUFA, Polyunsaturated fatty acid.



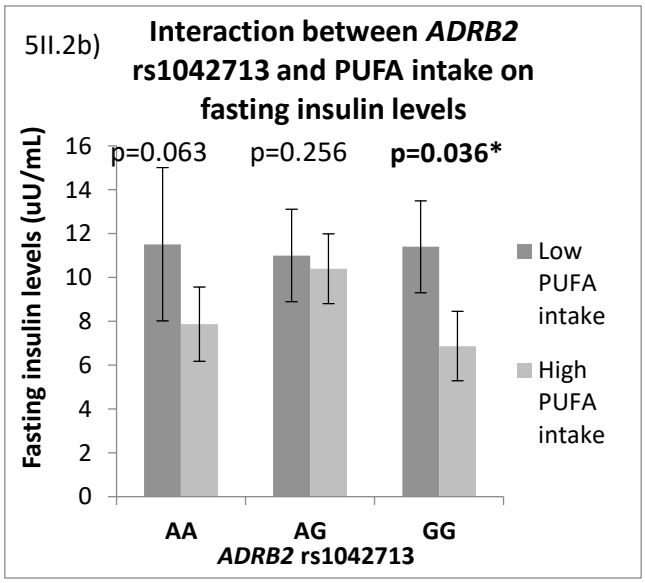


Figure 5II.2: The effect of the interaction between *ADRB2* rs1042713 and PUFA intake on (5II.2a) HOMA-IR and (5II.2b) fasting insulin levels. PUFA intake was dichotomised into two groups by using the median value of 6% of TE/day (for all participants) for analysis.

Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, BMI, physical activity status, smoking status, alcohol consumption and total energy intake.

*p<0.05 was considered statistically significant.

PUFA, Polyunsaturated fatty acid; HOMA-IR, Homeostatic model assessment -insulin resistance.

5II.5 Discussion

Single nucleotide polymorphism (SNP) in *ADRB2* gene such as rs1042713 and rs1042714 cause alterations in the structural conformation of the receptor which eventually affect the function of β-adrenergic receptors (Green et al., 1994). This may influence the binding of catecholamines to the beta-2 adrenoceptors and hence alter lipolysis. A meta-analysis involving 18 published articles revealed that there was an association between rs1042714 and susceptibility to obesity, but no association was found in rs1042713 (Zhang, Wu, & Yu, 2014). Contrary to the latter, studies on the Saudi population reported significant association between *ADRB2* rs1042713 and rs1042714 polymorphisms and the development of obesity, as also with insulin resistance and dyslipidemia (Daghestani et al., 2010; Daghestani et al., 2012). However, findings from Asian populations (Japanese and Korean) reported negative association between obesity and *ADRB2* gene polymorphisms (Hayakawa et al., 2000; Kim et al., 2002). Moreover, these studies did not investigate interaction of gene variants, phenotypes and dietary nutrients. To date, there is no data reported on *ADRB2* rs1042713 and rs1042714 polymorphisms in the Malaysian population. To the best of our knowledge, this is the first study that investigates the effect of *ADRB2* rs1042713 and rs1042714 on obesity and obesity related metabolic parameters and its interaction with dietary nutrients in Malaysian adults.

5II.5.1 Association between *ADRB2* gene variants and ethnicity

In our study, we found no significant difference in the genotype distribution of *ADRB2* rs1042713 between ethnic groups. However, we found that Malaysian Chinese had significantly lower frequency of the risk allele (G) of rs1042714 compared to Malaysian Malays and Malaysian Indians combined (11.9% vs 28.7%). This finding indicated that difference in gene variants in *ADRB2* does exist in various ethnic groups.

5II.5.2 Association between *ADRB2* gene variants and obesity and insulin resistance

In our study, we found no association between *ADRB2* gene variants (rs1042713 and rs1042714) and odds of obesity (obesity was defined as BMI≥27.5 kg/m^2). However, *ADRB2* gene variants (rs1042713 and rs1042714) were associated with insulin resistance (using a cut off of HOMA-IR≥1.7). We found that the carriers of G allele of rs1042713 had increased odds of insulin resistance compared to the non-carriers (AA), in the dominant model, even after adjusting for potential confounders. These findings suggest that variations in *ADRB2* gene may interfere with glucose homeostasis and cause insulin resistance. Prior, Goldberg, & Ryan, 2011

reported that *ADRB2* rs1042713–rs1042714 haplotype was associated with glucose intolerance and insulin resistance in obese post-menopausal women. A possible explanation for this observation could be, the alteration in the structural conformation of the receptor, which may have enhanced sympathetic stimulation leading to increased lipolysis (Masuo & Lambert, 2011). This overstimulation of *ADRB2* is found to be associated with the pathogenesis of insulin resistance as it inhibits the insulin-induced translocation of GLUT4 and reduces glucose uptake via the cAMP-dependent protein kinase A-dependent pathways (Mangmool et al., 2016).

We found that HOMA-IR in the obese individuals carrying G allele of *ADRB2* rs1042713 was above the diagnostic cut off (1.7). It is now an established fact that higher levels of non-esterified fatty acids (NEFA) in the blood can induce preferential use of free fatty acids over glucose to generate ATP even in the presence of insulin in muscle and adipose tissue resulting in hyperglycaemia (Koves et al., 2008; Zhang et al., 2010). Stimulation by noradrenaline of adipose tissue *ADRB2* increases the release of NEFA. In addition, free fatty acids (FFAs) can stimulate hepatic gluconeogenesis and alter pancreatic insulin release and subsequent metabolism in individuals with impaired glucose metabolism (Boden & Shulman, 2002).

5II.5.3 Association between *ADRB2* rs1042713 and blood lipid levels

In the present study, our results revealed that HDL cholesterol levels in obese individuals carrying the G allele of rs1042713 and rs1042714were positively, significantly higher than the diagnostic cut off (1mmol/L) and triglyceride levels were significantly lower than the diagnostic cut off (1.7mmol). However, we found that obese individuals carrying the G allele of rs1042713 had significantly higher total cholesterol and LDL cholesterol levels compared to the non-carriers (AA). Total cholesterol (5.7±0.1mmol/L) and LDL cholesterol levels (3.6±0.1mmol/L) in obese individuals carrying the G allele were above the diagnostic cut offs, indicating dyslipidaemia (5.2mmol/L and 2.6mmol/L, respectively). LDL cholesterol levels in individuals carrying the G alleles of *ADRB2* rs0142714 were also significantly higher than the diagnostic cut offs, indicating dyslipidaemia. However, these differences in biochemical parameters were not observed in non-obese individuals. In our participants, excess FFAs in circulation in obese individuals carrying the gene variants of *ADRB2* may have driven dyslipidaemia. Increased lipolysis due to the polymorphisms of *ADRB2* gene may have caused increased levels of NEFA induced hepatic production of VLDL and hence higher LDL levels in our participants (Jung & Choi, 2014).

5II.5.4 Interaction between dietary fats and *ADRB2* rs1042713 on glycaemic indices

We report that the level of fasting glucose was modulated by the types of dietary fatty acids such as SFA and PUFA in the carriers of GG genotype of *ADRB2* rs1042713. The carriers of GG genotype of rs1042713 had significantly lower fasting glucose levels with intake of relatively higher PUFA: SFA ratio (≥0.8/day) (Fig 1b) and lower SFA intake (<7.3% of TE/day) (Fig 1a). Moreover, the carriers of GG genotype of rs1042713 had significantly lower fasting insulin levels and HOMA-IR when consuming higher PUFA intake (≥6% of TE/day).

These findings suggest that carriers of GG genotype of *ADRB2* rs1042713 consuming higher percentage of PUFA demonstrated better homeostatic control of fasting blood glucose and insulin sensitivity. A meta-analysis of 102 randomised controlled feeding trials with 4,200 subjects has reported that, PUFA had the most beneficial effects in improving glycaemia, insulin resistance, and insulin secretion in comparison to dietary carbohydrate, SFA and MUFA (Imamura et al., 2016). The anti-inflammatory properties of PUFA increase the production of adiponectin via PPARα activation and alleviate adipose tissue inflammation via GPR120 and resolvins/protectins, which favour insulin sensitivity. It also suppresses oxidative stress, pancreatic lipotoxicity, reduces toxicity of tissue free fatty acids and increases membrane fluidity (Coelho et al., 2017). This body of evidence indicates that the composition of dietary fatty acid intake plays an important role in affecting glucose metabolism and insulin sensitivity.

As per Malaysian recommendations, 10% of total energy should come from SFA and 3 to 8% of total energy should come from PUFA (National Coordinating Committee on Food and Nutrition, 2010). On an average, dietary intake of our participants with respect to SFA and PUFA were within these ranges. However, individuals with G allele of rs1042713 may need to consume higher quantity of PUFA to combat diet-related non-communicable diseases. Replacing isocaloric quantity of foods rich in SFA with PUFA may improve homeostatic control of blood glucose and enhance insulin sensitivity (HOMA-IR) in such individuals.

5II.6 Summary

The aim of this chapter is to evaluate i) the differences in genotype distributions of *ADRB2* gene variants (rs1042713 and rs1042714) between the adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians), ii) the association of *ADRB2* rs1042713 and rs1042714 with obesity and insulin resistance; iii) the association between *ADRB2* rs1042713 and rs1042714 and obesity-related phenotypes in obese and non-obese

individuals; and iv) the interaction between *ADRB2* rs1042713 and rs1042714 and dietary nutrients on obesity-related phenotypes, in the Malaysian adults. *ADRB2* genotyping, dietary, physical activity, anthropometric and biochemical data were collected from 79 obese and 99 non-obese individuals. Our results revealed that Malaysian Chinese had significantly lower frequency of the risk allele (G) of rs1042714 compared to the Malaysian Malays and Indians combined. No significant difference was found in the genotype distribution of *ADRB2* rs1042713 between the ethnic groups.

Logistic regression revealed no association between *ADRB2* gene variants and obesity (Bonferroni corrected $p>0.025$). However, we found that the carriers of GG genotype of rs1042713 had increased odds of insulin resistance, compared to AA genotype in the additive model, after adjusting for potential covariates. In addition, we found that obese individuals carrying the G allele of rs1042713 had significantly higher total cholesterol and LDL cholesterol levels compared to the non-carriers (A), whereas obese individuals carrying the G allele of rs1042714 had significantly higher WHR compared to the non-carriers (C). However, such differences were not observed in non-obese individuals.

Gene-diet interaction analysis revealed that individuals carrying the risk allele of *ADRB2* rs1042713 (G) had significantly lower fasting glucose levels with intake of relatively higher PUFA: SFA ratio (≥0.8/day) and lower SFA intake (<7.3% of TE/day). Moreover, the latter had significantly lower fasting insulin levels and HOMA-IR when consuming higher PUFA intake (≥6% of TE/day). Food sources with high PUFA content may improve homeostatic control of blood glucose and enhance insulin sensitivity in individuals with homozygous mutant variants of *ADRB2* rs1042713.

5.3 Limitations

There are few limitations exist in the current study. The sample size of the current study is moderate, and hence limiting the statistical power to detect the true gene-diet interactions on obesity-related phenotypes. Our study sample is not representative of the Malaysian population, and hence the findings of current experiments may not be generalisable to the Malaysian population as a whole. Participants of this study were predominantly middle-aged females with sedentary lifestyle living in Beranang, Jalan Broga and Bandar Kajang. Therefore, the results are reported as the associations found in the participants of the current study, and may not be generalisable to the Malaysian population at large. Large scale future studies are required to replicate the findings of this study in the Malaysian population from other geographical

locations. We have carefully controlled for confounding lifestyle factors and baseline values in the analyses. However, unmeasured or unknown confounders such as behaviour, socioeconomic and sociodemographic indicators may also exist. Therefore, the associations and interactions observed in the current study could arise as a result of the unmeasured confounding factors. This study is preliminary, and more studies with larger sample size are warranted.

Chapter 6

An intervention programme to manage body weight and reduce metabolic risk factors in overweight and obese Malaysian adults: A 6-month individualised Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention

6.1 Introduction

The prevalence of obesity is increasing worldwide (Upadhyay et al., 2018). Obesity is a major public health problem and has clinical implications in increasing the prevalence of comorbid conditions such as hypertension, hyperglycaemia, dyslipidaemia, insulin resistance and inflammation (Finer, 2015). National Health and Morbidity Survey (NHMS) 2015 reported that 5.6 million Malaysian adults aged 18 and above were overweight and 3.3 million were obese (Institute for Public Health, Malaysia, 2015). According to Tan et al., 2011, the treatment of obesity and its primary comorbidities are estimated to cost the Malaysian health care system approximately US$162 million per year. Therefore, the recent challenge is to develop an effective and sustainable weight loss intervention programme to combat high prevalence of obesity in the Malaysian population.

Previous evidence has illustrated that a moderate weight loss of 5-10% in body weight (Brown et al., 2016; Wing et al., 2011), particularly in visceral adipose tissue (Goodpaster et al., 1999; Kwon, Kim, & Kim, 2017; Pascale et al., 1992) had beneficial effects in insulin sensitivity, glycaemic control and cardiovascular disease risk. However, over the years, strategies to combat obesity remained ineffective, particularly in long term weight loss programmes (Bombak, 2014). Past studies have investigated interventions based on various dietary patterns by varying the dietary macronutrient composition (Goss et al., 2013; Sacks et al., 2009). Other strategies that have been used to facilitate weight management include reduction in energy density, portion control through meal replacement, and improved diet quality (Davis et al., 2010; Fung et al., 2015; Heymsfield et al., 2003; Rolls et al., 2005). A key strategy for weight management that can be applied across the dietary patterns is to reduce energy density of meals. Clinical trials show that reducing energy density is effective for weight loss and weight loss maintenance (Ello-Martin et al., 2007; Ledikwe et al., 2007).

The most popular among the weight loss diets emphasise reduction of carbohydrate intake, and hence encouraging high protein intake, as high fat diets are generally avoided due to higher energy density. A high protein diet with 25-30% of total energy from protein showed greater reduction in weight and waist circumference and greater improvement in glycaemic control and cardio-metabolic profile in Asian Indians, Iranian and Australian populations (Azadbakht et al., 2013; Farnsworth et al., 2003; Gulati et al., 2017). High protein diet is potentially effective in promoting satiety, reducing energy intake, inducing weight loss and maintaining lean muscle tissue (Astrup et al., 2015; Clifton, 2012). A meta-analysis of randomised

controlled trials reported that by comparing with a standard protein, low-fat diet, low-carbohydrate, high-protein diets provided greater reduction in body weight, blood triglyceride levels and preservation of fat-free mass (Wycherley et al., 2012). Elevated protein diet was associated with increased satiety, thermogenesis and glycogenesis which may lead to greater weight loss and improvement in insulin sensitivity (Westerterp-Plantenga et al., 2006).

Atkin diet, a high-protein, very low-carbohydrate, very high-fat diet promoted by Atkins (60% of energy from fat, 35% of energy from protein and 5% of energy from carbohydrate) is perhaps the most popular high-protein diet in America for weight loss. However, healthcare professionals do not recommend long-term adherence to an Atkins-like, high-protein diet due to the high fat content of the diet (~60% energy), particularly the saturated fat (~25% of energy from SFA), which may eventually lead to elevations in total cholesterol and LDL cholesterol levels (Astrup, Meinert Larsen, & Harper, 2004). Low carbohydrate-high protein diets may have short-term beneficial effects on weight control, however concerns have also been expressed on long-term effects, notably with respect to cardiovascular outcomes. Long-term high protein or high meat intake in humans may lead to hyperuricemia (leading to joint pain and gout), hypercalcuria (leading to kidney stones, hypocalcemia, and osteoporosis), renal malfunction, increased cancer risk, liver malfunction, and increased risk of coronary artery disease (Delimaris, 2013). Extremely low-carbohydrate diets may also lead to ketosis, nausea, dizziness, constipation, headache, fatigue, and smelly breath.

Diets high in protein (30% of energy from protein) but low in fat (<30% of energy from fat) and moderate in carbohydrate (40% of energy from carbohydrate) are as effective at promoting weight loss as Atkins diets, and reduce total cholesterol and LDL cholesterol as well as triglycerides (Baba et al., 1999; Johnston, Tjonn, & Swan, 2004; Parker et al., 2002). However, these diets have not received the same attention as the Atkins diet. Johnston et al., 2004 reported that energy-restricted, low-fat diets of varying protein content (15 or 30% of energy from protein) were equally effective at promoting steady weight loss over 6 weeks of the intervention period, but diet satisfaction was greater in those consuming the high-protein, low-fat diet. The authors reported that the biomarkers of health and disease, including blood lipid profile, insulin sensitivity, uricosuria, hypercalciuria, and glomerular filtration rates, were not adversely affected by the high-protein, low-fat diet. Farnsworth et al., 2003 also reported that high-protein, moderate-carbohydrate diets which replace carbohydrate with protein from meat, poultry, and dairy foods (HP diet: 27% of energy from protein, 44% of energy from carbohydrate, and 29% of energy from fat) has beneficial metabolic effects and no adverse effects on markers of bone

turnover or calcium excretion compared to a standard-protein diet (SP diet: 16% of energy from protein, 57% of energy from carbohydrate, and 27% of energy from fat). Therefore, high-protein diets that are low in fat and moderate in carbohydrate may represent an appealing diet plan for healthful weight loss and deserve further investigation.

Dietary fibre is known to be a key component in healthy eating. A review of studies reported that higher dietary fibre intake was associated with increased satiety and decreased hunger (Howarth, Saltzman, & Roberts, 2001). Previous studies have reported a favourable metabolic profile with high fibre diet (Jung & Choi, 2017; Kabir et al., 2002). Large prospective cohort studies consistently reported significant associations between high dietary fibre intake (>25 g/d in women and >38 g/d in men) and reduced risk of developing type 2 diabetes (T2D) (20-30% of reduced risk) (Weickert & Pfeiffer, 2018). The proposed mechanisms for this relationship remain unclear, but the type of fibre may play a role. In the gut, certain soluble fibres which form a viscous gel matrix is believed to slow gastric emptying and lead to a greater feeling of fullness (Slavin & Green, 2007). Soluble fibres slow absorption of glucose further in the small intestine and lead to lower postprandial glycaemic and insulinemic responses (Yu et al., 2014). Therefore, high soluble fibres may contribute to the improvement in glycaemic control and insulin sensitivity. Moreover, in both the cross-sectional and longitudinal analyses, fibre intake was found to be protective against high inflammation levels (Griffith, et al., 2006). It was suggested that dietary fibre decreases lipid oxidation, which in turn associated with decrease in inflammation (King, 2005). Therefore, incorporating food sources that are high in fibre such as whole grains, vegetables, fruits, peas and nuts into our diets may help to improve metabolic health.

General recommendations based on dietary guidelines, e.g. recommended nutrient intake (RNI) or recommended dietary allowance (RDA), are often ineffective and end in unsuccessful weight management programmes as there is no one-size-fit-all diet that is more effective than others. A recent review by (Koliaki et al., 2018) reported that a dietary regime to manage obesity should be safe, efficacious, nutritionally adequate, culturally sensitive, cost effective, adoptable and should ensure long term maintenance of weight loss. Setting a realistic weight loss goal and following a balanced dietary plan tailored according to an individual's need and food preference, are key to facilitating sustainable weight loss and reduction in metabolic risk in obese individuals. Ethnic diversity in Malaysia is unique (Bumiputera – 69.1%, Chinese – 23%, Indians – 6.9% and others – 1%) (Department of Statistics, Malaysia, 2018). According to a current study, Malaysians of different ethnic background continue to practice traditional

meal patterns, habitually (Abdullah et al., 2016). Individualised dietary intervention according to individual's food preference and cultural habit could be more effective in establishing the individual's adherence to the intervention diet and promote successful and sustainable weight management.

6.2 Objectives

The aim of this chapter is to assess the effect of an individualised Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet, an energy-restricted, high-protein diet that is low in fat, moderate in carbohydrate and high in vitamin E (calorie restriction of 300-500kcal/day, 30% of energy from protein, <30% of energy from fat, 40% energy from carbohydrate, ≥15mg/d of vitamin E and ≥25g/d of fibre) on obesity-related anthropometric and blood biochemical parameters (e.g. fasting glucose, fasting insulin, HOMA-IR, hsCRP and lipid levels) in overweight and obese Malaysian adults compared to the control diet group who received generalised dietary advice on weight loss according to Malaysian Dietary Guidelines (MDG) 2010 (<1500kcal/day with a macronutrient composition of approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate). In the intervention diet group, individual diet charts were prescribed based on their personal preferences, likes/dislikes, traditional customs and meal times. They were responsible for obtaining their own food sources and preparing their own meals, following the formulated diet chart as guidance.

6.3 Methodology

6.3.1 Study design

This study was designed as a 6 month, parallel-arm, randomised controlled trial to assess the effect of Hipcref diet on anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults compared to the control diet group who received generalised dietary advice on weight loss based on MDG 2010.

6.3.2 Participant selection

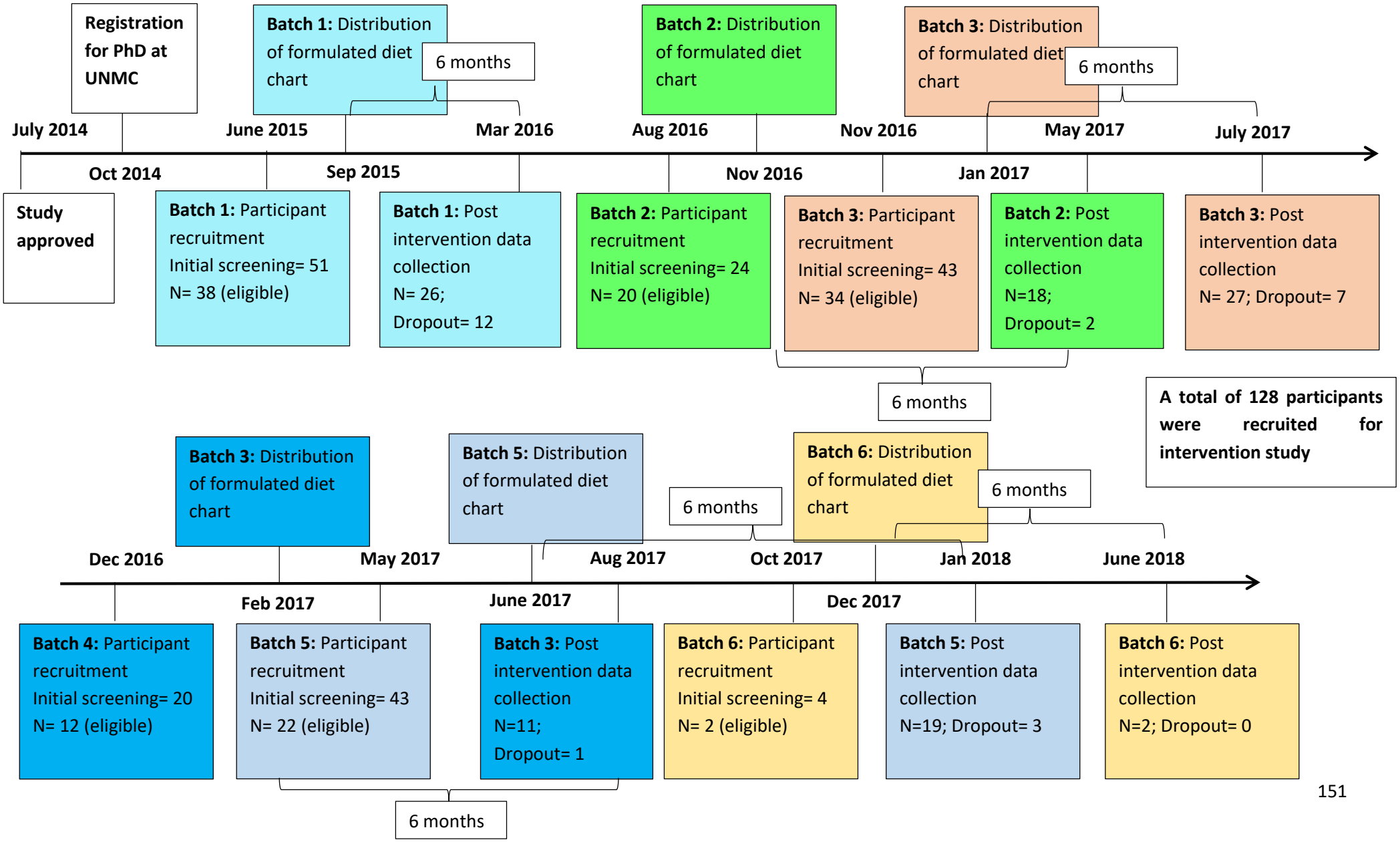
Detailed information on the participant selection has been previously described in Chapter 3. Briefly, a total of 128 apparently healthy overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) with a BMI≥23kg/m^2 aged 18 years and above, who met the specific inclusion and exclusion criteria, were recruited to participate in the current intervention

study. Six waves of recruitment (cohorts) had staggered start dates between June 2015 to June 2018 and the timeline for participant recruitment, distribution of diet chart and post-intervention assessment are illustrated in Figure 6.1.

6.3.3 Randominsation of the study participants

Covariate adaptive randomisation was performed to minimise the imbalance of sample size between the two treatments (Hipcref diet group and control diet group) across a set of covariates without compromising randomness (Zagoraiou, 2017; Zhao, 2014). There is evidence from previous literature that in response to nutritional interventions, significant differences existed between gender and ethnic groups (Haughton et al., 2018; Leblanc et al., 2014; Lovejoy et al., 2001). Therefore, in the current study, eligible participants were stratified based on two covariate stratum a) by gender (female and male); b) by ethnicity (Malaysian Chinese, Malays and Indians), thus a total of 6 (2x3) strata was constructed. First participant was truly randomly allocated to the appropriate strata of one of the treatments; then each subsequent participant was assigned sequentially to the two treatments according to the appropriate strata. Therefore, 128 participants were randomly assigned to one of the two treatments: Hipcref diet group (n=65) or control diet group (n=63). Each participant was briefed about the weight reduction programme (Appendices 4 & 5) and written informed consent was requested and obtained from each participant (Appendices 6 & 7).

Figure 6.1: Timeline for participant recruitment, distribution of diet chart and post-intervention assessment in the RCT/dietary intervention study (six batches of recruitment)



6.3.4 Hipcref diet (intervention diet)

6.3.4.1 Mode of delivery

At baseline, diet intake was assessed as reported earlier. Attention was paid to details of personal preferences (vegetarian, non-vegetarian), likes/dislikes, food customs and meal times and documented. Based on the baseline energy intake, a reduction of 300-500 kcal/day of total energy intake (depending on the individual energy intake and basal metabolic rate) was computed. The composition of the macronutrients of the revised diet provided 30% of energy from protein, 30% of energy from fat and 40% energy from carbohydrate. An example menu for a typical day was planned and charted. The food sources listed in the formulated diet chart for each participant were tailored according to individual's likes, dislikes, personal preferences and cultural habits. All participants were responsible for obtaining their own food sources and preparing their own meals, following the formulated diet chart as guidance. In general, diet charts were revised and new recipes were suggested once a month to introduce variety and to minimise monotony. Ideal BMI was calculated for each participant and they were encouraged to reach that goal.

a) Composition of the macronutrients

Protein intake

30% calories of protein energy of the revised energy intake were converted to protein intake in grams per day. This quantity was then translated to protein rich food sources. Chicken, pork, egg and fish such as salmon, mackerel, sardines, tuna, anchovy, but not red meat (to reduced saturated fat intake) were charted for the non-vegetarian participants. Plant-based foods such as soya bean and products, tofu, chickpea and yellow/red lentils and dairy products such as low fat/skimmed milk, low fat-plain yogurt and soft cream cheese were charted for both the vegetarian and non-vegetarian participants.

For instance, a) in case of non-vegetarian preference, 120g/day of steamed chicken breast (approximately 29g of protein), 120g/day of salmon (approximately 27g of protein), 120g/day of pork (approximately 26g of protein) and 120g/day of white pomfret (approximately of 22g of potein) were advised. Individuals were requested to consume at least 2 servings of the latter per day. In case of b) vegetarian preference, 400g/day of steamed tofu (approximately 32g of protein), 100g/day of lentils, (approximately 22g of protein), 200ml/day of low fat yogurt (approximately 10g of protein), 200ml/day of skimmed milk (approximately 7g of protein) and

100g/day of green pea (approximately 7g of protein) were advised. Individuals were requested to consume at least 3 servings of the latter per day. Dietary counselling was provided to Hipcref participants on examples of prescribed portion sizes with the aid of photographs. The total protein in grams on the formulated diet charts ranged from 73.1-98.5g (995-1273 kcal/day; 28-31% of energy from protein).

Fat intake

A similar approach was taken with respect to fat intake. 30% calories of fat energy of the revised energy intake were converted to fat intake in grams per day. The fats and oils used to cook meals were recorded in the structured questionnaire for all individuals. The number of family members the food was cooked for was recorded as well. The per day use of cooking oil/butter/margarine was quantified for the participant in the formulated diet chart. As per the formulated diet chart, the quantity of oil/butter/margarine per day formulated to the individual was multiplied by the number of family member times 30 to arrive at a total quantity of cooking medium per month to be used for the whole family. The participants were advised to adhere to the above quantity of fats and oils, and not go beyond the formulated quantity. The total fat in grams on the formulated diet charts ranged from 27.6-43.1g (995-1273 kcal/day; 25-30.6% of energy from fat).

Carbohydrate intake

Same approach was taken with respect to formulation of energy from carbohydrate. 40% calories of carbohydrate energy of the revised energy intake were converted to carbohydrate intake in grams per day. This quantity was then translated to high fibre, complex carbohydrate-rich foods with low glycemic index and entered in the individual diet charts and menu plans. The total carbohydrate in grams on the formulated diet charts ranged from 100-143.1g (995-1273 kcal/day; 38.8-45.7% of energy from carbohydrate). Participants were requested to consume whole grains such as whole meal bread, whole oats, and brown rice rather than refined grains such as white rice, white bread and white flour, to increase fibre intake and reduce postprandial glucose response.

b) Formulation of fibre ≥25g/day

Previous studies have reported on a favourable metabolic profile with high fibre diet (Jung & Choi, 2017; Kabir et al., 2002). Therefore, we introduced fibre intake of ≥25g/day in the formulated diet chart as per Malaysian Dietary Guidelines (MDG) 2010 (National Coordinating Committee on Food and Nutrition, 2010). We included leafy green vegetables (broccoli, spinach and carrot etc.), legumes (chickpeas, peas and lentils etc), fruits (apple, pear and guava etc.), muesli, whole grain cereals, and oat products to increase soluble and non-soluble dietary fibre to the Hipcref diet. 1 serving of vegetables (3 tablespoons) such as broccoli and kale contain 5.3g and 3.2g of fibre, respectively. Therefore, participants in the intervention group were requested to consume at least 3 servings of vegetables per day. Moreover, other food groups may contribute to the fibre consumption, for example, 1 serving of lentil (50g; ½ cup) such as chickpea contains 7g of fibre and 1 serving of brown rice (120g) and contains 4g of fibre.

c) Formulation of vitamin E ≥15mg/day

Calorie-restricted, high-protein diet with normal fibre intake was designed prior to the intervention study. Participants from the intervention group in batches 1 & 2 (intervention diet group n=33; control diet group n=25) received personalised diet charts, which comprised of a reduction of 300-500 kcal/day (calorie-restricted), 30% of energy from protein (high-protein), 40% of energy from carbohydrate, 30% of energy from fat and fibre intake ⩾25g/day. Vitamin E intake⩾15mg/day was introduced to the intervention diet group starting from batches 3-6 (intervention diet group n=32; control diet group n=38), after significant interaction was found between *FTO* gene variants and vitamin E on hsCRP levels (Chapter 5) (pg 118). Based on the experiments of gene-diet interactions at baseline (n=136; using baseline data from batches 1-3), individuals carrying the risk allele of *FTO* rs9930506 had significantly lower hsCRP levels with higher vitmin E intake. Therefore, we decided to introduce vitamin E intake ≥15mg/day into the formulated diet charts, according to the recommendation of Recommended Dietary Allowance (RDA) US, to improve their metabolic health. Food sources which are high in vitamin E such as cooking oils (including sunflower oil, palm oil and canola oil), nuts (including almonds, peanuts, hazelnuts and walnuts), seeds (including sunflower seeds) and green leafy vegetables (including broccoli and spinach) were introduced into the Hipcref diet chart to increase participant's vitamin E intake. 1 serving of cooking oils (10ml; 1 tablespoon) such as sunflower oil and palm oil contain 5mg and 3mg of vitamin E, respectively. 1 serving

of seeds and nuts (30g; one handful) such as sunflower seed, hazelnut and almond contain 11.3mg, 7.5mg and 7.2mg of vitamin E, respectively.

6.3.4.2 Regular monitoring throughout the intervention period

Regular monitoring and dietary counselling was provided to both intervention diet group and control diet group. The monthly meetings took place at UNM or at a designated venue. In each of the dietary consultation session, supporting materials for nutritional education which covered a variety of topics related to weight loss, behavioural change and healthy food choices (e.g. low glycaemic index foods, refined vs complex carbohydrate, good fats vs bad fats, reduction in sugar and salt consumption) were distributed to each participant. Participants were advised to choose healthier meal preparation techniques, such as steaming, boiling, roasting, grilling and baking. Healthier recipe options were listed in the formulated diet chart and were given to each of the individual in the Hipcref diet group. Consumption of raw salad was advised. When dining out, individuals were advised to avoid fried foods (e.g. fried noodle, fried rice and deep-fried chicken), sambal (chili and palm sugar paste in oil), oily gravy and oily soup (e.g. curry and asam laksa) to reduce total calorie intake and fat intake. They were advised to avoid eating out and prepare their own meals at home to reduce their total energy intake and fat consumption. They were also encouraged to limit the intake of the processed foods and sweetened beverages.

To increase participant's motivation and adherence to the intervention, all participants were registered on our WhatsApp group, Facebook group (Lose to gain; Appendix 20) or Instagram page dedicated for this project, to convey necessary messages (e.g. nutritional-related article and motivational quote) to them from the research team at the shortest time possible. For those participants who requested to keep themselves anonymous and refused to join any of the aforementioned social media groups, our research team will private message the same content to them. For those participants who did not own a smartphone or who were inactive in social media, our research team will contact with them through message or call them once a week to keep them motivated so that we do not lost contact with the participants. Furthermore, to keep them motivated, Dr Soma Mitra had visited their working places (e.g. schools) to deliver talks on healthy diet and nutrition.

6.3.5 Control diet

6.3.5.1 Mode of delivery

Participants allocated to the control diet group (n=63) did not receive an individualised formulated diet chart. Generalised dietary advice on weight loss was provided to them based on the Malaysian Dietary Guidelines (MDG) 2010 (<1500kcal/day with a macronutrient composition of approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate) (National Coordinating Committee on Food and Nutrition, 2010). Control diet participants were advised to reduce their total energy intake and consume no more than 1500kcal of energy per day, which is the energy intake recommended by MDG 2010 for individuals with sedentary activity. This quantity of total energy intake per day was translated to food-based dietary counseling as follows: 4 servings of cereals and grains (30g of carbohydrate per serving), 2 servings of fruits, 3 servings of vegetables, 1 serving of fish (14g of protein per serving), ½ serving of meat/poultry (14g of protein per serving), ½ serving of legumes (7g of protein per serving) and 1 serving of milk and dairy products (7g of protein per serving) per day. Participants were also advised to consume low fat food, less sugar and salt in general. All participants were responsible for obtaining their own food sources and preparing their own meals, following the MDG 2010 as guidance.

6.3.6 No change in physical activity status

Majority of the study participants were categorised as physically inactive at baseline (90.8-98.4%; Table 6.2). All participants were asked to maintain their usual levels of physical activity and to refrain from drinking and smoking during the intervention period.

6.3.7 Measurement of anthropometric parameters

Measurements of anthropometric parameters were taken at baseline, at month 3 and at month 6 of the intervention period. Height of the individual (barefoot) was measured with a standard height rod. Body composition including weight, fat mass (kg), skeletal muscle mass (kg), fat free mass (kg) and percent body fat (%) were measured using a body composition analyzer DSM-BIA (InBody 230, Seoul, Korea). Body mass index (BMI) was arrived at by using the formula, weight in kilograms divided by the square of height in meters (kg/m^2). Overweight and obesity was defined as BMI 23kg/m^2-27.4kg/m^2, and BMI≥27.5kg/m^2, respectively (WHO Expert Consultation, 2004). Waist circumference (WC) was measured at the midpoint between lower margin of palpable rib and the top of iliac crest. Hip circumference was measured at the

widest portion of the buttocks (WHO Expert Consultation, 2008). Waist–hip ratio (WHR) was calculated using the ratio of the circumference of the waist to the hip. Systolic and diastolic blood pressure was measured using a digital blood pressure monitor, following the recommendation from American Heart Association (AHA) (Pickering et al., 2005).

6.3.8 Blood collection and biochemical analysis

Blood biochemical markers were only collected and assessed at baseline and at month 6 of the intervention. Fasting blood samples were collected from each participant early in the morning between 7am to 10am following an overnight fast, at baseline and at month 6 of the intervention period. Fasting venous blood was drawn by an experienced phlebotomist from the antecubital vein into vacutainer tubes containing fluoride oxalate for plasma glucose analysis and vacutainer tube with clot activator and gel for serum separation (Becton Dickinson, Oxford, United Kingdom) for serum lipids (including total cholesterol, triglyceride and HDL-cholesterol), serum insulin and hsCRP analysis. All the aforementioned biochemical analysis was assessed using Abbott Architect CI8200 Automatic System according to manufacturer's instructions. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as the product of fasting plasma glucose (mmol/L) and fasting serum insulin (uU/ml) divided by 22.5 according to homeostatic model assessment (HOMA) (Wallace et al., 2004). Low-density lipoprotein (LDL) cholesterol was calculated from values of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride using the Friedewald formula: LDL cholesterol = Total cholesterol – [(Triglyceride/5) + HDL cholesterol] (Friedewald et al., 1972).

6.3.9 Assessment of dietary intake

Dietary data were collected and assessed at baseline and at month 6 of the intervention period only. Detailed information on the assessment of dietary intake has been described in an earlier chapter (Chapter 3). The intakes of macronutrients including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and trans fatty acid (TFA) were expressed as a percentage of total energy (TE) consumed. The intakes of micronutrients were energy adjusted to unit/total energy intake(kcal)x1000kcal.

6.3.10 Assessment of physical activity level

Physical activity data were collected through an interviewer administered structured questionnaire at baseline and at month 6 of the intervention period. Detailed information on the assessment of physical activity level has been described in an earlier chapter (Chapter 3).

According to the Ministry of Health Malaysia, individuals who accumulate at least 150 minutes/week of moderate intensity physical activity or 60 minutes/week of vigorous intensity physical activity are considered as physically active, else they are considered as physically inactive (National Coordinating Committee on Food and Nutrition, 2010). Accordingly, participants of the current study were then categorised into two groups; physically active and physically inactive for statistical analysis.

6.3.11 Measure of dietary adherence

Dietary adherence was assessed as the difference between participant's respective macronutrient goal as per the assigned diet and their reported dietary intake at the end of the 6 months of the intervention period. For participants assigned to the intervention diet group, dietary adherence was calculated as the difference between the reported and recommended distribution of percent energy from protein:fat:carbohydrate, which was 30:30:40, by using the 'Mahalanobis distance equation' (Alhassan et al., 2008). The equation used to calculate distance from the recommended goal was

$$\sqrt{\frac{[(XPRO-30)^2+(XFAT-30)^2+(XCHO-40)^2]}{3}}$$

where X_{PRO}, X_{FAT} and X_{CHO} were defined as participant's reported percent energy from protein, fat and carbohydrate, respectively. For participants assigned to the control diet group, dietary adherence was calculated as the difference between the reported and recommended distribution of percent energy from protein:fat:carbohydrate, which was 15:30:55.

$$\sqrt{\frac{[(XPRO-15)^2+(XFAT-30)^2+(XCHO-55)^2]}{3}}$$

The adherence score measured a degree of deviation from the recommended macronutrient goal. Thus, a lower score indicated better adherence and a higher score indicated poorer adherence. However, the measure of dietary adherence to the individual's recommended nutrient goal was only assessed at the end of the intervention. Participant's compliance and adherence to the formulated diet was not assessed periodically throughout the 6 months of the intervention period. This may have limited the validity of the research trial.

6.3.12 Power and sample size calculation

Sample size was computed using the formula $n = [2SD^2(Z_{1-\alpha/2} + Z_{1-\beta})^2]/d^2$, according to Charan and Biswas, 2013. The primary outcome measure was the change in body weight. Assuming 10% reduction in body weight was expected, an effect size (d) of 7.6 (76kg-68.4kg=7.6kg; with a SD of 11.2 (Ambak et al., 2018)) was calculated. To detect this difference with a significance level of 5% ($1-\alpha/2 = 1.96$) and power of 80% ($1- \beta = 0.84$), 34 participants were required in each of the study arm. Assuming attrition rate of 20%, a total of 41 participants were required in each of the study arm. A total of 82 participants should be enrolled in this study. However, we decided to recruit as many participants as possible to secure a good sample size in case attrition rate went up later.

6.3.13 Statistical analysis

Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). Data were expressed as mean ± standard error (SE) or number (percentage). Log transformation was performed to transform non-normally distributed data into normally distributed data. The current study was designed to test an approach applicable to primary care and hence the analysis was conducted on an intention-to-treat basis rather than on compliance to treatment (per protocol), meaning all the participants who have been randomised into the two diet groups were included for analysis regardless of non-completers. For the non-completers, the last observations/measurements on the anthropometric, blood biochemical and dietary parameters were carried forward as the post-intervention measurements for analysis. Chi square test was performed to assess the differences on categorical variables at baseline, whereas ANCOVA was performed to assess the differences on continous variables at baseline, after adjusting for covariates age, gender, physical activity status, alcohol consumption, smoking status and intervention group.

A two-way mixed ANOVA was performed to assess the effect of treatment group x time on anthropometric parameters at baseline, at month 3 and at month 6 between Hipcref diet group and control diet group on an 'intention-to-treat' (ITT) basis. Adjustment for covariates such as age, gender, physical activity status, smoking status and alcohol consumption was applied. With a significant F test, post hoc pairwise comparison was performed to assess the differences within the treatment groups at different time points. A two-way mixed ANOVA was performed to assess the changes in blood biochemical and dietary parameters at baseline and at month 6 between Hipcref diet group and control diet group. Adjustment for covariates such as age,

gender, physical activity status, smoking status and alcohol consumption was applied. A statistical probability level of $p<0.05$ (two-sided) was considered significant.

6.4 Results

6.4.1 General characteristics: Differences between the completers and dropouts in Hipcref diet group and control diet group

A total of 128 participants (84% women) completed the baseline assessment. They were randomly assigned to the Hipcref diet group (n=65; female=54 and male=11) or the control diet group (n=63; female=54 and male=9). Of the initial 128 participants, 7 participants dropped out from the Hipcref diet group and 18 participants dropped out from the control diet group due to job relocation (n=9), retirement (n=5), accident (n=2), volunteer withdrawal of consent (n=5) and 4 were lost to follow up (Figure 6.2). Therefore, the study was completed with 103 participants. Hipcref diet group = 58 participants (female=47 and male=11); control diet group = 45 participants (female=41 and male=4). There was no significant difference in age, height, weight, BMI, distribution in gender, ethnicity and physical activity status between the completers and dropouts, after adjusting for covariates age, gender, physical activity status, alcohol consumption, smoking status and intervention group ($p>0.05$) (Table 6.1). However, we found that dropouts had significantly higher frequency of smoking and alcohol consumption compared to the completers ($p=0.014$ and $p=0.015$, respectively).

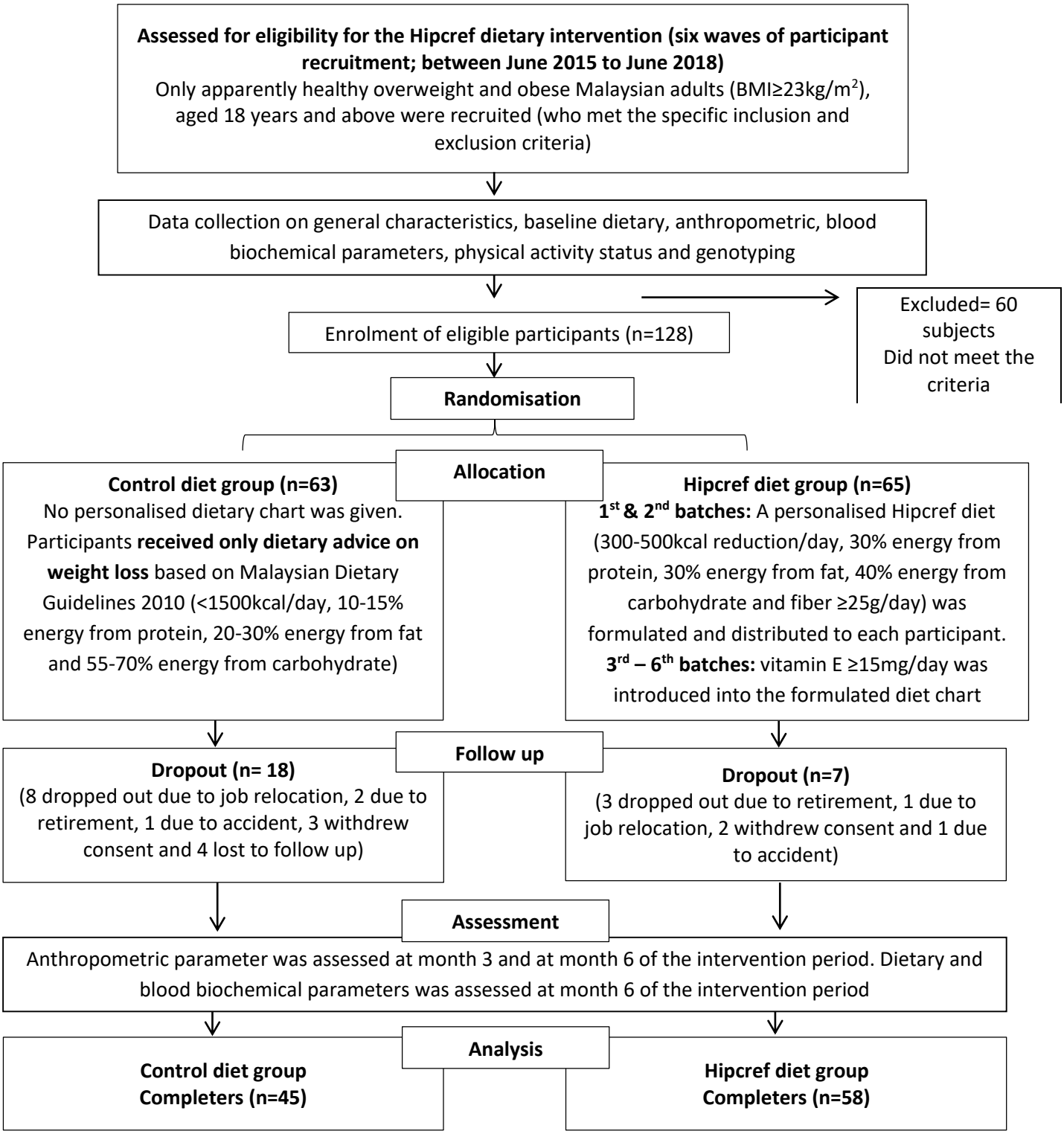


Figure 6.2: Flow chart of the intervention study: Enrolment, random allocation, follow up, withdrawal and assessment of the study participants

Table 6.1: General characteristics: Differences between the completers and dropouts

Variables	Completers (n=103)	Dropouts (n=25)	p value
Age (y)	44.7 ± 1.1	40.9 ± 2.2	0.236 [a]
Gender			
Female (n)	88 (85.4%)	20 (80.0%)	0.502 [b]
Male (n)	15 (14.6%)	5 (20.0%)	
Ethnicity			
Chinese	16 (15.5%)	8 (32.0%)	0.086 [b]
Malays	50 (48.5%)	7 (28.0%)	
Indians	37 (36.0%)	10 (40.0%)	
Physical activity status			
Physically inactive	97 (94.2%)	24 (96.0%)	0.719 [b]
Physically active	6 (5.8%)	1 (4.0%)	
Smoking status			
Never	102 (99.0%)	23 (92.0%)	**0.014 [b]***
Former	1 (1.0%)	0	
Current	0	2 (8.0%)	
Alcohol consumption status			
Never	103 (100%)	23 (92.0%)	**0.015 [b]***
Former	0	1 (4.0%)	
Current	0	1 (4.0%)	
Height (cm)	157.6 ± 0.7	159.5 ± 1.7	0.378 [a]
Weight (kg)	72.4 ± 1.2	80.0 ± 5.2	0.087 [a]
BMI (kg/m2)	29.2 ± 0.4	31.0 ± 1.3	0.147 [a]

Data were presented as mean (± SE) or number (percentage)
[a] p value based on ANCOVA after adjusting for age, gender, physical activity status, alcohol consumption, smoking status and intervention group.
[b] p value based on Chi square test.
*p<0.05 was considered as significant.

6.4.2 General characteristics: Differences between Hipcref diet group and control diet group on an intention-to-treat (ITT) analysis

The statistical analysis on the comparisons of the general characteristics between the Hipcref diet group and control diet group was conducted on an intention-to-treat basis (Table 6.2). The gender distribution was not significantly different in both Hipcref diet group and control diet group, females (83% vs 86%) and males (17% vs 14%) (p=0.681). The mean age (y) in the 2 arms of the study did not differ statistically (Hipcref diet group: 44.8±1.3y and control diet group: 43.1±1.5y; p=0.437). There was no significant difference in the distribution of the three ethnic groups in the two arms of the study (p=0.996). No difference in physical activity status (p=0.057), smoking status (p=0.219) and alcohol consumption status (p=0.351) as well. No significant difference was found in height (p=0.564), body weight (p=0.747) and BMI (p=0.950) between the two groups. No significant difference was found in the frequency of non-vegetarians and vegetarians between the Hipcref diet group (98.5% and 1.5%, respectively) and control diet group (98.4% and 1.6%, respectively) (p=0.982).

Table 6.2: General characteristics: Differences between Hipcref diet group and control diet group on an intention-to-treat (ITT) analysis

Variables	Hipcref diet group (n=65)	Control diet group (n=63)	p value
Age (y)	44.8 ± 1.3	43.1 ± 1.5	0.437 [a]
Gender			
Female (n)	54 (83.1%)	54 (85.7%)	0.681 [b]
Male (n)	11 (16.9%)	9 (14.3%)	
Ethnicity			
Malays	29 (44.6%)	28 (44.5%)	0.996 [b]
Chinese	24 (36.9%)	12 (19.0%)	
Indians	12 (18.5%)	23 (36.5%)	
Physical activity status			
Physically inactive	59 (90.8%)	62 (98.4%)	0.057 [b]
Physically active	6 (9.2%)	1 (1.6%)	
Smoking status			
Never	64 (98.5%)	61 (96.8%)	0.219 [b]
Former	1 (1.5%)	0	
Current	0	2 (3.2%)	
Alcohol consumption status			
Never	65 (100%)	61 (96.8%)	0.351 [b]
Former	0	1 (1.6%)	
Current	0	1(1.6%)	
Dietary preference			
Non-vegetarian	64 (98.5%)	62 (98.4%)	0.982 [b]
Vegetarian	1 (1.5%)	1 (1.6%)	
Height (cm)	158.3 ± 0.8	157.5 ± 1.0	0.564 [a]
Weight (kg)	74.2 ± 1.5	73.6 ± 2.4	0.747 [a]
BMI (kg/m2)	29.6 ± 0.6	29.4 ± 0.7	0.950 [a]

Data were presented as mean (± SE) or number (percentage)

[a] p value was based on ANCOVA, after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption.

[b] p value based on Chi square test.

$p<0.05$ was considered as significant.

6.4.3 Differences in anthropometric parameters from baseline, at month 3 and month 6 between Hipcref diet group and control diet group

A two-way mixed ANOVA analysis (Figure 6.3) (Appendix 21) revealed significant treatment group x time effect on body weight ($p<0.001$), BMI ($p<0.001$), WC ($p<0.001$), WHR ($p=0.001$), fat mass ($p<0.001$), fat-free mass ($p=0.010$) and percent body fat ($p<0.001$), even after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption. Further, pairwise comparisons revealed that Hipcref diet group had significant reduction in body weight, BMI, WC, fat mass and percent body fat at month 3 and at month 6 compared to the baseline values ($p<0.05$). However, pairwise comparisons revealed that control diet group had significant increase in body weight and BMI at month 3 and at month 6 compared to the baseline values ($p<0.05$). Moreover, control diet group had significant increase in WC, WHR and fat mass at month 6 compared to the baseline values ($p<0.05$).

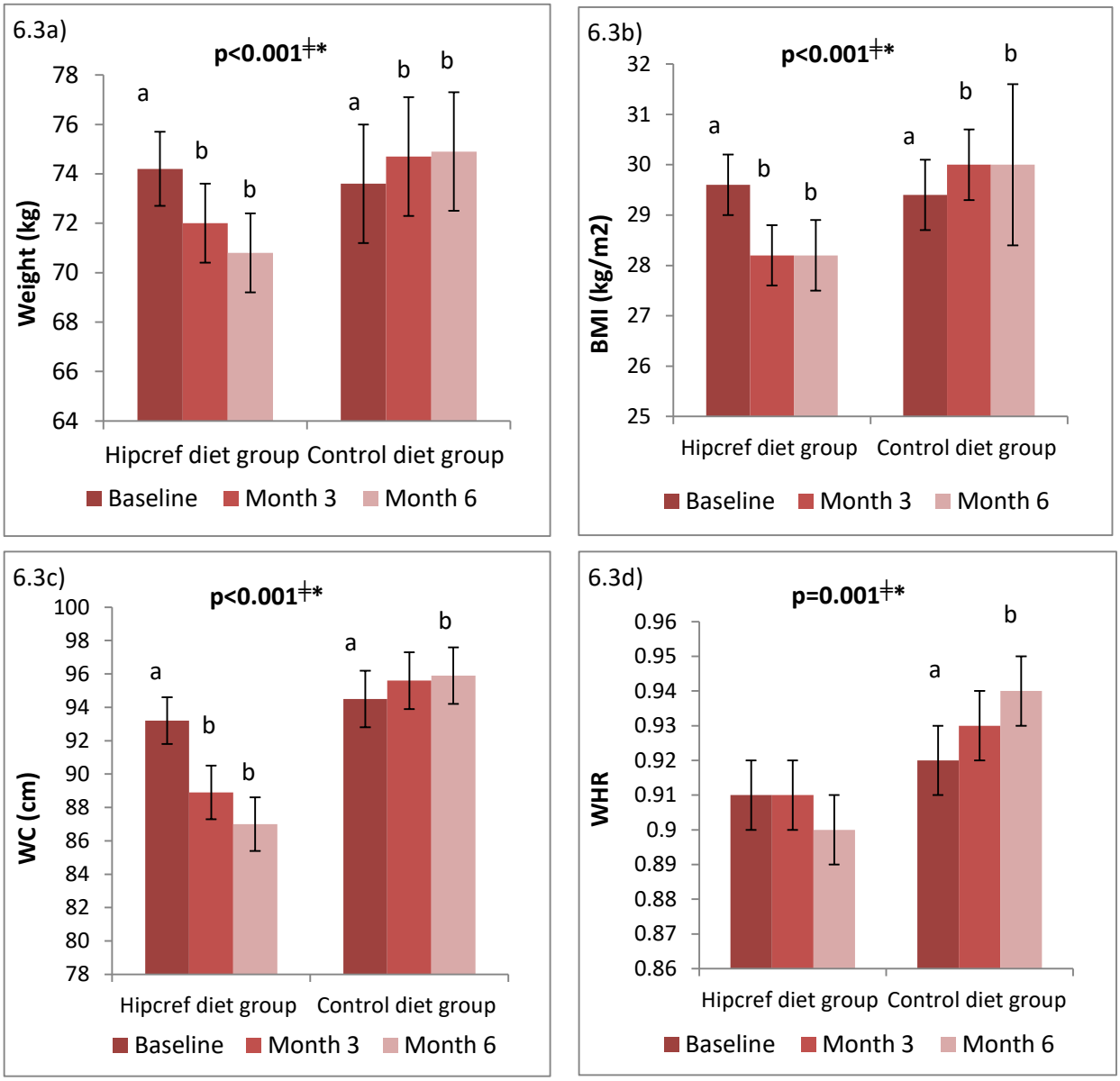


Figure 6.3 (a-h): Differences in anthropometric parameters from baseline, at month 3 and month 6 between Hipcref diet group (n=65) and control diet group (n=63) (Appendix 21).

ǂA two-way mixed ANOVA was performed to assess the effect of treatment group x time on anthropometric parameters at month 3 and month 6 between Hipcref diet group and control diet group. Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

*p<0.05 was considered as significant.

With a significant F test, post hoc pairwise comparisons were performed to assess the difference at month 3 and month 6 compared to baseline values within the treatment group, after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption.

a, b Figures not sharing common letter was significantly different (p<0.05) compared to baseline.

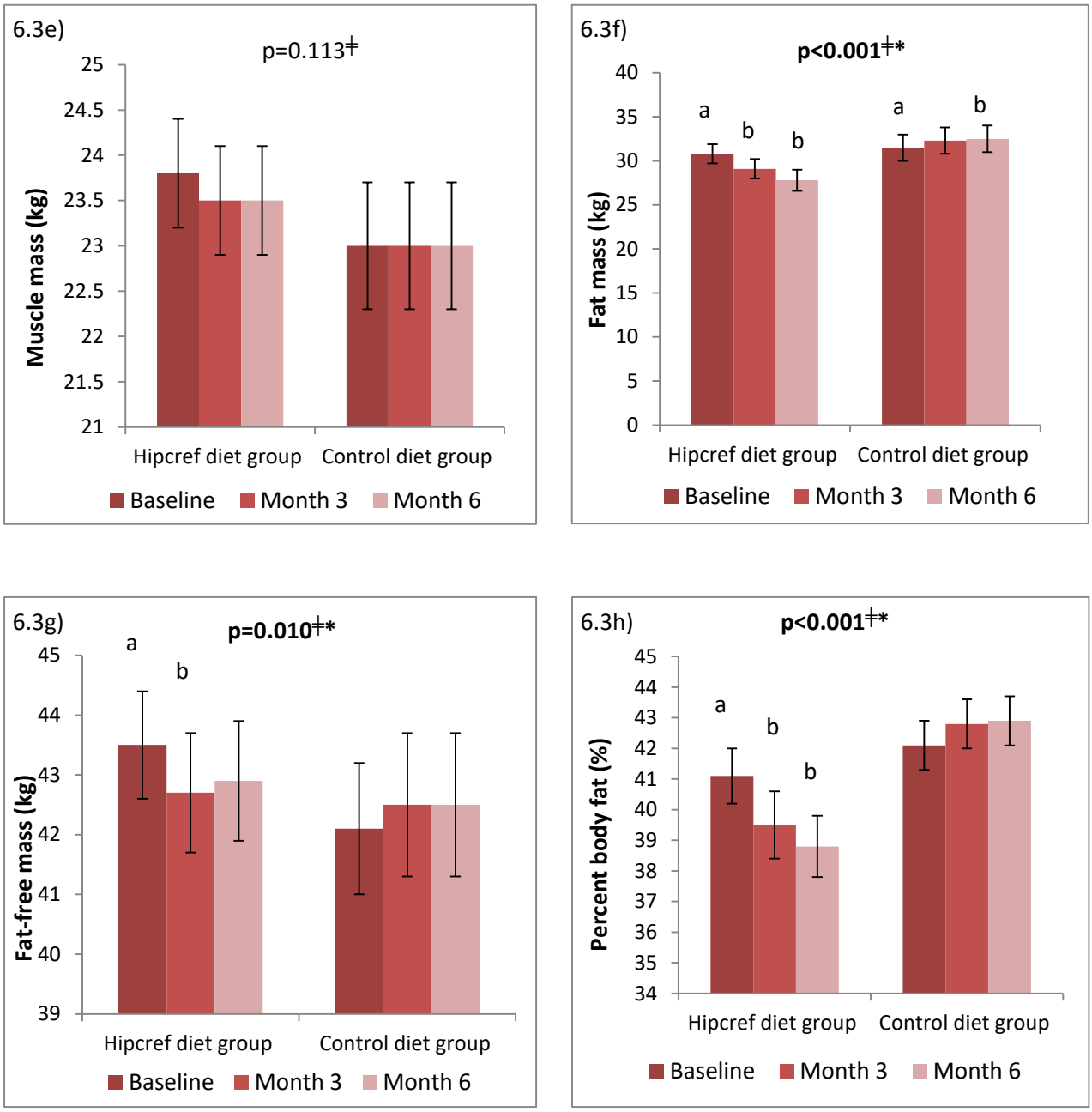


Figure 6.3 (a-h): Differences in anthropometric parameters from baseline, at month 3 and month 6 between Hipcref diet group (n=65) and control diet group (n=63).

ǂA two-way mixed ANOVA was performed to assess the effect of treatment group x time on anthropometric parameters at month 3 and month 6 between Hipcref diet group and control diet group. Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

*$p<0.05$ was considered as significant.

With a significant F test, post hoc pairwise comparisons were performed to assess the difference at month 3 and month 6 compared to baseline values within the treatment group, after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption.

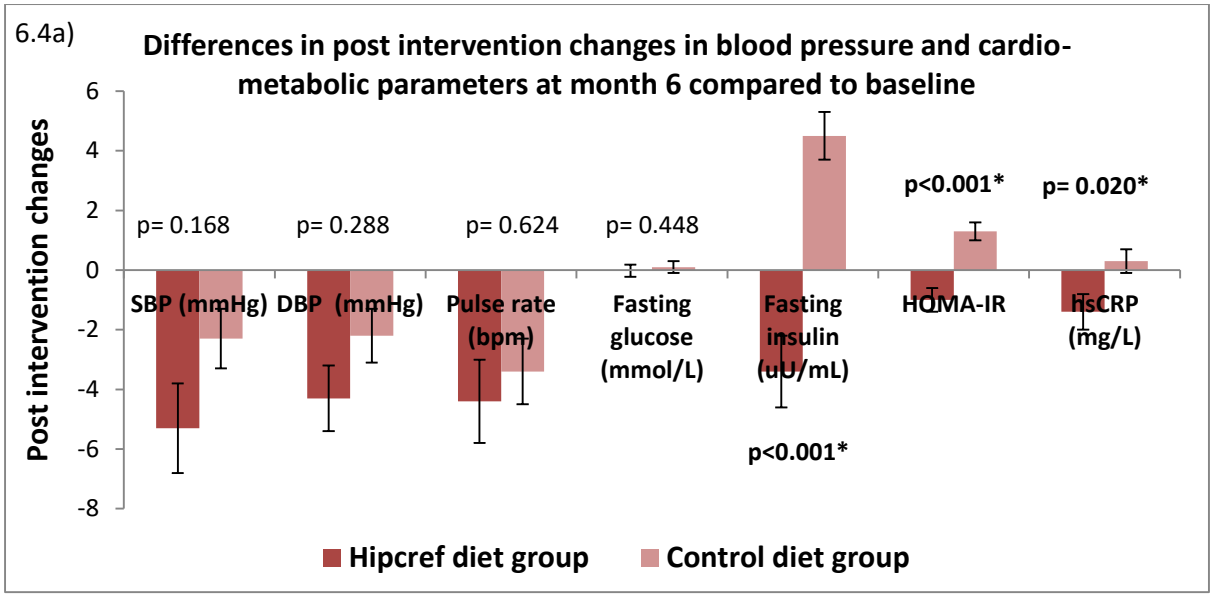
a, b Figures not sharing common letter was significantly different ($p<0.05$) compared to baseline.

6.4.4 Differences in post intervention changes in blood pressure and blood biochemical parameters from baseline and at month 6 between Hipcref diet group and control diet group

A two-way mixed ANOVA analysis revealed that the Hipcref diet group had significantly higher reduction in fasting insulin ($p<0.001$), HOMA-IR ($p<0.001$) and hsCRP levels ($p=0.020$) compared to the control diet group at month 6, even after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption (Figures 6.4a & 6.4b) (Appendix 22). No significant difference was found in blood pressure, fasting glucose and blood lipid levels between the two groups ($p>0.05$).

6.4.5 Differences in post intervention changes in dietary parameters from baseline and at month 6 between Hipcref diet group and control diet group

Results from a two-way mixed ANOVA analysis revealed that the Hipcref diet group consumed significantly higher percent energy from protein ($p<0.001$), percent energy from PUFA ($p=0.009$), energy-adjusted vitamin E (mg) ($p<0.001$), energy-adjusted fibre (g) ($p<0.001$) and had lower intake of total energy ($p<0.001$), percent energy from fat ($p=0.001$) and percent energy from carbohydrate ($p=0.007$) compared to the control diet group at month 6, even after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption (Figures 6.5a & 6.5b) (Appendix 23).



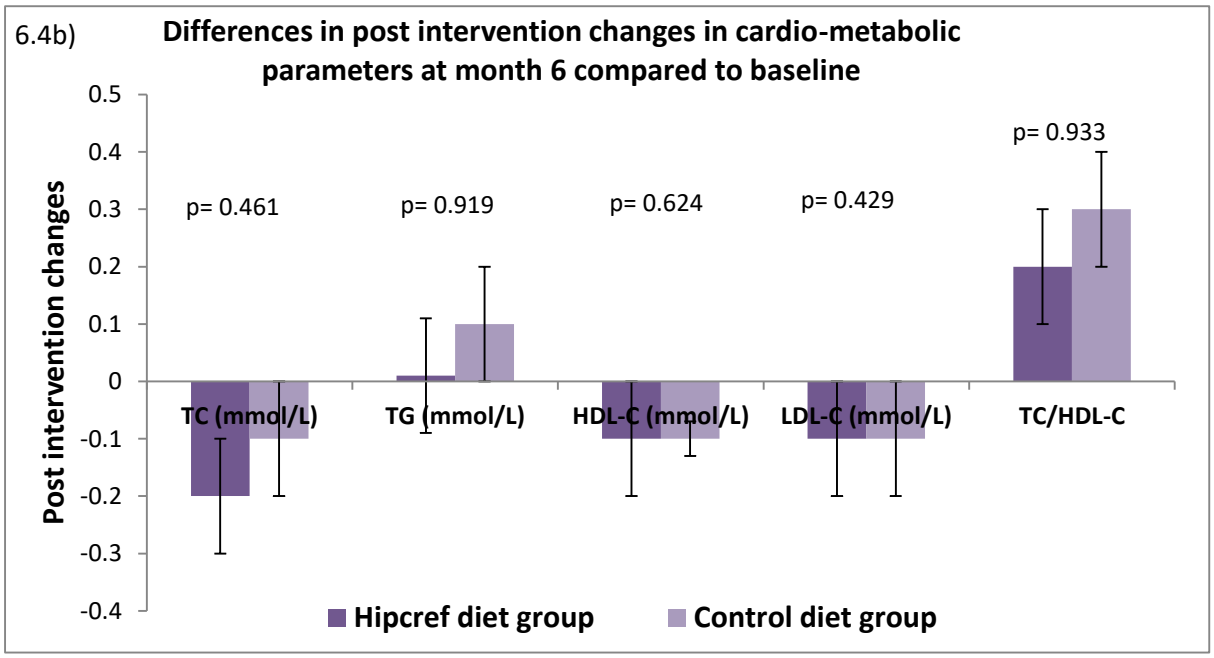


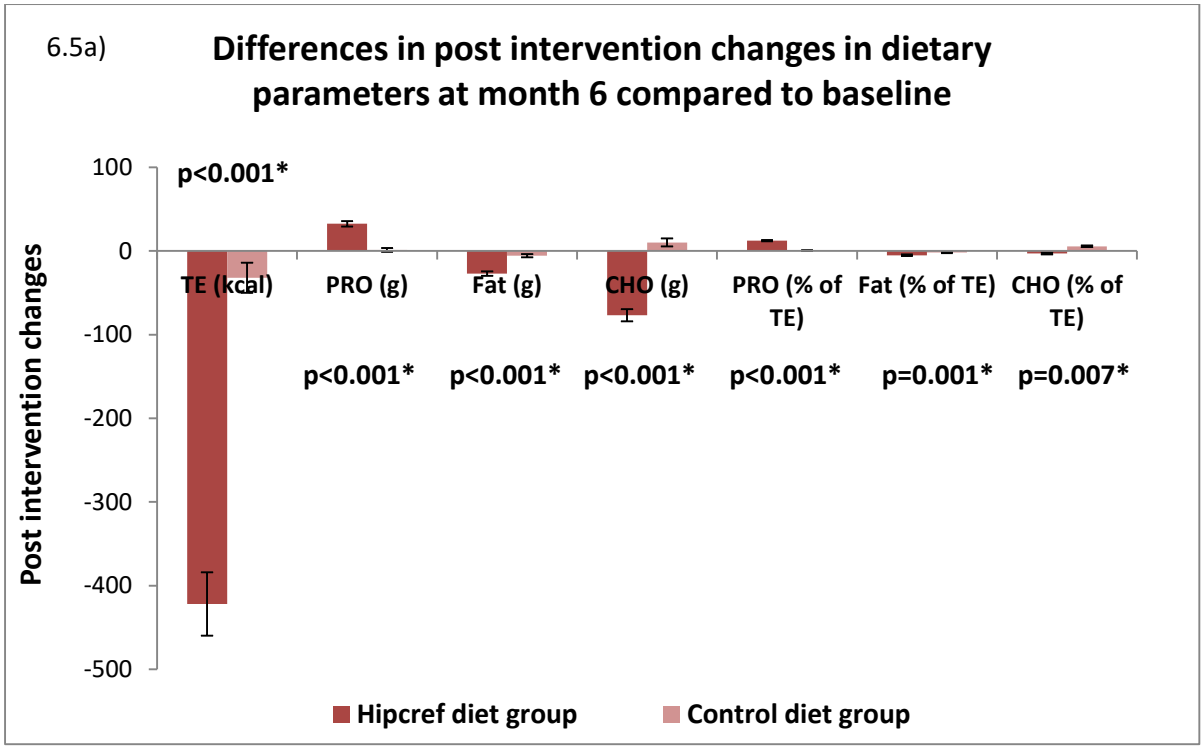
Figure 6.4 (a & b): Differences in post intervention changes in blood pressure and cardio-metabolic parameters at month 6 compared to baseline between the Hipcref diet group (n=65) and control diet group (n=63) (Appendix 22).

A two-way mixed ANOVA was performed to assess the effect of treatment group x time on blood pressure and blood biochemical parameters between the Hipcref diet group and control diet group.

Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

*p<0.05 was considered as significant.

SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment -insulin resistance; hsCRP, high-sensitivity C-reactive protein.



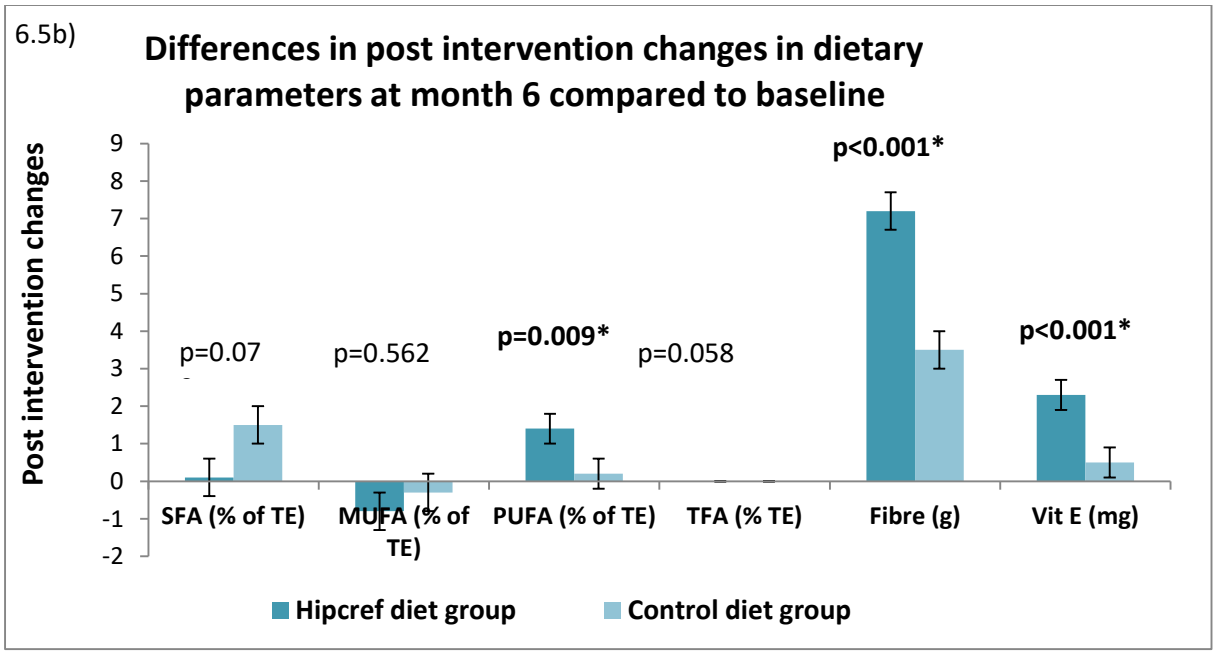


Figure 6.5 (a & b): Differences in post intervention changes in dietary parameters at month 6 compared to baseline in Hipcref diet group (n=65) and control diet group (n=63) (Appendix 23)

A two-way mixed ANOVA was performed to assess the effect of treatment group x time on blood pressure and blood biochemical parameters between the Hipcref diet group and control diet group, after adjusting for covariates, age, gender, physical activity, smoking and alcohol consumption.

*p<0.05 was considered as significant.

TE, total energy intake; PRO, protein; CHO, carbohydrate; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TFA, trans fatty acid; Vit, vitamin.

6.4.6 Dietary adherence score: Difference between Hipcref diet group and control diet group

The adherence score for both the Hipcref diet group and the control diet group is presented in Table 6.3. No significant difference was found in the dietary adherence score between the two groups on per protocol basis (p=0.489). However, significant difference in adherence score was found between the two groups on an intention-to-treat (ITT) basis. Control diet group had significantly higher score (7.4 ± 0.8) compared to Hipcref diet group (4.6 ± 0.4) (p=0.002), suggesting poor dietary adherence compared to the Hipcref diet group.

Table 6.3: Adherence score: Difference between Hipcref diet group and control diet group

	Per protocol analysis			Intention-to-treat (ITT) analysis		
Adherence score	**Hipcref diet group (n=58)**	**Control diet group (n=45)**	**p value**	**Hipcref diet group (n=65)**	**Control diet group (n=63)**	**p value**
Mean (± SE)	3.8± 0.2	4.1± 0.4	0.489	4.6 ± 0.4	7.4 ± 0.8	**0.002***
Minimum	1.1	1.5	-	1.1	1.5	
Maximum	8.2	11.3	-	16.4	22.7	

Independent t test was performed to assess the difference in adherence score between Hipcref diet group and control diet group.
*p<0.05 was considered as significant.

6.5 Discussion

Despite the large number of weight loss intervention programmes, long term maintenance of weight loss has been unsuccessful in populations, and regain of lost weight is a common phenomenon (Dombrowski et al., 2014). Majority of earlier weight loss intervention studies, targeted calorie restriction and reduced carbohydrate or fat intake (Strychar, 2006). In this study, we aimed to develop a dietary strategy for weight management which is easy to adhere to. The formulated diet was based on habitual food intake and hence was affordable. To the best of our knowledge, this is the first study conducted in overweight and obese Malaysian adults that investigates the effectiveness of a 6-month individualised, calorie-restricted diet with high protein, high vitamin E and normal fibre intake. The aim of this study was to investigate the effect of Hipcref diet on body composition and blood biochemical parameters compared to the control diet at the end of the intervention period. As far as we know, our approach is new in the Malaysian population.

Our findings revealed that overweight and obese Malaysian adults following Hipcref diet had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the generalised dietary instruction on weight loss. Participants following

Hipcref diet had successfully reduced 3.5kg of body weight and lost 2.3% of percent body fat in 6 months, which translated to a reduction of 0.6kg of body weight and 0.4% of percent body fat per month. The beneficial effect of Hipcref diet on obesity-related anthropometric parameters (e.g. BMI, WC, fat mass and percent body fat) was observed at month 3 and was maintained at month 6. Insulin sensitivity improved significantly in the participants following Hipcref diet as reflected in the reduced fasting insulin levels and reduced HOMA-IR. Baseline fasting blood glucose levels in the Hipcref diet group was below the diagnostic cut off (5.6mmol/L) (Alberti, Zimmet, & Shaw, 2006) defining metabolic syndrome, and it did not reduce after intervention, suggesting that intervention diet resulted in weight loss and improvement in insulin sensitivity without leading to hypoglycaemia.

Individuals following MDG 2010 dietary guidelines (control diet group) showed opposite effects. They had significant increase in obesity-related anthropometric parameters (e.g. BMI, WC, fat mass and percent body fat), HOMA-IR and hsCRP levels after 6 months. These results suggest that traditional dietary counselling for weight management based on MDG 2010, National Coordinating Committee of Food and Nutrition (NCCFN) (not more than 1500kcal/day with a macronutrient composition - approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate) seem to be ineffective in promoting weight loss in overweight and obese Malaysian adults.

6.5.1 High attrition rate in control diet group

Our findings revealed that there was relatively higher attrition rate in the control diet group compared to the intervention diet group. This may be due to demotivation in the latter individuals, to pursue the programme due to failure to lose weight. Furthermore, our results revealed that the dropouts were associated with certain characteristic traits such as higher frequency of cigarette smoking and alcohol drinking compared to the completers. Therefore, it is important for the individuals to refrain from alcohol and cigarette consumption during weight management programmes for better weight loss outcomes.

In addition, tailoring dietary interventions to an individual's like, dislike and food preference may lead to better adherence to dietary prescriptions. Previous evidence suggested that the degree of adherence is highly influenced by how different a dietary intervention is from an individual's usual diet (Gibson & Sainsbury, 2017). Therefore, Hipcref diet which tailored according to individual's food preference and cultural habit may lead to higher compliance, adherence and lower attrition rate compared to the control diet group.

6.5.2 Regular monitoring throughout the intervention period

The success of this dietary intervention could be attributed to the regular monitoring throughout the intervention period. Mettler et al., 2014 reported that guided weight management programmes resulted in greater motivational improvement for behaviour change compared to unsupervised weight management programmes (Mettler et al., 2014). A weight loss programme which provides regular supervision and monitoring may allow participants to access health-care provider knowledge and feedback, and hence improve self-efficacy and self-motivation. A good adherence to healthy weight loss behaviours is essential for initial and long-term weight loss. Losing weight without guidance throughout the weight management programmes can be demotivating and discouraging.

6.5.3 Intervention diet - Calorie restriction

Our findings revealed significant reduction in total energy intake in individuals following Hipcref diet (an average reduction of 422kcal/day). Portion control is key to managing weight, however requesting people simply to 'eat less' may not be the best approach. Smaller portions of high-energy-dense food may disproportionately increase energy intake compared to consumption of low energy foods (Rolls, 2014). Liquid meal replacements and solid pre-portioned foods (PPFs) for weight loss and weight loss maintenance may have been successful in weight loss (Heymsfield et al., 2003) but it is doubtful whether or not such artificial diets lead to better understanding of individual portions during meal times post intervention. Under real life situations, consumers do not retain the motivation to continue with such foods which do not relate to the cereals, fruits, vegetables, egg or meat that they eat habitually. As part of the intervention process, counselling was provided to the Hipcref diet group on choosing portions that were charted to the participants, using household utensils (scales, plates, cups and spoons) and portion size photographs. We envisaged that this support would lead to improved understanding of the portions consumed and the corresponding calorific value.

With respect to blood biochemical parameters, our results revealed significant reduction in hsCRP and HOMA-IR with the Hipcref diet. High sensitivity C-reactive protein (hsCRP) is known to be the best systemic marker for inflammation and it is a strong independent predictor of future cardiovascular events (Devaraj et al., 2009). It has been demonstrated that increase in hsCRP levels was highly associated with increased adipose tissue. Adipose tissue is involved in the regulation and production of inflammatory cytokines that induce the production of hsCRP (Maachi et al., 2004). Therefore, a reduction in the excess body fat stores can

significantly reduce the inflammatory state (Petelin et al., 2014). A systematic review reported that reduction of hsCRP levels may be due to the consequence of weight loss following a moderate reduction in calorie intake (Selvin et al., 2007a). Previous studies have reported that calorie restriction was a significant factor in improving glycaemic control and insulin sensitivity with weight loss (Wing et al., 1994). In our participants, favourable effect on the above biochemical parameters in individuals on Hipcref diet may due to the calorie restriction and reduction in body fat mass.

6.5.4 Intervention diet - Higher intake of protein

High-protein diets promote weight loss, reduce abdominal obesity, reduce triglyceride levels, and improve glucose metabolism (Gannon et al., 2003; Papakonstantinou et al., 2010; Parker et al., 2002; Pesta & Samuel, 2014). We envisaged that higher protein intake could prevent loss of muscle mass as the participants lost weight through calorie reduction. Johnston et al., 2017 reported that high protein diet with modest caloric restriction was effective in reducing HOMA-IR in healthy adults compared to lower protein, standard diet, in U.S. population. Conflicting theories have been reported by other studies. Wycherley et al., 2012 reported that, high protein and standard protein diets resulted in similar weight loss and reduction in HOMA-IR. These investigators concluded that reduction in body weight was the factor in improving insulin sensitivity compared to the quantity of dietary protein.

Several studies have investigated the effect of high protein diet on hsCRP (Azadbakht et al., 2013; Gulati et al., 2017; Noakes et al., 2005). Gulati et al., 2017 reported that a high protein diet (29% PRO, 24% FAT and 47% CHO) was found to be effective in reducing hsCRP levels compared to the control diet group (15% PRO, 25% FAT and 60% CHO) in an Indian population. Contrary to that, Azadbakht et al., 2013 and Noakes et al., 2005 reported that hsCRP level was significantly reduced with weight loss, independent of dietary protein content, in an Iranian population. In our participants, perhaps the positive impact on insulin sensitivity was due to the combined effect of both caloric restriction and high protein intake.

6.5.5 Macronutrient intake and intervention diet

Participants in the Hipcref diet group had significantly lower consumption of total energy intake, percent energy from fat and percent energy from carbohydrate and significantly higher intake of percent energy from protein, PUFA and fibre compared to control diet group after a 6-month of dietary intervention.

Despite widespread consensus that a reduced intake of saturated fat lowers cardiovascular disease (CVD) risk, the optimal type of macronutrient (protein, unsaturated fat, or carbohydrate) that should replace saturated fat is uncertain (Briggs, Petersen, & Kris-Etherton, 2017). Many past studies have focused on fat consumption and metabolic syndrome (Freire et al., 2005; Riccardi, Giacco, & Rivellese, 2004). In particular, the consumption of saturated fat was believed to influence insulin resistance and cardiovascular disease by increasing serum LDL cholesterol levels (DiNicolantonio, Lucan, & O'Keefe, 2016). In recent years, there has been an increasing interest in the effect of carbohydrate on metabolic disease. In the past, it was not considered a concern if 55-65% of the total energy was sourced from carbohydrate rich foods. However, recently there is concern that excessive intake of carbohydrates, particularly refined carbohydrates may contribute to metabolic diseases (Siri-Tarino et al., 2010). High carbohydrate consumption has been reported to increase serum triglyceride and lower HDL cholesterol and lead to metabolic syndrome (Ma et al., 2006). Further, excess carbohydrates are converted to triglycerides, and the correlation between elevated serum triglyceride levels and cardiovascular diseases cannot be disputed (Nordestgaard & Varbo, 2014). Lower consumption of carbohydrate and increased consumption of protein may, in fact, be the most effective way to reduce serum triglyceride levels.

In our study, in Hipcref diet group, we observed a reduction in total cholesterol and LDL cholesterol levels from baseline although these reductions did not reach statistical significance. No change in triglyceride levels from baseline was found in the participants following Hipcref diet, and the triglyceride levels were lower than the diagnostic cut off defining metabolic syndrome, 1.7mmol/L (Alberti, Zimmet, & Shaw, 2006). There was a small reduction in HDL cholesterol level in the Hipcref diet group, this difference from baseline did not reach statistical significance and was above the diagnostic cut off defining metabolic syndrome (1.03mmol/L for male; 1.29mol/L for female; Alberti, Zimmet, & Shaw, 2006). These results may be explained by the food sources selected for the Hipcref diet participants. The food sources to increase protein intake in the current study were mainly animal proteins. Animal food sources are known to increase saturated fat intake.

A systematic review reported that soy protein (with isoflavones) or other plant proteins (pea and lupine protein, wheat gluten) had greater decrease in total cholesterol and LDL cholesterol levels compared to animal sourced protein (Chalvon-Demersay et al., 2017). Diets that induce negative energy balance continue to be the cornerstone of weight management. However, long-term volitional reduction in energy intake is challenging. With transformation of Malaysia's

economy in the recent decades, the food consumption pattern of the population has shifted from that of a traditional cereal and legume based one to that which is rich in meat and meat products (Soon, 2014). Animal products not only provide high quality protein, but also are important sources of essential micronutrients (iron, zinc and vitamin A). To maximise outcome, our Hipcref diet sourced protein mainly from food of animal origin e.g. milk and milk products, egg, fish and chicken, but not red meat.

Two major goals of dietary recommendations are to lower blood pressure and improve serum lipids, two of the primary determinants of cardiovascular disease risk. Early research documented that the DASH diets (carbohydrate-rich diet that emphasises fruits, vegetables, and low-fat dairy products) reduced blood pressure in study subjects (Conlin, 1999). In addition to lowering blood pressure, the DASH diet lowered total cholesterol and LDL cholesterol levels (Sacks et al., 2001). It is uncertain, whether or not partial replacement of carbohydrate with either unsaturated fat or protein can improve blood pressure and lipid profile (Sacks et al., 2001). In OmniHeart randomised trial, a diet that partially replaced carbohydrates with protein, about half from plant sources, lowered blood pressure, LDL cholesterol levels, and triglyceride levels, as well as HDL cholesterol levels among adults with prehypertension or stage 1 hypertension (Appel et al., 2005). A diet that partially replaced carbohydrates with unsaturated fatty acids, predominantly monounsaturated fatty acids, lowered blood pressure and triglyceride levels and increased HDL cholesterol levels, but had no significant effect on LDL cholesterol levels.

The Hipcref diet contributed to 32% energy from fat of which 8% of TE was from SFA, 11% of TE from MUFA, 8% of TE from PUFA and 0.1% of TE from TFA. These changes from the baseline were within the favourable range of the recommended guidelines (National Coordinating Committee on Food and Nutrition, 2010). The Hipcref diet was successful in increasing the PUFA intake from 6% of TE to 8% of TE, which is on the higher range of the recommendation (4-8% of TE) (National Coordinating Committee on Food and Nutrition, 2010). We report that moderate energy restriction (average reduction of 422kcal/day), carbohydrate restriction (43% energy from carbohydrate; 175g/day) and higher protein intake (an average of 27% energy from protein; 108g/day), with fat intake within the NCCFN guidelines have been successful in managing weight in our Malaysian adult participants.

6.5.6 Intervention diet - ≥15mg/day of vitamin E

Dietary micronutrients, especially with anti-inflammatory and antioxidant properties are known to have beneficial effects on cardiovascular outcome (Pham-Huy, He, & Pham-Huy, 2008). Vitamin E is a micronutrient with anti-oxidant and immune modulating properties which can reduce oxidative stress and inflammation (Singh & Jialal, 2004). A significant inverse association of vitamin E and hsCRP has been demonstrated in other studies (Devaraj et al., 2007; Scheurig et al., 2008). A study by Devaraj et al., 2008, demonstrated that the combination of alpha and gamma-tocopherol supplementation showed beneficial effect on markers of inflammation in patients suffering from metabolic syndrome, suggesting the potential of vitamin E in ameliorating oxidative stress and inflammation in such patients.

Previous studies demonstrated that vitamin E supplementation (900 mg/d for 4 months) reduced oxidative stress and improved insulin action in healthy and non-insulin-dependent diabetic patients (Paolisso et al., 1993). Manning et al., 2004 reported that overweight individuals supplemented with high-dose vitamin E (800IU of vitamin E per day for 3 months and 1200IU for further 3 months) improved insulin action and decreased plasma fasting insulin and glucose levels by reducing cellular oxidative stress, altering membrane properties, and decreasing inflammatory activity. However, contradictory findings have been found in other studies. A meta-analysis involved 135,967 participants from 19 clinical trials reported that high-dosage (> or =400 IU/d) vitamin E supplements may increase all-cause mortality (Chew & Milton, 2005). Numerous studies reported that vitamin E supplementation (400 IU/day) to individuals with diabetes and at high risk for cardiovascular disease events had a neutral or no adverse effect on cardiovascular disease outcomes, on microvascular complications, and on glycaemic control (Heart Outcomes Prevention Evaluation Study, 2000; Lonn et al., 2002).

The effects of vitamin E supplementation are marginal. However, there are clear benefits of long-term consumption of vitamin E through diets containing oils, nuts and seeds (Abdali et al., 2015; Ros, 2010). Moreover, there is no sufficient evidence of toxic effects from vitamin E found naturally in foods (Monsen, 2000). These data provide no support for the use of these supplements for the prevention of CVD, but antioxidant-rich foods are recommended to be incorporated into healthy diets for better metabolic health. Moreover, a recent study suggests that obese people with metabolic syndrome face an unexpected quandary when it comes to vitamin E as they need more than normal levels of the vitamin because their weight and other problems are causing increased oxidative stress, but those same problems actually cause their

effective use of vitamin E to be reduced (Mah et al., 2015). These findings support higher dietary α-tocopherol requirements for obese adults. In our study the intake of vitamin E in both the Hipcref diet group and control diet group met the Malaysian dietary recommendations (7.5mg/day for women and 10mg/day for men). However, reduction in hsCRP levels was only observed in the Hipcref diet group.

6.5.7 Intervention diet - ≥25g/day of dietary fibre

A review of studies examining the effects of fibre on body weight found that higher dietary fibre intake was associated with increased satiety and decreased hunger (Howarth et al., 2001). Large prospective cohort studies consistently reported significant associations between high dietary fibre intake (>25 g/d in women and >38 g/d in men) and reduced risk of developing type 2 diabetes (T2D) (20-30% of reduced risk) (Weickert & Pfeiffer, 2018). The proposed mechanisms for this relationship remain unclear, but the type of fibre may play a role. In the gut, certain soluble fibres which form a viscous gel matrix is believed to slow gastric emptying and lead to a greater feeling of fullness (Slavin & Green, 2007). Soluble fibres slow absorption of glucose further in the small intestine and lead to lower postprandial glycaemic and insulinemic responses (Yu et al., 2014). Therefore, high soluble fibres may contribute to the improvement in glycaemic control and insulin sensitivity. Moreover, in both the cross-sectional and longitudinal analyses, Ma et al., 2006 reported that fibre intake was protective against high hsCRP levels. It was suggested that dietary fibre decrease lipid oxidation, which in turn is associated with decrease inflammation (King, 2005). Therefore, we report that the beneficial effect on cardio-metabolic parameters in our Hipcref diet group may be due to their higher fibre intake.

6.5.8 Strengths and limitations

The dietary intervention is novel, practical and easily adoptable in day to day life. Our intervention programme did not include any physical activity regimens. This dietary intervention strategy could be readily tested in primary care services with minimal cost. Furthermore, our study participants represent the major ethnic groups of Malaysia (Malaysian Malays, Chinese and Indians) and this study has tested a dietary strategy that included ethnic food preferences and popular traditional recipes practiced in all the three major ethnic groups. This study has successfully proved that simple modification of traditional recipes can bring a modest change in body composition and metabolic parameters in overweight and obese Malaysian adults.

However, few limitations exist in this study. Our study participants were recruited from the supermarkets and schools in a specific geographical location (Beranang, Jalan Broga and Bandar Kajang) and they were predominantly middle-aged women with sedentary lifestyle. Hence, the findings of the current experiments may not be generalisable to the Malaysian population as a whole. Therefore, large scale studies are required to replicate the findings of the studies in the Malaysian population from other geographical locations. Moreover, there was relatively higher attrition rate in the control diet group compared Hipcref diet group. This may be due to demotivation to pursue the programme because of failure to lose weight. However, if we assume that the non-completers in the control diet group lost less weight compared to the completers, our estimate of treatment effect would remain unaffected. In other words, it is unlikely that the missing data would have changed our main results from positive to negative. It is understandable that individuals motivated to lose weight participated in the current study willingly. Another limitation exists in our study is the measurement of body composition using bioelectrical impedance analysis (BIA). Many factors such as the food and fluid intake, environment, ethnicity and phase of menstrual cycle may affect the BIA estimates.

We have carefully controlled for baseline values and other lifestyle factors in the analyses. However, other unmeasured or unknown confounders such as behaviour, socioeconomic and sociodemographic parameters may also exist. It is undeniable that the effect size estimate of the dietary intervention observed in our current study may due to the aforementioned confounding factors, and hence causes bias in our current findings. With respect to the assessment of dietary intake and physical activity, assessment using questionnaires (FFQ and activity record) may prone to measurement errors, and hence caused bias in the current findings.

In addition, participant's compliance and adherence to the formulated diet was not assessed periodically throughout the 6 months of the intervention period. This may have limited the validity of the research trial. Future studies should review the individuals' food diaries at regular intervals or measure specific blood, urine or faecal biomarkers of exposure during the intervention to assess adherence. Moreover, prescription of vitamin E ≥15mg/day was introduced to the intervention diet group (formulated diet chart) starting from batches 3-6 (intervention diet group n=32; control diet group n=38), after significant interaction was found between FTO gene variants and vitamin E on hsCRP levels (Chapter 5). As per the dietary strategy of this study, calorie restriction, higher protein, vitamin E and dietary fibre intake had a combined effect on the success of the intervention diet. We cannot attribute the success of the Hipcref diet to any one of the latter dietary components. The differences in the nutrient

composition of the formulated Hipcref diet charts make it difficult to interpret the cause-effect relationships between Hipcref dietary intervention and the weight loss outcomes. More clinical trials with larger sample size and longer duration are required to confirm the long-term effect of Hipcref diet on anthropometric and cardio-metabolic parameters.

6.6 Summary

The aim of this chapter is to investigate the effect of a 6-month Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention in the Malaysian adults with respect to body composition and metabolic parameters, using a 6-month randomised controlled trial. 128 overweight and obese Malaysian adults (BMI≥23kg/m^2) were randomised to Hipcref diet group (n=65) or control diet group (n=63). Intervention group received formulated Hipcref diet based on their personal preferences. Participants were responsible for obtaining their own food sources and preparing their own meals, following the formulated diet chart as guidance. The control diet group followed a generalised dietary advice based on Malaysian Dietary Guidelines, 2010. Two-way mixed ANOVA analysis revealed significant treatment group x time effect on anthropometric parameters ($p<0.05$) on an intention-to-treat basis. Further, pairwise comparisons revealed that Hipcref diet participants had significant reduction in body weight, BMI, WC, fat mass and percent body fat at month 6 compared to baseline values, even after adjusting for covariates. Two-way mixed ANOVA analysis revealed Hipcref diet group had significantly higher reduction in fasting insulin, HOMA-IR and hsCRP levels compared to the control diet group at month 6. However, opposite effects on the aforementioned parameters were found in the control diet group. At the end of the intervention period, compared to the control diet group, Hipcref diet group were found to consume significantly higher percent energy from protein, higher percent energy from PUFA, higher vitamin E, higher fibre, lower total energy intake, lower percent energy from fat and lower percent energy from carbohydrate. We opine that the success of the Hipcref diet on overweight/obese Malaysian adults may be due to the combined effect of the nutrient composition of the Hipcref diet. We cannot attribute the success of the Hipcref diet to any one of the latter dietary components. Further, we emphasise that personal dietary preferences should be considered as an important factor when developing strategies for weight management in any population, especially a multi-ethnic population as of Malaysia. However, the findings of current experiments may not be generalisable to the Malaysian population as a whole. More clinical trials with larger sample size and longer duration are required to confirm the long-term effect of Hipcref diet on anthropometric and cardio-metabolic parameters.

Chapter 7

Post-intervention differences in dietary, anthropometric and cardio-metabolic parameters: Gene-diet interaction between Hipcref diet and *FTO* and *ADRB2* gene variants

7.1 Introduction

The prevalence of obesity has reached epidemic proportions globally. Based on previous National Health and Morbidity Surveys (NHMSs) carried out in 2006, 2011 and 2015, an increasing trend of the prevalence of overweight and obesity was observed among the Malaysian adults aged 18 years and older, 29.1% and 14.5% in 2006 (Noor Safiza et al., 2008), 29.4% and 15.1% in 2011 (Institute for Public Health, Malaysia, 2011), 30.0% and 17.7% in 2015 (Institute for Public Health, Malaysia, 2015), respectively. Obesity has now become the major public health problem due to its clinical implications in increasing the prevalence of comorbid conditions such as hypertension, hyperglycaemia, dyslipidaemia, insulin resistance and inflammation (Finer, 2015). There are numerous well-established obesity treatments and recommendations for its prevention and control. However, over the years, strategies to combat obesity remained ineffective, with only less than one out of five patients have successfully reduced and maintained their lost weight (Wing & Phelan, 2005). Although several weight loss strategies reported reduction in weight and improvement in metabolic risk factors in obese individuals, each individual has shown huge variability in response to the same intervention (Bray, 2008) These inter-individual variations in response to weight loss interventions suggesting that genetic factors may play an important role in modulating weight regulation (Loos & Rankinen, 2005).

Gene-environment interaction is defined as "a different effect of an environmental exposure on disease risk in persons with different genotypes," or "a different effect of a genotype on disease risk in persons with different environmental exposures" (Ottman, 1996). Genetic predisposition to obesity may have interacted with such environments in determining the obesity epidemic. This has been proven in recent randomised controlled trials (RCTs), which reported that individual genetic profile was found to modify the effect of dietary interventions on weight loss and post-intervention differences in obesity-related anthropometric and metabolic risk parameters (Qi, 2014). This finding supported that individual genetic background may have affected the success of the dietary interventions in the prevention and treatment of obesity. Therefore, there is no one diet that should be recommended universally to reduce body weight and improve metabolic health. Personalised nutrition which tailored nutritional advice or recommendations based on individual's genetic makeup should be introduced to optimise individual's response to particular interventions (Deram & Villares, 2009).

To date, studies investigating the interactions between gene variants and weight loss outcomes in response to dietary interventions are still limited (Martínez, 2014). This has been a limiting factor for developing and establishing a personalised diet according to individual's genetic make up for weight management to combat the prevalence of obesity and its complications. It is important to identify and understand the interplay between genetic and lifestyle factors in the development of obesity and obesity-related traits. Early identification of the candidate gene variants and its interactions with dietary treatment may allow for the provision of good quality personalised recommendation to achieve effective weight loss and successful maintenance of weight.

In Chapter 6, our findings revealed that overweight and obese Malaysian adults following Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the generalised dietary instruction on weight loss. Participants following Hipcref diet had successfully reduced 3.5kg of body weight and lost 2.3% of percent body fat in 6 months, which translated to a reduction of 0.6kg of body weight and 0.4% of percent body fat per month. The success of the Hipcref diet on overweight and obese Malaysian adults may be due to the combined effect of higher percent energy from protein, percent energy from PUFA, Vitamin E, fibre and lower total energy, lower percent energy from fat and percent energy from carbohydrate. Therefore, in this chapter, we further analyse the modulatory effect of gene variants on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet intervention.

7.2 Objectives

The aim of this chapter is to investigate the response of *FTO* and *ADRB2* gene variants on the post-intervention differences in anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults, in response to a 6-month Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet intervention.

Chapter 7-Section-I: *FTO* gene

7I.1 Introduction

Fat-mass and obesity associated (*FTO*) gene was the first successfully determined candidate gene in genome-wide association studies (GWAS) which was associated with increased risk of obesity (Loos & Bouchard, 2008). It is positioned at chromosome region 16q12.211, encoding a Fe (II)- and 2-oxoglutarate (OG) dependent nucleic acid demethylase (Gerken et al., 2007). It is highly expressed in the arcuate nucleus of the hypothalamus (Fredriksson et al., 2008). Initial observations suggested that *FTO* gene polymorphisms play an important role in regulating energy homeostasis by signalling the hunger and satiety centre in hypothalamus, but it is unknown whether it through feeding behaviour or energy expenditure (Scuteri et al., 2007). Polymorphisms in *FTO* gene were linked to appetite responses, and therefore influence energy intake and body weight regulation (Fawcett & Barroso, 2010). Previous evidence reported that individuals carrying the AA genotype of *FTO* rs9930609 was found to weight 3kg more and had a 1.7 fold increased risk of obesity compared to individuals carrying TT genotype (Frayling et al., 2007). Other studies also reported that the carriers of the risk allele (G) of *FTO* rs9930506 was associated with increased odds of obesity in the Polish and Italian population (Sentinelli et al., 2012; Wrzosek et al., 2016). A recent study reported that the haplotype of three adjacent *FTO* gene polymorphisms (rs9930501, rs9930506 and rs9932754) had a strong association with obesity indices (e.g. BMI and body fat percent) in Iranian male adolescents (Kalantari et al., 2018).

FTO was the most investigated gene for gene-lifestyle interactions in clinical interventions. Between 2009 and 2015, there were a total of 6 studies investigating the interactions between *FTO* rs9939609, rs1558902 and rs8050136 SNPs with various weight loss strategies (Grau et al., 2009; Labayen et al., 2015; Matsuo et al., 2012; Mitchell et al., 2010; Zhang et al., 2012; Zheng et al., 2015). Individuals carrying the risk alleles of *FTO* SNPs may be benefited by various weight loss strategies including calorie restriction diets (Matsuo et al., 2012), hypocaloric low fat diets (K Grau et al., 2009) and hypocaloric high protein diets (Zhang et al., 2012). This evidence suggested that dietary intervention does not affect individuals with *FTO* minor and major alleles differentially. Similar finding was reported from a systematic review and meta-analysis of 9563 individuals, which showed that individuals carrying the minor allele (A) of *FTO* rs9939609 did not respond differently to dietary, physical activity, or drug based weight loss interventions compared to the major allele (T). The authors suggested

that genetic predisposition to obesity associated with *FTO* gene variants may be attenuated or counteracted with the above interventions (Livingstone et al., 2016). To date, the gene-diet interactions of the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 on the post-intervention differences in obesity-related phenotypes have not been identified in any clinical intervention trial.

7I.2 Objectives

The aim of this section is to investigate the response of the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in overweight and obese Malaysian adults, in response to a 6-month Hipcref diet intervention.

7I.3 Methodology

7I.3.1 Study design

The detailed information on the study design and methods have been described in an earlier chapter (Chapter 6). Hipcref dietary intervention is a 6-month, parallel-arm, randomised controlled trial, which assessed the effect of a Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet on dietary, anthropometric and cardio-metabolic parameters, compared to the control group who received generalised dietary instruction on weight loss based on Malaysian Dietary Guidelines 2010, in overweight and obese Malaysian adults.

7I.3.2 Participant selection

Detailed information on the participant selection have been described in an earlier chapter (Chapter 6). Briefly, a total of 128 eligible, apparently healthy overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above with a BMI≥23kg/m^2 were recruited to participate in the RCT. Participants (n=128) were randomly assigned to one of the two treatments: intervention diet group (Hipcref diet, n=65) or control diet group (control diet, n=63), using covariate adaptive randomisation technique. Eligible participants were stratified based on two covariate stratum a) by gender (female and male); b) by ethnicity (Malaysian Chinese, Malays and Indians), thus a total of 6 (2x3) strata was constructed.

Of the initial 128 participants, 7 participants dropped out from the Hipcref diet group and 18 participants dropped out from the control diet group due to job relocation (n=9), retirement (n=5), accident (n=2), volunteer withdrawal of consent (n=5) and 4 were lost to follow up

(Figure 6.1). Therefore, the study was completed with 103 participants. Hipcref diet group = 58 participants (female=47 and male=11); control diet group = 45 participants (female=41 and male=4).

7I.3.3 Assessment of anthropometric, dietary, blood biochemical parameters, physical activity level, substance abuse and dietary adherence

Detailed information on the assessment of anthropometric parameters, dietary nutrients analysis, blood biochemical parameters, physical activity levels, substance abuse and dietary adherence have been described in an earlier chapter (Chapter 6).

7I.3.4 Hipcref diet and control diet

Detailed information on the delivery mode of Hipcref diet and control diet have been described in an earlier chapter (Chapter 6).

7I.3.5 DNA extraction and genotyping assay

Detailed information on the DNA extraction and genotyping assay for *FTO* gene variants have been described in an earlier chapter (Chapter 5).

7I.3.6 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). Genotype distributions and allele frequencies of *FTO* rs9930501, rs9930506 and rs9932754 were estimated by gene counting and Chi-square test was used to assess the deviation from Hardy-Weinberg equilibrium (HWE) (Namipashaki et al., 2015). Data were expressed as mean ± standard error (SE) or number (percentage). Log transformation was performed to transform non-normally distributed data into normally distributed data. Data analysis was conducted on an 'intention-to-treat' basis rather than on compliance to treatment (per protocol), meaning all the participants who have been randomised into the two diet groups were included for analysis. For the non-completers, the last observations/measurements on the dietary, anthropometric and blood biochemical parameters were carried forward as the post-intervention measurements for statistical analysis. Linear regression was performed to determine the model best fits the association between *FTO* gene variants and post-intervention difference in body weight.

Chi square test was performed to assess the differences in the categorical variables at baseline, whereas ANCOVA was performed to assess the differences in the continous variables at baseline, after adjusting for age, gender, physical activity status, alcohol consumption and smoking status. The main effects of *FTO* genotype and dietary intervention on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters at month 6 were analysed using general linear regression models, with the adjustment for covariates including age, gender, physical activity status, alcohol consumption, smoking status, and the baseline value for the respective variable. To analyse the potential interactions between genotype and dietary intervention, an interaction product term of *FTO* genotype x dietary group was included in the models. A statistical probability level of $p<0.05$ (two-sided) was considered significant.

7I.4 Results

7I.4.1 Genotype distributions and allele frequencies of *FTO* gene variants

The allele frequencies for minor allele (G) of *FTO* rs9930501 and rs9930506 were 0.40 and 0.39 respectively, and the allele frequency for minor allele (C) of *FTO* rs9932754 was 0.40, which did not deviate from Hardy-Weinberg equilibrium as tested by Chi square test (χ^2=1.84, χ^2= 0.84 and χ^2= 0.98, repectively) (Table 7I.1). Due to the similar findings between the three *FTO* SNPs (rs9930501, rs9930506 and rs9932754) which were in complete linkage disequilibrium (LD), we reported only rs9930506 as the tag SNP for the following analysis.

Table 7I.1: Genotype distributions and allele frequencies of *FTO* gene variants

SNPs	***FTO* rs9930501**			***FTO* rs9930506**			***FTO* rs9932754**		
	Genotype	**Total n=128**	**χ2**	**Genotype**	**Total n=128**	**χ2**	**Genotype**	**Total n=128**	**χ2**
Additive model	**AA**	50 (39.1%)	1.84	**AA**	50 (39.1%)	0.84	**TT**	49 (38.3%)	0.98
	AG	54 (42.2%)		**AG**	56 (43.7%)		**TC**	56 (43.7%)	
	GG	24 (18.7%)		**GG**	22 (17.2%)		**CC**	23 (18.0%)	
Dominant model	**AA**	50 (39.1%)		**AA**	50 (39.1%)		**TT**	49 (38.3%)	
	AG + GG	78 (60.9%)		**AG + GG**	78 (60.9%)		**TC + CC**	79 (61.7%)	
Recessive model	**AA + AG**	104 (81.3%)		**AA + AG**	106 (82.8%)		**TT + TC**	105 (82.0%)	
	GG	24 (18.7%)		**GG**	22 (17.2%)		**CC**	23 (18.0%)	
Allele frequency	**A**	154 (60.2%)		**A**	156 (60.9%)		**T**	154 (60.2%)	
	G	102 (39.8%)		**G**	100 (39.1%)		**C**	102 (39.8%)	

The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with χ^2<3.841 considered as no deviation from Hardy Weinberg Equilibrium.

7I.4.2 Model best fits the association between *FTO* gene variants and post-intervention difference in body weight

Linear regression was performed to determine the model best fits the association between *FTO* gene variants and post-intervention difference in body weight.Based on the linear regression analysis, our results revealed that the additive model of rs9930506 (r^2=0.060, p=0.504) showed the best fit to the post-intervention difference in body weight, compared to the dominant model (r^2=0.050, p=0.577) and the recessive model (r^2=0.049, p=0.583). Therefore, we reported only the additive model for rs9930506 for statistical analysis.

7I.4.3 General characteristics: Differences between the *FTO* genotypes at baseline

The differences in general characteristics between the *FTO* genotypes at baseline are reported in Table 7I.2. No significant difference was found in age (p=0.286), height (p=0.994), body weight (p=0.208) and BMI (p=0.182) between the *FTO* genotypes. There was no significant difference in the distribution of gender (p=0.267), physical activity status (p=0.681), smoking status (p=0.734) and alcohol consumption (p=0.583) between the two genotype groups. However, our results revealed significant difference in the distribution of ethnicity between the *FTO* genotypes (p=0.015). On further analysis, ethnicity of 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Chinese vs Malaysian Malays and Indians) for further analysis. Our results revealed that Chinese had significantly lower frequency of the risk allele (G) of rs9930506 compared to the Malays and Indians combined (41.7% vs 65.4%, p=0.032) (Table 7I.3).

Table 7I.2: General characteristics: Differences between the *FTO* genotypes at baseline

SNPs	***FTO* rs9930506**			
	AA (n=50)	**AG (n=56)**	**GG (n=22)**	**p value**
Age	42.6 ± 1.5	45.9 ± 1.5	42.5 ± 2.4	0.286[a]
Height	157.6 ± 1.0	158.5 ± 1.1	157.5 ± 1.4	0.994[a]
Weight	71.4 ± 1.6	74.4 ± 2.1	78.4 ± 5.0	0.208[a]
BMI	28.8 ± 0.6	29.5 ± 0.7	31.2 ± 1.3	0.182[a]
Gender				
Female	44 (88.0%)	44 (78.6%)	20 (90.9%)	0.267[b]
Male	6 (12.0%)	12 (21.4%)	2 (9.1%)	
Ethnicity				
Malays	26 (52.0%)	20 (35.7%)	11 (50.0%)	**0.015[b]**
Chinese	14 (28.0%)	8 (14.3%)	2 (9.1%)	
Indians	10 (20.0%)	28 (50.0%)	9 (40.9%)	
Physical activity status				
Physical inactive	2 (4.0%)	3 (5.4%)	2 (9.1%)	0.681[b]
Physical active	48 (96.0%)	53 (94.6%)	20 (90.0%)	
Smoking Status				
Never	48 (96.0%)	55 (98.2%)	22 (100.0%)	0.734[b]
Current	1 (2.0%)	0	0	
Former	1 (2.0%)	1 (1.8%)	0	
Alcohol consumption				
Never	49 (98.0%)	55 (98.2%)	22 (100.0%)	0.583 [b]
Current	0	1 (1.8%)	0	
Former	1 (2.0%)	0	0	

Data were expressed as mean (±SE) or number (percentage).
[a] p value based on ANCOVA after adjusting for age, gender, physical activity status, alcohol consumption and smoking status.
[b] p value based on Chi square test.
p<0.05 was considered as significant.

Table 7I.3: Differences in *FTO* genotype distribution between Chinese versus Malays and Indians

***FTO* SNPs**	**rs9930506**		
Ethnicity	**AA (n=50)**	**AG+GG (n=78)**	**p value**
Chinese (n=24)	14 (58.3%)	10 (41.7%)	**0.032***
Malay & Indians (n=104)	36 (34.6%)	68 (65.4%)	

Ethnicity with 3 categories (Malays, Chinese and Indians) was then recoded into 2 categories (Chinese versus Malays and Indians) for further analysis. Data were expressed in total number and percentages. The difference in genotype frequency between ethnic groups was assessed by Chi square test.
*p<0.05 was considered significant.

7I.4.4 Dietary adherence score: Difference between the *FTO* genotypes

No significant difference was found in the adherence score between the *FTO* genotypes (p=0.999) after adjusting for covariates (Table 7I.4).

Table 7I.4: Dietary adherence score: Difference between the *FTO* genotypes

SNPs	***FTO* rs9930506**			
Adherence score	**AA (n=50)**	**AG (n=56)**	**GG (n=22)**	**p value**
Mean (± SE)	36.2 ± 0.8	5.8 ± 0.7	5.8 ± 1.1	0.966
Minimum	1.2	1.1	1.5	-
Maximum	22.7	22.3	22.2	-

p<0.05 was considered as significant based on ANCOVA after adjusting for age, gender, physical activity status, alcohol consumption, smoking status and dietary group.

Table 7I.5: Distribution of *FTO* genotypes: Difference between the intervention diet group and control diet group

Genotypes	**Intervention diet group (n=65)**	**Control diet group (n=63)**	**p value**
AA (n=50)	23 (35.4%)	27 (42.9%)	0.382
AG (n=56)	28 (43.1%)	28 (44.4%)	
GG (n=22)	14 (21.5%)	8 (12.7%)	

p<0.05 was considered as significant based on Chi square test.

7I.4.5 Distribution of *FTO* genotypes: Difference between the intervention diet group and control diet group

No significant difference was found in the distribution of *FTO* genotypes between the intervention diet group and control diet group (p=0.382) (Table 7I.5).

Table 7I.6: Effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in dietary parameters, in response to a 6-month Hipcref diet or control diet

Dietary parameters	Group	Intervention diet group (n=65)			Control diet group (n=63)			p interaction (genotype x intervention group) ǂ
		AA (n=23)	AG (n=28)	GG (n=14)	AA (n=27)	AG (n=28)	GG (n=8)	
Total energy intake (kcal)	**Baseline**	2075 ± 354	1968 ± 411	2039 ± 357	1936 ± 40	2048 ± 74	2013 ± 79	0.443
	Month 6	1637 ± 52	1574 ± 52	1588 ± 50	1901 ± 46	2005 ± 65	2036 ± 77	
	Changes	-438 ± 70	-394 ± 58	-451 ± 64	-36 ± 28	-43 ± 29	23 ± 12	
Protein intake (g)	**Baseline**	78.9 ± 5.3	72.7 ± 4.8	75.7 ± 6.1	74.6 ± 4.0	72.0 ± 5.0	79.5 ± 6.2	0.900
	Month 6	112.5 ± 4.3	104.1 ± 5.0	108.4 ± 3.9	75.1 ± 2.9	75.2 ± 3.9	77.1 ± 3.7	
	Changes	33.7 ± 7.0	31.4 ± 4.7	32.8 ± 6.0	0.5 ± 3.6	3.2 ± 3.6	-2.4 ± 5.4	
Fat intake (g)	**Baseline**	85.7 ± 5.0	84.3 ± 4.0	83.7 ± 6.4	78.1 ± 2.8	80.6 ± 4.1	91.0 ± 9.0	0.727
	Month 6	58.1 ± 2.8	581 ± 3.1	55.7 ± 2.7	71.4 ± 3.3	77.1 ± 3.4	81.2 ± 9.4	
	Changes	-27.6 ± 5.1	-26.2 ± 3.8	-28.0 ± 6.2	-6.7 ± 2.9	-3.5 ± 2.9	-9.7 ± 4.1	
Carbohydrate intake (g)	**Baseline**	254.5 ± 16.1	247.1 ± 14.3	247.3 ± 12.1	244.4 ± 8.8	272.2 ± 12.7	235.5 ± 18.8	0.775
	Month 6	177.8 ± 7.5	172.8 ± 8.0	175.3 ± 8.6	262.3 ± 8.7	269.4 ± 10.2	265.4 ± 12.2	
	Changes	-76.7 ± 13.2	-74.4 ± 12.6	-82.0 ± 10.4	17.9 ± 7.1	-2.8 ± 5.8	30.0 ± 17.1	
Percent energy from protein (%)	**Baseline**	15.5 ± 1.0	14.3 ± 0.7	14.8 ± 0.9	15.5 ± 0.9	13.9 ± 0.7	15.7 ± 1.5	0.885
	Month 6	27.7 ± 0.8	26.8 ± 1.1	27.3 ± 0.5	15.7 ± 05	14.9 ± 0.5	15.2 ± 0.6	
	Changes	12.2 ± 1.3	12.5 ± 1.3	12.6 ± 1.1	0.3 ± 0.8	1.0 ± 0.6	-0.6 ± 1.2	
Percent energy from fat (%)	**Baseline**	37.6 ± 1.7	37.7 ± 1.4	36.4 ± 1.7	36.2 ± 1.0	34.8 ± 1.4	39.6 ± 3.0	0.995
	Month 6	31.4 ± 0.9	33.3 ± 1.2	31.7 ± 1.4	37.7 ± 1.7	37.1 ± 1.5	38.6 ± 3.6	
	Changes	-5.7 ± 1.6	-4.7 ± 1.2	-4.7 ± 2.3	-2.5 ± 1.1	-0.6 ± 1.1	-4.3 ± 2.0	
Percent energy from carbohydrate (%)	**Baseline**	44.7 ± 2.2	46.6 ± 1.6	47.8 ± 2.0	47.3 ± 1.2	49.7 ± 1.5	43.4 ± 3.4	0.628
	Month 6	43.6 ± 0.8	42.8 ± 0.9	44.0 ± 1.5	50.5 ± 2.4	50.1 ± 2.0	48.1 ± 4.1	
	Changes	-1.6 ± 2.0	-3.5 ± 1.5	-3.8 ± 2.0	7.2 ± 1.5	3.3 ± 1.2	8.0 ± 3.6	
SFA (% of TE)	**Baseline**	7.8 ± 0.7	8.2 ± 0.7	8.5 ± 1.4	7.8 ± 0.6	6.2 ± 0.6	11.3 ± 1.5	0.885
	Month 6	7.6 ± 0.4	8.7 ± 0.5	8.2 ± 0.8	8.8 ± 0.7	9.1 ± 0.6	9.3 ± 1.3	
	Changes	-0.2 ± 0.7	0.6 ± 0.6	-0.3 ± 1.7	1.0 ± 0.7	2.9 ± 0.7	-2.0 ± 0.8	
MUFA (% of TE)	**Baseline**	11.7 ± 0.9	12.1 ± 0.9	9.5 ± 1.0	10.4 ± 0.6	10.4 ± 0.8	14.7 ± 1.4	0.584
	Month 6	10.2 ± 0.5	11.2 ± 0.6	9.8 ± 0.8	10.2 ± 0.5	10.9 ± 0.6	11.1 ± 1.2	

	Changes	-1.5 ± 0.8	-0.9 ± 0.8	0.4 ± 1.0	-0.3 ± 0.6	0.5 ± 0.9	-3.5 ± 0.5	
PUFA (% of TE)	**Baseline**	7.5 ± 0.8	6.6 ± 0.7	5.3 ± 0.5	5.8 ± 0.6	6.2 ± 0.6	8.2 ± 1.1	0.503
	Month 6	8.3 ± 0.6	7.9 ± 0.5	7.9 ± 0.6	6.9 ± 0.7	5.9 ± 0.5	7.0 ± 0.8	
	Changes	0.9 ± 0.7	1.3 ± 0.6	2.7 ± 0.7	1.0 ± 0.6	-0.2 ± 0.9	-1.3 ± 0.9	
TFA (% TE)	**Baseline**	0.1 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	0.1 ± 0.02	0.1 ± 0.1	0.2 ± 0.02	0.593
	Month 6	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.02	
	Changes	-0.02 ± 0.04	-0.1 ± 0.02	0.01 ± 0.03	0.1 ± 0.03	0.01 ± 0.1	-0.03 ± 0.02	
Energy-adjusted fibre intake (g)	**Baseline**	5.8 ± 0.6	7.4 ± 0.7	4.9 ± 0.7	4.7 ± 0.5	5.9 ± 0.7	6.5 ± 1.7	0.817
	Month 6	13.2 ± 0.9	137 ± 0.6	135 ± 1.0	8.7 ± 0.7	9.1 ± 0.6	9.6 ± 1.8	
	Changes	7.5 ± 0.9	6.3 ± 0.8	8.6 ± 1.2	4.0 ± 0.6	3.2 ± 1.2	3.2 ± 1.2	
Energy adjusted vitamin E intake (mg)	**Baseline**	6.5 ± 0.8	5.6 ± 0.6	4.6 ± 0.6	6.6 ± 0.5	4.7 ± 0.5	7.8 ± 1.3	0.622
	Month 6	8.2 ± 0.4	8.0 ± 0.5	7.6 ± 0.3	6.5 ± 0.4	6.3 ± 0.4	6.2 ± 0.8	
	Changes	1.6 ± 0.8	2.5 ± 0.5	3.0 ± 0.7	-0.03 ± 0.5	1.5 ± 0.6	-1.6 ± 1.1	

╪ General linear regression model was performed to assess the effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in dietary parameters after adjusting for age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for respective variable.
*$p<0.05$ was considered as significant.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, trans fatty acids; TE, total energy intake.

Table 7I.7: Effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet or control diet

Anthropometric parameters	Group	Intervention diet group (n=65)			Control diet group (n=63)			p interaction (genotype x intervention group) ǂ
		AA (n=23)	AG (n=28)	GG (n=14)	AA (n=27)	AG (n=28)	GG (n=8)	
Weight (kg)	**Baseline**	73.7 ± 2.6	73.6 ± 2.6	76.4 ± 2.2	69.4 ± 1.8	75.2 ± 3.3	82.0 ± 13.7	0.199
	Month 6	70.7 ± 2.7	69.9 ± 2.9	72.7 ± 2.2	70.3 ± 1.8	76.6 ± 3.3	84.3 ± 13.4	
	Changes	-2.9 ± 0.7	-3.8 ± 0.8	-3.7 ± 1.1	1.0 ± 0.4	1.4 ± 0.6	2.3 ± 0.7	
BMI (kg/m2)	**Baseline**	29.7 ± 1.1	29.0 ± 0.9	30.6 ± 0.8	28.0 ± 0.	30.0 ± 1.0	32.2 ± 3.4	0.324
	Month 6	28.4 ± 1.1	27.5 ± 1.0	29.2 ± 0.8	28.4 ± 0.7	30.7 ± 1.0	33.1 ± 3.3	
	Changes	-1.2 ± 0.3	-1.5 ± 0.3	-1.4 ± 0.5	0.4 ± 0.2	0.6 ± 0.3	1.0 ± 0.3	
WC (cm)	**Baseline**	92.7 ± 2.4	92.3 ± 2.2	95.6 ± 0.6	90.0 ± 1.8	97.1 ± 2.2	100.0 ± 8.4	0.464
	Month 6	86.7 ± 2.7	86.3 ± 2.7	88.9 ± 2.9	91.2 ± 1.9	98.7 ± 2.2	102.3 ± 8.2	
	Changes	-5.9 ± 1.1	-6.1 ± 1.0	-7.0 ± 1.2	1.1 ± 0.7	1.5 ± 0.6	2.4 ± 0.7	
WHR	**Baseline**	0.89 ± 0.02	0.91 ± 0.01	0.93 ± 0.9	0.91 ± 0.01	0.94 ± 0.01	0.90 ± 0.02	0.731
	Month 6	0.89 ± 0.01	0.90 ± 0.01	0.92 ± 0.02	0.92 ± 0.01	0.96 ± 0.02	0.93 ± 0.03	
	Changes	-0.01 ± 0.01	-0.01 ± 0.01	-0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	
Muscle mass (kg)	**Baseline**	23.8 ± 1.1	24.1 ± 1.0	23.8 ± 0.6	22.0 ±0.6	23.6 ± 1.1	24.2 ± 3.3	0.133
	Month 6	23.7 ± 1.1	23.7 ± 1.0	22.7 ± 0.7	22.0 ± 0.6	23.6 ± 1.1	24.7 ± 3.3	
	Changes	-0.1 ± 0.2	-0.3 ± 0.3	-0.7 ± 0.4	-0.03 ± 0.3	-0.01 ± 0.2	0.6 ± 0.2	
Fat mass (kg)	**Baseline**	30.2 ± 2.0	30.0 ± 1.7	33.3 ± 1.5	28.9 ± 1.2	32.1 ± 2.1	37.8 ± 8.3	0.286
	Month 6	27.6 ± 2.1	26.5 ± 2.0	30.9 ± 1.4	29.5 ± 1.2	33.6 ± 2.1	39.2 ± 8.2	
	Changes	-2.6 ± 0.7	-3.5 ± 0.8	-2.4 ± 0.8	0.6 ± 0.4	1.4 ± 0.5	1.4 ± 0.5	
Fat-free mass (kg)	**Baseline**	43.5 ± 1.8	43.7 ± 1.6	43.1 ± 1.0	40.6 ± 1.0	43.0 ± 1.8	44.2 ± 5.6	0.297
	Month 6	43.1 ± 1.8	43.2 ± 1.6	41.7 ± 1.1	41.2 ± 1.2	43.0 ± 1.8	45.1 ± 5.4	
	Changes	-0.4 ± 0.3	-0.4 ± 0.5	-1.4 ± 0.8	0.5 ± 0.6	0.01 ± 0.2	0.9 ± 0.3	
Percent body fat	**Baseline**	40.5 ± 1.8	40.5 ± 1.3	43.3 ± 1.0	41.4 ± 0.9	42.2 ± 1.3	44.3 ± 2.4	0.168
	Month 6	38.4 ± 1.9	37.3 ± 1.5	42.4 ± 0.9	41.9 ± 1.1	43.4 ± 1.4	44.8 ± 2.3	
	Changes	-2.2 ± 0.7	-3.2 ± 0.8	-1.0 ± 0.7	0.5 ± 0.5	1.2 ± 0.4	0.5 ± 0.4	
SBP (mmHg)	**Baseline**	120.8 ± 3.0	126.5 ± 2.9	123.6 ± 3.7	120.4 ± 2.9	122.4 ± 2.9	122.7 ± 5.0	0.957
	Month 6	116.4 ± 2.7	120.1 ± 3.1	119.4 ± 3.2	118.9 ± 3.0	118.8 ± 3.0	121.8 ± 4.8	
	Changes	-4.4 ± 2.4	-6.5 ± 2.1	-4.2 ± 3.6	-1.5 ± 2.3	-3.5 ± 1.6	-0.9 ± 3.3	
DBP (mmHg)	**Baseline**	81.0 ± 2.2	81.6 ± 2.2	86.2 ± 2.9	77.6 ± 1.6	80.4 ± 1.8	81.9 ± 3.2	0.260
	Month 6	76.1 ± 2.6	78.9 ± 2.1	79.6 ± 2.2	75.6 ± 1.6	76.6 ± 2.1	84.6 ± 3.1	
	Changes	-5.0 ± 1.4	-2.7 ± 1.6	-6.5 ± 3.1	-2.0 ± 1.3	-3.8 ± 1.3	2.7 ± 2.5	
Pulse rate (bpm)	**Baseline**	76.1 ± 2.7	77.1 ± 2.1	79.1 ± 1.9	77.1 ± 2.3	74.3 ± 2.2	75.8 ± 1.5	0.241
	Month 6	72.6 ± 3.2	74.4 ± 2.3	70.1 ± 2.8	71.8 ± 2.3	72.3 ± 2.2	74.4 ± 2.1	

	Changes	-3.5 ± 2.4	-2.8 ± 2.1	-9.0 ± 2.8	-5.3 ± 22.0	-2.0 ± 1.6	-1.4 ± 1.6	
Blood biochemical parameters	**Group**	**AA (n=23)**	**AG (n=28)**	**GG (n=14)**	**AA (n=27)**	**AG (n=28)**	**GG (n=8)**	**p interaction (genotype x intervention group) ╪**
Fasting glucose (mmol/L)	**Baseline**	5.1 ± 0.2	5.2 ± 0.3	5.2 ± 0.3	4.9 ± 0.2	5.6 ± 0.7	5.6 ± 0.6	0.603
	Month 6	5.2 ± 0.3	5.1 ± 0.4	5.2 ± 0.3	5.0 ± 0.2	5.8 ± 0.5	5.5 ± 0.4	
	Changes	0.1 0.2	-0.2 ± 0.4	-0.1 ± 0.3	0.2 ± 0.3	0.1 ± 0.3	-0.1 ± 0.4	
Fasting insulin (uU/mL)	**Baseline**	11.4 ± 1.9	12.0 ± 2.9	12.2 ± 2.9	7.1 ± 1.4	8.1 ± 0.8	13.5 ± 3.1	0.302
	Month 6	8.3 ± 0.9	7.8 ± 1.1	9.5 ± 1.6	11.2 ± 2.4	13.0 ± 1.4	17.9 ± 2.9	
	Changes	-3.0 ± 1.8	-4.2 ± 2.1	-2.7 ± 2.2	4.1 ± 1.3	4.9 ± 1.4	4.4 ± 1.8	
HOMA-IR	**Baseline**	2.6 ± 0.5	3.4 ± 1.0	3.1 ± 1.0	1.5 ± 0.3	1.9 ± 0.2	3.7 ± 1.4	0.160
	Month 6	1.9 ± 0.2	2.0 ± 0.4	2.3 ± 0.4	2.7 ± 0.7	3.4 ± 0.5	4.6 ± 1.1	
	Changes	-0.7 ± 0.4	-1.4 ± 0.8	-0.9 ± 0.8	1.2 ± 0.5	1.5 ± 0.4	0.9 ± 0.6	
TC (mmol/L)	**Baseline**	5.7 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	5.5 ± 0.2	5.5 ± 0.3	5.9 ± 0.3	0.658
	Month 6	5.5 ± 0.2	5.4 ± 0.2	5.0 ± 0.3	5.4 ± 0.2	5.4 ± 0.2	5.2 ± 0.2	
	Changes	-0.2 ± 0.1	-0.1 ± 0.2	0.3 ± 0.3	-0.1 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.2	
Triglyceride (mmol/L)	**Baseline**	1.6 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	0.263
	Month 6	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	1.7 ± 0.2	1.1 ± 0.2	
	Changes	-0.02 ± 0.01	-0.04 ± 0.3	0.2 ± 0.1	0.01 ± 0.04	0.2 ± 0.1	0.2 ± 0.1	
HDL-C (mmol/L)	**Baseline**	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	0.317
	Month 6	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.3 ± 0.04	1.3 ± 0.1	
	Changes	-0.2 ± 0.03	-0.04 ± 0.1	-0.2 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.03	-0.2 ± 0.1	
LDL-C (mmol/L)	**Baseline**	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.1	3.2 ± 0.2	3.4 ± 0.2	3.8 ± 0.2	0.904
	Month 6	3.4 ± 0.2	3.2 ± 0.2	3.0 ± 0.3	3.3 ± 0.2	3.3 ± 0.2	3.4 ± 0.2	
	Changes	-0.1 ± 0.1	-0.1 ± 0.1	-0.3 ± 0.3	0.1 ± 0.1	-0.1 ± 0.1	-0.4 ± 0.2	
TC/HDL-C	**Baseline**	3.8 ± 0.2	3.9 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	4.0 ± 0.2	3.9 ± 0.1	0.361
	Month 6	4.2 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	3.6 ± 0.2	4.3 ± 0.2	4.0 ± 0.2	
	Changes	0.3 ± 0.1	0.03 ± 0.3	0.2 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.2	
hsCRP (mg/L)	**Baseline**	4.3 ± 0.9	5.6 ± 1.3	9.9 ± 3.3	3.1 ± 0.9	8.2 ± 2.1	3.0 ± 0.6	0.340
	Month 6	3.8 ± 0.8	2.8 ± 0.7	6.8 ± 2.0	3.6 ± 0.9	8.0 ± 1.5	3.9 ± 1.5	
	Changes	0.2 ± 1.0	-2.5 ± 0.8	-1.9 ± 1.3	0.7 ± 0.5	-0.2 ± 0.6	0.9 ± 1.2	

╪ General linear regression model was performed to assess the effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet or control diet, after adjusting for age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable.

*p<0.05 was considered as significant.

BMI, Body mass index; WC, Waist circumference; WHR, Waist-hip ratio; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low –density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment -insulin resistance; hsCRP, high-sensitivity C-reactive protein.

7I.4.6 Effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet or control diet

General linear regression analysis revealed no significant effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet or control diet, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable ($p>0.05$) (Tables 7I.6 & 7I.7).

7I.5 Discussion

In Chapter 6, we reported that the participants following Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the control diet group who received generalised dietary advice based on Malaysian Dietary Guidelines 2010. Participants following Hipcref diet had successfully reduced 3.5kg of body weight and lost 2.3% of percent body fat in 6 months, which translated to a reduction of 0.6kg of body weight and 0.4% of percent body fat per month. With respect to per day intakes, participants on Hipcref diet had, reduced energy intake, higher percent energy from protein, lower percent energy from fat, lower percent energy from carbohydrate, higher percent energy from PUFA, higher vitamin E and higher fibre intake. We opined that the success of the Hipcref diet may be due to the combined effect of the above dietary composition of the macro and micronutrients of the Hipcref diet.

Earlier evidence has suggested that genetic variations may modulate the effect of dietary intervention on weight loss outcomes. Individuals with different genetic background may respond differently to the same intervention. Therefore, in this chapter, we aimed to further analyse the response of the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet intervention, in overweight and obese Malaysian adults.

7I.5.1 *FTO* gene variants and post-intervention differences in dietary parameters

Our results revealed no significant effect of the interaction between *FTO* genotype x dietary group on the post-intervention difference in dietary parameters, after 6 months Hipcref diet intervention, in overweight and obese Malaysian adults. A previous large-scale meta-analysis of 177 330 adults from 40 studies reported that the presence of risk allele (A) of *FTO* rs9930609 was associated with higher dietary protein intake and lower carbohydrate intake compared to the non-carriers (Qi et al., 2014). Similar finding was reported in another study where the risk allele carriers of *FTO* rs1421085 (C) were associated with higher dietary protein intake, independent of BMI (Tanaka et al., 2013). It should be noted that the current study is an intervention programme with a formulated diet which was designed for the participants to consume formulated quantity of protein per day. Therefore, the current study differs from Qi et al., 2014 and Tanaka et al., 2013 where dietary intake was computed retrospectively. Given that the food choices are affected by environment, traditions, behaviour and stress, it is not surprising that no significant association was found between *FTO* gene variants and post-intervention differences in energy and macro/micronutrient intake.

7I.5.2 *FTO* gene variants and post-intervention differences in anthropometric and cardio-metabolic parameters

Several *FTO* gene variants (such as rs9930609, rs1558902 and rs8050136) have been explored in gene-diet interaction studies (Zhang et al., 2012; De Luis et al., 2015; Mitchell et al., 2010). However, to date, no study has investigated the interaction between *FTO* rs9930501, rs9930506 and rs9932754 and dietary intervention on the post-intervention differences in obesity-related phenotypes. In the current study, we found no significant effect of the interaction between *FTO* genotype x dietary group on the post-intervention differences in obesity-related anthropometric and blood biochemical parameters, after 6 months Hipcref diet intervention.

Our findings are in line with a recent systematic review and meta-analysis of 9563 participants involving 8 randomised controlled trials which reported that the post-intervention differences in body weight, waist circumference and adiposity after weight loss interventions were not significantly different between the minor allele (A) and major allele carriers (T) of *FTO* rs9939609 (Livingstone et al., 2016). The authors reported that individuals carrying the minor allele of *FTO*

gene variants responded equally well to dietary, physical activity or drug based weight loss interventions, regardless of the intervention length, age, ethnicity, sample size, gender, and baseline body mass index. Moreover, no significant association between rs9939609 and weight loss or post-intervention differences in metabolic risk factors was found in other populations such as obese Japanese and obese Spanish women after a calorie-restricted dietary intervention (Labayen et al., 2015; Matsuo et al., 2012). However, Zhang et al., 2012 reported that individuals carrying the risk allele (A) of *FTO* rs1558902 may have positive impact on weight loss, body composition and fat distribution after a high-protein diet compared to low-protein diet in an overweight and obese American population.

7I.5.3 *FTO* gene variants and insulin resistance

In the current study, we found no significant effect of the interaction between *FTO* genotype x dietary group on the post-intervention difference in insulin resistance after 6 months of dietary intervention. Similar finding was found in a 2-year POUNDS LOST trial which reported no significant difference in the improvements of body weight and insulin sensitivity were observed between the risk allele carriers (A) and the non-risk allele carriers (T) of *FTO* rs9939609 in an American population (Zheng et al., 2015). However, a 10-week randomised controlled trial, NUGENOB, conducted by Grau et al., 2009, reported that TT genotype carriers of the same gene and SNP had greater reduction in HOMA-IR and HOMA-β after 10 weeks of low fat diet compared to high fat diet in the European population. The beneficial effect of low fat diet on insulin sensitivity was not found in the risk allele carriers (AA) (K Grau et al., 2009). It is interesting to note that the average age of the NUGENOB participants were significantly younger (age ranged 20-50 years) compared to the POUNDS LOST participants, 50.1 years (±9.9 SD) (age ranged 20-70 years). Similar to POUNDS LOST participants, our study participants had a higher average age of 44 years (age ranged 18-70 years). Therefore, such differences in the gene-diet interactions may be explained by the advanced age in our participants and POUNDS LOST participants.

7I.5.4 Mechanism of *FTO* gene variants

Mechanism of action of *FTO* in mediating satiety and hunger signals is yet to be elucidated. It is known that *FTO* is a DNA/RNA demethylase which influences splicing, stability and export of mRNA (Lesbirel & Wilson, 2018). Animal studies provided evidence that *FTO* is expressed in the

arcuate nucleus of the hypothalamus and is associated with appetite and energy intake. A study conducted by Karra et al., 2013, showed that *FTO* gene variants were associated with obesity and obesity-prone behaviour due to higher level of circulating 'hunger hormone', ghrelin which increases food intake and a preference for energy-dense foods. A new mechanism was proposed, that rather than affecting *FTO* itself, *FTO* SNPs may remotely alter the expression of other downstream genes such as genes involved in body mass regulation by changes in epigenetic mechanisms including methylation of other genes (Almén et al., 2012; Zhou et al., 2017). The presence of *FTO* variants increased adipocyte-specific expression of IRX3 and IRX5 during early adipocyte differentiation, causing a reduction in mitochondrial thermogenesis and increased lipid storage (Claussnitzer et al., 2015).

Interestingly, a recent study by Kalantari et al., 2018 reported that a complete linkage disequilibrium (LD) was found between the three neighbouring SNPs of intron 1 region of the *FTO* gene (rs9930501, rs9930506 and rs9932754). This haplotype in the first intron of the *FTO* gene (rs9930501, rs9930506 and rs9932754) with complete linkage disequilibrium (LD), had a strong association with obesity indices (e.g. BMI and body fat percent) in Iranian male adolescents (Kalantari et al., 2018). The authors suggested that the effect of *FTO* gene (rs9930501, rs9930506 and rs9932754) on obesity-related anthropometric variables was exerted through haplotypes (and not single SNPs). This finding was supported by an earlier study which reported that the haplotype in the first intron of the *FTO* gene (rs9930501, rs9930506 and rs9932754) was highly associated with increased odds of obesity (in BMI) and body fat percent in adolescent boys even after the adjustment for age, total energy intake and physical activity (Kalantari et al., 2018). The authors suggested that *FTO* gene polymorphisms (rs9930501, rs9930506 and rs9932754) may exert their effects on obesity indices as a haplotype, and not as single SNPs.

7I.6 Summary

The aim of this section is to investigate the response of the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, after a 6-month Hipcref diet intervention, in overweight and obese Malaysian adults. *FTO* rs9930501, rs9930506 and rs9932754 gene variants were genotyped. The post-intervention differences in dietary intakes, anthropometric parameters, blood lipids levels, fasting insulin and glucose levels and hsCRP levels were assessed by *FTO* genotypes. General

linear regression analysis revealed no significant effect of interaction between *FTO* genotype x dietary group on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable. Our results suggest that the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 do not lead to greater or lesser weight loss or post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to dietary intervention.

Chapter 7- Section-II: *ADRB2* gene

7II.1 Introduction

Obesity is caused by positive energy balance when energy consumption exceeds energy expenditure and resulted in excess body fat storage (Spiegelman & Flier, 2001). Energy expenditure is an important component in energy balance system and hence affects the regulation of body weight. The components of total energy expenditure are i) obligatory energy expenditure which required to perform cellular and organ functions; ii) adaptive thermogenesis which induced by diet or cold exposure and iii) physical activity (Lowell & Bachman, 2003). At the cellular level, biochemical processes involved in energy expenditure that happen in the mitochondria hold a key to the modulation of adaptive thermogenesis in order to expend excess energy and prevent obesity. Therefore, identification of target tissue and intracellular mechanisms mediating adaptive thermogenesis is an area of intense interest.

The sympathetic nervous system is the efferent pathway to the brain in controlling adaptive thermogenesis. The evidence for this is as follows: i) cold exposure and diet increase sympathetic nerve activity, ii) exogenous administration of neurotransmitters (norepinephrine and epinephrine) stimulate energy expenditure, and iii) the thermogenic target tissue, brown adipose tissue is heavily innervated by sympathetic nerves. *ADRB2* gene coding for beta-2 adrenergic receptor has received significant attention in this respect. These receptors are part of the adrenergic system, which transmit the thermogenic signal to peripheral target tissues to stimulate thermogenesis. It stimulates lipid mobilisation in adipose tissue through the action of catecholamines (Kurokawa et al., 2008).

Previous studies reported that no significant interaction was observed between *ADRB2* gene variants (rs1042714 and rs1042713) and weight loss outcomes after a low fat dietary intervention or a combination of dietary and exercise intervention in overweight and obese Americans and Brazilian populations (Saliba et al., 2014; Gardner et al., 2018). However, other study reported that individuals carrying risk allele (G) of *ADRB2* rs1042714 had greater reduction in body weight compared to the non- carriers (CC), after 3 months of energy-restricted diet, in obese Spanish women (Ruiz et al., 2011). To the best of our knowledge, there is no study investigating the effect of *ADRB2* gene variants on weight loss outcomes in the Malaysian population.

7II.2 Objectives

The aim of this section is to investigate the response of *ADRB2* rs1042713 and rs1042714 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults, after a 6-month Hipcref diet intervention.

7II.3 Methodology

7II.3.1 Study design

The detailed information on the study design and methods have been described in an earlier chapter (Chapter 6). Hipcref dietary intervention is a 6-month, parallel-arm, randomised controlled trial, which assessed the effect of a 6-month of Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) diet on dietary, anthropometric and cardio-metabolic parameters, compared to the control group who received generalised dietary instruction on weight loss based on Malaysian Dietary Guidelines 2010, in overweight and obese Malaysian adults.

7II.3.2 Participant selection

Detailed information on the participant selection have been described in an earlier chapter (Chapter 6). Briefly, a total of 128 eligible, apparently healthy overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above with a BMI≥23kg/m^2 were recruited to participate in this randomised dietary intervention. Participants (n=128) were randomly assigned to one of the two treatments: intervention diet group (Hipcref diet, n=65) or control diet group (control diet, n=63), using covariate adaptive randomisation technique. Eligible participants were stratified based on two covariate stratum a) by gender (female and male); b) by ethnicity (Malaysian Chinese, Malays and Indians), thus a total of 6 (2x3) strata was constructed.

Of the initial 128 participants, 7 participants dropped out from the Hipcref diet group and 18 participants dropped out from the control diet group due to job relocation (n=9), retirement (n=5), accident (n=2), volunteer withdrawal of consent (n=5) and 4 were lost to follow up (Figure 6.1). Therefore, the study was completed with 103 participants. Hipcref diet group = 58 participants (female=47 and male=11); control diet group = 45 participants (female=41 and male=4).

7II.3.3 Assessment of anthropometric, dietary, blood biochemical parameters, physical activity level, substance abuse and dietary adherence

Detailed information on the assessment of anthropometric parameters, dietary nutrients analysis, blood biochemical parameters, physical activity levels, substance abuse and dietary adherence have been described in an earlier chapter (Chapter 6).

7II.3.4 Hipcref diet and control diet

Detailed information on the delivery mode of Hipcref diet and control diet have been described in an earlier chapter (Chapter 6).

7II.3.5 DNA extraction and genotyping assay

Detailed information on the DNA extraction and genotyping assay for *ADRB2* gene variants have been described in an earlier chapter (Chapter 5).

7II.3.6 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). Genotype distributions and allele frequencies of *ADRB2* rs1042713 and rs1042714 were estimated by gene counting and Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE) (Namipashaki et al., 2015). Data were expressed as mean ± standard error (SE) or number (percentage). Log transformation was performed to transform non-normally distributed data into normally distributed data. Data analysis was conducted on an 'intention-to-treat' basis rather than on compliance to treatment (per protocol), meaning all the participants who have been randomised into the two diet groups were included for analysis. For the non-completers, the last observations/measurements on the dietary, anthropometric and blood biochemical parameters were carried forward as the post-intervention measurements for statistical analysis. Linear regression was performed to determine the model best fits the association between *ADRB2* gene variants and post-intervention difference in body weight.

Chi square test was performed to assess the differences in the categorical variables at baseline, whereas ANCOVA was performed to assess the differences in the continous variables at baseline,

after adjusting for age, gender, physical activity status, alcohol consumption and smoking status. The main effects of *ADRB2* genotype and dietary intervention on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters at month 6 were analysed using general linear regression models, with the adjustment for covariates including age, gender, physical activity status, alcohol consumption, smoking status, and the baseline value for the respective variable. To analyse the potential interactions between genotype and dietary intervention, an interaction product term of *ADRB2* genotype x dietary group was included in the models. Bonferroni correction was applied to account for muliple comparisons and a Bonferroni corrected p value of <0.025 ($\alpha = 0.05/2$) was considered significant.

7II.4 Results

7II.4.1 Genotype distributions and allele frequencies of *ADRB2* rs1042713 and rs1042714

In the total population, the minor allele frequencies of *ADRB2* rs1042713 (A allele) and *ADRB2* rs1042714 (G allele) were 0.48 and 0.13, respectively, which did not deviate from Hardy-Weinberg equilibrium as tested by Chi square test (χ^2= 0.03 and χ^2= 0.79, respectively) (Table 7II.1).

Table 7II.1: Genotype distributions and allele frequencies of *ADRB2* rs1042713 and rs1042714

SNPs	***ADRB2* rs1042713**			***ADRB2* rs1042714**		
	Genotype	**Total n=128**	**χ2**	**Genotype**	**Total n=128**	**χ2**
Additive model	**AA**	30 (23.4%)	0.03	**CC**	96 (75.0%)	0.79
	AG	63 (49.3%)		**CG**	31 (24.2%)	
	GG	35 (27.3%)		**GG**	1 (0.8%)	
Dominant model	**AA**	30 (23.4%)		**CC**	96 (75.0%)	
	AG+ GG	98 (76.6%)		**CG + GG**	32 (25.0%)	
Recessive model	**AA + AG**	93 (72.7%)		**CC + CG**	127 (99.2%)	
	GG	35 (27.3%)		**GG**	1 (0.8%)	
Allele frequency	**A**	123 (48.0%)		**C**	223 (87.1%)	
	G	133 (52.0%)		**G**	33 (12.9%)	

The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with χ^2<3.841 considered as no deviation from Hardy Weinberg Equilibrium.

7II.4.2 Model best fits the association between *ADRB2* gene variants and post-intervention difference in body weight

Linear regression was performed to determine the model best fits the association between *ADRB2* gene variants and post-intervention difference in body weight. Based on the linear regression analysis, our results revealed that dominant models of both rs1042713 and rs1042714 (r^2=0.072,

p=0.421 and r^2=0.051, p=0.571, respectively) showed the best fit to the post-intervention differences in body weight, compared to the additive models (r^2=0.051, p=0.565 and r^2=0.042, p=0.638, respectively) and the recessive models (r^2=0.014, p=0.878 and r^2=0.032, p=0.723, respectively). Therefore, we reported only the dominant models for both *ADRB2* rs1042173 and rs1042714 for statistical analysis.

Table 7II.2: General characteristics: Differences between the *ADRB2* genotypes

SNPs	***ADRB2* rs1042713**			***ADRB2* rs1042714**		
	AA (n=30)	**AG+GG (n=98)**	**p value**	**CC (n=96)**	**CG+GG (n=32)**	**p value**
Age	46.4 ± 1.7	43.3 ± 1.1	0.164	44.0 ± ± 1.1	43.9 ± 1.9	0.780[a]
Height	158.5 ± 1.4	157.8 ± 0.7	0.756	158.2 ± 0.8	157.1 ± 1.3	0.297[a]
Weight	72.0 ± 1.9	74.5 ± 1.7	0.276	74.2 ± 1.8	73.1 ± 1.9	0.780[a]
BMI	28.6 ± 0.6	29.8 ± 0.5	0.311	29.5 ± 0.7	29.6 ± 0.7	0.880[a]
Gender						
Female	23 (76.7%)	85 (86.7%)	0.184	81 (84.4%)	27 (84.4%)	1.000[b]
Male	7 (23.3%)	13 (13.3%)		15 (15.6%)	5 (15.6%)	
Ethnicity						
Malays	13 (43.3%)	44 (44.9%)	0.978	43 (44.8%)	14 (43.8%)	0.201[b]
Chinese	6 (20.0%)	18 (18.4%)		21 (21.9%)	3 (9.4%)	
Indians	11 (36.7%)	36 (36.7%)		32 (33.3%)	15 (46.8%)	
Physical activity status						
Physical inactive	27 (90.0%)	94 (95.9%)	0.212	91 (94.8%)	30 (93.8%)	0.822[b]
Physical active	3 (10.0%)	9 (4.3%)		5 (5.2%)	2 (6.2%)	
Smoking Status						
Never	30 (100%)	95 (98%)	0.625	94 (97.9%)	31 (96.9%)	0.160[b]
Current	0	1 (1.0%)		0	1 (3.1%)	
Former	0	2 (2%)		2 (2.1%)	0	
Alcohol consumption						
Never	30 (100%)	96 (98.0%)	0.733	94 (98.0%)	32 (100%)	0.713
Current	0	1 (1.0%)		1 (1.0%)	0	
Former	0	1 (1.0%)		1 (1.0%)	0	

Data were expressed as mean (±SE) or number (percentage).
[a] p value based on ANCOVA after adjusting for age, gender, physical activity status, alcohol consumption and smoking status.
[b] p value based on Chi square test.
p<0.025 was considered as significant after applied for Bonferroni correction.

7II.4.3 General characteristics: Differences between the *ADRB2* genotypes

No significant difference was found in age, height, body weight and BMI between the two genotype groups of *ADRB2* rs1042713 (AG+GG vs AA) and *ADRB2* rs1042714 (CG+GG vs CC) at baseline (p>0.05) (Table 7II.2). There was no significant difference in the distribution of ethnicity, physical activity status, smoking status and alcohol consumption between the two genotype groups of *ADRB2* rs1042713 (AG+GG vs AA) and *ADRB2* rs1042714 (CG+GG vs CC) (p>0.05).

7II.4.4 Dietary adherence score: Difference between the *ADRB2* genotypes

No significant difference was found in the adherence score between the *ADRB2* genotypes for rs1042713 (p=0.911) and rs1042714 (p=0.329), after 6 months of dietary intervention (Table 7II.3).

Table 7II.3: Dietary adherence score: Difference between the *ADRB2* genotypes

SNPs	***ADRB2* rs1042713**			***ADRB2* rs1042714**		
Adherence score	**AA (n=30)**	**AG+GG (n=98)**	**p value**	**CC (n=96)**	**CG+GG (n=32)**	**p value**
Mean (± SE)	5.5 ± 0.8	6.1 ± 0.6	0.911	5.8 ± 0.5	6.6 ± 1.0	0.329
Minimum	1.1	1.2	-	1.1	1.5	-
Maximum	19.9	22.7	-	22.3	22.7	-

p<0.025 was considered as significant based on ANCOVA after adjusting for age, gender, physical activity status, alcohol consumption and smoking status.

Table 7II.4: Distribution of *ADRB2* genotypes: Difference between the intervention diet group and control diet group

SNPs	**Genotypes**	**Intervention diet group (n=65)**	**Control diet group (n=63)**	**p value**
rs1042713	**AA (n=30)**	16 (24.6%)	14 (22.2%)	0.749
	AG+GG (n=98)	49 (75.4%)	49 (77.8%)	
rs1042714	**CC (n=96)**	53 (81.5%)	43 (68.3%)	0.083
	CG+GG (n=32)	12 (18.5%)	20 (31.7%)	

p<0.05 was considered as significant based on Chi square test.

7I.4.5 Distribution of *ADRB2* genotypes: Difference between the intervention diet group and control diet group

No significant difference was found in the distribution of *ADRB2* genotypes between the intervention diet group and control diet group (rs1042713; p=0.749 and rs1042714; p=0.083) (Table 7I.4).

7I.4.6 Effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet intervention

Bonferroni correction was applied to account for multiple comparisons (α=0.05/2=0.025). General linear regression analysis revealed significant effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in SFA intake (p interaction =0.007) and MUFA intake (p interaction =0.015), in response to a 6-month Hipcref diet intervention, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for respective variable (Table 7II.5). Individuals carrying the risk

allele of rs1042713 (AG+GG) had significantly greater reduction in SFA intake after a Hipcref diet compared to a control diet (p=0.006) (Figure 7.1a). Furthermore, individuals carrying the risk allele of rs1042713 (AG+GG) had significantly greater reduction in MUFA intake after a Hipcref diet compared to the individuals carrying the non-risk allele (AA) (p=0.019) (Figure 7.1b).

With respect to cardio-metabolic parameters, our results revealed significant effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in fasting insulin (p interaction =0.012) and HOMA-IR (p interaction =0.021), in response to a 6-month Hipcref diet intervention, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable (Table 7II.6). Individuals carrying both the risk allele (CG+GG) and the non-risk allele (CC) of rs1042714 had significantly greater reduction in fasting insulin (p<0.001 and p=0.001, respectively) (Figure 7.2a) and HOMA-IR (p<0.001 and p=0.006, respectively) (Figure 7.2b) after a Hipcref diet compared to a control diet, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable. No significant effect of the interaction between *ADRB2* genotype x dietary group was found in other anthropometric and cardio-metabolic parameters (p>0.05).

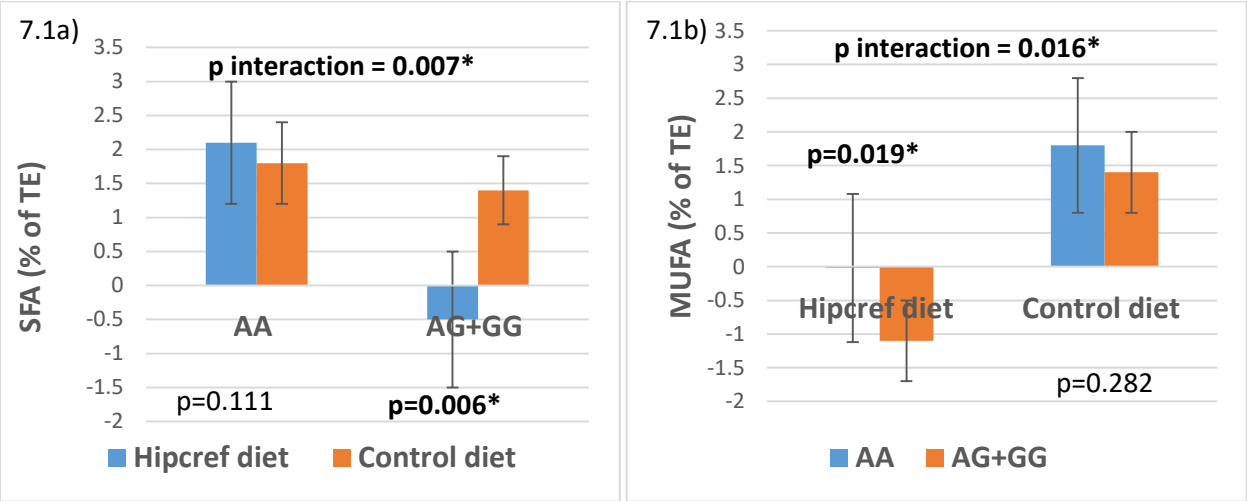


Figure 7.1: Effect of the interaction between *ADRB2* genotype (rs1042713) x dietary group on the post-intervention differences in (a) SFA and (b) MUFA intake, in response to Hipcref diet vs control diet.
Age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for SFA were adjusted for.
*p<0.025 was considered significant was based on general linear regression model.

Table 7II.5: Effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention difference in dietary parameters, in response to a 6-month Hipcref diet or control diet

SNPs		*ADRB2* rs1042713					*ADRB2* rs1042714				
Dietary parameters	**Group**	**Intervention diet group (n=65)**		**Control diet group (n=63)**		**p interaction (genotype x intervention group) ǂ**	**Intervention diet group (n=65)**		**Control diet group (n=63)**		**p interaction (genotype x intervention group) ǂ**
		AA (n=16)	**AG+GG (n=49)**	**AA (n=14)**	**AG+GG (n=49)**		**CC (n=53)**	**CG+GG (n=12)**	**CC (n=43)**	**CG+GG (n=20)**	
Total energy intake (kcal)	**Baseline**	2085 ± 121	2000 ± 49	2019 ± 80	1989 ± 44	0.067	1994 ± 52	2143 ± 111	2011 ± 49	1962 ± 62	0.204
	Month 6	1616 ± 59	1593 ± 36	2057 ± 81	1938 ± 40		1609 ± 36	1556 ± 60	1997 ± 49	1937 ± 46	
	Changes	-469 ± 89	-407 ± 41	38 ± 25	-51 ± 21		-385 ± 81	-587 ± 81	-34 ± 22	-27 ± 28	
Protein intake (g)	**Baseline**	74.3 ± 6.1	75.9 ± 3.5	74.2 ± 4.7	74.1 ± 3.5	0.311	76.5 ± 3.5	71.4 ± 5.3	76.4 ± 3.5	69.2 ± 4.9	0.726
	Month 6	107.7 ± 6.4	108.1 ± 3.1	76.6 ± 3.8	75.1 ± 2.6		107.4 ± 3.2	110.7 ± 5.5	75.4 ± 2.6	75.4 ± 3.1	
	Changes	33.4 ± 6.2	32.2 ± 4.0	2.4 ± 5.0	1.0 ± 2.6		31.0 ± 3.9	39.3 ± 5.9	-0.9 ± 3.0	6.2 ± 3.4	
Fat intake (g)	**Baseline**	85.1 ± 6.0	84.5 ± 3.2	85.8 ± 5.0	79.4 ± 2.8	0.493	81.0 ± 2.7	100.9 ± 7.8	81.0 ± 3.2	80.4 ± 3.8	0.077
	Month 6	60.2 ± 4.3	56.8 ± 1.9	82.6 ± 5.7	73.0 ± 2.5		58.1 ± 2.1	55.6 ± 2.1	75.4 ± 2.6	73.6 ± 3.3	
	Changes	-24.9 ± 5.4	-27.8 ± 3.2	-3.2 ± 3.1	-6.4 ± 2.2		-22.9 ± 2.6	-45.3 ± 7.8	-5.1 ± 2.2	-6.9 ± 3.5	
Carbohydrate intake (g)	**Baseline**	267.1 ± 22.4	247.0 ± 8.9	251.2 ± 16.7	256.9 ± 8.2	0.148	250.4 ± 10.1	258.9 ± 15.3	257.2 ± 9.0	252.3 ± 12.8	0.567
	Month 6	173.8 ± 8.8	175.6 ± 5.5	272.2 ± 13.1	264.0 ± 6.8		176.7 ± 5.5	168.7 ± 7.7	266.4 ± 6.8	264.7 ± 12.2	
	Changes	-93.3 ± 19.3	-71.4 ± 7.5	21.0 ± 9.0	7.1 ± 5.5		-73.7 ± 8.6	-90.7 ± 13.4	9.2 ± 6.1	12.4 ± 7.1	
Percent energy from protein (%)	**Baseline**	14.2 ± 0.7	15.0 ± 0.6	14.6 ± 1.1	14.9 ± 0.6	0.815	15.2 ± 0.6	13.1 ± 0.9	15.1 ± 0.6	14.1 ± 1.0	0.266
	Month 6	26.8 ± 1.3	27.4 ± 0.6	14.7 ± 0.5	15.5 ± 0.4		26.9 ± 0.7	28.4 ± 0.6	15.2 ± 0.3	15.6 ± 0.8	
	Changes	12.5 ± 1.5	12.4 ± 0.9	0.1 ± 1.0	0.6 ± 0.5		11.8 ± 0.8	15.3 ± 1.3	0.03 ± 0.6	1.5 ± 0.7	
Percent energy from fat (%)	**Baseline**	36.8 ± 2.1	37.5 ± 1.0	37.8 ± 1.5	35.4 ± 1.0	0.737	36.5 ± 1.0	41.3 ± 2.0	35.6 ± 1.1	36.7 ± 1.4	0.299
	Month 6	33.0 ± 1.5	32.1 ± 0.7	37.1 ± 1.8	37.7 ± 1.3		32.3 ± 0.8	32.3 ± 1.1	37.3 ± 1.5	38.1 ± 2.1	
	Changes	-3.5 ± 1.8	-5.6 ± 1.0	-2.0 ± 1.3	-1.9 ± 0.9		-4.2 ± 1.0	-8.9 ± 2.2	-1.6 ± 0.9	-2.5 ± 1.4	
Percent energy from carbohydrate (%)	**Baseline**	47.6 ± 2.0	45.7 ± 1.3	46.1 ± 1.9	48.4 ± 1.1	0.922	47.1 ± 1.2	42.2 ± 2.9	47.7 ± 1.2	48.2 ± 1.8	0.436
	Month 6	42.9 ± 1.4	43.5 ± 0.6	51.0 ± 2.3	49.7 ± 1.7		43.4 ± 0.7	43.2 ± 0.9	50.1 ± 1.7	49.7 ± 2.8	
	Changes	-5.0 ± 2.0	-2.2 ± 1.2	6.2 ± 2.1	5.4 ± 1.1		-3.8 ± 1.0	1.0 ± 3.0	5.7 ± 1.2	5.4 ± 1.6	
SFA (% of TE)	**Baseline**	7.6 ± 1.0	8.3 ± 0.6	6.4 ± 0.8	7.8 ± 0.6	**0.007***	8.1 ± 0.5	8.3 ± 1.1	7.6 ± 0.6	7.2 ± 0.8	0.501
	Month 6	9.6 ± 0.8	7.7 ± 0.3	8.2 ± 1.1	9.2 ± 0.5		8.2 ± 0.4	8.4 ± 0.7	8.6 ± 0.5	9.9 ± 0.7	
	Changes	2.1 ± 0.9	-0.5 ± 0.6	1.8 ± 1.0	1.4 ± 0.5		0.1 ± 0.6	0.1 ± 1.2	0.9 ± 0.6	2.7 ± 0.8	

MUFA (% of TE)	**Baseline**	12.0 ± 1.2	11.2 ± 0.6	11.5 ± 1.0	10.8 ± 0.6	**0.016***	11.6 ± 00.6	110.6 ± 1.5	11.0 ± 0.6	10.8 ± 0.9	0.807
	Month 6	12.0 ± 0.9	10.1 ± 0.3	10.1 ± 0.7	10.8 ± 0.4		10.5 ± 0.4	10.6 ± 0.7	10.4 ± 0.4	11.0 ± 0.7	
	Changes	-0.02 ± 1.1	-1.1 ± 0.6	-1.4 ± 1.0	-0.03 ± 0.6		-1.0 ± 0.6	0.01 ± 1.1	-0.6 ± 0.7	0.2 ± 0.8	
PUFA (% of TE)	**Baseline**	6.8 ± 0.8	6.6 ± 0.5	8.1 ± 1.1	5.8 ± 0.4	0.221	6.4 ± 0.4	7.5 ± 1.8	6.5 ± 0.5	5.9 ± 0.6	0.470
	Month 6	7.3 ± 0.6	8.3 ± 0.4	7.7 ± 1.0	6.1 ± 0.4		8.1 ± 0.3	8.0 ± 0.9	6.7 ± 0.5	6.0 ± 0.5	
	Changes	0.5 ± 0.6	1.8 ± 0.5	-0.5 ± 0.8	0.4 ± 0.4		1.7 ± 0.4	0.4 ± 1.5	0.2 ± 0.5	0.1 ± 0.6	
TFA (% TE)	**Baseline**	0.1 ± 0.02	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.04	0.928	0.1 ± 0.02	0.1 ± 0.04	0.1 ± 0.1	0.1 ± 0.02	0.543
	Month 6	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.2 ± 0.02		0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	
	Changes	0 ± 0.02	-0.04 ± 0.02	0.1 ± 0.02	0.03 ± 0.1		-0.04 ± 0.02	0.01 ± 0.1	0 ± 0.1	0.1 ± 0.04	
Energy-adjusted fibre intake (g)	**Baseline**	7.0 ± 0.8	6.1 ± 0.5	5.6 ± 0.7	5.4 ± 0.5	0.549	6.6 ± 0.4	5.0 ± 1.1	5.7 ± 0.5	5.0 ± 0.8	0.166
	Month 6	13.2 ± 0.6	13.6 ± 0.6	9.4 ± 0.9	8.9 ± 0.5		13.3 ± 0.5	14.5 ± 0.8	9.1 ± 0.6	8.8 ± 0.8	
	Changes	6.3 ± 0.9	7.5 ± 0.7	3.8 ± 0.9	3.5 ± 0.5		6.7 ± 0.6	9.5 ± 1.2	3.5 ± 0.6	3.7 ± 0.6	
Energy adjusted vitamin E intake (mg)	**Baseline**	6.5 ± 0.8	5.5 ± 0.5	7.3 ± 0.8	5.5 ± 0.4	0.629	5.5 ± 0.4	6.5 ± 1.8	6.1 ± 0.5	5.4 ± 0.5	0.458
	Month 6	8.3 ± 0.6	7.9 ± 0.3	6.9 ± 0.5	6.2 ± 0.3		7.9 ± 0.3	8.5 ± 0.7	6.3 ± 0.3	6.5 ± 0.5	
	Changes	1.8 ± 0.6	2.4 ± 0.5	-0.4 ± 0.8	0.7 ± 0.4		2.3 ± 0.3	2.0 ± 1.5	0.2 ± 0.5	1.1 ± 0.6	

╪ General linear regression model was performed to assess the effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in dietary parameters after adjusting for age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for respective variable.

*p<0.025 was considered as significant after applied for Bonferroni correction.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, trans fatty acids; TE, total energy intake.

Table 7II.6: Effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to Hipcref diet or control diet

SNPs		*ADRB2* rs1042713					*ADRB2* rs1042714				
Anthropometric parameters	Group	Intervention diet group (n=65)		Control diet group (n=63)		p interaction (genotype x intervention group) ǂ	Intervention diet group (n=65)		Control diet group (n=63)		p interaction (genotype x intervention group) ǂ
		AA (n=16)	AG+GG (n=49)	AA (n=14)	AG+GG (n=49)		CC (n=53)	CG+GG (n=12)	CC (n=43)	CG+GG (n=20)	
Weight (kg)	**Baseline**	73.3 ± 2.6	74.6 ± 1.8	70.5 ± 2.7	74.5 ± 3.0	0.586	73.8 ± 1.8	76.2 ± 2.1	74.7 ± 3.3	71.2 ± 2.6	0.146
	Month 6	69.4 ± 2.6	71.2 ± 2.0	71.4 ± 2.9	75.9 ± 2.9		70.8 ± 1.9	70.5 ± 2.4	76.1 ± 3.3	72.3 ± 2.6	
	Changes	-3.9 ± 0.8	-3.3 ± 0.6	1.0 ± 0.4	1.4 ± 0.4		-3.0 ± 0.5	-5.7 ± 1.2	1.4 ± 0.4	1.1 ± 0.4	
BMI (kg/m^2)	**Baseline**	28.5 ± 0.7	29.9 ± 0.7	28.8 ± 1.0	29.6 ± 0.8	0.594	29.5 ± 0.7	30.0 ± 0.7	29.5 ± 0.9	29.4 ± 1.1	0.267
	Month 6	27.0 ± 0.8	286 ± 0.7	29.2 ± 1.0	30.2 ± 0.8		28.3 ± 0.7	27.8 ± 1.0	30.1 ± 0.9	29.9 ± 1.1	
	Changes	-1.5 ± 0.3	-1.3 ± 0.2	0.4 ± 0.2	0.6 ± 0.2		-1.2 ± 0.2	-2.2 ± 0.5	0.6 ± 0.2	0.5 ± 0.2	
WC (cm)	**Baseline**	94.8 ± 2.6	92.7 ± 1.6	92.8 ± 2.8	94.9 ± 2.0	0.953	92.7 ± 1.6	95.6 ± 3.1	93.4 ± 2.2	96.7 ± 2.4	0.435
	Month 6	88.0 ± 3.1	86.7 ± 1.9	94.2 ± 2.8	96.4 ± 2.8		86.8 ± 1.8	87.9 ± 3.7	94.9 ± 1.1	98.2 ± 2.4	
	Changes	-6.8 ± 1.0	-6.0 ± 0.7	1.4 ± 0.5	1.5 ± 0.5		-5.9 ± 0.7	-7.7 ± 1.3	1.5 ± 0.6	1.5 ± 0.4	
WHR	**Baseline**	0.91 ± 0.01	0.91 ± 0.01	0.90 ± 0.02	0.93 ± 0.01	0.830	0.91 ± 0.01	0.92 ± 0.02	0.91 ± 0.01	0.94 ± 0.01	0.077
	Month 6	0.89 ± 0.01	0.90 ± 0.01	0.91 ± 0.02	0.95 ± 0.01		0.90 ± 0.01	0.90 ± 0.01	0.93 ± 0.01	0.96 ± 0.01	
	Changes	-0.02 ± 0.02	-0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01		-0.01 ± 0.01	-0.02 ± 0.01	0.01 ± 0.03	0.02 ± 0.01	
Muscle mass (kg)	**Baseline**	25.0 ± 1.3	23.4 ± 0.6	22.2 ± 0.8	23.2 ± 0.8	0.045	23.7 ± 0.7	24.5 ± 1.3	23.4 ± 0.9	22.1 ± 0.9	0.067
	Month 6	25.0 ± 1.4	23.0 ± 0.6	21.9 ± 0.9	23.4 ± 0.8		23.5 ± 0.7	23.4 ± 1.2	23.5 ± 0.9	22.1 ± 0.9	
	Changes	0.01 ± 0.4	-0.4 ± 0.2	-0.3 ± 0.4	0.2 ± 0.1		-02 ± 0.2	-1.1 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	
Fat mass (kg)	**Baseline**	27.8 ± 1.6	31.7 ± 1.3	30.0 ± 1.8	31.9 ± 1.9	0.978	30.6 ± 1.2	31.5 ± 1.8	31.8 ± 2.0	30.7 ± 2.0	0.661
	Month 6	23.8 ± 1.7	29.2 ± 1.4	30.6 ± 1.9	33.1 ± 1.9		27.9 ± 1.4	27.7 ± 1.9	32.9 ± 2.0	31.7 ± 1.9	
	Changes	-3.9 ± 1.0	-2.6 ± 0.5	0.6 ± 0.5	1.2 ± 0.3		-2.7 ± 0.5	-3.8 ± 0.8	1.1 ± 0.4	1.0 ± 0.3	
Fat-free mass (kg)	**Baseline**	45.6 ± 2.2	42.8 ± 1.0	40.5 ± 1.4	42.3 ± 1.3	0.860	43.2 ± 1.1	44.7 ± 2.1	42.8 ± 1.5	40.6 ± 1.5	0.177
	Month 6	45.4 ± 2.3	42.0 ± 1.0	41.6 ± 1.8	42.8 ± 1.4		42.9 ± 1.1	42.5 ± 2.1	43.4 ± 1.5	40.6 ± 1.5	
	Changes	-0.2 ± 0.6	-0.8 ± 0.4	1.2 ± 1.0	0.2 ± 0.2		-0.3 ± 0.8	-2.2 ± 0.8	0.6 ± 0.4	0.04 ± 0.3	
Percent body fat (%)	**Baseline**	37.8 ± 1.9	42.2 ± 0.9	42.3 ± 1.3	42.1 ± 0.9	0.563	41.0 ± 1.0	41.4 ± 2.1	41.9 ± 0.8	42.5 ± 1.6	0.834
	Month 6	34.4 ± 2.1	40.2 ± 1.0	42.9 ± 1.4	42.9 ± 1.0		38.7 ± 1.1	39.2 ± 2.2	42.7 ± 1.0	43.5 ± 1.6	
	Changes	-3.5 ± 1.1	-2.0 ± 0.5	0.6 ± 0.4	0.9 ± 0.3		-2.4 ± 0.6	-2.2 ± 0.6	0.8 ± 0.4	1.0 ± 0.3	
SBP (mmHg)	**Baseline**	126.5 ± 4.6	123.0 ± 1.9	120.1 ± 3.0	122.0 ± 2.3	0.372	123.1 ± 2.1	127.1 ± 3.9	121.0 ± 2.3	122.7 ± 3.2	0.181
	Month 6	119.9 ± 4.5	118.2 ± 1.8	121.4 ± 2.8	118.6 ± 2.2		119.1 ± 2.0	116.6 ± 3.8	119.1 ± 2.3	112.0 ± 3.0	
	Changes	-6.6 ± 3.2	-4.8 ± 1.6	1.3 ± 2.8	-3.3 ± 1.4		-4.1 ± 1.6	-10.5 ± 2.9	-2.0 ± 1.7	-3.1 ± 1.9	
DBP (mmHg)	**Baseline**	84.3 ± 2.9	81.8 ± 1.6	77.9 ± 1.4	79.8 ± 1.4	0.635	81.9 ± 1.4	84.5 ± 4.0	79.1 ± 1.4	80.1 ± 2.1	0.737
	Month 6	80.1 ± 3.8	77.4 ± 1.3	76.9 ± 1.4	77.3 ± 1.6		78.4 ± 1.5	76.4 ± 2.9	78.0 ± 1.5	75.4 ± 2.1	
	Changes	-4.2 ± 2.1	-4.4 ± 1.3	-1.0 ± 1.5	-2.6 ± 1.1		-3.5 ± 1.1	-8.1 ± 2.8	-1.0 ± 1.0	-4.7 ± 1.8	
Pulse rate (bpm)	**Baseline**	74.2 ± 3.5	78.2 ± 1.4	77.0 ± 2.8	75.3 ± 1.6	0.229	77.1 ± 2.6	77.5 ± 2.6	74.5 ± 1.7	78.2 ± 2.4	0.402

	Month 6	71.4 ± 3.6	73.3 ± 1.8	69.8 ± 2.2	73.0 ± 1.7		72.8 ± 1.9	73.1 ± 2.9	70.7 ± 1.7	75.9 ± 2.4	
	Changes	-2.8 ± 3.2	-4.9 ± 1.5	-7.2 ± 2.5	-2.3 ± 1.3		-4.3 ± 1.6	-4.5 ± 3.0	-3.9 ± 1.	-2.3 ± 2.2	
Blood biochemical parameters	**Group**	**AA (n=16)**	**AG+GG (n=49)**	**AA (n=14)**	**AG+GG (n=49)**	**p interaction (genotype x intervention group) ╪**	**CC (n=53)**	**CG+GG (n=12)**	**CC (n=43)**	**CG+GG (n=20)**	**p interaction (genotype x intervention group) ╪**
Fasting glucose (mmol/L)	**Baseline**	5.2 ± 0.3	5.1 ± 0.2	4.9 ± 0.2	5.4 ± 0.4	0.516	5.2 ± 0.2	5.2 ± 0.2	4.9 ± 0.2	6.1 ± 0.9	0.582
	Month 6	5.4 ± 0.6	5.1 ± 0.2	5.1 ± 0.2	5.5 ± 0.3		5.2 ± 0.2	5.0 ± 0.3	5.1 ± 0.2	6.0 ± 0.7	
	Changes	0.2 ± 0.5	-0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.2		0.01 ± 0.2	-0.2 ± 0.3	0.2 ± 0.2	-0.1 ± 0.4	
Fasting insulin (uU/mL)	**Baseline**	12.3 ± 2.9	11.6 ± 1.7	5.7 ± 0.6	9.1 ± 1.0	0.135	11.2 ± 1.4	14.6 ± 4.9	8.0 ± 0.9	9.2 ± 1.9	**0.012***
	Month 6	9.1 ± 1.5	8.1 ± 0.7	8.8 ± 1.3	14.0 ± 1.6		8.5 ± 0.8	7.6 ± 1.1	11.6 ± 1.1	15.6 ± 3.3	
	Changes	-3.2 ± 2.6	-3.5 ± 1.3	3.1 ± 1.2	4.9 ± 1.0		-2.7 ± 1.0	-7.1 ± 4.5	3.6 ± 0.8	6.4 ± 2.0	
HOMA-IR	**Baseline**	3.2 ± 0.9	3.0 ± 0.6	1.2 ± 0.2	2.2 ± 0.3	0.073	2.9 ± 0.5	3.5 ± 1.1	1.8 ± 0.3	2.3 ± 0.4	**0.021***
	Month 6	2.5 ± 0.7	1.9 ± 0.2	2.0 ± 0.3	3.6 ± 0.5		2.1 ± 0.3	1.8 ± 0.3	2.8 ± 0.3	4.4 ± 1.1	
	Changes	-0.7 ± 0.7	-1.1 ± 0.5	0.8 ± 0.3	1.5 ± 0.4		-0.9 ± 0.5	-1.7 ± 0.7	0.9 ± 0.2	2.1 ± 0.7	
TC (mmol/L)	**Baseline**	5.2 ± 0.2	5.6 ± 0.1	5.2 ± 0.3	5.6 ± 0.2	0.624	5.5 ± 0.1	5.6 ± 0.3	5.4 ± 0.2	5.8 ± 0.3	0.823
	Month 6	5.3 ± 0.2	5.3 ± 0.2	5.0 ± 0.3	5.5 ± 0.3		5.3 ± 0.2	5.2 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	
	Changes	0.1 ± 0.2	-0.3 ± 0.1	-0.1 ± 0.2	0.1 ± 0.1		-0.2 ± 0.1	-0.4 ± 0.3	-0.03 ± 0.1	0.4 ± 0.2	
Triglyceride (mmol/L)	**Baseline**	1.6 ± 0.3	1.4 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	0.463	1.4 ± 0.1	1.8 ± 0.4	1.2 ± 0.1	1.4 ± 0.1	0.364
	Month 6	1.4 ± 0.3	1.5 ± 0.1	1.4 ± 0.3	1.4 ± 0.1		1.5 ± 0.1	1.5 ± 0.4	1.3 ± 0.1	1.5 ± 0.2	
	Changes	-0.2 ± 0.2	-0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1		0.1 ± 0.1	-0.2 ± 0.5	0.1 ± 0.1	0.1 ± 0.1	
HDL-C (mmol/L)	**Baseline**	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	0.074	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	0.883
	Month 6	1.6 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1		1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	
	Changes	0.1 ± 0.1	-0.1 ± 0.04	-0.1 ± 0.1	-0.1 ± 0.1		-0.1 ± 0.1	0.01 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.1	
LDL-C (mmol/L)	**Baseline**	3.1 ± 0.3	3.4 ± 0.1	3.0 ± 0.2	3.5 ± 0.1	0.642	3.3 ± 0.1	3.2 ± 0.4	3.3 ± 0.1	3.6 ± 0.3	0.639
	Month 6	3.1 ± 0.3	3.3 ± 0.1	3.0 ± 0.2	3.4 ± 0.1		3.2 ± 0.1	3.3 ± 0.2	3.3 ± 0.1	3.3 ± 0.2	
	Changes	0.1 ± 0.2	-0.2 ± 0.1	-0.04 ± 0.1	-0.1 ± 0.1		-0.1 ± 0.1	-0.3 ± 0.3	0.1 ± 0.1	-0.3 ± 0.2	
TC/HDL-C	**Baseline**	3.6 ± 0.2	3.8 ± 0.1	3.4 ± 0.2	3.8 ± 0.2	0.397	3.7 ± 0.1	4.2 ± 0.4	3.5 ± 0.1	4.0 ± 0.3	0.182
	Month 6	3.7 ± 0.3	4.0 ± 0.2	3.5 ± 0.2	4.1 ± 0.2		4.0 ± 0.2	3.9 ± 0.3	3.9 ± 0.1	4.1 ± 0.3	
	Changes	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.1		0.3 ± 0.1	-0.4 ± 0.5	0.3 ± 0.1	0.2 ± 0.2	
hsCRP (mg/L)	**Baseline**	3.6 ± 1.3	7.1 ± 1.3	4.6 ± 1.5	5.7 ± 1.4	0.508	6.4 ± 1.2	5.1 ± 1.4	4.2 ± 1.0	7.8 ± 2.5	0.116
	Month 6	2.9 ± 1.1	4.4 ± 0.7	4.7 ± 1.5	6.0 ± 1.0		4.5 ± 0.7	2.2 ± 0.6	4.9 ± 0.9	7.3 ± 2.0	
	Changes	-0.4 ± 0.3	-1.9 ± 0.8	0.1 ± 0.5	0.4 ± 0.5		-1.1 ± 0.7	-2.7 ± 1.2	0.2 ± 0.5	0.6 ± 0.8	

╪ General linear regression model was performed to assess the effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to Hipcref diet or control diet, after adjusting for age, gender, physical activity, smoking, alcohol consumption and the baseline value for the respective variable.

*p<0.025 was considered as significant after applied for Bonferroni correction.

BMI, Body mass index; WC, Waist circumference; WHR, Waist-hip ratio; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low –density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment -insulin resistance; hsCRP, high-sensitivity C-reactive protein.

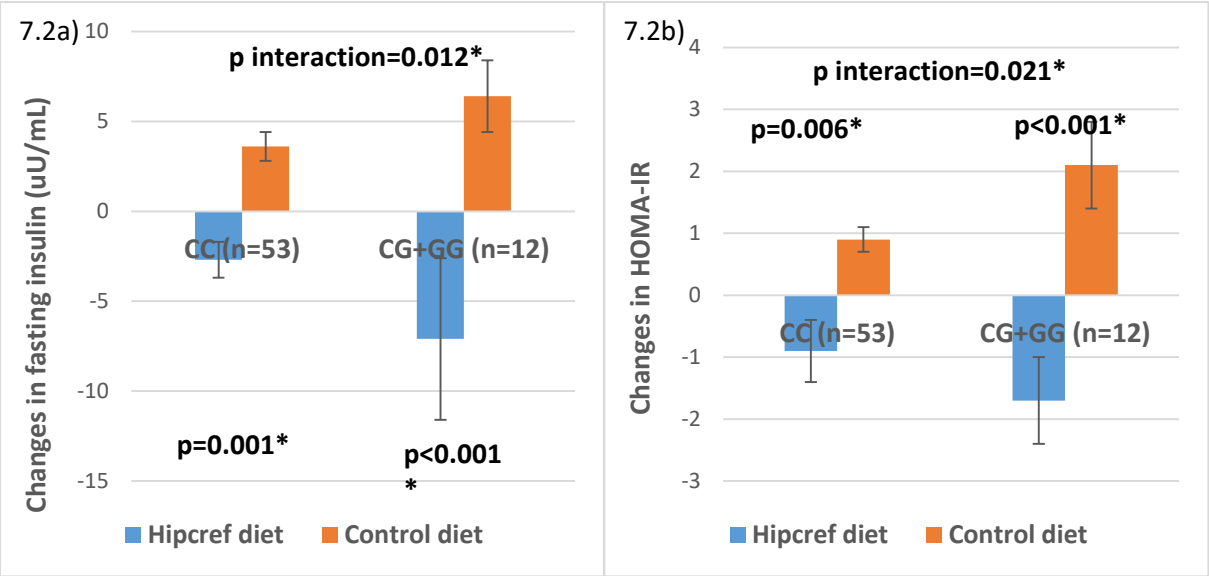


Figure 7.2: Effect of the interaction between *ADRB2* genotype (rs1042714) x dietary group on the post-intervention differences in (a) fasting insulin and (b) HOMA-IR, in response to Hipcref diet vs control diet.
Age, gender, physical activity, smoking, alcohol consumption and the baseline value for the respective variable were adjusted for.
*p<0.025 was considered significant was based on general linear regression model.

7II.5 Discussion

In Chapter 6, we reported that participants receiving Hipcref (high-protein, calorie-restricted, high Vitamin E and normal fibre) diet had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the control diet group who received dietary advice based on Malaysian Dietary Guidelines 2010. Therefore, in this study, we aimed to investigate the response of *ADRB2* rs1042713 and rs1042714 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet intervention.

7II.5.1 *ADRB2* gene variants and post-intervention differences in dietary parameters

Our results revealed significant effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in SFA and MUFA intake. Individuals carrying the risk allele of rs1042713 (AG+GG) had significantly greater reduction in SFA intake after a Hipcref diet compared to a control diet. Furthermore, individuals carrying the risk allele of rs1042713 (AG+GG) had significantly greater reduction in MUFA intake after a Hipcref diet compared to the individuals carrying the non-risk allele (AA). These findings suggest that individuals carrying the risk allele of rs1042713 (AG+GG) had greater reduction in SFA and MUFA intake following a Hipcref diet compared to a control diet.

7II.5.2 *ADRB2* gene variants and post-intervention differences in anthropometric parameters

Our results revealed no significant effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in body weight and obesity-related anthropometric parameters. This finding suggests that *ADRB2* gene variants do not lead to greater or lesser weight loss or post-intervention differences in anthropometric parameters, in response to a Hipcref diet or control diet. Our findings are in line with an earlier study of 109 obese Brazilian women by Saliba et al., 2014, which reported that no significant association was found between *ADRB2* gene variants (rs1042714 and rs1042713) and weight loss, after 7 weeks of lifestyle modification with a combination of dietary and exercise intervention. The dietary intervention consists of 600 kcal deficit per day based on individualised estimated energy requirements, with no specific prescription regarding macronutrient intake (Saliba et al., 2014). The authors concluded that the mutations in aforementioned genes do not lead to greater or lesser weight loss in response to lifestyle intervention (Saliba et al., 2014). A 2-year DIETFITS intervention study also reported no significant gene-diet interaction was found on weight loss between the two alleles of *ADRB2* rs1042714 (C>G), in response to two hypocaloric diets (low carbohydrate diet vs low fat diet) in overweight and obese Americans (Gardner et al., 2018).

Another study with calorie restriction as dietary intervention by Ruiz et al., 2011, reported that both genotypes of *ADRB2* rs1042713 (G>A) showed similar reduction in body weight. However, obese Spanish women carrying risk allele (G) of *ADRB2* rs1042714 had greater reduction in body weight compared to the non- carriers (CC), after 3 months of energy-restricted diet. This finding suggests that there may be a gender-specific genetic association between rs1042714 and intervention employed on the changes in body weight. However, due to the small sample size of male participants in our study, we did not stratify our participants by gender for analysis. Therefore, further large scale studies are required to confirm the gender-specific genetic association between rs1042714 and post-intervention differences in obesity-related phenotypes.

7II.5.3 *ADRB2* gene variants and post-intervention differences in cardio-metabolic parameters

β-adrenergic receptors which involved in catecholamine function are known to regulate diverse physiological processes including glucose metabolism. Prior, Goldberg, & Ryan, 2011 reported that *ADRB2* rs1042713–rs1042714 haplotype was associated with glucose intolerance and

insulin resistance in obese post-menopausal women. A possible explanation for this observation could be, the alteration in the structural conformation of the receptor, which may have enhanced sympathetic stimulation (Masuo & Lambert, 2011). This overstimulation of *ADRB2* is found to be associated with the pathogenesis of insulin resistance as it inhibits the insulin-induced translocation of GLUT4 and reduces glucose uptake via the cAMP-dependent protein kinase A-dependent pathways (Mangmool et al., 2016). Our results revealed significant effect of the interaction between *ADRB2* genotype x dietary group on the post-intervention differences in fasting insulin and insulin resistance (HOMA-IR), in response to a 6-month Hipcref diet intervention. Individuals carrying both the risk allele (CG+GG) and non-risk allele (CC) of rs1042714 had significantly greater reduction in fasting insulin and HOMA-IR after a 6-month of Hipcref diet compared to a control diet, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable. These findings suggest that Hipcref diet may improve homeostatic control of blood insulin and enhance insulin sensitivity (HOMA-IR) in individuals carrying both the risk allele (CG+GG) and non-risk allele (CC) of rs1042714 compared to control diet. However, the mechanistic link between the Hipcref diet and *ADRB2* gene variants on the homeostatic control of fasting insulin and insulin sensitivity and is unknown. Further researches are required to understand the role of genetic variants and Hipcref diet on the underlying molecular mechanisms of insulin resistance. No significant effect of the interaction between *ADRB2* genotype x dietary group was found on the other cardio-metabolic parameters.

7II.6 Summary

The aim of this section is to investigate the response of *ADRB2* rs1042713 and rs1042714 on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, after a 6-month Hipcref diet intervention, in overweight and obese Malaysian adults. *ADRB2* rs1042713 and rs1042714 gene variants were genotyped. The post-intervention differences in dietary intakes, anthropometric parameters, blood lipids levels, fasting insulin levels and fasting glucose levels and hsCRP levels were assessed by *ADRB2* genotypes. Bonferroni correction was applied to account for multiple comparisons ($\alpha=0.05/2=0.025$). General linear regression analysis revealed that individuals carrying the risk allele of *ADRB2* rs1042713 (AG+GG) had significantly greater reduction in SFA intake after a Hipcref diet compared to a control diet. Furthermore, individuals carrying the risk allele of *ADRB2* rs1042713 (AG+GG) had significantly greater reduction in MUFA intake after a Hipcref diet compared to the individuals carrying the non-risk allele (AA). With respect to cardio-metabolic parameters,

individuals carrying both the risk allele (CG+GG) and non-risk allele (CC) of *ADRB2* rs1042714 had significantly greater reduction fasting insulin and HOMA-IR, after a 6-month of Hipcref diet compared to a control diet, after adjusting for potential confounders. No significant effect of the interaction between *ADRB2* genotype x dietary group was found on other anthropometric and cardio-metabolic parameters.

7.3 Limitations

Randomised controlled trial is known to be the gold standard method to examine cause-and-effect relationship between an intervention and outcome. However, there are several limitations exist in the current study. The sample size of the current study is moderate, and hence limiting the statistical power to detect the true gene-diet interactions on the weight loss outcomes. Single gene analysis remained to be too simplistic. Potential false positive results may occur due to multiple testing, and hence further study should assess the combined effect of multiple gene variants by computing polygenic risk scores (PRS). Our participants were recruited from the supermarkets and schools of a specific geographical location (Beranang, Jalan Broga and Bandar Kajang) and they were predominantly middle-aged women with sedentary lifestyle. These factors may have influenced the study outcomes and the findings of current experiments may not be generalisable to the Malaysian population as a whole. Therefore, large scale studies are required to replicate the findings of this study in the Malaysian population from other geographical locations.

We have carefully controlled for confounding lifestyle factors and baseline values in the analyses. However, unmeasured or unknown confounders such as behaviour, socioeconomic and sociodemographic indicators may also exist. Therefore, the associations and gene-diet interactions observed in the current study could arise as a result of the unmeasured confounding factors. With respect to the assessment of dietary intake and physical activity, assessment using questionnaires (FFQ and activity record) may prone to measurement errors, and hence caused bias in the current findings. This study is preliminary, and more trials with larger sample size are warranted.

Chapter 8

Polygenic risk scoring: The combined effect of *FTO* and *ADRB2* gene variants on the post-intervention differences in body composition and cardio-metabolic parameters

8.1 Introduction

Genome wide association studies have identified a large number of genes and gene variants that are associated with the development of obesity in many populations worldwide (Speliotes et al., 2010). Genes involved in energy homeostasis, adaptive thermogenesis, lipoprotein metabolism, appetite control and insulin signaling are some of the main candidate genes that have been studied in various populations. Further, it is now an accepted fact that obesity is a polygenic disorder (rarely monogenic), with many candidate gene variants contributing to the risk of increased body weight and co-morbidities (Loos, 2009). As individual genetic variants generally confer only a moderate risk to a trait, analyzing multiple risk alleles simultaneously can be more informative and can enhance predictive power, particularly in polygenic conditions. A significant genotype effect between the number of risk alleles and risk of abdominal obesity was identified in the LIPGENE-SU.VI.MAX study, with approximately a 2.5 fold increased risk in individuals carrying two or more risk alleles compared to individuals carrying 1 or no risk alleles (Weedon et al., 2006). For most individual's genetic predisposition to metabolic disease has a polygenic basis.

Family and twin studies indicate that up to 80% of the variance in body mass index (BMI) is attributable to genetic factors. Genetic factors also contribute approximately 50% towards the risk of type 2 diabetes mellitus (T2DM). Heritability rates of 10%–30% for the metabolic syndrome (MetS) have been estimated (Bellia et al., 2009; Henneman et al., 2008), indicating that these conditions are partly heritable. Nutrition and physical activity are key environmental factors, which potentially interact with genetic predisposition, to promote the progression and pathogenesis of these combined environmental and polygenic, diet-related diseases. Excessive caloric intake and sedentary lifestyle promote the obese phenotype. More than half of adults in Europe and the US are overweight or obese, leading to the MetS, which in turn greatly enhances subsequent risk of cardio-metabolic disease (Eckel, 2005). There is no doubt that a genetic component can also impact on the risk of insulin resistance, the sensitivity to which may be further amplified by poor diet.

Emerging evidence from gene-diet interactions from observational studies and from randomised controlled trials have supported the fact that the epidemic of obesity and its co-morbidities are the consequences of the interactions between multiple gene variant and lifestyle factors (Qi, 2012). For example, low-fat dietary intervention was associated with more weight loss among overweight and obese individuals with *IRS1* rs2943641 CC genotype compared to

the non-carriers (CT and TT genotypes) (Qi et al., 2011). Overweight and obese individuals carrying the T allele of *PPM1K* rs1440581 lost more in weight with a low-carbohydrate diet compared to the non-carriers (C allele) (Xu et al., 2013). The above is evidence for the fact that individuals with different genetic background may respond differently to the same intervention program (Deram & Villares, 2009). Greater understanding of potential nutrient-gene interaction, can aid in manipulating diet in a way that minimises the metabolic risk of obesity, attenuates insulin resistance and reduces risk of cardio-metabolic disease. At a public health level, more attention must be given to modification of lifestyles of the general public to reduce risk of obesity and T2DM and to increase physical activity. At a clinical level, individual patients with increased metabolic risk need to be identified so that their multiple risk factors can be reduced. Early identification of at risk individuals is of paramount importance. Considering the long asymptomatic period often preceding the manifestation of T2DM and CVD, early diagnosis could enable earlier targeted interventions such as implementation of healthy lifestyle changes in nutritional behavior and exercise or pharmacotherapy, thus reducing disease development.

Inter-individual differences in response to dietary factors and interventions highlight the role of genetics and the potential of a nutrigenetics approach based on identification of nutrient sensitive or responsive genotypes, whereby nutrient intake is manipulated or optimised based on an individuals' genetic profile to reduce disease risk or improve effectiveness of dietary recommendations (Joffe & Houghton, 2016). Early identification of the candidate gene variants that have the potential to influence dietary nutrients metabolically and/or at the molecular level may allow for the provision of good quality personalised recommendation of nutrient intake to achieve effective weight loss and successful maintenance of weight.

Recent evidence suggests that the use of polygenic risk scores (PRS) including multiple gene variants that predispose an individual to obesity may predict the degree of risk for obesity and strategically contribute to weight management programs (Shabana, Shahid, & Hasnain, 2018). PRS summarises the effect of multiple gene variants into a single score to measure the genetic susceptibility to a certain disease (Lewis & Vassos, 2017; Torkamani, Wineinger, & Topol, 2018). It is calculated by summing all the risk alleles of the selected gene variants, weighted by the corresponding effect size estimate. Single SNP analyses are often underpowered due to the small effect size of individual SNP. Moreover, over adjustment for multiple comparisons when testing multiple independent SNPs for associations between variables, may increase the likelihood of type II errors (the chance that the effective treatment was undiscovered) (Feise,

2002). Previous studies have reported that the inclusion of large number of SNPs into PRS model have had larger effect size and hence greater predictive power of risk (Dudbridge, 2013).

8.2 Objectives

In this chapter, we aimed to investigate the combined effect of *FTO* rs9930501, rs9930506 and rs9932754 and *ADRB2* rs1042713 and rs1042714, by computing polygenic risk scores (PRS), on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet intervention, in overweight and obese Malaysian adults.

8.3 Methodology

8.3.1 Study design

Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention was a 6-month, parallel-arm, randomised controlled trial, which assessed the effect of a 6-month intervention of Hipcref (an energy deficit of 300-500kcal/day, 30% energy from protein, 30% energy from fat, 40% energy from carbohydrate, vitamin E≥15mg/day and fibre≥25g/day) diet on obesity-related anthropometric and cardio-metabolic parameters in overweight and obese Malaysian adults (Chapter 6). The control group received generalised dietary advice on weight loss, based on the Malaysian Dietary Guidelines 2010 (<1500kcal/day with a macronutrient composition of approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate).

8.3.2 Participant selection

Detailed information on the participant selection has been previously described in an earlier chapter (Chapter 6). Briefly, a total of 128 eligible, apparently healthy overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) aged 18 years and above with a BMI≥23kg/m^2 were recruited to participate in this randomised dietary intervention study. Participants (n=128) were randomly assigned to one of the two treatments: intervention diet group (Hipcref diet, n=65; female=54 and male=11) or control diet group (control diet, n=63; female=54 and male=9), using covariate adaptive randomisation method. Eligible participants were stratified based on two covariates a) by gender (female and male); and b) by ethnicity (Malaysian Chinese, Malays and Indians), hence a total of 6 (2x3) strata was constructed. Of the initial 128 participants, 7 participants dropped out from the Hipcref diet group and 18 participants dropped out from the control diet group due to job relocation (n=9), retirement

(n=5), accident (n=2), volunteer withdrawal of consent (n=5) and 4 were lost to follow up (Figure 6.1). Therefore, the study was completed with 103 participants. Hipcref diet group = 58 participants (female=47 and male=11); control diet group = 45 participants (female=41 and male=4).

8.3.3 Assessment of anthropometric, dietary, blood biochemical parameters, physical activity level, substance abuse and dietary adherence

Detailed information on the assessment of anthropometric parameters, dietary nutrients analysis, blood biochemical parameters, physical activity levels, substance abuse and dietary adherence have been described in an earlier chapter (Chapter 6).

8.3.4 Hipcref diet and control diet

Detailed information on the delivery mode of Hipcref diet and control diet have been described in an earlier chapter (Chapter 6).

8.3.4 DNA extraction and genotyping assay

Detailed information on *FTO* and *ADRB2* genotyping has been described in an earlier chapter (Chapter 7).

8.3.5 Computation of polygenic risk scores (PRS)

PRS was calculated as the weighed sum of the risk alleles possessed by each individual, in which the weight was taken as the natural log of the odds ratio (OR) associated with the risk

$$PRS = \sum_{i=1}^{n} W_i \, X_i$$

allele of each SNP (Marees et al., 2018; Reisberg et al., 2017; Yin et al., 2015). The formula to evaluate PRS is as follows:

where X_i represents the number of the risk alleles; W_i represents the natural log of the odds ratio of obesity associated with the risk allele of the SNP; n represents the number of the SNPs included in PRS. All the 5 SNPs (*FTO* rs9930506, rs9930506 and rs9932754 and *ADRB2* rs1042713 and *ADRB2* rs1042714) were included in the PRS model. The risk alleles of each SNP had been reported in previously published GWAS studies, *FTO* rs9930501 (G allele), rs9930506 (G allele) and rs9932754 (C allele) and *ADRB2* rs1042713 (G allele) and *ADRB2* rs1042714 (G allele) (Daghestani et al., 2012; Kalantari et al., 2018; Wrzosek et al., 2016;

Zhang et al., 2014). The odds ratio for each SNP were computed from our previously published studies and unpublished data (Chapter 5) and were substituted in the PRS formula (Table 8.1). Participants were then stratified into three equal groups as the first tertile (Tertile 1, PRS 0-0.64), second tertile (Tertile 2, PRS 0.65-3.59) and third tertile (Tertile 3, PRS 3.60-8.18) of polygenic risk scores (PRS) with scores increasing from the first to the third tertile.

Table 8.1: The odds ratio (OR) of obesity associated with each SNP obtained from our previously published/unpublished data

Gene	SNP	Risk allele	RAF	OR (95%CI)	Natural log (OR)
FTO	**rs9930506**[a]	G	0.37	2.87 (1.14-7.19)	1.05
	rs9930501[b]	G	0.37	3.03 (1.23-7.49)	1.11
	rs9932754[b]	C	0.37	3.04 (1.22-7.59)	1.11
ADRB2	**rs1042713**[c]	G	0.13	1.38 (0.08-23.93)	0.32
	rs1042714[d]	G	0.51	1.00 (0.40-2.23)	0

SNP, single nucleotide polymorphisms; RAF, risk allele frequency; OR, odds ratio.
[a] Mitra, Tan & Amini, 2018
[b] Unpublished data (Appendix 17)
[c] Mitra, Tan & Amini, 2019
[d] Unpublished data (Chapter 5; Table 5II.3)

8.3.6 Data analysis

Statistical analysis was performed using the Statistical package for social sciences (IBM SPSS statistic, Chicago, IL, USA, version 22). Data were expressed as mean ± standard error (SE) or number (percentage). Data analysis was conducted on an 'intention-to-treat' basis rather than on compliance to treatment (per protocol), meaning all the participants who have been randomised into the two diet groups were included for analysis regardless of non-completers. For the non-completers, the last observations/measurements on the anthropometric, blood biochemical and dietary parameters were carried forward as the post-intervention measurements for statistical analysis. Log transformation was performed to transform non-normally distributed data into normally distributed data.

One-way ANCOVA was performed to assess the differences in the continous variables at baseline between the tertiles of PRS, after adjusting for covariates age, gender, physical activity status, alcohol consumption, smoking status and BMI. Chi square tests were performed the differences in categorical variables at baseline between the tertiles of PRS. The dietary adherence scores between the tertiles of PRS and the difference in PRS between the ethnic groups were assessed using one-way ANCOVA. The main effects of polygenic risk score and dietary intervention on the post-intervention difference anthropometric and cardio-metabolic parameters at month 6 were analysed using general linear regression models, with the

adjustment for covariates including age, gender, physical activity status, alcohol consumption, smoking status, and the baseline value for the respective variable. Moreover, to analyse the potential interactions between genotype and dietary intervention, an interaction product term of PRS x dietary group was included in the models. A statistical probability level of $p<0.05$ (two-sided) was considered significant.

8.4 Results

8.4.1 General characteristics: Differences between the tertiles of PRS at baseline

Statistical analyses on the comparisons of the general characteristics between the tertiles of PRS at baseline were conducted on an intention-to-treat basis (Table 8.2). Our results revealed no significant difference in age ($p=0.716$), height ($p=0.494$), weight ($p=0.195$), BMI ($p=0.233$), gender ($p=0.879$), physical activity status ($p=0.850$), smoking status ($p=0.590$) and alcohol consumption ($p=0.392$) between the tertiles of PRS. Significant difference in PRS was found between the ethnic groups. In further analysis (Table 8.3), our results revealed that, in our study participants, Indians (3.9 ± 0.3) had significantly higher PRS compared to Chinese (2.1 ± 0.4) ($p=0.008$) (Table 8.3), indicating Indians were more likely to be genetically predisposed to obesity compared to Chinese.

Table 8.2: General characteristics: Differences between the tertiles of PRS at baseline

Variables	Tertile 1 (n=38)	Tertile 2 (n=47)	Tertile 3 (n=43)	p value
Age (y)	42.7 ± 1.7	45.3 ± 1.7	43.7 ± 1.6	0.716
[1] **Gender**				
Female	33 (86.8%)	39 (83.0%)	36 (83.7%)	0.879
Male	5 (213.2%)	8 (17.0%)	7 (16.3%)	
[1] **Ethnicity**				
Malays	20 (52.6%)	19 (40.4%)	18 (41.9%)	**0.016 ***
Chinese	12 (31.6%)	7 (14.9%)	5 (11.6%)	
Indians	6 (15.8%)	21 (44.7%)	20 (46.5%)	
[1] **Physical activity status**				
Physical inactive	36 (94.7%)	45 (95.7%)	40 (93.0%)	0.850
Physical active	2 (5.3%)	2 (94.3%)	3 (7.0%)	
[1] **Smoking Status**				
Never	37 (97.4%)	45 (95.8%)	43 (100%)	0.590
Current	0	1 (2.1%)	0	
Former	1 (2.6%)	1 (2.1%)	0	
[1] **Alcohol consumption**				
Never	37 (97.4%)	46 (97.9%)	43 (100%)	0.392
Current	0	1 (2.1%)	0	
Former	1 (2.6%)	0	0	
[2] **Height (cm)**	157.6 ± 1.2	158.7 ± 1.1	157.5 ± 1.1	0.494
[2] **Weight (kg)**	70.6 ± 1.8	75.9 ± 2.4	74.7 ± 2.8	0.195
[2] **BMI (kg/m^2)**	28.5 ± 0.7	29.9 ± 0.7	30.0 ± 0.8	0.233

Data were expressed as mean (±SE) or number (percentage).
[1] p value based on Chi square test.
[2] p value based on ANCOVA, after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption.
*p<0.05 was considered as significant.
BMI, Body mass index.

Table 8.3: Differences between the ethnic groups in the mean (±SE) values of PRS

Ethnicity	PRS	p value
Chinese (n=24)	2.1 ± 0.4*	0.008*
Malays (n=57)	3.0 ± 0.4	
Indians (n=47)	3.9 ± 0.3*	

*p<0.05 was considered as significant based on ANCOVA, after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption**.**

8.4.2 Dietary adherence score: Difference between the tertiles of PRS

No significant difference was found in the adherence score between the tertiles of PRS (p=0.544) (Table 8.4).

Table 8.4: Dietary adherence scores: Differences between the tertiles of PRS

Adherence score	Tertile 1 (n=38)	Tertile 2 (n=47)	Tertile 3 (n=43)	p value
Mean (± SE)	5.8 ± 0.8	6.6 ± 0.9	5.5 ± 0.7	0.501
Minimum	4.1	4.9	4.1	-
Maximum	7.4	8.4	6.8	-

*p<0.05 was considered as significant based on ANCOVA, after adjusting for covariates age, gender, physical activity, smoking and alcohol consumption.

8.4.3 The effect of the interaction between PRS x dietary group on the post-intervention differences in dietary parameters

General linear regression analysis revealed no significant effect of the interaction between PRS x dietary group on the post-intervention differences in dietary parameters, after adjusting for covariates age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for respective variable ($p>0.05$) (Table 8.5).

8.4.4 The effect of the interaction between PRS group x dietary intervention on the post-intervention differences in anthropometric and cardio-metabolic parameters

General linear regression analysis revealed no significant effect of the interaction between PRS group x dietary intervention on the post intervention differences in body weight and obesity-related anthropometric parameters (e.g. BMI, WC, WHR, body fat mass, percent body fat and muscle mass), at month 6 of the dietary intervention, in overweight and obese Malaysian adults ($p>0.05$), after adjusting for covariates, age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable (Table 8.6). However, with respect to cardio-metabolic parameters our results revealed significant PRS group x dietary intervention effect on the post-intervention changes in hsCRP levels (p interaction=0.048), after adjusting for age, gender, physical activity, smoking, alcohol consumption and the baseline value for hsCRP levels. Participants from the second tertile of PRS had significantly greater reduction in hsCRP levels with Hipcref diet compared to the control diet (-2.5 ± 0.9mg/L vs -0.03 ± 0.6mg/L, p=0.025), after 6 months of intervention period (Fig 8.1). Similarly, participants from the third tertile of PRS had significantly greater reduction in hsCRP levels with Hipcref diet compared to the control diet (-2.4 ± 1.0mg/L vs 0.8 ± 1.0mg/L, p=0.025), after 6 months of intervention period (Fig 8.1). Such difference was not observed in the first tertile of PRS ($p>0.05$).

Table 8.5: Effect of the interaction between PRS x dietary group on the post-intervention differences in dietary parameters, in response to a 6-month Hipcref diet or control diet

Dietary parameters	Group	Intervention diet group (n=65)			Control diet group (n=63)			p interaction (PRS x intervention group) ǂ
		Tertile 1 (n=20)	Tertile 2 (n=22)	Tertile 3 (n=23)	Tertile 1 (n=18)	Tertile 2 (n=25)	Tertile 3 (n=20)	
Total energy intake (kcal)	**Baseline**	2003 ± 72	2017 ± 74	2041 ± 96	1904 ± 50	2038 ± 62	2025 ± 81	0.185
	Month 6	1629 ± 58	1618 ± 54	1556 ± 49	1880 ± 64	1988 ± 63	2010 ± 60	
	Changes	-374 ± 66	-400 ± 61	-485 ± 68	-25 ± 39	-50 ± 25	-15 ± 30	
Protein intake (g)	**Baseline**	80.4 ± 6.0	70.6 ± 4.0	76.0 ± 5.7	76.4 ± 4.3	73.8 ± 5.0	72.3 ± 5.6	0.962
	Month 6	111.1 ± 4.8	105.9 ± 5.5	107.3 ± 4.2	73.4 ± 3.0	76.9 ± 4.4	76.4 ± 3.2	
	Changes	30.7 ± 7.7	35.4 ± 5.4	31.4 ± 4.7	-3.0 ± 4.6	3.1 ± 3.4	3.0 ± 4.3	
Fat intake (g)	**Baseline**	81.2 ± 4.5	85.8 ± 4.8	86.6 ± 5.1	76.1 ± 3.0	80.4 ± 4.5	80.8 ± 2.5	0.293
	Month 6	58.0 ± 3.2	59.5 ± 3.7	55.5 ± 2.2	70.7 ± 4.1	75.1 ± 3.8	79.3 ± 4.4	
	Changes	-23.2 ± 4.5	-26.3 ± 4.8	-31.2 ± 4.9	-5.4 ± 3.2	-5.4 ± 3.5	-6.3 ± 2.9	
Carbohydrate intake (g)	**Baseline**	245.4 ± 17.5	252.6 ± 12.3	256.9 ± 15.8	241.2 ± 10.2	264.8 ± 12.8	257.2 ± 14.0	0.926
	Month 6	177.2 ± 8.6	176.7 ± 8.8	171.8 ± 7.3	258.9 ± 8.6	271.2 ± 11.1	265.4 ± 10.5	
	Changes	-68.2 ± 14.2	-76.0 ± 10.7	-85.2 ± 13.9	17.7 ± 9.7	6.4 ± 6.9	8.2 ± 8.6	
Percent energy from protein (%)	**Baseline**	16.1 ± 1.1	13.9 ± 0.7	14.5 ± 0.7	15.9 ± 1.1	14.6 ± 0.9	14.1 ± 0.9	0.596
	Month 6	27.5 ± 0.9	26.6 ± 1.3	27.6 ± 0.6	15.4 ± 0.3	15.5 ± 0.7	15.0 ± 0.4	
	Changes	11.4 ± 1.4	12.7 ± 1.4	13.0 ± 1.1	-0.6 ± 1.0	0.9 ± 0.7	0.9 ± 0.8	
Percent energy from fat (%)	**Baseline**	37.0 ± 1.9	37.4 ± 1.5	37.7 ± 1.5	35.6 ± 1.0	35.0 ± 1.7	37.6 ± 1.4	0.555
	Month 6	31.4 ± 1.0	33.1 ± 1.4	32.3 ± 1.0	37.7 ± 2.0	37.7 ± 1.8	37.3 ± 1.8	
	Changes	-5.0 ± 1.7	-4.6 ± 1.3	-2.9 ± 1.0	-2.0 ± 1.1	-1.5 ± 1.4	-2.2 ± 1.2	
Percent energy from carbohydrate (%)	**Baseline**	45.8 ± 2.1	46.0 ± 2.1	46.7 ± 1.7	47.1 ± 1.2	49.0 ± 1.8	47.2 ± 1.8	0.496
	Month 6	43.7 ± 0.9	42.8 ± 1.1	43.5 ± 1.0	50.5 ± 2.8	49.5 ± 2.6	50.2 ± 2.1	
	Changes	-2.7 ± 1.7	-3.0 ± 2.0	-3.0 ± 1.7	75 ± 1.8	4.7 ± 1.6	5.0 ± 1.6	
SFA (% of TE)	**Baseline**	7.8 ± 0.8	8.5 ± 0.7	8.0 ± 0.7	8.0 ± 0.8	6.2 ± 0.5	8.7 ± 1.1	0.935
	Month 6	7.6 ± 0.5	8.4 ± 0.6	8.5 ± 0.6	8.4 ± 0.8	8.7 ± 0.7	9.9 ± 0.7	
	Changes	-0.2 ± 0.8	-0.1 ± 0.7	0.5 ± 1.1	0.4 ± 0.9	2.6 ± 0.6	1.1 ± 1.0	
MUFA (% of TE)	**Baseline**	12.2 ± 1.0	11.8 ± 0.9	10.3 ± 1.0	11.0 ± 0.7	9.8 ± 0.8	12.4 ± 1.0	0.683
	Month 6	10.2 ± 0.6	10.9 ± 0.7	10.5 ± 0.5	10.2 ± 05	10.2 ± 0.6	11.6 ± 0.7	
	Changes	-1.9 ± 0.8	-0.9 ± 0.9	0.2 ± 0.8	-0.9 ± 0.7	0.4 ± 0.8	-0.8 ± 1.2	

PUFA (% of TE)	**Baseline**	7.1 ± 0.6	6.9 ± 0.9	5.9 ± 0.8	6.4 ± 0.7	5.7 ± 0.6	6.9 ± 0.7	0.572
	Month 6	8.3 ± 0.5	8.2 ± 0.6	7.7 ± 0.5	7.4 ± 0.9	5.9 ± 0.6	6.4 ± 0.4	
	Changes	1.2 ± 0.7	1.3 ± 0.7	1.8 ± 0.8	1.0 ± 0.8	0.2 ± 0.6	-0.6 ± 0.7	
TFA (% TE)	**Baseline**	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 01	0.1 ± 0.02	0.726
	Month 6	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.02	
	Changes	-0.1 ± 0.04	-0.02 ± 0.03	-0.01 ± 0.03	0.1 ± 0.04	0.03 ± 0.1	0.04 ± 0.03	
Energy-adjusted fibre intake (g)	**Baseline**	6.2 ± 0.6	7.0 ± 0.8	5.6 ± 0.7	5.6 ± 0.6	5.0 ± 0.8	6.0 ± 0.7	0.571
	Month 6	130 ± 0.9	13.7 ± 0.7	13.8 ± 0.8	9.5 ± 0.8	8.0 ± 0.7	9.8 ± 0.8	
	Changes	6.8 ± 0.9	6.6 ± 1.0	8.2 ± 0.9	3.9 ± 0.8	3.0 ± 0.8	3.9 ± 0.7	
Energy adjusted vitamin E intake (mg)	**Baseline**	5.8 ± 0.6	6.7 ± 0.9	4.8 ± 0.7	6.9 ± 0.6	5.1 ± 0.5	6.1 ± 0.8	0.761
	Month 6	8.0 ± 0.3	8.4 ± 0.5	7.6 ± 0.4	6.8 ± 0.6	5.8 ± 0.4	6.8 ± 0.5	
	Changes	2.2 ± 0.7	1.7 ± 0.7	2.9 ± 0.6	-0.9 ± 0.6	0.7 ± 0.5	0.5 ± 0.4	

╪ General linear regression model was performed to assess the effect of the interaction between PRS x dietary group on the post-intervention differences in dietary parameters after adjusting for age, gender, physical activity, smoking, alcohol consumption, total energy intake and the baseline value for respective variable.
*$p<0.05$ was considered as significant.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, trans fatty acids; TE, total energy intake.

Table 8.6: Effect of the interaction between PRS x dietary group on the post-intervention differences in anthropometric and cardio-metabolic parameters, in response to a 6-month Hipcref diet or control diet

Anthropometric parameters	Group	Intervention diet group (n=65)			Control diet group (n=63)			p interaction (PRS x dietary group) ǂ
		Tertile 1 (n=20)	Tertile 2 (n=22)	Tertile 3 (n=23)	Tertile 1 (n=18)	Tertile 2 (n=25)	Tertile 3 (n=20)	
Weight (kg)	**Baseline**	72.4 ± 2.9	75.2 ± 3.3	74.9 ± 1.6	68.5 ± 1.9	76.4 ± 3.6	74.5 ± 5.8	0.058
	Month 6	69.9 ± 3.0	72.1 ± 3.4	70.3 ± 1.9	69.5 ± 1.9	77.6 ± 3.6	76.3 ± 5.7	
	Changes	-2.5 ± 0.7	-3.2 ± 0.7	-4.6 ± 1.0	1.0 ± 0.6	1.1 ± 0.6	1.8 ± 0.5	
BMI (kg/m2)	**Baseline**	29.3 ± 1.2	29.6 ± 1.1	29.8 ± 0.6	27.6 ± 0.7	30.2 ± 0.9	30.1 ± 1.6	0.125
	Month 6	28.2 ± 1.2	28.4 ± 1.2	28.0 ± 0.8	28.0 ± 0.8	30.7 ± 1.0	30.9 ± 1.6	
	Changes	-1.1 ± 0.3	-1.3 ± 0.3	-1.8 ± 0.4	0.4 ± 0.9	0.5 ± 0.3	0.8 ± 0.2	
WC (cm)	**Baseline**	91.1 ± 2.6	94.4 ± 2.7	93.9 ± 2.1	87.5 ± 1.8	98.1 ± 2.4	96.1 ± 3.7	0.224
	Month 6	85.9 ± 3.0	88.4 ± 3.0	86.7 ± 2.5	88.6 ± 2.1	99.3 ± 2.5	98.2 ± 3.5	
	Changes	-5.2 ± 1.1	-6.1 ± 1.0	-7.2 ± 1.1	1.1 ± 1.1	1.2 ± 0.6	2.1 ± 0.5	
WHR	**Baseline**	0.89 ± 0.02	0.92 ± 0.01	0.92 ± 0.01	0.90 ± 0.01	0.94 ± 0.02	0.93 ± 0.01	0.369
	Month 6	0.89 ± 0.01	0.91 ± 0.01	0.90 ± 0.01	0.9 ± 0.01	0.94 ± 0.02	0.95 ± 0.02	
	Changes	-0.01 ± 0.01	-0.01 ± 0.01	-0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	
Muscle mass (kg)	**Baseline**	23.6 ± 1.2	24.1 ± 1.2	23.7 ± 0.7	22.2 ± 0.8	23.4 ± 1.1	23.1 ± 1.5	0.068
	Month 6	23.5 ± 1.1	24.0 ± 1.2	23.0 ± 0.6	22.3 ± 0.8	23.3 ± 1.1	23.4 ± 1.5	
	Changes	-0.1 ± 0.2	-0.1 ± 0.3	-0.8 ± 0.3	0.02 ± 0.4	-0.1 ± 0.2	0.3 ± 0.2	
Fat mass (kg)	**Baseline**	29.4 ± 2.2	31.5 ± 2.0	31.3 ± 1.3	27.6 ± 1.2	33.6 ± 2.1	32.2 ± 3.7	0.234
	Month 6	27.0 ± 2.4	28.3 ± 2.3	28.2 ± 1.5	28.0 ± 1.4	34.9 ± 2.1	33.6 ± 3.6	
	Changes	-2.4 ± 0.8	-3.2 ± 0.7	-3.2 ± 0.8	0.4 ± 0.6	1.2 ± 0.5	1.4 ± 0.4	
Fat-free mass (kg)	**Baseline**	43.4 ± 1.9	43.8 ± 2.0	43.5 ± 1.1	40.9 ± 1.2	42.8 ± 1.8	42.3 ± 2.5	0.069
	Month 6	42.8 ± 1.9	43.9 ± 2.0	41.9 ± 1.1	42.1 ± 1.5	42.7 ± 1.8	42.7 ± 2.5	
	Changes	-0.3 ± 0.3	0.1 ± 0.6	-1.7 ± 0.6	1.2 ± 0.9	-0.1 ± 0.2	0.4 ± 0.3	
Percent body fat	**Baseline**	40.0 ± 1.9	41.5 ± 1.4	41.7 ± 1.2	40.2 ± 1.1	43.6 ± 1.0	42.0 ± 1.7	0.468
	Month 6	37.9 ± 2.1	38.6 ± 1.8	39.7 ± 1.3	40.4 ± 1.4	44.6 ± 1.1	43.0 ± 1.7	
	Changes	-2.1 ± 0.8	-2.9 ± 0.8	-2.0 ± 0.8	0.2 ± 0.7	1.1 ± 0.4	1.0 ± 0.4	
SBP (mmHg)	**Baseline**	121.5 ± 3.3	126.5 ± 3.3	123.4 ± 3.0	119.6 ± 3.1	124.0 ± 3.6	120.2 ± 2.8	0.843
	Month 6	116.6 ± 3.1	121.1 ± 3.5	118.0 ± 2.6	117.6 ± 3.8	122.6 ± 2.9	116.5 ± 3.0	
	Changes	-5.0 ± 2.6	-5.4 ± 2.3	-5.3 ± 2.7	-2.0 ± 3.2	-1.4 ± 1.8	-3.7 ± 1.9	
DBP (mmHg)	**Baseline**	81.9 ± 2.5	80.8 ± 2.2	84.3 ± 2.5	76.9 ± 1.9	81.3 ± 1.9	79.3 ± 2.1	0.843
	Month 6	77.3 ± 2.9	78.1 ± 2.4	78.7 ± 1.9	75.3 ± 2.0	78.9 ± 2.0	76.7 ± 2.5	
	Changes	-4.6 ± 1.5	-2.8 ± 1.7	-5.5 ± 2.3	-1.6 ± 1.8	-2.4 ± 1.0	-2.6 ± 2.0	
Pulse rate (bpm)	**Baseline**	76.5 ± 3.0	77.7 ± 2.4	77.4 ± 1.9	75.0 ± 3.0	75.3 ± 2.1	76.9 ± 2.4	0.105
	Month 6	72.9 ± 3.6	74.5 ± 2.6	71.2 ± 2.2	68.9 ± 2.5	71.9 ±2.2	76.0 ±2.4	
	Changes	-3.5 ± 2.7	-3.2 ± 2.3	-6.2 ± 2.2	-6.0 ± 2.4	-3.4 ± 1.9	-0.9 ± 1.5	
Blood biochemical parameters	**Group**	**Tertile 1 (n=20)**	**Tertile 2 (n=22)**	**Tertile 3 (n=23)**	**Tertile 1 (n=18)**	**Tertile 2 (n=25)**	**Tertile 3 (n=20)**	**p interaction (PRS x dietary group) ǂ**

Fasting glucose (mmol/L)	**Baseline**	5.0 ± 0.3	5.3 ± 0.4	5.1 ± 0.2	4.6 ± 0.1	5.1 ± 0.2	6.2 ± 0.9	0.381
	Month 6	5.3 ± 0.3	5.0 ± 0.5	5.2 ± 0.2	4.9 ± 0.2	5.3 ± 0.3	6.0 ± 0.7	
	Changes	0.2 ± 0.2	-0.4 ± 0.5	0.1 ± 0.2	0.3 ± 0.2	0.3 ± 0.3	-0.2 ± 0.3	
Fasting insulin (uU/mL)	**Baseline**	11.4 ± 2.2	11.5 ± 2.3	12.5 ± 3.0	5.4 ± 0.6	9.0 ± 1.6	10.2 ± 1.5	0.121
	Month 6	8.3 ± 1.0	±	8.5 ± 1.1	9.0 ± 1.3	13.2 ± 2.6	15.9 ± 2.0	
	Changes	-3.2 ± 2.1	-3.2 ± 1.3	-4.0 ± 2.6	3.6 ± 1.3	4.1 ± 1.4	5.7 ± 1.7	
HOMA-IR	**Baseline**	2.6 ± 0.5	3.4 ± 1.1	3.0 ± 0.8	1.1 ± 0.1	1.9 ± 0.3	2.8 ± 0.6	0.122
	Month 6	1.9 ± 0.3	2.1 ± 0.5	2.0 ± 0.3	2.0 ± 0.3	3.3 ± 0.7	4.4 ± 0.8	
	Changes	-0.6 ± 0.5	-1.3 ± 0.9	-1.0 ± 0.7	0.9 ± 0.3	1.3 ± 0.5	1.6 ± 0.6	
TC (mmol/L)	**Baseline**	5.7 ± 0.2	5.3 ± 0.2	5.5 ± 0.2	5.5 ± 0.3	5.3 ± 0.2	5.9 ± 0.3	0.944
	Month 6	5.5 ± 0.2	5.4 ± 0.2	5.1 ± 0.2	5.4 ± 0.3	5.4 ± 0.2	5.4 ± 0.2	
	Changes	-0.2 ± 0.1	0.1 ± 0.2	-0.4 ± 0.2	-0.04 ± 0.2	0.1 ± 0.1	-0.5 ± 0.2	
Triglyceride (mmol/L)	**Baseline**	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.0 ± 0.1	1.3 ± 0.2	1.5 ± 0.2	0.456
	Month 6	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.0 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	
	Changes	-0.1 ± 0.1	0 ± 0.1	0 ± 0.3	-0.02 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	
HDL-C (mmol/L)	**Baseline**	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	0.527
	Month 6	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	
	Changes	-0.1 ± 0.1	-0.03 ± 0.1	-0.1 ± 0.1	-0.2 ± 0.1	-0.02 ± 0.03	-0.2 ± 0.04	
LDL-C (mmol/L)	**Baseline**	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.8 ± 0.2	0.672
	Month 6	3.4 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.4 ± 0.2	3.2 ± 0.2	3.4 ± 0.2	
	Changes	-0.1 ± 0.1	0 ± 0.2	-0.3 ± 0.2	0.2 ± 0.1	-0.01 ± 0.1	-0.3 ± 0.2	
TC/HDL-C	**Baseline**	3.8 ± 0.2	3.7 ± 0.2	3.8 ± 0.3	3.1 ± 0.2	3.7 ± 0.2	4.2 ± 0.2	0.055
	Month 6	4.1 ± 0.3	4.2 ± 0.2	3.7 ± 0.2	3.5 ± 0.2	3.9 ± 0.2	4.4 ± 0.2	
	Changes	0.3 ± 0.1	-0.1 ± 0.3	-0.1 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	
hsCRP (mg/L)	**Baseline**	3.8 ± 1.0	6.1 ± 1.5	8.3 ± 2.3	1.7 ± 0.3	7.2 ± 1.7	6.2 ± 2.2	**0.048***
	Month 6	3.8 ± 0.9	3.4 ± 0.9	4.9 ± 1.4	2.1 ± 0.6	7.6 ± 1.3	6.6 ± 1.8	
	Changes	0.9 ± 1.0	-2.5 ± 0.9	-2.4 ± 1.0	0.3 ± 0.6	-0.03 ± 0.6	0.8 ± 0.8	

╪ General linear regression model was performed to assess the effect of the interaction between PRS x dietary group on the post-intervention difference in anthropometric and cardio-metabolic parameters, after adjusting for age, gender, physical activity, smoking, alcohol consumption and the baseline value for respective variable.

*$p<0.05$ was considered as significant.

BMI, Body mass index; WC, Waist circumference; WHR, Waist-hip ratio; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low –density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment -insulin resistance; hsCRP, high-sensitivity C-reactive protein.

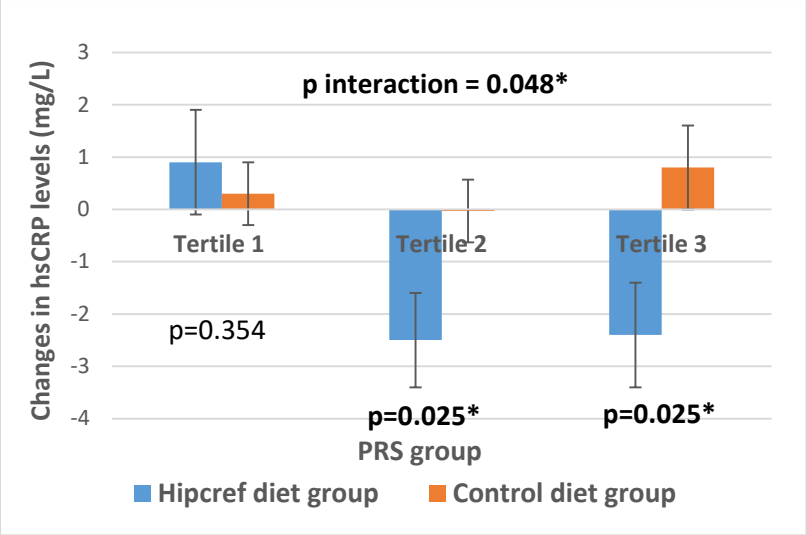


Figure 8.1: Effect of the interaction between PRS x dietary group on the post-intervention difference in hsCRP levels in response to 6-month Hipcref diet vs control diet.
Age, gender, physical activity, smoking, alcohol consumption and the baseline value for hsCRP levels were adjusted for.
*p<0.05 was considered significant based on general linear regression model.

8.5 Discussion

Polygenic risk scoring accounts for the combined effect of multiple SNPs on disease outcomes, and may have a larger combined effect size and hence higher power to detect association with diseases than an individual SNP (Baker et al., 2018). Therefore, quantitative measure of the effect of multiple gene variants is essential to detect the modulatory effect of gene variants on weight loss outcomes. In this chapter, we aimed to investigate the combined effect of *FTO* (rs9930501, rs9930506 and rs9932754) and *ADRB2* (rs1042713 and rs1042714) gene variants on the post intervention differences in anthropometric and cardio-metabolic parameters, using polygenic risk scores (PRS), in overweight and obese Malaysian adults.

8.5.1 Polygenic risk scores and ethnicity

This study reports that with respect to *FTO* and *ADRB2* genes and their respective gene variants, significant differences exist between the three major Malaysian ethnic groups. Malaysian Indians had significantly higher polygenic risk scores compared to Malaysian Chinese. An earlier study reported that Malaysian Indians had significantly higher frequency of the risk allele (G) of *FTO* rs9930506 compared to the Malaysian Chinese and Malays (Mitra et al., 2018). This finding adds to the existing evidence that the differences in the allele frequencies and genotype distributions of gene variants do exist in various ethnic groups. This fact should be accounted for when designing intervention programmes in multi-ethnic populations.

8.5.2 Polygenic risk scores (PRS) and post-intervention differences in body weight and anthropometric parameters

At the month 6 of the Hipcref dietary intervention, our data shows a positive linear trend in the reduction of body weight (-2.5kg, -3.2kg and -4.6kg), BMI (-1.1, -1.3 and -1.8), WC (-5.2cm, -6.1cm and -7.2cm) and fat mass (-2.4kg, -3.2kg and -3.2kg) across the PRS tertiles (first, second and third), respectively. However, such post-intervention differences in body weight and anthropometric parameters (e.g. WC, WHR, body fat mass and muscle mass) did not reach statistical significance. These findings suggest that individuals who are genetically predisposed to obesity, do not lead to greater or lesser body weight loss, in response to a 6-month Hipcref diet or a control diet.

Our findings are in line with a recent systematic review and meta-analysis of 9563 participants involving 8 randomised controlled trials which reported that the post-intervention differences in body weight, waist circumference and adiposity after weight loss interventions were not significantly different between the minor allele (A) and major allele carriers (T) of *FTO* rs9939609 (Livingstone et al., 2016). The authors reported that individuals carrying the minor allele of *FTO* gene variants responded equally well to dietary, physical activity or drug based weight loss interventions, regardless of the intervention length, age, ethnicity, sample size, gender, and baseline body mass index. Moreover, no significant association between rs9939609 and weight loss or post-intervention differences in metabolic risk factors was found in other populations such as obese Japanese and obese Spanish women after a calorie-restricted dietary intervention (Labayen et al., 2015; Matsuo et al., 2012).

An earlier study of 109 obese Brazilian women conducted by Saliba et al., 2014, reported that no significant association was found between *ADRB2* gene polymorphisms (rs1042714 and rs1042713) and weight loss, after a 7-week of lifestyle modification with a combination of dietary and exercise intervention. The dietary intervention consists of 600 kcal deficit per day based on individualised estimated energy requirements, with no specific prescription regarding macronutrient intake (Saliba et al., 2014). The authors concluded that the mutations in aforementioned genes do not lead to greater or lesser weight loss in response to lifestyle intervention (Saliba et al., 2014). A 2-year DIETFITS intervention study also reported no significant gene-diet interaction between the two alleles of *ADRB2* rs1042714 (C>G), in weight loss, in response to two hypocaloric diets (low carbohydrate diet vs low fat diet) in overweight

and obese Americans (Gardner et al., 2018). These findings showed that *ADRB2* gene variants did not affect the weight loss outcomes in response to dietary interventions.

8.5.3 Polygenic risk scores (PRS) and post intervention differences in cardio-metabolic parameters

With respect to the cardio-metabolic parameters, we found no significant effect of PRS x dietary group interaction on the post-intervention difference in blood lipid levels, insulin resistance and glycaemic indices. However, our results revealed that individuals in the second and third tertiles of PRS had significantly greater post-intervention reduction in hsCRP levels after 6 months of Hipcref diet compared to the control diet, after adjusting for potential confounders. Such effect was not observed in the first tertile of PRS. hsCRP is a common systemic marker for investigation of inflammation and also an independent predictor of future cardiovascular events (Devaraj, Singh, & Jialal, 2009). Previous epidemiological studies have consistently reported low-grade chronic and systemic inflammation as a major characteristic of obesity (Emanuela et al., 2012). Our finding suggests that Hipcref diet was able to attenuate and weaken the effect of PRS on inflammation (e.g. hsCRP levels), and that higher risk individuals benefited the most. The Look AHEAD study reported that a moderate improvement in body weight and fitness after 1 year of lifestyle intervention through calorie restriction and physical activity, was not able to mitigate the unfavorable cardio-metabolic effects (e.g. hsCRP and triglyceride levels) associated with GCKR Leu446Pro in overweight and obese individuals with diabetes mellitus (Belalcazar et al., 2016). It is interesting to note that the average age (±SD) of the Look AHEAD study was 59.0 ± 6.8 and our study participants had an average age (±SE) of 43.7 ± 1.6 years. We opine that the advanced age in the Scottish population may have precipitated the comorbidities in their population in the group with the risk allele. Moreover, our participants were apparently healthy overweight and obese adults without any clinical metabolic conditions.

8.5.4 Hipcref diet, polygenic risk scores and hsCRP levels

i) Calorie restriction, hsCRP and PRS

Our data shows a positive linear trend in the reduction of total energy intake (-374kcal/day, -400kcal/day and -485kcal/day) across the tertiles of PRS (first, second and third), respectively. It is common knowledge that increase in hsCRP levels is associated with increased adipose tissue (Maachi et al., 2004). Therefore, a reduction in the excess body fat stores can

significantly reduce the inflammatory state of individuals (Petelin et al., 2014). Reduction of hsCRP levels in individuals may be due to the consequence of weight loss following a moderate reduction in calorie intake (Selvin, Paynter, & Erlinger, 2007). Further reduction in total energy intake through calorie restricted diet resulted in significant reduction in hsCRP levels and the risk of atherosclerosis in various populations (Fontana et al., 2004; Svendsen et al., 2012). The current study reveals that individuals with higher scores i.e. at higher risk, had lower energy intake through the 6 months of the intervention trial. Further, individuals at high risk benefited most, metabolically, at least with respect to hsCRP levels.

ii) High protein intake and PRS

According to Merritt, Jamnik, & El-Sohemy, 2018, individuals carrying the risk allele (A) of rs1558902 of *FTO* gene had higher BMI and WC with lower dietary protein intake compared to the non-carriers (T). This effect was not found in the higher protein intake risk group, suggesting that high protein intake may be protective against the effect of *FTO* gene on BMI and WC. Huang et al., 2014 reported that the risk allele carriers (A) of *FTO* rs9930609 had significant reduction in food craving and appetite with a hypocaloric high protein weight loss diet. *FTO* is highly expressed in the arcuate nucleus of the hypothalamus. Animal and in vitro studies suggest that *FTO* plays an important role in sensing cellular amino acids (Gulati et al., 2013). The sensing of amino acid levels in the brain is known to mediate orexigenic and anorexigenic pathways to control food intake and energy balance. Studies have suggested that the availability of essential amino acids may be one of the factors regulating *FTO* levels. *FTO* mRNA and protein levels were found to be dramatically downregulated by total amino-acid deprivation (Cheung et al., 2013). In case of *ADRB2* gene, a study by Fernandez-Twinn et al., 2006 reported that rodent offspring on 'low protein diet' demonstrated reduced beta-adrenergic responsiveness and attenuated adrenergic and insulin signaling, which increased the risk of heart failure and insulin resistance in the animals. This finding corroborates with the current findings as well. Therefore, it is suggested that the participants with highest PRS benefited most with high protein intake through Hipcref diet.

iii) Vitamin E, hsCRP and PRS

Vitamin E is a micronutrient with anti-oxidant and immune modulating properties, which can reduce oxidative stress and influence inflammatory processes (Singh & Jialal, 2004). A significant inverse association of Vitamin E and hsCRP has been demonstrated in some studies (Devaraj et al., 2007; Scheurig et al., 2008). The authors reported that Vitamin E may have

modulated the expression of *FTO* gene through the deactivation of PKC signaling, which in turn attenuated weight gain. Moreover, Qin et al., 2006 also reported that a combined treatment of vitamins C and E resulted in diminished oxidative stress and prevented the loss of β-adrenergic receptor activity due to overstimulation in rabbits with myocardial infarction. Increased consumption of vitamin E may be an effective weight management strategy to improve inflammatory status in individuals carrying higher number of risk alleles.

iv) PUFA and PRS

Cell and animal studies have demonstrated beneficial effects of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) on inflammation and insulin sensitivity (Oliver et al., 2012; Storlien et al., 1996; Storlien et al., 1991). The PPARγ Pro12Ala polymorphism is an example of the relevance of gene-nutrient interactions in the development of obesity, the MetS and T2DM. As the ratio of total PUFA to SFA increased, a significant inverse relationship was shown for both fasting insulin concentrations and BMI in the Ala carriers, suggesting that the potential protective effect of the Ala allele may be lost in the presence of a high SFA diet (Luan et al., 2001). More recently an inverse relationship between Ala frequency and T2DM prevalence has been observed in populations where energy from lipids exceeded 30% of the total energy intake (Scacchi et al., 2007). The current study reports that study participants with risk alleles of *FTO* and *ADRB2,* and possessing higher PRS, benefited most with increased PUFA through Hipcref diet intervention.

v) High fibre intake and PRS

Hosseini-Esfahani et al., 2017 suggested that dietary fibre may modify the association of *FTO* SNPs (using genetic risk score) and obesity. The authors reported that in a middle-eastern population, individuals with high genetic risk score and high fibre intake had the lowest risk of obesity compared to those with low genetic risk score and low intake of fibre. Moreover individuals carrying the AA genotype of *FTO* rs11076023 in the third tertile of dietary fibre intake had significantly lower waist circumference compared to the non-carriers (AT and TT genotypes) (Vimaleswaran et al., 2016). Consumption of a high fibre diet may be an effective weight management strategy for individuals carrying higher number of risk alleles.

8.5.5 Limitations

There are several limitations exist in the current study. The sample size of the current study is moderate, limiting the statistical power of detecting a gene-diet interaction. We acknowledge

that the study is exploratory and due to the small sample size, is underpowered to detect the gene-diet interactions. However, from the current investigation, we have generated a hypothesis. Moreover, our study participants were recruited from the supermarkets and schools in a specific geographical location (in the areas of Beranang, Jalan Broga and Bandar Kajang) and they were predominantly middle-aged women with sedentary lifestyle. These factors may have influenced the study outcomes and hence the findings of current experiments may not be generalisable to the Malaysian population as a whole. Therefore, large scale studies are required to replicate the findings from our studies in the Malaysian population from other geographical locations.

We have carefully controlled for baseline and other lifestyle factors in the analyses. However, unmeasured or unknown confounders such as other behaviour, socioeconomic and sociodemographic indicators may also exist. Therefore, the associations and gene-diet interactions observed in the current study could arise as a result of the unmeasured confounding factors. With respect to the assessment of dietary intake and physical activity, assessment using questionnaires (FFQ and activity record) may prone to measurement errors, and hence caused bias in the current findings. Body composition was assessed using bioelectrical impedance analysis (BIA). This is one of the limitations of this study. Many factors such as the food and fluid intake, environment, ethnicity and phase of menstrual cycle affect BIA estimates. As per the dietary strategy of this study, we opine that calorie restriction, higher protein, PUFA, vitamin E and dietary fibre intake had a combined effect on the success of the intervention diet (Hipcref diet). We cannot attribute the success of the Hipcref diet to any one of the latter dietary components, due to the nature of the study design. Nonetheless, this study is still quite preliminary, and more trials with larger sample size are warrant.

8.6 Summary

This chapter aimed to investigate the combined effect of *FTO* (rs9930501, rs9930506 and rs9932754) and *ADRB2* (rs1042713 and rs1042714) gene variants using polygenic risk score (PRS), on the post-intervention differences in anthropometric and cardio-metabolic parameters, after 6 months of Hipcref diet or control diet, in overweight and obese Malaysian adults. PRS was calculated as the weighted sum of the risk alleles possessed by each individual, in which the weight was taken as the natural log of the odds ratio (OR) associated with the risk allele of each SNP. Participants were then stratified into three equal groups using first (Tertile 1), second (Tertile 2) and third (Tertile 3) tertiles of polygenic risk scores (PRS). Our results revealed no

significant PRS x dietary group effect on the post-intervention differences in body weight, obesity-related anthropometric parameters (e.g. BMI, WC, WHR, body fat mass, muscle mass and percent body fat), blood lipid levels and glycemic indices, after a 6-month of Hipcref diet or control diet. However, we found significant PRS x dietary group effect on post- intervention difference in hsCRP levels after 6 months of dietary intervention. Individuals from the second and third tertiles of PRS had greater reduction in hsCRP levels after 6 months of Hipcref diet compared to a control diet, after adjusting for covariates. However, such difference was not observed in the first tertile of PRS. This study lays out a strategy to manage inflammation for overweight and obese individuals who are genetically predisposed to obesity. It can be suggested that, Hipcref diet can deliver favorable inflammation outcomes at least in our study participants. However, more trials are required to confirm the current findings in other geographical locations and other population groups.

Chapter 9

General discussion and conclusion

9.1 General discussion

The prevalence of obesity and its co-morbidities in the Malaysian population has been increasing dramatically since the past decade. It has now become the leading preventable cause of morbidity and mortality in the Malaysian population according to the Department of statistics, Malaysia, 2018. Interestingly, disparities in the prevalence of obesity within the ethnic groups in the Malaysian population had been reported earlier. The prevalence of obesity and metabolic syndrome was highest in Malaysian Indian, followed by Malays and Chinese (Institute for Public Health, Malaysia, 2015; Rampal et al., 2012). Previous evidence suggested that the differences in dietary pattern, lifestyle and genetic factors may have contributed to the differences in the metabolic risk between the ethnic groups (McKeigue et al., 1988; Tan, Dunn, & Yen, 2011; Tan et al., 2011). The related health care costs are substantially high and has increased the economic burden of the Malaysia government on health care system (Tan et al., 2011). Immediate action is necessary to combat the high prevalence of obesity in the Malaysian population.

To date, there are only a few weight management intervention studies which have been experimented in the Malaysian population using different strategies such as dietary or physical activity-based programmes or behavior modification programmes (Soon et al., 2013; Kassim et al., 2017; Moy, Sallam, & Wong, 2006; Ramli et al., 2013). However, the results were conflicting and unsatisfactory. Generalised advice on weight loss in multi-ethnic Malaysian population seem to be less effective in increasing individual's compliance to the intervention and hence end in unsuccessful weight management programme (Loos & Rankinen, 2005). An optimal weight loss diet should be personalised, cost effective, culturally sensitive and practical under real life situation for long term weight control (Koliaki et al., 2018). Therefore, the main aim of this doctoral thesis was to assess the efficiency of an individualised dietary intervention programme tailored according to an individual's food preference and cultural habit in managing body weight and reducing metabolic risk factors, in overweight and obese Malaysian adults (Malaysian Malays, Chinese and Indians). Further, this study aimed to investigate the effect of the interaction between gene variants x dietary group on the post-intervention differences in dietary intake, anthropometric and cardio-metabolic parameters, in response to the dietary intervention.

A total of 178 Malaysian adults were recruited to examine the differences in dietary intake, physical activity level and obesity-related anthropometric and blood biochemical parameters

between the three different ethnic groups (Malaysian Malays, Chinese and Indians) (Chapter 4). In addition, this project aimed to investigate the association between gene variants (*FTO* and *ADRB2* genes) and obesity and obesity-related traits, and to assess the interaction between gene variants and dietary factors in modulating the obesity-related anthropometric and biochemical parameters (Chapter 5).

A 6-month two parallel-arm, randomised controlled trial was conducted to assess the effect of the Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention on the dietary intake, anthropometric and cardio-metabolic parameters in the study participants (Chapter 6). The control diet group received generalised dietary advice on weight loss based on MDG 2010. Further, the effect of the interaction between variants of the same gene (*FTO* and *ADRB2* genes) x dietary group on the post-intervention differences in dietary intake, anthropometric and cardio-metabolic parameters was assessed, after 6 months of dietary intervention (Chapter 7). Moreover, the combined effect of *FTO* and *ADRB2* gene variants on the above aspect was assessed by computing polygenic risk scores (PRS) (Chapter 8). We conclude that gene-diet interaction studies on obesity phenotypes may identify sub-groups of individuals who may benefit from tailored dietary modifications such as the Hipcref dietary intervention.

9.2 Main findings

9.2.1 Are there any differences in dietary intake, physical activity level and obesity-related anthropometric and blood biochemical parameters between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians)? (Research question 1)

Baseline investigation of 178 multi-ethnic Malaysian adults revealed that, in our study participants, Malaysian Indians had significantly higher BMI, WC, fat mass, body fat percent, fasting insulin, HOMA-IR and lower HDL cholesterol levels, compared to the Chinese and Malays (Chapter 4). These results supported the previous findings which reported that obesity and its complications disproportionately affected Indians in the Malaysia population compared to the Malays and the Chinese (Institute for Public Health, Malaysia, 2015; Rampal et al., 2012). Tan, Dunn, & Yen, 2011, reported that Malaysian Indians were more likely to exhibit central obesity, elevated fasting glucose levels and lower HDL cholesterol levels compared to the Chinese and the Malays. The authors also reported that compared to the Malaysian Malays and Chinese, Indians were less likely to engage in physical activity and had lower consumption of

fruits and vegetables, which may contribute towards insulin resistance and metabolic syndrome. Interestingly, dietary analysis in our study revealed that Malaysian Indians consumed lower dietary cholesterol and higher fibre, compared to their Malaysian Chinese and Malay counterparts. Therefore, more attention should be paid to Malaysian Indians in attempt to reduce body weight and metabolic parameters in the Malaysian population as a whole. However, our study sample is not representative of the Malaysian population, and hence the findings of the current experiments may not be generalisable to the Malaysian population as a whole. Large scale future studies are required to replicate the findings of this study in the Malaysian population from other geographical locations.

9.2.2 How do gene variants affect the phenotypes of obesity-related traits of Malaysian adults? Are there any differences in genotype distributions and allele frequencies of gene variants between adult Malaysians of the three different ethnic groups (Malaysian Malays, Chinese and Indians)? (Research questions 2 & 3)

It is an established fact that obesity and related chronic diseases are multifactorial in nature and arise from the complex interaction between multiple gene variants, physical activity and dietary factors (Marti et al., 2004). Multiple genetic loci have been determined by genome-wide association studies (GWAS) that are associated with increased body mass index (BMI) and the susceptibility to obesity in a cumulative manner (Speliotes et al., 2010). Single nucleotide polymorphisms in *FTO* gene was the first reported gene variant, to be associated with obesity in the European population; with AA genotype of rs9930609 having a 1.7 fold increased risk of obesity compared to TT genotype (Frayling et al., 2007). Studies on Caucasian population have found significant association between *FTO* rs9930506 and obesity (Sentinelli et al., 2012; Wrzosek et al., 2016). In recent, Kalantari et al., 2018 reported that the haplotype of *FTO* rs9930501, rs9930506 and rs9932754 with complete linkage disequilibrium (LD), had a strong association with obesity indices (e.g. BMI and body fat percent) in the Iranian male adolescents. However, relatively little is known with respect to the interaction between dietary nutrients and *FTO* gene variants on obesity and obesity related metabolic phenotypes in the Asian populations

With respect to the risk of obesity and gene variants of *FTO* and *ADRB2*, our results revealed contrasting differences between the same (Chapter 5). In case of *FTO* gene variants (n=178), the risk alleles of *FTO* rs9930501 (G), rs9930506 (G), and rs9932754 (C) were associated with increased odds of obesity compared to the AA, AA and TT genotypes, respectively, in the

additive model. Moreover, the risk allele carriers of *FTO* rs9930501 (G), rs9930506 (G) and rs9932754 (C) had significantly higher BMI, WC, WHR, fat mass, body fat percent, hsCRP levels and lower HDL cholesterol levels compared to the non-carriers (AA, AA and TT, respectively). These results replicate the previous findings from the European population such as Polish and Italian populations, which report that risk allele of *FTO* rs9930506 was associated with lower HDL-C levels (Franczak et al., 2017; Sentinelli et al., 2012). Fisher et al., 2009 also reported that the risk allele (A allele) of *FTO* rs9939609 contributed to higher levels of plasma hsCRP, independent of its effect on BMI and fatness. It is suggested that *FTO* gene variations may not only contribute to the increase in the size of fat mass, but may as well enhance the inflammatory state of adipose tissue stores thus increasing systemic inflammation. Interestingly, we found that Malaysian Indians expressed significantly higher frequency of the risk alleles of *FTO* rs9930501 (G), rs9930506 (G) and rs9932754 (C) when compared to the Chinese and the Malays. Our findings add to the existing evidence that differences in gene variants in *FTO* do exist in various ethnic groups.

The function of the FTO protein is still largely elusive. It is known that *FTO* is a DNA/RNA demethylase which influences splicing, stability and export of mRNA out of the nucleus (Lesbirel & Wilson, 2018). Initial observation suggested that it plays an important role in regulating energy homeostasis by signalling the hunger and satiety centre in brain, but it is unknown whether it influences eating behaviour or energy expenditure (Scuteri et al., 2007). Recently, a new mechanism was proposed, suggesting variants in the *FTO* gene exert effect on the genes located nearby and determining obesity (Smemo et al., 2014). It is assumed that SNPs located within the first intron may affect obesity by altering *FTO* expression directly, or, these SNPs may also remotely alter the expression of other downstream genes such as genes involved in body mass regulation by changes in epigenetic mechanisms including methylation of other genes (Almén et al., 2012; Zhou et al., 2017).

In the recent years, attention has also been focused on the adrenergic receptor system for its role in the stimulation of thermogenesis and in activating lipid mobilisation from the fat stores. With respect to this, the beta-2 adrenoceptor gene is a candidate gene since it is the dominating lipolytic receptor in the human white adipose tissue (Arner & Hoffstedt, 1999). It stimulates lipid mobilisation through lipolysis in adipocytes, regulates body fat accumulation and energy expenditure (Taylor, 2007). A meta-analysis involving 18 published articles revealed significant association between *ADRB2* rs1042714 and susceptibility to obesity (Zhang, Wu,

& Yu, 2014). These findings have suggested that gene variants play a critical role in weight management. Studies from Saudi Arabia also reported significant association between *ADRB2* rs1042713 and rs1042714 polymorphisms and the development of insulin resistance, dyslipidemia, overweight, and obesity (Daghestani et al., 2010; Daghestani et al., 2012). However, findings from Asian populations (Japanese and Korean) reported negative association between obesity and *ADRB2* gene polymorphisms. Moreover, these studies did not investigate interaction of gene variants with dietary nutrients (Hayakawa et al., 2000; Kim et al., 2002).

With respect to *ADRB2* (n=178), our results revealed that *ADRB2* rs1042713 and rs1042714 were not associated with increased odds of obesity ($p>0.025$). However, we found that the homozygous mutant carriers of *ADRB2* rs1042713 (GG) were associated with increased odds of insulin resistance compared to the non-carriers (AA), in the additive model. These findings suggest that variations in *ADRB2* gene may interfere with glucose homeostasis and cause insulin resistance. Prior et al, 2011 reported that *ADRB2* rs0142713–rs1042714 haplotype was associated with glucose intolerance and insulin resistance in obese post-menopausal women (Prior et al., 2011). A possible explanation for this observation could be, the alteration in the structural conformation of the receptor, which may have enhanced sympathetic stimulation leading to increased lipolysis (Masuo & Lambert, 2011). This overstimulation of *ADRB2* is found to be associated with the pathogenesis of insulin resistance as it inhibits the insulin-induced translocation of GLUT4 and reduces glucose uptake via the cAMP-dependent protein kinase A-dependent pathways (Mangmool et al., 2016).

Furthermore, obese individuals carrying the G allele of rs1042713 had significantly higher total cholesterol and LDL cholesterol levels compared to the non-carriers (A), whereas obese individuals carrying the G allele of rs1042714 had significantly higher WHR compared to the non-carriers (C). However, such differences were not observed in non-obese individuals. We opine that the excess FFAs in circulation in obese individuals carrying the gene variants of *ADRB2* may have driven dyslipidaemia. Increased lipolysis due to the polymorphisms of *ADRB2* gene may have caused increased levels of NEFA induced hepatic production of VLDL and hence higher LDL levels in our participants (Jung & Choi, 2014). Interestingly, we found that Malaysian Chinese had significantly lower frequency of the risk allele (G) of *ADRB2* rs1042714 compared to Malaysian Malays and Malaysian Indians combined (11.9% vs 28.7%).

9.2.3 How do gene variants interact with dietary factors and modulate the effect of obesity-related phenotypes in Malaysian adults? (Research question 4)

Previous evidence has suggested that individual or population differences in the development of obesity related metabolic diseases may result not only from genetic variation, but may also due to the modulatory effect of dietary nutrients on gene and gene variants (Heianza & Qi, 2017). Therefore, exploring gene-diet interactions on obesity-related phenotypes is important in understanding the pathogenesis of obesity and its comorbidities. Our findings from the gene-diet interaction analysis revealed that the haplotype of *FTO* rs9930506, rs9930501 and rs9932754 may interact with dietary protein intake and Vitamin E intake and modulate hsCRP levels (Chapter 5). Individuals carrying the risk alleles of rs9930501 (G), rs9930506 (G) and rs9932754 (C) showed significantly lower hsCRP levels with high vitamin E intake and higher percent energy from protein. It is common knowledge that vitamin E (a micronutrient with anti-oxidant and immune modulating properties) can reduce oxidative stress and influence inflammatory processes (Singh & Jialal, 2004). A significant inverse association of vitamin E and hsCRP has been demonstrated in some studies (Devaraj et al., 2007; Scheurig et al., 2008). A randomised weight loss intervention trial has reported that the carriers of the risk allele of *FTO* gene exhibited greater reduction in food cravings with a calorie restricted and high-protein diet (Huang et al., 2014). Zhang et al., 2012, reported that a high protein diet led to weight loss and improvement in body composition in the individuals carrying the risk allele of the *FTO* gene. No significant association was found between *FTO* SNPs and other dietary nutrients on obesity-related traits.

Furthermore, our results also revealed that the level of fasting glucose was modulated by the types of dietary fatty acids such as SFA and PUFA in the carriers of GG genotype of *ADRB2* rs1042713. The carriers of GG genotype of rs1042713 had significantly lower fasting glucose levels with intake of relatively higher PUFA: SFA ratio (≥0.8/day) and lower SFA intake (<7.3% of TE/day). Moreover, the carriers of GG genotype of rs1042713 had significantly lower fasting insulin levels and HOMA-IR when consuming higher PUFA intake (≥6% of TE/day). These findings suggest that carriers of GG genotype of *ADRB2* rs1042713 consuming higher percentage of PUFA demonstrated better homeostatic control of fasting blood glucose and insulin sensitivity. A meta-analysis of 102 randomised controlled feeding trials with 4,200 subjects has reported that, PUFA had the most beneficial effects in improving glycaemia, insulin resistance, and insulin secretion in comparison to dietary carbohydrate, SFA and MUFA

(Imamura et al., 2016). Therefore, we suggest that replacing isocaloric quantities of foods rich in SFA with PUFA may improve homeostatic control of blood glucose and enhance insulin sensitivity (HOMA-IR) in individuals carrying the G allele of *ADRB2* rs1042713. No significant gene-diet interaction on obesity-related traits was found in the individuals carrying *ADRB2* rs1042714. We have carefully controlled for confounding lifestyle factors and baseline values in the analyses. However, unmeasured or unknown confounders such as behaviour, socioeconomic and sociodemographic indicators may also exist. These factors may contribute to the variations in the gene-diet interactions on obesity-related phenotypes in our study participants.

9.2.4 How does individualised Hipcref dietary intervention benefit the overweight and obese Malaysian adults with respect to obesity-related anthropometric and blood biochemical parameters? (Research question 5)

Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention was formulated and developed to reduce body weight and cardio-metabolic risk factors in the overweight and obese Malaysian adults (Chapter 6). A 6-month two parallel-arm, RCT was conducted to assess the effect of the Hipcref diet (an energy deficit of 300-500kcal/day, 30% energy from protein, 30% energy from fat, 40% energy from carbohydrate, vitamin E≥15mg/day and fibre≥25g/day) on obesity-related anthropometric and blood biochemical parameters (e.g. fasting glucose, fasting insulin, HOMA-IR, hsCRP and lipid levels) in the study participants. The control diet group received generalised dietary advice on weight loss according to Malaysian Dietary Guidelines (MDG) 2010 (<1500kcal/day with a macronutrient composition of approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate).

Our results revealed that overweight and obese Malaysian adults following Hipcref (High-protein, calorie-restricted, high Vitamin E and normal fibre) diet had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to the generalised dietary advice on weight loss. Participants following Hipcref diet had successfully reduced 3.5kg of body weight and lost 2.3% of percent body fat in 6 months, which translated to a reduction of 0.6kg of body weight and 0.4% of percent body fat per month. The beneficial effect of Hipcref diet on obesity-related anthropometric parameters (e.g. BMI, WC, fat mass and percent body fat) was observed at month 3 and was maintained at month 6. Insulin sensitivity improved significantly in the participants following Hipcref diet as reflected

in the reduced fasting insulin levels and reduced HOMA-IR. Baseline fasting blood glucose levels in the Hipcref diet group was below the diagnostic cut off (5.6mmol/L) defining metabolic syndrome, and it did not reduce after intervention, suggesting that intervention diet resulted in weight loss and improvement in insulin sensitivity without leading to hypoglycaemia. Although not significant, small reductions were achieved in total cholesterol and LDL cholesterol levels. After 6 months of intervention, Hipcref diet group was found to consume significantly higher percent energy from protein, higher percent energy from PUFA, higher vitamin E (mg), higher fibre (g), lower total energy, lower percent energy from fat and lower percent energy from carbohydrate. We opine that the success of the Hipcref diet in achieving positive outcomes in overweight/obese Malaysian adults may be, due to the combined effect of the nutrient composition of the Hipcref diet.

Hipcref diet: Is it safe?

Unlike the 'Atkin diet' which is a very popular high-protein diet with extremely low carbohydrate intake and high fat intake (60% of energy from fat, 35% of energy from protein and 5% of energy from carbohydrate), our Hipcref diet is high in protein (30% of energy from protein) but low in fat (<30% of energy from fat) and moderate in carbohydrate (40% of energy from carbohydrate). Healthcare professionals do not recommend long-term adherence to an Atkins-like, high-protein diet due to the high fat content of the diet (~60% energy), particularly the saturated fat (~25% of energy from SFA), which may eventually lead to elevations in total cholesterol and LDL cholesterol levels (Astrup, Meinert Larsen, & Harper, 2004). Previous evidence suggested that diets high in protein (30% of energy from protein) but low in fat (<30% of energy from fat) and moderate in carbohydrate (40% of energy from carbohydrate) are as effective at promoting weight loss as Atkins-type of diets, and reduce total cholesterol and LDL cholesterol as well as triglycerides (Baba et al., 1999; Johnston, Tjonn, & Swan, 2004; Parker et al., 2002). Farnsworth et al., 2003 reported that this high-protein diet (HP diet: 27% of energy from protein, 44% of energy from carbohydrate, and 29% of energy from fat) has beneficial metabolic effects and no adverse effects on markers of bone turnover or calcium excretion. Therefore, Hipcref diet that is high in protein, low in fat and moderate in carbohydrate may represent an appealing diet plan for healthful and safe weight loss and deserve further investigation. However, long-term high protein or high meat intake in humans may lead to hyperuricemia (leading to joint pain and gout), hypercalcuria (leading to kidney stones, hypocalcaemia, and osteoporosis), renal malfunction, increased cancer risk, liver malfunction,

and increased risk of coronary artery disease (Delimaris, 2013). Individuals suffering from kidney diseases are not encouraged to consume a high-protein diet (e.g. Hipcref diet).

Moreover, our Hipcref diet introduces high vitamin E intake (≥15mg/day) for better metabolic health in overweight and obese individuals. Vitamin E is a potent antioxidant with anti-inflammatory and immune-modulating properties, which can reduce oxidative stress and influence inflammatory processes (Singh & Jialal, 2004). Inverse association between Vitamin E and biomarkers of oxidative stress and inflammation (e.g. C-reactive protein, LDL oxidation, monocyte function) and carotid atherosclerosis in patients with coronary artery disease (Devaraj et al., 2007). A recent study suggests that obese people with metabolic syndrome need more than normal levels of vitamin E because their weight and other problems are causing increased oxidative stress, and those same problems actually cause their effective use of vitamin E to be reduced (Mah et al., 2015). These findings support higher dietary α-tocopherol requirements for obese adults. In a large long-term trial, vitamin E supplementation did not show reduction in the risk of major cardiovascular events (Sesso et al., 2008). In addition, a meta-analysis involved 135,967 participants from 19 clinical trials reported that high-dosage (> or =400 IU/d) vitamin E supplements may increase all-cause mortality (Chew & Milton, 2005). However, there are clear benefits of long-term consumption of vitamin E through diets containing oils, nuts and seeds, but the effects of vitamin E supplementation are marginal (Abdali et al., 2015; Ros, 2010). Moreover, there is no sufficient evidence of toxic effects from vitamin E found naturally in foods (Monsen, 2000). These data provide no support for the use of these supplements for the prevention of CVD, but antioxidant-rich foods are recommended to be incorporated into healthy diets for better metabolic health. More clinical trials with longer duration and larger sample size are required to confirm the long-term effect of Hipcref diet on human health.

Effectiveness and sustainability of weight management programmes

It is interesting to note that our Hipcref intervention programme did not include any physical activity regimens. The beneficial effects of Hipcref dietary intervention on anthropometric and cardio-metabolic parameters (e.g. blood lipid levels, insulin sensitivity and inflammation marker) observed in this study was depended on diet alone. Previous evidence suggests that calorie restriction is necessary for initial weight loss, however there is a necessity to include exercise in combination with diet to effectively improve body composition and blood biomarkers of metabolic risk factors for long-term weight loss (Clark, 2015). A systematic

review and meta-analysis of weight-loss clinical trials reported that dietary interventions resulted in a mean weight loss of approximately 5 to 8.5 kg (5% to 9%) at the first 6 months compared to the baseline (Franz et al., 2007). However, a weight-loss plateau appears at approximately 6 months and stabilised to a weight loss of approximately 4.5 to 7.5 kg (4.8% to 8%) at month 12. Therefore, the focus of a weight management programme at this point in time should evolve from weight loss only, to weight loss with continued weight-loss maintenance. The authors suggest that to break through the weight loss plateau, individuals should maintain lower-calorie diet, and they are highly encouraged to perform regular physical activity to maintain calorie deficit, in order to prevent weight regain or to lose more weight. Weight regain is likely to occur if weight-loss interventions are discontinued entirely at this point in time.

Previous evidence demonstrated that when combined with diet, exercise interventions were more effective at improving body composition and blood biomarkers compared to either an exercise, or diet, alone option for intervention. Exercise-alone interventions or advice-only interventions, did not result in successful weight loss, and experienced minimal weight loss at any time point (Franz et al., 2007). This is supported by a meta-analysis of 66-population based studies and 162-studywise groups which reported that the combination of diet and resistance training (e.g. progressive training volume of 2-to-3 sets for 6-to-10 reps at an intensity of ≥75% maximal sustainable heart rate, utilizing whole body and free-weight exercises) is more effective in inducing weight loss and lowering blood lipid levels and insulin resistance compared to endurance training or the combination of resistance training and endurance training (Clark, 2015). The author also reported that among all the dietary regimes, a hypocaloric high-protein diet (>1.5g of protein*kg^{-1}body mass or >25% of energy from protein) appears to produce greater effect on body compositional changes (e.g. loss of body mass, fat mass and retention of FFM) and improvement in glycaemic control compared to all hypocaloric diets (Clark, 2015). Therefore, a combination of calorie-restricted diet (preferably high protein diets) and regular physical activity is highly encouraged for successful and sustainable weight management outcomes.

Hipcref diet vs general dietary guidelines

We opine that dietary guidelines by MDG 2010 (macronutrient composition - approximately 10-15% energy from protein, 20-30% energy from fat and 55-70% energy from carbohydrate), may be applicable to healthy Malaysians with healthy BMI. However, for overweight and

obese Malaysian adults, we recommend Hipcref diet for successful weight management, and most importantly for favourable metabolic health. Hipcref dietary intervention can be an effective weight management strategy to manage body weight and improve body composition, insulin sensitivity and inflammatory markers in overweight and obese individuals in any population group. However, more clinical trials are necessary to examine the long-term effect of Hipcref diet on weight loss and metabolic health.

Furthermore, dietary guidelines should recommend a variety of popular traditional recipes specific to ethnic groups as practiced in Malaysia. At present, the guidelines are generalised dietary advice. Nutrition Society of Malaysia can raise awareness on the elements of Hipcref diet for weight management. A policy brief can be framed and the Ministry of Health Malaysia can be approached for revised recommendations for overweight and obese individuals as per the results obtained from the current project. Hopefully in the near future the revised recommendations would be accepted and documented in the guidelines.

Personalisation in weight management programmes

Further, we emphasise that personal dietary preferences should be considered as an important factor when developing strategies for weight management in any population, especially in a multi-ethic population. This study has tested a dietary strategy that included ethnic food preferences and popular traditional recipes practiced in all the three major ethnic groups and has successfully proved that simple modification of traditional recipes can bring a modest change in body composition and metabolic parameters. The degree of adherence is highly influenced by how different a dietary intervention is from an individual's usual diet, and hence tailoring dietary interventions to an individual's food preference and cultural habit may lead to better adherence to dietary prescriptions (Gibson & Sainsbury, 2017). Therefore, it is recommended to introduce a variety of popular traditional recipes practiced in all the three major ethnic groups into Malaysian Dietary Guidelines. This could have increased the efficacy in establishing individual's adherence to the intervention diet, and hence can be successful and sustainable weight management in the Malaysian population.

9.2.5 Do individuals with different gene variants respond differently to the dietary intervention programme with respect to body composition and cardio-metabolic parameters (Research questions 6 & 7)

Several weight loss strategies have shown reduction in weight and improvement in metabolic risk factors in obese individuals, where study participants demonstrated huge variability of phenotypes in response to the same intervention (Bray, 2008) These inter-individual variations in response to weight loss interventions suggest that genetic factors may play an important role in modulating weight regulation (Loos & Rankinen, 2005). Therefore, inter-individual variances at the genetic level cannot be ignored when developing strategies for weight management in overweight and obese population as there is no one-size-fits-all diet that can suit all individuals (Ferguson et al., 2016). Personalised nutrition tailored according to individual's genetic background, age, gender, physical activity level, cultural habits and food preferences has been proposed as a new therapeutic approach to optimise health and to prevent obesity (Gaboon, 2011). Early identification of the candidate gene variants that have potential to influence dietary nutrients may allow for the provision of good quality personalised recommendation to achieve effective weight loss and successful maintenance of weight.

In the individual SNP analysis, general linear regression analysis revealed no significant effect of the interaction between *FTO* gene variants x dietary intervention on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters, after 6 months of dietary intervention ($p>0.05$) (Chapter 7). With respect to *ADRB2* gene variants, our results revealed significant effect of the interaction between *ADRB2* genotype x dietary intervention on the post-intervention differences in fasting insulin and HOMA-IR, in response to a 6-month Hipcref diet intervention, after adjusting for covariates. Individuals carrying both the risk alleles (CG+GG) and the non-risk allele (CC) of rs1042714 had significantly greater reduction in fasting insulin and HOMA-IR after 6 months of Hipcref diet compared to a control diet. With respect to post-intervention differences in dietary intake, individuals carrying the risk allele of rs1042713 (AG+GG) had significantly greater reduction in SFA intake and MUFA intake after 6 months of Hipcref diet.

A recent study has reported that polygenic risk scoring accounts for the combined effect of multiple SNPs on disease outcomes, and may have a larger combined effect size and hence higher power to detect association between gene variants and risk of diseases than individual SNP. The use of polygenic risk scores (PRS) including multiple gene variants that predispose

an individual to obesity may have better prediction of the obesity risk and aid in weight management programmes (Shabana, Shahid, & Hasnain, 2018). Single SNP analyses are often underpowered due to the small effect size of an individual SNP (Feise, 2002). Moreover, over adjustment for multiple comparisons when testing multiple independent SNPs for associations between variables, may increase the likelihood of type II errors (the chance that the effective treatment was not discovered) (Feise, 2002). Therefore, we assessed the combined response of *FTO* and *ADRB2* gene variants on the post-intervention differences in dietary, anthropometric and cardio-metabolic parameters by computing polygenic risk scores (PRS) (Chapter 8). PRS summarises the effect of multiple gene variants into a single score to measure the genetic susceptibility to a certain disease (Lewis & Vassos, 2017). It is calculated by summing all the risk alleles of the selected gene variants, weighted by the corresponding effect size estimated from the data from GWAS (Torkamani, Wineinger, & Topol, 2018).

General linear regression analysis revealed significant effect of the interaction between PRS x dietary intervention on the post-intervention difference in hsCRP levels, in response to a 6-month dietary intervention. Individuals from the second and third tertiles of PRS had significantly greater reduction in hsCRP levels after 6 months of Hipcref diet compared to a control diet. This finding suggests that individuals carrying higher polygenic risk scores (PRS), i.e. higher risk, were associated with greater reduction in hsCRP levels with a Hipcref diet compared to a control diet. Therefore, overweight and obese Malaysian adults carrying the risk alleles of *FTO* and *ADRB2* genes, may benefit from Hipcref diet. However, large scale studies are required to confirm the current findings in a wider population from other geographical locations and population groups. As per the dietary strategy of this study, calorie restriction, higher protein, PUFA, vitamin E and dietary fibre intake had a combined effect on modulating the effect of gene variants on the post intervention changes in body composition and cardio-metabolic parameters. We cannot attribute the variations in post-intervention differences in anthropometric and cardio-metabolic parameters between the PRS groups to any of the latter dietary components of the Hipcref diet.

Future direction - The use of precision nutrition

There is an increasing interest in using genetic testing for precision nutrition or personalised nutrition in healthcare settings. The goal of precision nutrition is not to substitute the general dietary guidelines, but to enhance and modify the dietary guidelines according to individual's genetic information to improve long term weight management, if there is sufficient evidence

to do so. However, the use of genotypic information in personalised nutrition has been questioned due to various reasons. The major issues to be addressed regarding to genotype-based personalised dietary advice are as below (Görman et al., 2013):

- Does our current understanding and evidence on gene-diet interactions sufficiently to offer personalised nutrition in public health settings?
- Are people more likely to make healthy changes in their eating habits if they are introduced to genotype-based dietary advice? Does personalised nutrition produce better, more sustained, more cost-effective and greater beneficial effect on human overall health and well-being that can be achieved by conventional dietary advice?

'Does our current understanding and evidence on gene-diet interactions sufficiently to offer personalised nutrition in healthcare settings?'

Nutrition-related research and genome-wide association studies have reported inconsistent and contradictory results over the years. This is not only due to the quality of the research, but the complexity of the effects of diet and nutrition on long-term health. The situation though is improving, as the genotyping costs have been decreased and the inclusion of genetics in many nutritional studies has been increased over the last few years. However, with the current data available, it is still premature to apply the use of genetic testing in the public health services or to be reimbursed by insurance. For precaution purposes, it is suggested that personalised dietary advice should be offered only when there is strong and consistent scientific evidence on the beneficial health effects, followed by stepwise evaluation of unforeseen behavioural and psychological effects.

The main challenge of the application of genetic testing using PRS is to determine which SNPs and the corresponding 'genotype effect sizes' are to be included into the model for the prediction of disease risk, as the parameters for generating an optimal PRS are unknown. There are also arguments that many disease-related gene variants were yet to be identified and risk prediction may be improved if more disease predisposing gene variants are included in the models (Kooperberg, LeBlanc, & Obenchain, 2010). Therefore, further studies should make better use of the whole-genome sequencing (which provides information on SNPs, small insertions/deletions, CNVs and other structural variants in a single dataset) or include a larger number of obesity-associated SNPs (positive associations with BMI have been highly replicated in many studies), into the PRS models to improve the prediction of the disease risk rather than the individual effect of a single gene variant. Different PRS models with the

inclusion of different combination of SNPs should be explored and investigated to determine a better model for the prediction of the disease risk and the outcome of the treatment.

However, there are some specific gene–diet interactions which the evidence of the possible benefits is consistent and sufficient, should be seriously considered by the experts or the health professionals. For example, genes that involved in insulin signalling pathway such as insulin receptor substrate 1 (*IRS1*), proprotein convertase subtilisin/kexin type 7 (*PCSK7*), transcription factor 7 like 2 (*TCF7L2)* and gastric inhibitory polypeptide receptor (*GIPR*) (T. Huang et al., 2015; Q. Qi, Bray, Hu, Sacks, & Qi, 2012; Q. Qi et al., 2011) were associated with insulin resistance and type 2 diabetes (Caruso et al., 2014). There is a large body of evidence showing that dietary carbohydrate and fat can modulate the effect of these genetic variants on insulin sensitivity. In a 2-year Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial, the risk allele carriers (CC) of *IRS1* rs2943641 (C>T) had greater reduction in weight and improvement in insulin sensitivity with a high-carbohydrate, low-fat diet compared to the non-risk allele carriers (T) in overweight and obese American population (Q. Qi et al., 2011). The mechanism underlying is unknown, but the author claimed that it may be due to lipid-induced insulin resistance. High fat diet may increase plasma free fatty acids and impair insulin signalling pathway by altering tyrosine/serine phosphorylation of IRS leading to decreased activation of IRS-associated PI3K activity (Belfort et al., 2005). Thus, *IRS1* associated PI3K activity may be enhanced by high-carbohydrate, low-fat diet in CC genotype. The risk allele carriers (T) of gastric inhibitory polypeptide receptor (*GIPR*) rs2287019 (C>T) showed greater reduction in weight, fasting glucose, fasting insulin and improvement in insulin sensitivity after 6 months of low-fat, high-carbohydrate, high fibre diet compared to high fat diet in overweight and obese American population (Q. Qi et al., 2012). Similar finding was observed in proprotein convertase subtilisin/kexin type 7 (*PCSK7*) gene polymorphism, risk allele carriers (GG) of *PCSK7* rs236918 (C>G) had greater reduction in fasting insulin and HOMA-IR by consuming a hypocaloric low-fat, high-carbohydrate diet compared to the non-carriers (C) in an overweight and obese American population (T. Huang et al., 2015). The risk allele carriers (T) of *TCF7L2* rs7903146 (C>T) and rs12255372 (G>T) had greater reduction in plasma glucose, insulin levels, HOMA-IR and glycaemic control after a low fat diet compared to high fat diet in overweight and obese European and American populations (Katrine Grau et al., 2010; Mattei, Qi, Hu, Sacks, & Qi, 2012) . Therefore, these findings provide strong evidence that the compositions of the diet with respect to the calories

from fat and carbohydrate play an important role in modulating the effect of genes involved in insulin signalling pathway.

'Are people more likely to make healthy changes in their eating habits if they are introduced to genotype-based dietary advice? Does personalised nutrition produce better, more sustained, more cost-effective and greater beneficial effect on human overall health and well-being that can be achieved by conventional dietary advice?'

As we known, behavior modification is key to success for any weight management programme. However, there is limited evidence to support that the genotype-based dietary advice will motivate appropriate behaviour changes. Preliminary studies suggest that incorporating genetic information in personalised nutrition may be useful in long-term weight loss. Arkadianos et al., 2007 examined weight loss and weight loss maintenance following a personalised calorie-controlled diet and exercise program, based on 24 SNPs in 19 genes involved in metabolism, in participants with a history of weight loss failure compared to control participants who just received generic dietary and exercise advice. The authors reported that the nutrigenetically tailored diet achieved better compliance, improvement in glucose levels and reduction in BMI not only during the weight loss period but also over the following year. A recent pan-European study investigated the attitudes of consumers towards genetic testing and personalised nutrition (Stewart-Knox et al., 2009). The results of this study were encouraging, with 66% of respondents willing to undergo genetic testing and 27% willing to adhere to a personalised diet. Interestingly, individuals with MetS and T2DM related health conditions were particularly positive toward nutrigenetic intervention. These findings are encouraging for the future application of genome-customised diets for obesity, MetS and T2DM prevention and therapy following personalised approaches. However, another personalised dietary intervention, based on 4 SNPs in four genes, with stratification of overweight/obese and control subjects into diet or diet and exercise groups, demonstrated that individuals were slow to take optimal health advice, particularly in the combined diet and exercise group (French, 2009). This was a small study based on a limited number of genetic variants, and it raises the issue of negative consumer opinion, which poses a potential barrier to the application of nutrigenetic based intervention. As the success or failure of any new technology is consumer driven, consumer research in the application of personalised nutrition is essential. Filling these gaps will require larger and better-designed RCTs.

The use of omics technologies

Recent studies have demonstrated that other than genotype-phenotype relationships, the existence of epigenetic markers such as DNA methylation or chromatin remodeling and gut microbiota may modify gene expression, and hence affect human health and disease (Hansen & Sams, 2018; Stöger, 2009). Therefore, a full molecular mechanistic insight will require coordinated sets of multiple omics data to implement more personalised recommendations for body weight management. Advances in omics technologies such as genomics, transcriptomics, proteomics and metabolomics, epigenetics, microRNA and gut microbiota are able to generate large amount of data sets from gene sequencing and gene expressions to proteins and metabolites (Karczewski & Snyder, 2018). The integration of multiple omics data is able to provide a more extensive analysis and comprehensive understanding on health and disease. Therefore, instead of single omics approach, future studies should integrate other 'omics' approaches to generate a better understanding on the casual relationships between genetic factors and lifestyle factors on the pathogenesis of obesity and its complications (Manzoni et al., 2018). These technologies have a great potential to be applied in health care system as an effective predictive or diagnostic tool in assessing the degree of involvement of genetics in the onset of obesity, type 2 diabetes and cardiovascular diseases as well as the development of personalised treatments to prevent obesity and its complications.

9.3 Impact on society

According to Tan et al., 2011, the treatment of obesity and its primary comorbidities are estimated to cost the Malaysian health care system approximately US$162 million per year. An effective weight management programme can not only improve the overall health and well-being of the Malaysian population, but can also reduce the burden of health cost of obesity-induced non-communicable diseases (NCD) incurred on the Ministry of Health, government of Malaysia. Therefore, introducing this dietary intervention programme (Hipcref diet) to the Malaysian population may have a strong impact on the individual, the society, the economy and benefit the nation holistically.

9.4 Potential application

The evidence based data generated in this study could provide useful insight to medical, health providers or policy makers involved in obesity management to consider adopting a holistic framework that sets therapeutic goals not only to reduce obesity but also to improve the

metabolic health of the individuals. This dietary intervention regime (Hipcref diet) can be used as a reference in prescribing a personalised diet chart to overweight and obese Malaysian adults (Malaysian Chinese, Malays and Indians) based on their differences in food habits and cultural attitudes toward food. This personalised weight reduction intervention which control weight in tandem with Malaysian food preferences and culture has potential to be used nationally in adults in order to control obesity and its complications in Malaysia. Subsequently, it may be modified in order to apply it in weight control in the Malaysian children.

Further, a better understanding on the interaction between multiple genes and lifestyle factors on obesity-related traits will definitely help to develop useful diagnostic and predictive tests, and to design and optimise new treatments to combat obesity and obesity-related chronic diseases in the Malaysian population. Early detection of candidate gene variants in the Malaysian populations prior to weight loss intervention may reveal the type of dietary intervention they should follow to provide a better outcome in weight reduction or reduction in metabolic risk factors. Thus, improving the effectiveness of a weight reduction strategy as it has the potential to modify metabolic and nuclear processes of susceptible individuals.

9.5 Conclusion

In summary, our results revealed significant differences in dietary intake, obesity-related anthropometric and cardio-metabolic parameters between the adult Malaysians of the three major ethnic groups (Malaysian Malays, Chinese and Indians). Malaysian Indians had significantly higher BMI, WC, fat mass, body fat percent, fasting insulin, HOMA-IR and lower HDL cholesterol levels, compared to the Chinese and Malays. Dietary analysis revealed that Malaysian Indians consumed lower dietary cholesterol and higher fibre, compared to their Malaysian Chinese and Malay counterparts. However, our study sample is not representative of the Malaysian population, and hence the findings of the current experiments may not be generalisable to the Malaysian population as a whole. With respect to gene variants (*FTO* and *ADRB2* genes), the findings from this project suggest that gene variants may affect individual's susceptibility to obesity-related phenotypes and it may interact with dietary intake and modulate the effect of obesity-related phenotypes, in the Malaysian adults. Interestingly, this study found significant differences in genotype distributions of gene variants (*FTO* and *ADRB2* genes) in the three major ethnic groups of Malaysia i.e. Malays, Chinese and Indians. However, it should be noted that the unmeasured or unknown confounders such as other behaviour, socioeconomic and sociodemographic indicators may contribute to the variations in the

associations or gene-diet interactions on obesity-related phenotypes observed in our study participants.

Hipcref (high-protein, calorie-restricted, high vitamin E and normal fibre) dietary intervention was formulated and developed to reduce body weight and cardio-metabolic risk factors in Malaysian adults. A 6-month, two parallel-arm, RCT revealed that individualised dietary intervention (Hipcref diet) tailored according to individual's food preferences and cultural habits had greater reduction in obesity-related anthropometric parameters (BMI, WC, WHR, fat mass and percent body fat) and better metabolic health outcomes (fasting insulin, HOMA-IR and hsCRP levels) compared to generalised weight loss advice based on Malaysian Dietary Guidelines (MDG) 2010. As per the dietary strategy of this study, calorie restriction, higher protein, vitamin E and dietary fibre intake had a combined effect on the success of the intervention diet. We cannot attribute the success of the Hipcref diet to any one of the latter dietary components. In addition, we propose that personalisation is an important factor when developing strategies for weight management, especially in multi-ethnic populations. Furthermore, individuals with different gene variants responded differently to the dietary intervention programme with respect to body composition and cardio-metabolic parameters. The sample size of the current study is moderate, and hence limiting the statistical power to detect the true associations or gene-diet interactions on obesity-related phenotypes. This study is preliminary, and more trials are required to confirm the current findings in a wider Malaysian population from other geographical locations and population groups.

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Appendix

Appendix 1: Ethical approval - University of Nottingham in Malaysia, Science and Engineering Research Ethics Committee (SEREC)

Science & Engineering Research Ethics Committee
Application Form (version 2, January 2014)

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Science & Engineering Research Ethics Committee (SEREC)
Application form: research studies involving human participants

PART A

APPLICANT DETAILS:

The applicant must be the person who will conduct the investigations; each application must be made by ONE applicant. The applicant may be a member of staff or a student (undergraduate or postgraduate) registered with the University of Nottingham.

The application form must be submitted from a University of Nottingham email account.

Applicant name:	**Soma Mitra**
Email address:	**soma.mitra@nottingham.edu.my**

If the applicant is an undergraduate OR postgraduate student please also complete the section below.

Student ID number:	**Not Applicable**
Course:	**Not Applicable**
Supervisor:	**Not Applicable**

For student applicants this form must be submitted via their supervisor's University of Nottingham email account. By doing this the supervisor is indicating that they support the application.

PROJECT DETAILS:

Title of project:	**Multidisciplinary Weight Reduction Intervention Programme for Malaysian Adults**
Abstract: Include a brief summary of the project (maximum of the 300 words)	Obesity is a complex physiological response to interactions between genetic predisposition of an individual, epigenetic factors and individual behavior and lifestyle (Campion et al, 2009). It is a major risk factor for insulin resistance, hypertension, type 2 diabetes, coronary heart disease, and increased incidence of certain forms of cancer (Cheung et al 2010, Deboer 2013). Khambalia and Seen 2010, report that in 10 years (1996 to 2006) the percentage of overweight adults in Malaysia has increased from 16.6% to 29.1%. Rates of obesity rose from 4.4% to 14% in the same period of time. The burden of overweight and obesity and the morbidity that is associated with it is a heavy burden on the health services of this country. Scientists agree that the total amount of dietary fat consumed is the single most potent food related risk factor for weight gain (Blundell and Cooling, 2000). Other studies incriminate saturated fat rather than total fat, yet others suggest that consumption of high energy density rather than fat per se (or high glycemic index of food) is a risk factor for positive energy balance and thus weight gain (Blundell and Cooling, 2000). Some individuals exhibit wise behavior in their food choices and in their physical activity patterns while others display a preference for fatty foods and often sweetness combined with fattiness in food and sedentary lifestyle. However such differences in food choices can be identified through simple food frequency questionnaires (Margets et al 1989); physical activity can be documented by structured questionnaire as well. Currently, there are no successful methods to control weight gain and obesity at the population level in Malaysia, the failure of a simple intervention program to manage weight gain has been acknowledged recently in different studies (Summerbell et al, 2005; Campbell et al, 2007; Hesketh et al, 2010; Garver, 2011). Screening for genetic defects leading to hyperphagia is cost intensive and impractical. In order to reduce the clinical complications and its consequences, intervention programs to control weight in tandem with cultural food preferences has to be introduced urgently at the community level in this country. Malaysia is an ethnically diverse population with each having a distinctly different food habit and cultural attitude towards food. However, general knowledge on healthy diet do improve eating behaviors (Shimokawa, 2013). We hope to generate data from different phases of this study. Based on the data the

	study will propose a proactive and effective model (Multidisciplinary Weight Reduction Intervention Programme) for weight gain control specific to Malaysian culture, food preference and physical activity levels of the individual.

PROJECT DETAILS

Application identification number*:	**SM190614**
Study start date (planned):	**30.06.14**
Study completion date (planned):	**30.06.15**

**Note: the application identification number is the applicant's initials followed by the date of submission. E.g. If John Wong submitted a proposal on 1st September 2013 then the application identification number would be JW010913.*

Previous applications:

Has a similar study been approved by SEREC? If YES, state the title, application identification number and the submission date in the boxes below. This may speed up the review process.

Title of similar study:	**Not Applicable**
Submission date of similar study:	**Not Applicable**
Application identification number:	**Not Applicable**

PART B

Minimal Risk Checklist

The purpose of this Checklist is to facilitate the review process and to identify projects that can be considered minimal risk.

If you answer "YES" to any of these questions you will need to complete Part C of the application form.

1. Will the population studied include any vulnerable groups, e.g. participants aged under 18, disabled or lacking capacity. (If YES, please give further details)

YES	NO
	√

2. Will any participants aged under 18 be involved without the consent of their parents, guardians or carers? (If YES, please give further details). SEREC will only approve 'opt-out' consent in very exceptional circumstances.

YES	NO
	√

3. Excluding consent forms, will it be possible to associate specific information in your records with specific participants on the basis of name, position or other identifying information contained in your records? (If YES, please give further details)

YES	NO
	√

4. Will persons participating in the study be subjected to physical or psychological discomfort, pain or aversive stimuli, which are more than expected from everyday life? (If YES, please give further details).

YES	NO
	√

5. Will any inducement, other than reasonable expenses / compensation for time, be given to participants? (If YES, please give further details).

YES	NO
	√

6. Will any participants involved in this study be doing so without their knowledge and consent, e.g. covert observation? (If YES, please give further details).

YES	NO
	√

7. Will the investigation involve any blood or tissue samples being taken from the participant? (If YES, please give further details including an explanation of how these samples will be stored and disposed of).

YES	NO
√	

8. Will the investigation involve any drugs, placebos or any other substances (supplements, vitamins) being administered to patients? (If YES, please give further details).

YES	NO
	√

9. Will the investigation require the participant to undergo any type of surgical / invasive procedure, physiological / psychophysiological monitoring, TMS or fMRI studies? (If YES, please give further details).

YES	NO
	√

10. Will the participant be exposed to any unpleasant, loud or prolonged (or multiple) stimuli/testing or deprivation or restriction, e.g. of food or sleep? (If YES, please give further details).

YES	NO
	√

11. Will participants engage in strenuous or unaccustomed physical activity? (If YES, please give further details).

YES	NO
	√

12. Will the investigation use procedures designed to induce participants to act contrary to their wishes or that will affect their ability to give continuing consent, e.g. hypnosis, alcohol use? (If YES, please give further details).

YES	NO
	√

13. Will the investigation involve any procedures which participants might not feel free to withdraw from or that they may regret taking part in? (If YES, please give further details).

YES	NO
	√

14. Is the investigation likely to intentionally or unintentionally induce embarrassment, humiliation, lowered self-esteem, guilt, conflict, anger, discouragement or other emotional reactions? (If YES, please give further details).

YES	NO
	√

15. Will participants be required to recall personal memories or induced to disclose information of an intimate or otherwise sensitive nature, e.g. relationships, feelings of success? (If YES, please give further details).

YES	NO
	√

16. Will participants be deceived or actively misled in any manner or will information be withheld from them that they might reasonably expect to receive? (If YES, please give further details).

YES	NO
	√

17. Are any participants being knowingly investigated for a problem which has received medical, psychiatric, clinical psychological or other similar attention? (If YES, please give further details).

YES	NO
	√

18. Will the research involve potentially sensitive topics, e.g. sexual, racial, religious or political attitudes? (If YES, please give further details).

YES	NO
	√

19. Is there the possibility of the disclosure of confidential information, by participants or researchers, during the course of the study, e.g. to other participants? (If YES, please give further details).

YES	NO
	√

20. Will any of the research activities be taking place outside of Malaysia or the UK? (If YES, please give further details).

YES	NO
	√

21. Are you aware of any other ethical concerns that are not identified in the application? (If YES, please give further details).

YES	NO
	√

If the application relates to an **undergraduate research project** or a research project which forms part of a **postgraduate taught course,** AND you answered "NO" to all of the questions in Part B then the research is considered minimal risk by SEREC.

By submitting this application I confirm that I understand the ethical requirements for my study and have read and complied with the University of Nottingham Code of Research Conduct and Research Ethics.

For "minimal risk" projects the applicant only needs to complete Part A and Part B before submitting the Application Form to their School Research Officer.

PART C

Part C should only be completed if the project is considered to greater than "minimal risk"

This section outlines the supporting information that should be submitted with your application IF it is considered to be greater than minimal risk.

Checklist of information to include with your application (please tick)

☐	A description of the study design (maximum 3000 words) which should cover, but is not limited to: – Rationale for the research – Number and type of participants – Number and duration of activities participants will be involved in – Equipment and procedures to be applied – Information about how participants will be recruited – Whether participants will be compensated (state how this will be done) – How participants will be informed that they may withdraw at any time – Plans to ensure participant confidentiality and anonymity – Plans for storage and handling of data – Information about what will happen to the data after the study – Information about how any data and images may be used
☐	Copies of any information to be given to participants. (This includes recruitment information e.g. adverts, posters, letters).
☐	A copy of the participant consent form.
☐	Copies of data collection sheets, e.g. questionnaires.
☐	Any other information (please state)

Where possible ALL supporting documents should be submitted as pdf files.

By submitting this application I confirm that I understand the ethical requirements for my study and have read and complied with the University of Nottingham Code of Research Conduct and Research Ethics.

If you answered "YES" to any of the questions in Part B then the completed form must be submitted to the Ethics Administrator.

Click HERE to submit the form.

The University of Nottingham
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Ethics Committee Reviewer Decision

This form must be completed by each reviewer. Each application will be reviewed by at least two members of the Ethics Committee. Reviews should be completed electronically and emailed to the **Ethics Administrator** (ethics@nottingham.edu.my) from a University of Nottingham email address.

Applicant full name:	Soma Mitra
Application identification number:	SM190614

REVIEWED BY:

Reviewer ID:	L
Date:	30.6.2014
Outcome:	Approved - see amendments/comments below

Major amendments required:

Minor amendments required:

1. Q7 of application form. Since blood samples are taken from the participants. There should be an explanation of how these samples will be stored and disposed of.

2. Healthy Volunteer's Information Sheet. Under section "Who can I complain to" - should contact Professor Dr Massawe Festo, Head School of Biosciences at 03-8924-8218 or festo.massawe@nottingham.edu.my and not to the Ethics Committee Secretary.

Comments:

The above adjustment to the form is required before initiation of the study.

Please note:

1. The approval only covers the participants and trials specified on the form and further approval must be requested for any repetition or extension to the investigation.
2. The approval covers the ethical requirements for the techniques and procedures described in the protocol but does not replace a safety or risk assessment.
3. Approval is not intended to convey any judgement on the quality of the research, experimental design or techniques.
4. Normally, all queries raised by reviewers should be addressed. In the case of conflicting or incomplete views, the ethics committee chair will review the comments and relay these to the applicant via email. All email correspondence related to the application must be copied to the Faculty research ethics administrator.
5. You have the right to appeal against a decision by the Research Ethics Committee. To do so please complete the appeal form and submit to ethics@nottingham.edu.my

Any problems which arise during the course of the investigation must be reported to the Research Ethics Committee

Appendix 2: Amendment to ethics approval - University of Nottingham in Malaysia, Science and Engineering Research Ethics Committee (SEREC)

The University of Nottingham
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Amendment Form: University of Nottingham Malaysia Campus Application for amendment to ethics approval for research study involving human participants

PART A

Applicant details

Student Applicant

If the applicant is an undergraduate or postgraduate taught or research student please complete the section below. The application must be approved by a supervisor in Part C and submitted via the supervisor's University of Nottingham email account.

Note: Research Assistants who are registered on a postgraduate programme should complete the Student Applicant section.

Student name:		Student ID #:	
Course:		Email address:	
Supervisor:			

Staff Applicant

If the applicant is a member of university research or academic staff, please complete the section below: For research staff, the application must be approved by the principal investigator (PI) and submitted via the PI's University of Nottingham email account.

Name of applicant:	Dr Soma Mitra	Name of PI (if different)	
Applicant email address:	Soma.Mitra@nottingham.edu.my	PI email address:	

Project details

Title of project:	Developing a model for an intervention programme for weight management in overweight and obese Malaysian adults

Application identification number:	SM220615
Study start date:	30.06.2014
Study completion date (planned):	30.07.2018

Date of original ethical approval:	30.06.2014

Previous applications:

Has a similar process to that covered by this amendment been approved by the UNMC Research Ethics Committee? If YES, state the title and the submission date in the boxes below. This may speed up the review process. Also, state how this application differs from the previous study.

Title of similar study:	Multidisciplinary Weight Reduction Intervention Programme for Malaysian Adults
Submission date of similar study	19.06.2014
How application differ:	In this above project, we request for an extension and we have included gene analysis in this study. Moreover, the title has been rephrased to 'Developing a model for an intervention programme for weight management in overweight and obese Malaysian adults'.

FOR OFFICE USE ONLY

Date form received:

	Name	**Date**
Passed to reviewer 1:	1.	
Passed to reviewer 2:	2.	

Date decision returned to applicant:

PART B

Changes since previous application

Please detail the ALL changes made to the submission that require an amendment, and the reasons for these changes.

My research is on a project titled 'Developing a model for an intervention programme for weight management in overweight and obese Malaysian adults'. As the title suggest it is an intervention study on Malaysian adults of 3 ethnic groups. Upon sample size calculation to achieve sufficient power of 80-90%, it was necessary to recruit 83 cases and 83 controls. Further as detailed in the initial application, this project investigates genotypic and phenotypic differences in Malaysian adults of all the three ethnic backgrounds. For purposes of statistical analysis, sufficient numbers of participants of all three backgrounds have to be recruited for effective outcome of data generated from this study. Recruitment of free living participants (as per inclusion criteria) has been difficult and is ongoing. Further upon recruitment as detailed in the application, an intervention diet is prescribed to cases, and both cases and controls are followed up for, adherence to study; diet and lifestyle data collection and blood collection and genetic analysis. Since the recruitment of participant cannot happen all at the same time, the timeline for the intervention of recruited participants is staggered as well. The attached schematic diagram will explain this process. Hence an extension is necessary for completion of the intervention programme and data collection. Therefore I request that you will consider my application favourably.

Moreover, the objectives of this study have been changed as the project goes on. Genetic analysis has been decided to be included in this study, making it a multidisciplinary weight reduction intervention programme. This has further extended the timeline of the study.

The aim and objectives of the study as recorded in the previous application are as follow:

1. To assess the habitual diet with respect to macro and micro nutrient intake.
2. To assess physical activity levels, using structured questionnaire.
3. To measure body composition using bio-electric impedance analysis (BIA, InBody 720) for quantification of whole body fat mass.
4. To test for relevant bio-chemical serum parameters : (a) Plasma glucose, (b) lipid profile, (c) serum insulin levels (d) serum leptin and (e) hsCRP levels.
5. Based on the baseline dietary and lifestyle data, to advice individualised modification on diet and lifestyle.
6. To conduct educational sessions related to weight management such as balanced diet.

The added specific objective is to identify the association of specific gene polymorphisms with dietary, anthropometric and serum biochemical parameters in overweight and obese Malaysian adults.

a) 15ml of blood will be collected for the analysis of fasting glucose, insulin, hsCRP and lipid levels.
b) Blood will also be investigated for specific genes (*FTO* and *ADRB2*) to evaluate the interaction between gene variants and dietary factors in modulating the obesity-related anthropometric and biochemical parameters,

PART C

Checklist of information to include with your application

Please tick the applicable boxes below to confirm that you have included the following information with your submission. Failure to include the required information may result in your ethics amendment and approval for the changes to your research to be delayed.

√	pdf of the original Ethics Application
√	A description of the changes to the study, with justification for these changes (maximum 2000 words)
√	Copies of any new information sheets to be given to participants. (This includes recruitment information e.g. adverts, posters, letters)
√	Copies of any new data collection sheets, e.g. questionnaires

I confirm that all of the above have been included in the application and are attached to the submission email.

Application name: Dr Soma Mitra

Date: 22.06.2015

√	*As the applicant I confirm that I have read and understand the ethical requirements for my study and have read and complied with the University of Nottingham Code of Research Conduct and Research Ethics.*

Supervisor / PI name (where applicable):

Supervisor / PI email address:

Date:

☐	*As supervisor / PI, I confirm that by submitting this application from my university email account I have checked the details of this application and that it has my approval.*

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Ethics Committee Reviewer Decision

This form must be completed by each reviewer. Each application will be reviewed by at least two members of the Ethics Committee. Reviews should be completed electronically and emailed to the **Ethics Administrator** (ethics@nottingham.edu.my) from a University of Nottingham email address.

Applicant full name:	Soma Mitra
Application identification number:	SM220615
REVIEWED BY:	
Reviewer ID:	L
Date:	07.07.15
Outcome:	Approved - no amendments required

Major amendments required:

Minor amendments required:

Comments:

Amendments accepted

Please note:

1. The approval only covers the participants and trials specified on the form and further approval must be requested for any repetition or extension to the investigation.
2. The approval covers the ethical requirements for the techniques and procedures described in the protocol but does not replace a safety or risk assessment.
3. Approval is not intended to convey any judgement on the quality of the research, experimental design or techniques.
4. Normally, all queries raised by reviewers should be addressed. In the case of conflicting or incomplete views, the ethics committee chair will review the comments and relay these to the applicant via email. All email correspondence related to the application must be copied to the Faculty research ethics administrator.
5. You have the right to appeal against a decision by the Research Ethics Committee. To do so please complete the appeal form and submit to ethics@nottingham.edu.my

Any problems which arise during the course of the investigation must be reported to the Research Ethics Committee

Appendix 3: Research registration under the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH)

Ver 3.0 September 2014 *NMRR/FORM/IAHODIA*

INVESTIGATOR'S AGREEMENT, HEAD OF DEPARTMENT AND ORGANISATIONAL / INSTITUTIONAL APPROVAL
PERSETUJUAN PENYELIDIK DAN KEBENARAN KETUA JABATAN DAN PENGARAH ORGANISASI/INSTITUSI

This document is intended for online submission for formal research registration. It is issued as the Investigator's Agreement to participate in the research as well as the investigator's **Head of Department and Director's Approva**l. Please upload this document in the required section in NMRR upon completion.
**Note: This form is NOT to be used for obtaining permission to conduct the research at the named / selected study site(s).

Dokumen ini adalah untuk penghantaran 'online' mengikut prosedur rasmi pendaftaran penyelidikan. Borang ini dikeluarkan sebagai pengakuan penyelidik untuk menjalankan penyelidikan dan persetujuan serta kebenaran daripada ***Ketua Jabatan dan Pengarah masing-masing****. Sila lengkapkan borang ini dan memuat naik ke dalam sistem NMRR di seksyen yang telah ditetapkan.*
***Nota : Borang ini BUKAN digunakan untuk tujuan mendapatkan keizinan untuk menjalankan penyelidikan di lokasi kajian yang dipilih.*

Research Title *[Tajuk Penyelidikan]*	Multidisciplinary Intervention Programme (MIP) for adults with non-syndromic obesity: Designing an appropriate intervention program for Malaysian Adults (Malaysian Malays, Malaysian Chinese and Malaysian Indians), based on their Diet, Lifestyle and presence of Obesogenes.		
Research ID *[Nombor Pendaftaran]*	25110	**Protocol Number (if available)** *[Nombor Protokol (jika ada)]*	

INVESTIGATOR'S AGREEMENT *[PERSETUJUAN PENYELIDIK]*

I have understood the above mentioned proposed research and I agree to participate as an investigator and being responsible to conduct the research.

Saya faham atas cadangan penyelidikan di atas dan bersetuju untuk mengambil bahagian serta bertanggungjawab untuk melaksanakan penyelidikan tersebut.

Name *[Nama]*	Soma Mitra
IC number *[Nombor K/P]*	50881372
Institute *[Institusi]*	The University of Nottingham Malaysia Campus
Signature and Official stamp *[Tandatangan dan Cop Rasmi]*	Soma Mitra FACULTY OF SCIENCE THE UNIVERSITY OF NOTTINGHAM MALAYSIA CAMPUS (Company No. 473520-K) JALAN BROGA, 43500 SEMENYIH SELANGOR DARUL EHSAN, MALAYSIA. TEL: 03-8924 8000 FAX: 03-8924 8018
Date *[Tarikh]*	

HEAD OF DEPARTMENT AGREEMENT *[PERSETUJUAN KETUA JABATAN]*

I agree to allow the above named investigator to conduct the above titled research.

Saya bersetuju dan membenarkan pegawai seperti bernama di atas untuk menjadi penyelidik di dalam projek penyelidikan tersebut di atas.

Name of Head : *[Nama Ketua Jabatan]*	Professor Festo Massawe
Signature and Official stamp *[Tandatangan dan Cop Rasmi]*	PROF. FESTO MASSAWE *Head of School, Biosciences* The University of Nottingham Malaysia Campus
Date *[Tarikh]*	1/4/15

ORGANISATIONAL / INSTITUTIONAL APPROVAL *[KEBENARAN ORGANISASI / INSTITUSI]*

I acknowledge and approve the named officer to conduct the above titled research.

Saya mengesahkan dan mengambil maklum penglibatan pegawai ini di dalam penyelidikan tersebut.

Name of Director *[Nama Pengarah]*	Professor Graham Kendall
Signature and Official stamp *[Tandatangan dan Cop Rasmi]*	*Professor Graham Kendall* *Vice Provost* *Research and Knowledge Transfer*
Date *[Tarikh]*	

This is computer generated. Borang ini adalah cetakan komputer. [25110/33737/100060]

Appendix 4: Participant's information sheet (English version)



University of Nottingham, School of Biosciences

Faculty of Science, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan

Title of Project: Multidisciplinary Intervention Programme (MIP) for adults with non-syndromic obesity: Designing an appropriate intervention program for Malaysian adults (Malaysian Malays, Malaysian Chinese and Malaysian Indians), based on their diet, lifestyle and presence of obesogenes

Principal Investigator: Dr Soma Mitra

Healthy Volunteer's Information Sheet

Invitation

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you decide to take part, you may keep this leaflet. Thank you for reading this.

Background

The prevalence of diet-enhanced chronic diseases has increased in both developed and developing countries during the last decades. Taking excess calories through diet cause weight gain, which if not **monitored** can result in an individual becoming overweight for his/her height. Overweight and obesity is a major basis for complications like hypertension, type 2 diabetes, coronary heart disease, and certain forms of cancer. Moreover, association between some obesity related genes and obesity related traits have been consistently observed in studies involving various populations worldwide. Studies have shown that different individuals carry variants of the same gene. Studying these differences may help the investigators find solutions to obesity and help manage excess weight of individuals.

Research has shown that individuals who were **counselled** in a primary care setting about the benefits of healthy eating and physical activity often took positive action to reduce weight. They altered their diet and behaviour more than individuals who did not receive counselling.

Malaysia is an ethnically diverse population with each ethnic group having a distinctly different food habit and cultural attitude towards food. However, general knowledge on healthy diet improve people's eating behaviours.

We hope to help you to reduce body weight through the following measures:

a) We will monitor your weight periodically through six months.

b) We will collect information on your food preferences and physical activity through a typical day and suggest changes in diet.
c) We will collect your body composition data by a simple and non-invasive technique to identify hidden fat in your body which may not be apparent just by measuring body weight.
d) It is important to check the blood sugar and lipid levels in your blood. This will increase the possibility of identifying if you are at risk of conditions like type 2 diabetes or cardiovascular diseases. Therefore, we will collect 12 mls of blood for analysis of blood sugar, lipid, leptin, hsCRP levels and genes, at the start of the study. We will collect same quantity of blood after 6 months again to study the differences between pre and post dietary changes that you will undergo through the six-month period. We will investigate gene variants of a few genes that have been reported to be obesogenic.
e) We will conduct educational sessions related to weight management such as healthy dietary habits, self-control techniques, coping with special situation (like party, holiday, breaks with friends and family).

With all the above information, our study will propose a proactive and effective model for weight gain control according to the culture, food preference and physical activity levels of you and other participants of this study.

What does the study involve?

Qualified scientists will take the measurements as mentioned above. Trained and qualified nutritionist will collect your dietary and physical activity data and advice you on changes. A qualified nurse from a diagnostic health centre will collect a small amount of blood for the tests mentioned above. All of the above procedure will take 30- 45 mins of your time.

We will contact you at your convenience through a) email or b) facebook or c) whatsApp to check whether or not you are having any difficulty in complying with our advice. Depending upon your response we will advise you on the next course of action.

Your responsibilities

Only to follow the changes suggested in your diet.

Do you have to take part?

Taking part in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

Who can take part?

If you are Malaysian and 18 years old and above
If you are **not** pregnant
If you are **not** suffering from cardiovascular diseases, stroke, diabetes, hypothyroidism, chronic liver disease, chronic kidney disease, psychological or psychiatric disorders
If your BP is **not** >160/100 mm Hg

If you are **not** an alcohol and/or drug abuser
If you are **not** on antidepressants, antipsychotics, steroids, chemotherapeutics.

Will my taking part in this study be kept confidential?

All information collected about you will be stored on a database which will be password protected and kept strictly confidential. Any information about you which leaves the research unit will have your name and address removed. Your data will be recorded against a number (ID) and you cannot be identified from it. This study has been reviewed and approved by the University of Nottingham in Malaysia Science and Engineering Research Ethics Committee.

How long we keep your data
The University may store your data for up to 25 years and for a period of no less than 7 years after the research project finishes.

Will I be paid for participating in this study?
There is no remuneration for participating in this study

Who can I complain to.
In case you have a complaint in your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator. If this achieves no satisfactory outcome, you can then contact the Ethics Committee Secretary, of the Faculty of Science Office, UNMC at vanitha.singaram@nottingham.edu.my. Additionally you can contact the head of the school Professor Festo Massawe at Festo.Massawe@nottingham.edu.my.

Contact for further information
If you have any questions regarding this study, or would like additional information about participation, please do not hesitate to contact me at 0122279026 or soma.mitra@nottingham.edu.my or Pui Yee at 0103662923 or khyx4tpe@nottingham.edu.my.

Thank you in advance for your interest and assistance with this research.

Yours sincerely
Dr Soma Mitra
Associate Professor
University of Nottingham Malaysia Campus
43500 Semenyih

Appendix 5: Participant's information sheet (Malay version)



University of Nottingham, Pusat Pengajian Biosains
Fakulti Sains, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan

Tajuk Projek: 'Program Intervensi Multidisiplin (MIP) bagi orang dewasa dengan obesiti bukan syndromik: Merancang program intervensi yang sesuai untuk orang dewasa Malaysia (Malaysian Malay, Malaysian Cina dan Malaysian Indian), berdasarkan diet, gaya hidup dan kehadiran obesogenes'
Penyiasat Utama: Dr Soma Mitra

Kunci Maklumat Sukarelawan yang Sihat

Jemputan

Anda telah dijemput untuk menyertai kajian penyelidikan. Sebelum anda membuat keputusan untuk menyertai kajian ini, ianya penting supaya anda memahami tujuan penyelidikan ini dilaksanakan dan keperluannya. Sila baca secara teliti informasi berlanjutan dan bincang bersama rakan dan saudara jika perlu. Anda boleh mengemukakan sebarang pertanyaan jikanya kurang jelas atau memerlukan lebih penjelasan. Sila buat keputusan sama ada anda ingin menyertai atau pun tidak. Sekiranya anda ingin meyertai kajian ini anda boleh simpan risalah ini. Terima kasih kerana mengambil masa untuk membaca kandungan ini.

Latar Belakang

Penularan diet yang dipertingkatkan penyakit kronik telah meningkat di negara maju dan membangun semenjak dekad yang lalu. Pengambilan kalori berlebihan melalui diet akan menyebabkan peningkatan berat badan, sekiranya tidak dipantau boleh menyebabkan seseorang individu menjadi terlebih berat badan untuk ketinggiannya. Berat badan berlebihan dan obesiti merupakan asas utama bagi komplikasi seperti tekanan darah tinggi, diabetes jenis 2, penyakit jantung koronari, dan beberapa bentuk kanser. Penyelidikan telah menunjukkan bahawa individu yang telah diberi kaunseling dalam suasana penjagaan yang utama tentang manfaat pemakanan sihat dan aktiviti fizikal yang kerap mengambil tindakan positif untuk mengurangkan berat badan. Mereka telah mengubah diet dan tingkah laku mereka lebih daripada individu yang tidak menerima kaunseling. Malaysia terdiri daripada penduduk yang pelbagai etnik dengan setiap satunya mempunyai tabiat pemakanan dan sikap budaya yang jelas berbeza ke arah makanan. Walau bagaimanapun, pengetahuan am tentang pemakanan sihat meningkatkan tingkah laku rakyat terhadap makanan.

Kami berharap dapat membantu anda untuk mengurangkan berat badan melalui langkah-langkah berikut:

a) Kami akan memantau berat badan anda dari masa ke semasa dalam tempoh 6 bulan.
b) Kami akan mengumpul maklumat mengenai pilihan makanan dan aktiviti fizikal hari biasa dan mencadangkan perubahan dalam pilihan makanan.
c) Kami akan mengumpul data komposisi badan anda dengan teknik yang mudah dan bukan invasif untuk mengenal pasti lemak tersembunyi dalam badan anda yang mungkin tidak jelas hanya dengan mengukur berat badan.
d) Adalah penting untuk secara berkala memeriksa gula darah, insulin, tahap lipid, leptin dan penanda keradangan dalam darah anda. Ini akan meningkatkan kemungkinan mengenal pasti jika anda mempunyai risiko keadaan seperti diabetes jenis 2 atau penyakit kardiovaskular. Oleh itu kami akan mengumpulkan 12 ml darah untuk analisis peringkat gula darah, insulin, lipid, leptin dan hsCRP. Kami akan mengumpul kuantiti darah yang sama sekali lagi selepas 6 bulan untuk mengkaji perbezaan yang anda menjalani sebelum dan selepas intervensi dalam tempoh enam bulan ini. Kami akan menyiasat beberapa gen variasi yang telah dilaporkan menjadi obesogenik.
e) Kami akan menjalankan sesi pendidikan yang berkaitan dengan pengurusan berat badan yang sihat seperti pemakanan, teknik kawalan diri, menghadapi keadaan khas (seperti parti, percutian, masa rehat bersama rakan-rakan dan keluarga).

Dengan semua maklumat di atas, kajian kami akan mencadangkan model proaktif dan berkesan untuk mengawal berat badan mengikut tahap budaya Malaysia, keutamaan makanan dan aktiviti fizikal peserta kajian.

Apakah yang terlibat dalam kajian?

Anda akan diberi tarikh, masa dan tempat bergantung kepada kemudahan anda melaporkan diri untuk pengumpulan data dan susulan. Ahli-ahli sains yang layak akan mengambil ukuran seperti yang dinyatakan di atas. Seorang jururawat yang bertauliah dari pusat kesihatan akan mengumpulkan darah untuk ujian tersebut di atas. Semua prosedur di atas akan mengambil masa antara 30 sehingga 45 minit. Kami akan menghubungi anda mengikut keselesaan anda melalui a) e-mel atau b) facebook untuk memeriksa sama ada atau tidak anda mempunyai apa-apa kesukaran dalam mematuhi nasihat kami. Bergantung kepada tindak balas anda, kami akan menasihati anda tentang kursus selanjutnya.

Tanggungjawab anda

Mengikuti perubahan yang dicadangkan dalam diet.

Adakah anda perlu mengambil bahagian?

Penyertaan dalam kajian adalah secara sukarela. Ianya terpulang kepada anda untuk memutuskan sama ada atau tidak untuk mengambil bahagian. Jika anda membuat keputusan untuk mengambil bahagian anda akan diberi lembaran maklumat ini untuk menyimpan dan diminta untuk menandatangani borang persetujuan. Jika anda membuat keputusan untuk mengambil bahagian,

anda masih bebas untuk menarik diri pada bila-bila masa tanpa memberi sebab. Tiada ganjaran kewangan atau yang berkaitan terlibat dengan kajian ini.

Siapa yang boleh mengambil bahagian?

Jika anda warganegara Malaysia dan berumur 18 tahun dan ke atas
Jika anda tidak hamil
Jika anda tidak mengalami penyakit jantung koronari, diabetes, strok, beberapa bentuk kanser, hipotiroidisme, penyakit hati kronik, penyakit buah pinggang kronik,
gangguan psikologi atau psikiatri
Jika BP anda tidak> 160/100 mm Hg
Jika anda bukan pendera alkohol dan / atau dadah
Jika anda tidak mengambil perubatan untuk antidepresan, antipsikotik, steroid, chemotherapeutucs.

Adakah pengambilan saya bahagian dalam kajian ini dirahsiakan?

Maklumat yang dikumpulkan tentang anda akan disimpan dalam pangkalan data yang akan dilindungi kata laluan dan dirahsiakan. Apa-apa maklumat tentang anda yang digunakan oleh unit penyelidikan tersebut tidak akan mengandungi nama dan alamat anda. Data anda akan direkodkan terhadap nombor (ID) dan anda tidak dapat dikenal pasti. Kajian ini telah dikaji semula dan diluluskan oleh Universiti Nottingham di Malaysia Sains dan Kejuruteraan Penyelidikan Jawatankuasa Etika.

Berapa lama kita menyimpan data anda

Universiti boleh menyimpan data anda sehingga 25 tahun dan untuk tempoh tidak kurang daripada 7 tahun selepas projek penyelidikan selesai.

Adakah saya akan dibayar untuk menyertai kajian ini?

Tidak ada imbuhan untuk mengambil bahagian dalam kajian ini.

Siapakah yang boleh saya membuat aduan kepada.

Dalam kes anda mempunyai aduan dalam rawatan anda oleh seorang anggota kakitangan atau apa-apa kaitan dengan kajian ini, anda boleh mengadu kepada penyiasat utama. Jika ini tidak mencapai keputusan yang memuaskan, anda boleh menghubungi Etika Jawatankuasa Pengajur Setiausaha, Fakulti Pejabat Sains, UNMC di vanitha.singaram@nottingham.edu.my. Selain itu, anda boleh menghubungi ketua departmen, Professor Festo Massawe di Festo.Massawe@nottingham.edu.my.

Hubungi untuk maklumat lanjut

Jika anda mempunyai sebarang soalan mengenai kajian ini, atau ingin maklumat tambahan mengenai penyertaan, sila hubungi saya di 01123298179 atau soma.mitra@nottingham.edu.my ataupun Tan Pui Yee di 0103662923 atau khyx4tpe@nottingham.edu.my. Terima kasih terlebih dahulu untuk minat dan bantuan anda dengan kajian ini.

Yang benar
Dr Soma Mitra
Profesor Madya
Universiti Nottingham Kampus Malaysia

Appendix 6: Participant's informed consent form (English version)

PARTICIPANT CONSENT FORM

I agree to participate in the research study titled:

Multidisciplinary Intervention Programme (MIP) for adults with non-syndromic obesity: Designing an appropriate intervention program for Malaysian adults (Malaysian Malays, Malaysian Chinese and Malaysian Indians), based on their diet, lifestyle and presence of obesogenes

1. I confirm that I have read and understood the participant information sheet and have been given the opportunity to ask questions and discuss this study. I have received satisfactory answers to my questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason and without my medical care or legal rights being affected.

3. I understand that even if I withdraw from the above study, the data collected from me will be used in analysing the results of the trial, unless I specifically withdraw consent for this.

4. In case of withdrawn consent, no new information will be collected or added to existing data, and I can request that all previously retained samples to be destroyed to prevent further analysis.

5. I consent to the storage, including electronic, of personal information for the purposes of this study. I understand that any information that could identify me will be kept strictly confidential and that no personal information will be included in the study report or other publication.

6. I agree to participate in the above study.

Use of Photographs — Form for individual consent
Masked faces of photographs of participants will be stored in the computers as evidence of the participants. Photographs will not be used for publication purposes, but for research only.
I hereby consent to the use of photographs of myself, taken by members of the University or by agents authorised on behalf of the University.

Name of the participant (print)	Date	Signature
Name of the person taking consent (print)	Date	Signature

Appendix 7: Participant's informed consent form (Malay version)

BORANG KEBENARAN PESERTA

Saya bersetuju untuk mengambil bahagian dalam kajian penyelidikan bertajuk:

Program Intervensi Multidisiplin (MIP) bagi orang dewasa dengan obesiti bukan syndromik: Merancang program intervensi yang sesuai untuk orang dewasa Malaysia (Malaysian Malay, Malaysian Cina dan Malaysian Indian), berdasarkan diet, gaya hidup dan kehadiran obesogenes

1. Saya mengesahkan bahawa saya telah membaca dan memahami lembaran maklumat peserta dan diberi peluang untuk bertanya dan membincangkan kajian ini. Saya telah menerima jawapan yang memuaskan terhadap soalan saya.

2. Saya faham bahawa penyertaan saya adalah secara sukarela dan saya bebas untuk menarik balik pada bila-bila masa tanpa memberi alasan dan tanpa penjagaan perubatan saya atau hak undang-undang yang terjejas.

3. Saya faham bahawa walaupun saya menarik diri daripada kajian di atas, data yang dikumpulkan daripada saya akan digunakan dalam menganalisis keputusan perbicaraan, kecuali saya menarik balik persetujuan ini.

4. Sekiranya persetujuan ditarik balik, tiada maklumat baru akan dikumpulkan atau ditambah kepada data sedia ada, dan saya boleh meminta semua sampel yang telah disimpan sebelum ini dimusnahkan untuk mengelakkan analisis lanjut.

5. Saya bersetuju dengan penyimpanan, termasuk elektronik, maklumat peribadi untuk tujuan kajian ini. Saya faham bahawa apa-apa maklumat yang boleh mengenal pasti saya akan disimpan secara sulit dan tidak akan ada maklumat peribadi dalam laporan kajian atau penerbitan lain.

6. Saya bersetuju untuk menyertai kajian di atas.

Penggunaan foto anda - Persetujuan individu

Gambar peserta (masked) akan disimpan di dalam komputer sebagai bukti peserta. Foto tidak akan digunakan untuk tujuan penerbitan, tetapi hanya untuk penyelidikan. Saya dengan ini bersetuju dengan penggunaan gambar-gambar saya sendiri, diambil oleh ahli-ahli Universiti atau oleh ejen-ejen yang diberi kuasa bagi pihak Universiti.

Nama peserta	Tarikh	Tandatangan
Nama orang yang mengambil persetujuan	Tarikh	Tandatangan

Appendix 8: Data collection sheet

Weight Reduction Project



Data Collection Schedule

ID No: _____ **Date of Birth: ___/___/_____** **Age: _____**

Race: _____ **Gender: _____**

Date of Interview: ___/___/_____

Surname: _____ **First Name: _____**

Contact Details:

_____

Signature of Interviewee

Body Composition Data

Age:_____ **Height:_____(cm)** **Weight: _____(kg)**

BMI: _____(kg/m^2) **Waist Circumference: _____(cm)**

Hip circumference: _____(cm) **WHR:_____**

Fat Mass (FM): _____(kg) **Fat Free Mass (FFM): _____(kg)**

Muscle mass (kg):_____ **Fat percentage (%): _____**

	Right hand	***Left hand***
Systolic blood pressure (mm Hg)		
Diastolic blood pressured (mm Hg)		
Pulse rate (beat per minute)		

Remarks:

Appendix 9: Preliminary Health and Lifestyle Questionnaire

Preliminary Health and Lifestyle Questionnaire

To be completed by all participants who have fulfilled the inclusion criteria

1. Ancestors details

	Race	Religion	Place of birth
Father			
Mother			
Paternal Grandfather			
Paternal Grandmother			
Maternal Grandfather			
Maternal Grandmother			

2. Cardiovascular History

Please encircle either (Yes or No)

Do you have diagnosed cardiovascular disease, atherosclerosis, previous heart attack, and/or previous stroke	Yes / No
Have you experienced angina (heart pain) within the last 3 months	Yes / No

3. Family History

Please encircle either (Yes or No)

Mother with Cardiovascular Disease at less than 65 years (high blood pressure, heart attack, angina, stroke, hardening of the arteries)	Yes / No
Father with Cardiovascular Disease at less than 55 years (high blood pressure, heart attack, angina, stroke, hardening of the arteries)	Yes / No
Parent with Type II Diabetes (adult-onset diabetes)	Yes / No

4. Lifestyle

a) Smoking

Please check the appropriate box with this symbol [X]

Never smoked	
Ex-smoker	
Current smoker less than 20 cigarettes/day	
Current smoker more than 20 cigarettes/day	

b) Alcohol

Please check the appropriate box with this symbol [X]

Average 0 drinks daily	
Average 1 drink daily or 7 units per week	
Average 2 drinks daily or 14 units per week	
Average 3 or more drinks daily or 21 or more units per week	

Questionnaire has been adapted from www.metagenics.co.nz

Appendix 10: FASTA sequence

a) The FASTA sequence of *TP53* rs1800371

```
CCGACCCCAA CCCCAGCCCC CTAGCAGAGA CCTGTGGGAA GCGAAAATTC CATGGGACTG
ACTTTCTGCT CTTGTCTTTC AGACTTCCTG AAAACAACGT TCTGGTAAGG ACAAGGGTTG
GGCTGGGGAC CTGGAGGGCT GGGGACCTGG AGGGCTGGGG GGCTGGGGGG CTGAGGACCT
GGTCCTCTGA CTGCTCTTTT CACCCATCTA CAGTCCCCCT TGCCGTCCCA AGCAATGGAT
GATTTGATGC TGTCCC
CGGACGATAT TGAACAATGG TTCACTGAAG ACCCAGGTCC AGATGAAGCT CCCAGAATGC
CAGAGGCTGC TCCCCGCGTG GCCCCTGCAC CAGCAGCTCC TACACCGGCG GCCCCTGCAC
CAGCCCCCTC CTGGCCCCTG TCATCTTCTG TCCCTTCCCA GAAAACCTAC CAGGGCAGCT
ACGGTTTCCG TCTGGGCTTC TTGCATTCTG GGACAGCCAA GTCTGTGACT TGCACGGTCA
GTTGCCCTGA GGGGC
```

b) The FASTA sequence of *LEP* rs7799039

```
AAAAGCTTCT TTAAGGATGG AGAGGCCCTA GTGGAATGGG GAGATTCTTC CGGGAGAAGC
GATGGATGCA CAGTTGGGCA TCCCCACAGA CGGACTGGAA AGAAAAAAGG CCTGGAGGAA
TCAATGTGCA ATGTATGTGT GTTCCCTGGT Tcaagggctg ggaactttct ctaaagggcc
aggtagaaaa cattttaggc tttctaagcc aaggcaaaat tgaggatatt acatgggtac
ttatacaaca agaataaaca atttacacaa ttttttgttg acagaattca aaaCTTTATA
GACACAGAAA TGCAAATTTC CTGTAATTTT CCCGTGAGAA CTATTCTTCT TTTGTTTTGT
TTTGCGACAG GGTTGCG
ctgatcctcc cgcctcagtc tccctaagtg ctgagatgtt gcaggaagtc agggaccccg
aacagagaga tcggctggag ccgtggcaga ggaacataaa ttttgaagat ttcatttaa
tatggacact tatcagttcc caaataatac ttttataatt ttttatgcct gtctttgctt
taatctctta atcctgttat cttcataagc taaggatgta cgtcacctca ggaccactgt
gataattgtg ttaactgtac agattgattg caaaacatgt gtgtttgaac aatatgaaat
cagtgcacct tgaaaaagag cagaataaca gcaattttta gggaacaagg gaagacaact
ataaggtctg actgcctgcg gggtcgggca aagggagcca tatttttctt cttgcagaga
gcctataaat agacctgcaa gtaggagaga tattgctaat ttcttttgct agcatggaat
attaatatta acaccctggg aaaggaatgc attcctgggg ggaggtctat aaatggccgc
tctgggaatg tctatcctat gcaatggaga taaggactga gatacgccct ggtctcctgc
agtaccctca ggcttactag ggtggtgaaa aactccgccc tggtaaattt gtggtcagac
cagttttctg ctctcgaaca ctgttttctg ttgtttaaga tgtttatcaa gacaatacgt
gcacctctga acacagaccc ttatcagtag ttctcctttt tgccctttga agcatgtgat
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gaggctcagg tgggcatcac agtactaccg atatgtgatg tcacccccgg cggcccagct
gtaaaattcc tctctttgta ctctctctct ttatttctca gccagctgac acttatggaa
aatagaaaga acctacgttg aaatattggg ggcaggttcc cccaataTCT GGTGcccaac
gtgggatact gagattacaa gcatgagcca ctgcatctgg cctcttcttt tgatttttt
ttttcaaact tttacaaatg tagaaaccgt tcttagcttt tgggcattac caaacccggc
agtggcaggc tcggttcacc aacgtcattt gcagttcccc gCTTTATGTT ATGGgttttg
ttttgttttg ttttttttAt tgagacagag tttcactctt gttgcccagg ctgtagtgc
```

c) The FASTA sequence of *FTO* rs9930501, rs9930506 and rs9932753

TTTTTTTTTT TTTGGAGACG GAGTCTCACT CTGTTGTGTA GGCTGGAGTG CAATGGCGCA
ATGCTGACTG CAACCTCCGC CTCCCAGGTT CAAGTGATTC TCCTGCCTCA GCCTCCCGAG
TAGCTGGGAT TACAGACATG TGCCACCATG CCCAGCTAAT TTTGAATTTT TAGTAGAGAT
GGGGTTTTGC AATGTCGGCC AGGCTAATCT GGAACTCCTG ACCTCAGGTG ATCGGCCCGC
ATTGGCCTCC CAAAGTGCCG GGACTGTAGG AGCGAGCCAC TGCTCCTGGC CTAGTTTTTA
ATTAAAACAA GTGAACAAAC AAAGGTGGGC ATAGAGATTG ATAAAGTGTA TTGGAAAATT
CACTAAGTTA TTGGTTTAAA TACTACTTTT TGTTCTAAAA TGAATATAAA GCTAAAACTC
TGGAGAATGA TGAGAATGTA AGAAGGGAGA AGTAAATTAT GTGTGATCCA ATATTAGGGA
CACAAAAAGG GACATACTACA
TGAATTACTA ATATCTAAGA AAATATGATA CATTTGAGAA CTTAGATGAA GTTGATACAT
ACCTAGAAAG ATAGGCATTA CTTAAATTGC TTCAAGAAGT AGAAAATCTG AATAAAACAA
TGAACGAAAT TGAATGATAA TGAAAAGTCC ATCTGTCAAA TGACAGCAGG CCCAGTTTTA
TAGGCACGTT AAACCAAATT TAAGTAATCC TTACTTACTC TTCTTCCCTT TTTAAAGAGT
TTTATTGGGG TATAATTGAT ATAGAATAAA CTGCACATAT TTAAAGTTTT GACATACGTA
TATACTGGTT AAACCACCAA AATCAAGATT AGGAGACATG TTCATTATCA CTCCTAATTT
GTGTCCCTCA GAAATCCTTT TCTCCTTCTC TCCCCTAGTG GTTCTGGGCA GCTACTAATC
TGCTTTCTGT CACTATTGAC TAGTTGGCAT TTTCTCGAAT TTTGCATAAA TGGAATCAAT
CAAACAGTAT GTGCTTTTGC

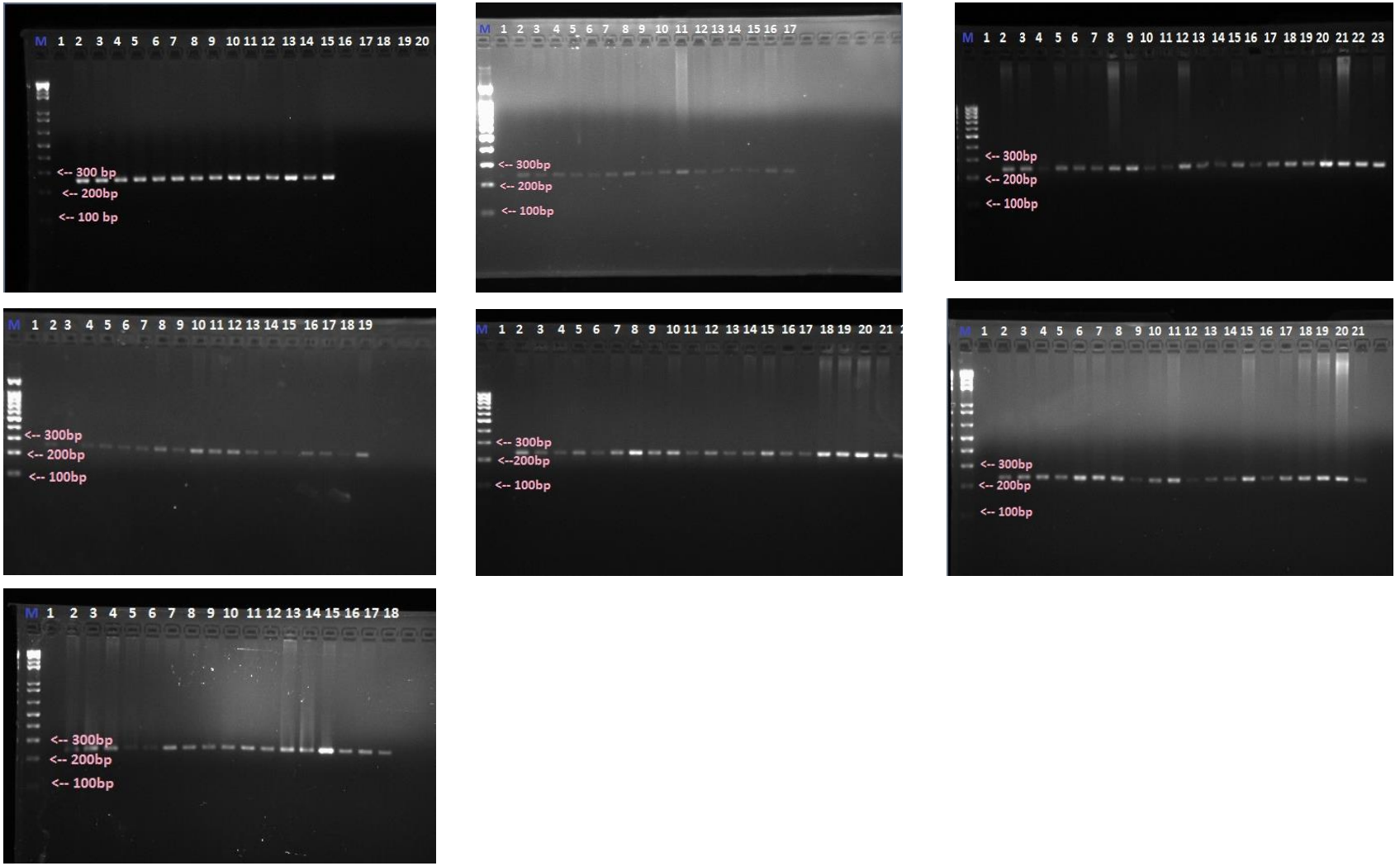
d) The FASTA sequence of *FTO* rs9939609

TGTGTTAGGC GAGATATGGT GAGTGGTTTC AGAGGCTTGT GTGAGAAAGT TGATACACTG
CCCCTACCCC ATGCAAACAC ACACTTTCTT TTCTTATGAT GACCTTTCTT TAAAAGCATT
TGTTGGAATA TGAGATTATA GGCCATAAGT GAGAAAACAA CTCTTTTTGA GCTGTGAGGA
ATACTAGGAG AGGAGAAAGT GAGCTGTGTG TGCTGCCCAT GGTGGTACGC TGCTATGGTT
CTACAGTTCC AGTCATTTTT GACAGCATGG ATTCAATGCA AAATGGCAAC ACACACTCTG
TATCTTTTGG CAGATCAGAA CATAATGAAA ATAAAATAAA AAAATTCAAA ACTGGCTCTT
GAATGAAATA GGATTCAGAA GAGATGATCT CAAATCTACT TTATGAGATA ATGTCCTTTT
TAAAAATAAA CACTAACATC AGTTATGCAT TTAGAATGTC TGAATTATTA TTCTAGGTTC
CTTGCGACTG CTGTGAATTTA
GTGATGCACT TGGATAGTCT CTGTTACTCT AAAGTTTTAA TAGGTAACAG TCAGAAATGG
AGTGGGAGAG CATAAAAGCA AACTGAAATG CAAATAGCTG GTACCCTGAA GCCATTAACT
TTAAGCTGGT TATTCCTGAC CTACTGTTTG GACATAAGAT GGTAGAGAGG CTGAGTGTGA
CTTGAACATT TGTTCCTTAG AAACACCATC CTTGGGCTGG GCGCAGTGGC TCACGCCTGT
GTTTCCAGGA CTTTGGGAGG CTGAGGCGGG CAGATCATGA GGTCAATAGA TCGAAACTAT
CCTGGCTAAC ACGGTGAAAT CCCATCTCTA CTAAAAATAC AAAAAATTAG CCAGGTGTGG
TGGCGGGTGC CTGTAGTCCC AGCTACTCGG GAGGCTGAGG CAGGAGAATT GCTTGAACCC
AGGAGGCAGA GGTTGCAGTG AGCCAAGGTC GCACCACTGC ACTCTAGCCT GGGCAACAGA
GTGAGACTCC TTCTCAAAAA

e) The FASTA sequence of *ADRB2* rs1042713 and rs1042714

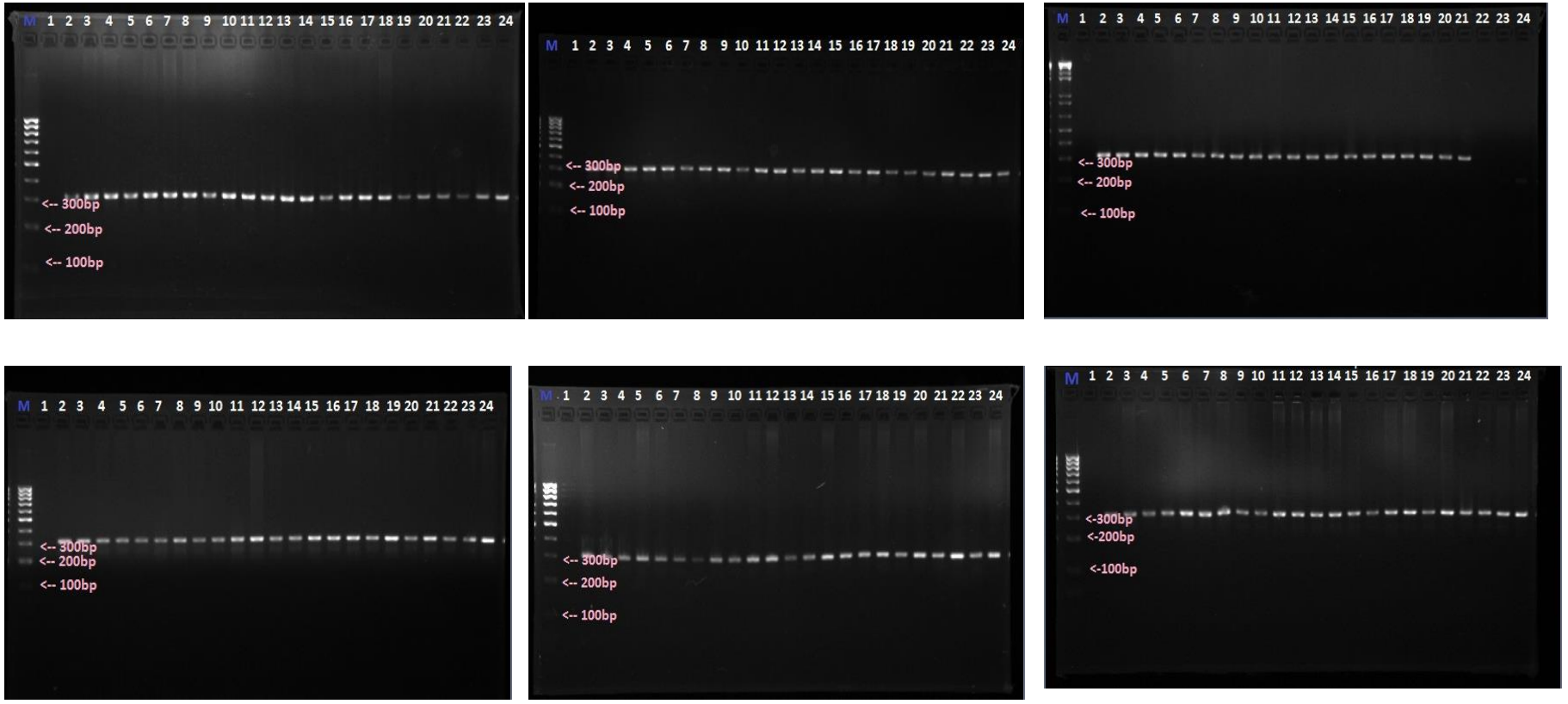
CTAGTCTGCG CACATAACGG TGCAGAACGC ACTCGCGAAG CGGCTTCTTC AGAGCACGGG
CTGGAACTGG CAG
GCACCGCGAG CCCCTAGCAC CCGACAAGCT GAGTGTGCAG GACGAGTCCC CACCACACCC
ACACCACAGC CGCTGAATGA GGCTTCCAGG CGTCCGCTCG CGGCCCGCAG AGCCCCGCCG
TGGGTCCGCC CGCTGAGGCG CCCCCAGCCA GTGCGCTCAC CTGCCAGACT GCGCGCCATG
GGGCAACCCG GGAACGGCAG CGCCTTCTTG CTGGCACCCA ATGGAAGCCA TGCGCCGGAC
CACGACGTCA CGCAGG
AAAGGGACGA GGTGTGGGTG GTGGGCATGG GCATCGTCAT GTCTCTCATC GTCCTGGCCA
TCGTGTTTGG CAATGTGCTG GTCATCACAG CCATTGCCAA GTTCGAGCGT CTGCAGACGG
TCACCAACTA CTTCATCACT TCACTGGCCT GTGCTGATCT GGTCATGGGC CTGGCAGTGG
TGCCCTTTGG GGCCGCCCAT ATTCTTATGA AAATGTGGAC TTTTGGCAAC TTCTGGTGCG
AGTTTTGGAC TTCCA
TTGATGTGCT GTGCGTCACG GCCAGCATTG AGACCCTGTG CGTGATCGCA GTGGATCGCT
ACTTTGCCAT TACTTCACCT TTCAAGTACC AGAGCCTGCT GACCAAGAAT AAGGCCCGGG
TGATCATTCT GATGGTGTGG ATTGTGTCAG GCCTTACCTC CTTCTTGCCC ATTCAGATGC
ACTGGTACCG GGCCACCCAC CAGGAAGCCA TCAACTGCTA TGCCAATGAG ACCTGCTGTG
ACTTCTTCAC GAACCAAGCC TATGCCATTG CCTCTTCCAT CGTGTCCTTC TACGTTCCCC
TGGTGATCAT GGTCTTCGTC TACTCCAGGG TCTTTCAGGA GGCCAAAAGG CAGCTCCAGA
AGATTGACAA ATCTGAGGGC CGCTTCCATG TCCAGAACCT TAGCCAGGTG GAGCAGGATG
GGCGGACGGG GCATGGACTC CGCAGATCTT CCAAGTTCTG CTTGAAGGAG CACAAAGCCC
TCAAGACGTT AGGCATCATC ATGGGCACTT TCACCCTCTG CTGGCTGCCC TTCTTCATCG
TTAACATTGT GCATGTGATC CAGGATAACC TCATCCGTAA GGAAGTTTAC ATCCTCCTAA
ATTGGATAGG CTATGTCAAT TCTGGTTTCA ATCCCCTTAT CTACTGCCGG AGCCCAGATT
TCAGGATTGC CTTCCAGGAG CTTCTGTGCC TGCGCAGGTC TTCTTTGAAG GCCTATGGCA
ATGGCTACTC CAGCAACGGC AACACAGGGG AGCAGAGTGG ATATCACGTG GAACAGGAGA
AAGAAAATAA ACTGCTGTGT GAAGACCTCC CAGGCACGGA AGACTTTGTG GGCCATCAAG
GTACTGTGCC TAGCGATAAC ATTGATTCAC AAGGGAGGAA TTGTAGTACA AATGACTCAC
TGCTGTAAAG CAGTTTTTCT ACTTTTAAAG ACCCCCCCCC GCCCAACAGA ACACTAAACA
GACTATTTAA CTTGAGGGTA ATAAACTTAG AATAAAATTG TAAAATTGTA TAGAGATATG
CAGAAGGAAG GGCATCCTTC TGCCTTTTTT ATTTTTTTAA GCTGTAAAAA GAGAGAAAAC
TTATTTGAGT GATTATTTGT TATTTGTACA GTTCAGTTCC TCTTTGCATG GAATTTGTAA
GTTTATGTCT AAAGAGCTTT AGTCCTAGAG GACCTGAGTC TGCTATATTT TCATGACTTT
TCCATGTATC TACCTCACTA TTCAAGTATT AGGGGTAATA TATTGCTGCT GGTAATTTGT
ATCTGAAGGA GATTTTCCTT CCTACACCCT TGGACTTGAG GATTTTGAGT ATCTCGGACC
TTTCAGCTGT GAACATGGAC TCTTCCCCCA CTCCTCTTAT TTGCTCACAC GGGGTATTTT
AGGCAGGGAT TTGAGGAGCA GCTTCAGTTG TTTTCCCGAG CAAAGTCTAA AGTTTACAGT
AAATAAATTG TTTGACCAG

Appendix 11: Visualisation of amplified PCR products containing *FTO* rs9930501, rs9930506 and rs9932754 (237bp)



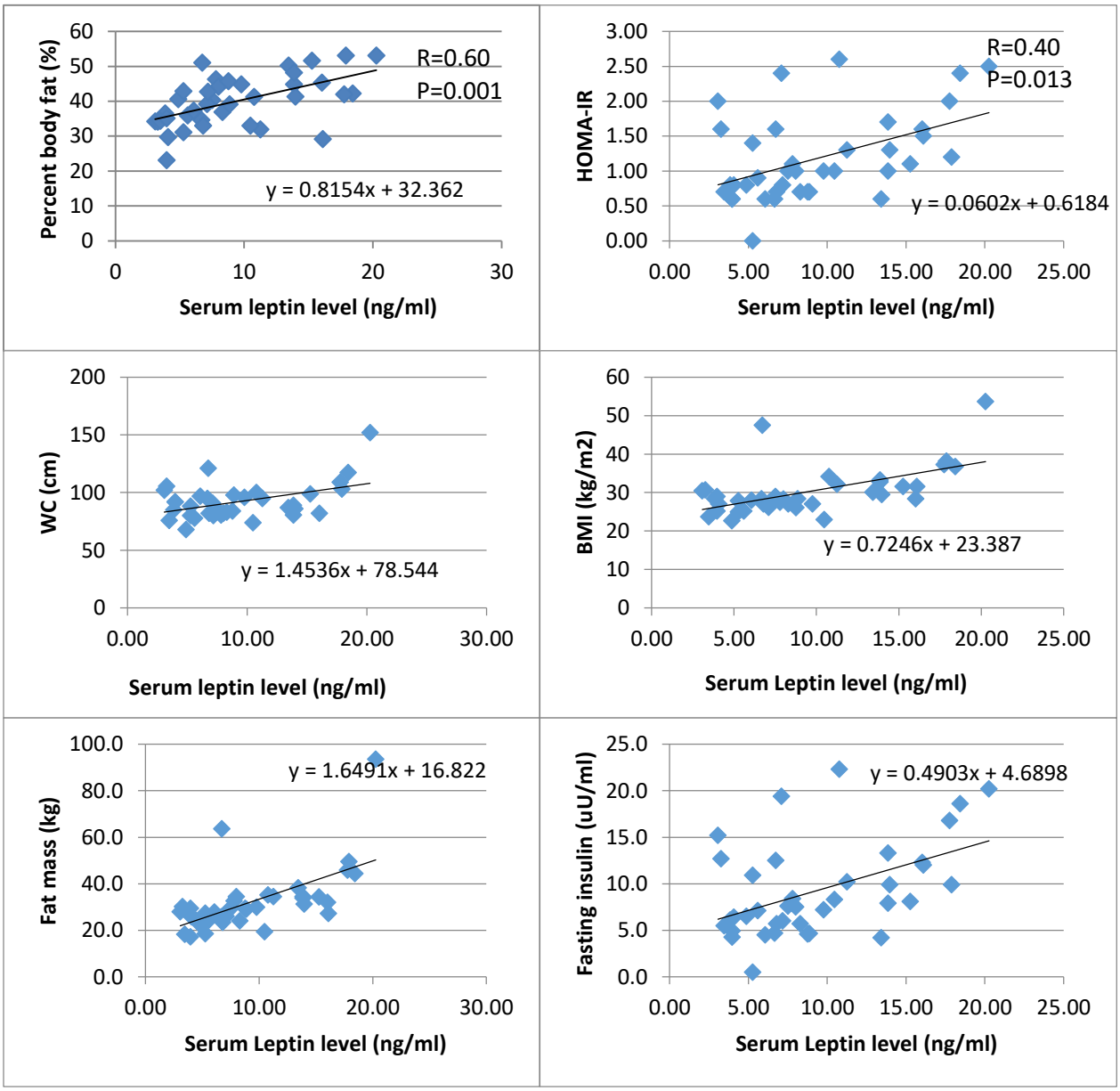
Note: Lane M: 100bp DNA marker; Lane 1: Negative control (no DNA samples); Lane 2-24: DNA samples from participants

Appendix 12: Visualisation of amplified PCR products containing *ADRB2* rs1042713 and rs1042714 (310bp).



Note: Lane M: 100bp DNA marker; Lane 1: Negative control (no DNA samples); Lane 2-24: DNA samples from participants

Appendix 13: Correlation between serum leptin levels and obesity-related phenotypes in the study participants (n=35)



P was based on Pearson's correlation test, with P was significant at <0.05

Appendix 14: Abstract accepted for oral presentation - 31st Annual Scientific Conference 2016 by Nutrition Society of Malaysia

Association of serum leptin levels and *LEP* gene variation with body composition and insulin resistance in obese Malaysian adults

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Effect of leptin on insulin resistance in obese Malaysians has seldom been reported. Leptin gene has been frequently studied in different populations for its contribution to genetic predisposition to obesity. The objective of our study was to identify correlation between *LEP* rs7799039 gene polymorphism with anthropometric variables, blood biochemical markers and serum leptin levels in overweight and obese Malaysian adults. Anthropometric parameters of 40 Malaysian adults with BMI >23kg/m2 was measured by bioelectrical impedance analysis. Serum leptin was measured using in-vitro enzyme-linked immunosorbent assay, Human Leptin ELISA Kit. Insulin resistance was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR, calculated as fasting glucose (mmol/L) * insulin (μIU/mL)/22.5). LEP rs7799309 was genotyped by using PCR-RFLP (n=34). Serum leptin level (9.0±5.0ng/ml) was positively correlated with BMI (29.7±5.7kg/m2) (r=0.583, p=0.001), waist circumference (90.6±15.1cm) (r=0.453, p=0.04), fat mass (31.5±12.5kg) (r=0.600, p=0.001), fat percentage (38.8±7.1%) (r=0.559, p=0.001), fasting insulin (8.0±6.0mmol/L) (r=0.385, p=0.014) and HOMA-IR (1.0±8) (r=0.391, p=0.013). Stepwise multiple linear regression revealed that percent fat and fasting insulin were the independent variables associated positively with serum leptin levels. With a 1-point increase in body fat percent, serum leptin increased by 0.4ng/ml. With a 1-point increase in fasting insulin, serum leptin increased by 0.33 ng/ml. In *LEP* (rs7799039), the frequency of minor allele A to major G was 0.46 and 0.54 respectively. It was found that overweight/obese participants with HOMA-IR <1.7 carrying AA allele compared to GA allele had significantly higher ($p<0.05$) blood triglyceride level (3.4±0.9 against 1.2±0.4); total cholesterol /HDL cholesterol ratio (6.2±2.6 against 3.6±0.9); lower HDL cholesterol (0.9±0.5 against 1.6±0.5mmol/L). GA carriers showed higher body fat percent than AA carriers (41.2±7.1 against 28.4±7.0) ($p<0.05$). Interaction of gene polymorphisms and obesity phenotypes can affect the outcome of weight reduction intervention programs. We suggest personalised intervention based on genetic makeup for successful outcome in weight management.

Appendix 15: Differences in the mean values (± SE) of dietary, anthropometric and biochemical parameters between the *FTO* genotypes (rs9930501 and rs9932754)

SNPs	rs9930501 (dominant model)			rs9932754 (dominant model)		
Genotype	**AA (n=76)**	**AG+GG(n=102)**	**p value**	**TT (n=75)**	**TC+CC(n=103)**	**p value**
Age (years)	41.0 ± 1.3	42.2 ± 1.2	0.686	41.1 ± 1.3	42.1 ± 1.2	0.781
[1]Weight (kg)	65.1 ± 1.8	69.2 ± 1.8	0.210	64.2 ± 1.5	69.8 ± 1.9	0.089
[1]Height (cm)	157.8 ± 0.8	157.8 ± 0.7	0.427	157.5 ± 0.8	158.0 ± 0.7	0.611
[1]BMI (c)	26.1 ± 0.6	27.7 ± 0.6	0.114	25.9 ± 0.6	27.7 ± 0.6	0.052
[1]WC (cm)	77.9 ± 2.4	85.2 ± 2.1	0.054	77.3 ± 2.4	85.5 ± 2.1	0.031
[1]WHR	**0.88 ± 0.01**	**0.90± 0.01**	**0.017**	**0.87 ± 0.01**	**0.90 ± 0.01**	**0.007**
[1]Muscle Mass (kg)	22.2 ± 0.6	22.6 ± 0.5	0.860	22.0 ± 0.6	22.7 ± 0.5	0.985
[1]Fat Mass (kg)	25.1 ± 1.2	27.8 ± 1.2	0.134	24.5 ± 1.0	28.2 ± 1.2	0.045
[1]Fat-free Mass (kg)	40.1 ± 0.8	41.5 ± 0.9	0.720	39.8 ± 0.8	41.7 ± 0.9	0.548
[1]% Body Fat	37.4 ± 0.9	39.1 ± 0.8	0.084	37.2 ± 0.8	39.2 ± 0.8	0.028
[1]Systolic Blood pressure (mmHg)	121.2 ± 2.2	123.5 ± 1.7	0.553	120.6 ± 2.2	123.9 ± 1.7	0.360
[1]Diastolic Blood pressure (mmHg)	79.7 ± 1.5	81.5 ± 1.7	0.439	79.1 ± 1.4	81.9 ± 1.2	0.242
[1]Pulse rate	76.5 ± 1.8	76.4 ± 1.2	0.885	76.7 ± 1.8	76.3 ± 1.1	0.956
Blood biochemical parameters	**AA (n=48)**	**AG+GG (n=78)**	**p value**	**TT (n=47)**	**TC+CC (n=79)**	**p value**
[2]Fasting Glucose (mmol/L)	4.93 ± 0.2	5.40 ± 0.3	0.161	4.95 ± 0.2	5.38 ± 0.3	0.202
Blood biochemical parameters	**AA (n=48)**	**AG+GG (n=78)**	**p value**	**TT (n=47)**	**TC+CC (n=79)**	**p value**
[2]Fasting Insulin (uU/mL)	8.96 ± 1.2	10.64 ± 1.2	0.293	8.77 ± 1.2	10.73 ± 1.2	0.268
[2]HOMA-IR	1.95 ± 0.3	2.80 ± 0.4	0.187	1.93 ± 0.3	2.80 ± 0.4	0.180
[2]Total cholesterol (mmol/L)	5.66 ± 0.2	5.43 ± 0.1	0.218	5.63 ± 0.2	5.45 ± 0.1	0.348
[2]Triglyceride (mmol/L)	1.38 ± 0.1	1.38 ± 0.1	0.976	1.33 ± 0.1	1.41 ± 0.1	0.688
[2]HDL Cholesterol (mmol/L)	**1.64 ± 0.1**	**1.46 ± 0.04**	**0.015***	**1.65 ± 0.1**	**1.45 ± 0.1**	**0.010***
[2]LDL cholesterol (mmol/L)	3.43 ± 0.1	3.32 ± 0.1	0.534	3.41 ± 0.1	3.33 ± 0.1	0.654
[2]Total cholesterol/HDL cholesterol	3.63 ± 0.2	3.81 ± 0.1	0.421	3.58 ± 0.2	3.84 ± 0.1	0.226
[2]hsCRP (mg/L)	3.47 ± 0.6	6.89 ± 1.0	0.019	3.47 ± 0.6	6.89 ± 1.0	0.019
Dietary nutrients intake	**AA (n=48)**	**AG+GG (n=78)**	**p value**	**TT(n=47)**	**TC+CC (n=79)**	**p value**
[2]Total energy intake (kcal)	2009.9 ± 43.3	2004.2 ± 41.7	0.442	1997.7±42.5	2011.5±41.9	0.454
[3]Total carbohydrate intake (g)	251.2 ± 9.1	255.6 ± 7.4	0.450	249.4 ± 9.1	256.6 ± 7.4	0.473
[3]Total protein intake (g)	75.6 ± 3.0	72.7 ± 2.6	0.493	74.4 ± 2.8	73.4 ± 2.7	0.658
[3]Total dietary fat intake (g)	82.2 ± 2.9	83.3 ± 7.4	0.735	82.1 ± 2.9	83.4 ± 2.5	0.759
[3]Percentage energy from carbohydrate (%)	46.2 ± 1.2	47.7 ± 0.9	0.385	46.2 ± 1.3	47.7 ± 0.9	0.415
[3]Percentage energy from protein (%)	15.2 ± 0.6	14.3 ± 0.4	0.343	15.1 ± 0.6	14.4 ± 0.4	0.479
[3]Percentage energy from dietary fat (%)	36.9 ± 1.0	36.7 ± 0.8	0.866	37.0 ± 4.9	31.9 ± 5.1	0.856
[3]Saturated fat (%TE)	7.6 ± 0.5	7.9 ± 0.5	0.910	7.7 ± 0.5	7.8 ± 0.5	0.976
[3]Monounsaturated fat (%TE)	11.1 ± 0.6	11.2 ± 0.5	0.976	11.1 ± 0.6	11.2 ± 0.5	0.916

[3]Polyunsaturated fat (%TE)	6.5 ± 0.5	6.4 ± 0.4	0.447	6.5 ± 0.5	6.4 ± 0.4	0.514
[3]Trans fat (%TE)	0.1 ± 0.02	0.1 ± 0.03	0.192	0.1 ± 0.02	0.1 ± 0.03	0.149
[3]Dietary cholesterol (%TE)	130.8 ± 12.9	90.8 ± 8.4	0.860	128.9 ± 13.1	92.4 ± 8.4	0.116
[3]Total sugar (%TE)	7.3 ± 0.6	7.3 ± 0.5	0.740	7.4 ± 0.6	7.2 ± 0.5	0.790
[3]Glucose (%TE)	1.0 ± 0.2	1.4 ± 0.2	0.298	1.0 ± 0.2	1.3 ± 0.2	0.323
[3]Fructose (%TE)	1.1 ± 0.2	1.3 ± 0.2	0.363	1.1 ± 0.2	1.3 ± 0.2	0.324
[3]Sucrose (%TE)	4.2 ± 0.4	4.0 ± 0.3	0.389	4.3 ± 0.4	3.9 ± 0.3	0.467
[3]Fibre (g)	**5.1 ± 0.4**	**6.3 ± 0.4**	**0.030***	**5.1 ± 0.4**	**6.3 ± 0.4**	**0.022***
[3]Vitamin E (mg)	**6.6 ± 0.5**	**5.3 ± 0.3**	**0.010***	**6.7 ± 0.5**	**5.3 ± 0.3**	**0.016***

One way ANCOVA was performed to determine the differences in the mean values of dietary, anthropometric and biochemical parameters between the *FTO* genotypes, after adjusting for covariates in different models [1]age, gender, physical activity status, smoking status and alcohol consumption; [2] model [1]+ BMI; and [3] model[2] + total energy intake.
*p<0.05 was considered significant.

Appendix 16: Differences in *FTO* genotype distributions between Malaysian Indians versus Malaysian Chinese and Malaysian Malays

***FTO* SNPs**	**rs9930501 (dominant model)**			**rs9932754 (dominant model)**		
Ethnicity	**AA (n=76)**	**AG+GG (n=102)**	**p value**	**TT (n=75)**	**TC+CC (n=103)**	**p value**
Indian (n=50)	10 (20.0%)	40 (80.0%)	**<0.001**	10 (20.0%)	40 (80.0%)	**<0.001**
Chinese & Malay (n=128)	66 (51.6%)	62 (48.4%)		65 (50.8%)	63 (49.2%)	

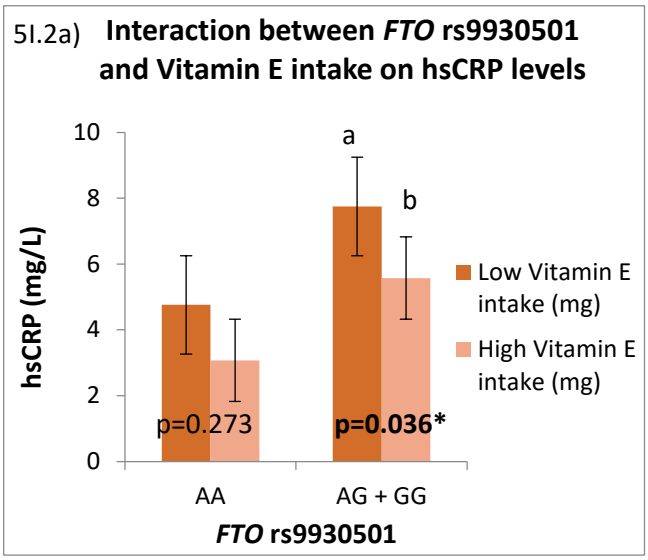
Ethnicity with 3 categories (Malaysian Malays, Chinese and Indians) was then recoded into 2 categories (Malaysian Indians versus Malaysian Chinese and Malaysian Malays) for further analysis. Data were expressed as total number and percentage. The differences in genotype frequencies between the ethnic groups were assessed by Chi square test. *p<0.05 was considered significant.

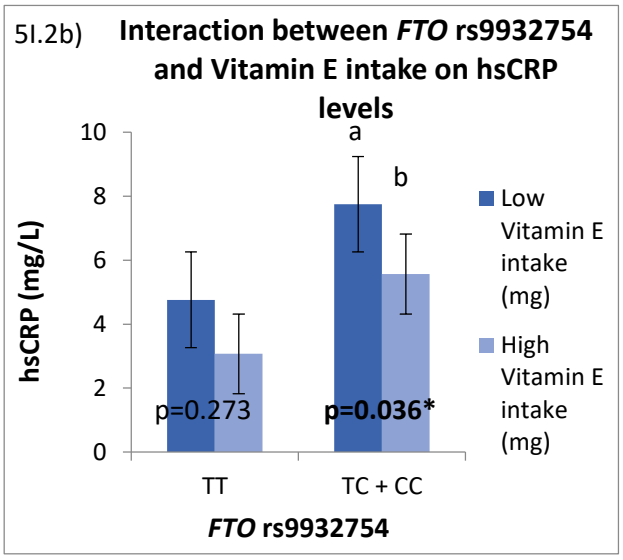
Appendix 17: Genotype distribution and allele frequency of *FTO* gene variants (rs9930501 and rs9932754) in obese and non-obese groups (in additive models)

SNPs	**Genotype**	**Overall n=178**	**X^2 Hardy-Weinberg**	**Obese BMI≥27.5 n=79**	**Non-obese BMI<27.5 n=99**	**Unadjusted OR (95%CI)**	**Unadjusted p value**	**Adjusted OR (95%CI) [a]**	**p value [a]**
rs9930501	**AA**	76 (42.7%)	3.15	29 (36.7%)	47 (47.5%)	1	-	1	-
	AG	72 (40.4%)		31 (39.2%)	41 (41.4%)	1.23 (0.64-2.36)	0.544	1.25 (0.65-2.48)	0.533
	GG	30 (16.9%)		19 (24.1%)	11 (11.1%)	**2.80 (1.17-6.72)**	**0.021***	**3.03 (1.23-7.49)**	**0.016***
rs9932754	**TT**	75 (42.1%)	2.12	28 (35.4%)	47 (47.5%)	1	-	1	-
	TC	74 (41.6%)		33 (41.8%)	41 (41.4%)	1.35 (0.70-2.60)	0.368	1.41 (0.71-2.82)	0.325
	CC	29 (16.3%)		18 (22.8%)	11 (11.1%)	**2.75 (1.14-6.65)**	**0.025***	**3.04 (1.22-7.59)**	**0.017***

Note: The top most gene variant denotes the wild variant of the SNP in each category, and the last gene variant denotes the mutant variant.
The agreement of genotype frequencies with Hardy Weinberg Equilibrium was tested by using chi Square test, with χ^2 < 3.841 considered as no deviation from Hardy Weinberg Equilibrium.
Logistic regression was conducted to determine the risk of obesity associated with gene variants. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated for each genotype. The homozygous wildtype was used as the reference group in additive model.
[a] adjusted for age, gender, physical activity status, smoking status and alcohol consumption.
*p<0.05 was considered as significant.

Appendix 18: The effect of the interaction between *FTO* gene variants (rs9930501 and rs9932754) and Vitamin E intake on hsCRP levels

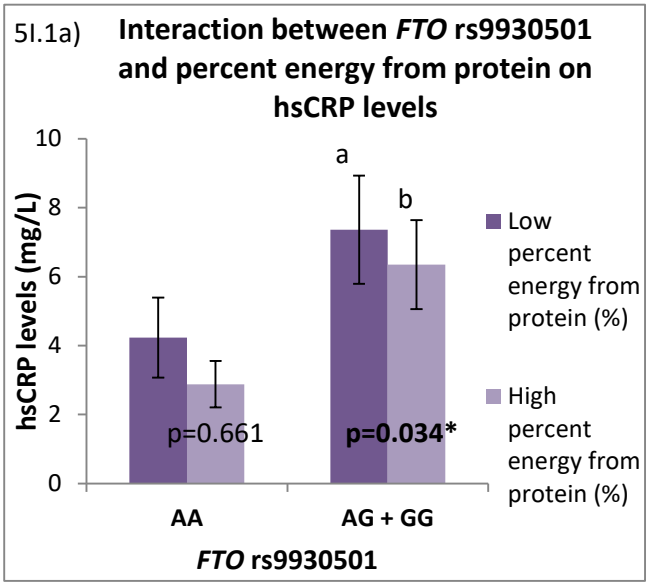


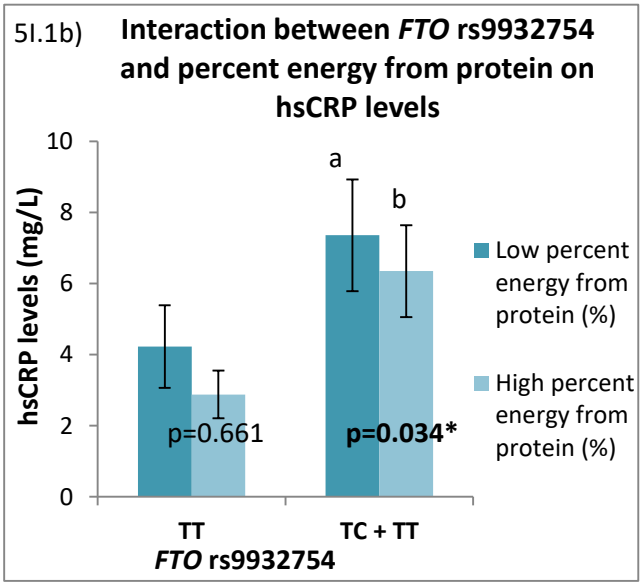


Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, physical activity status, smoking status, alcohol consumption, BMI and total energy intake. Energy adjusted Vitamin E intake was dichotomised into 2 groups by using the median value of 5.4mg/day (for all participants), for analysis.

*p<0.05 was considered significant.

Appendix 19: The effect of the interaction between *FTO* gene variants (rs9930501 and rs9932754) and percent energy from dietary protein on hsCRP levels





Gene-diet interaction was evaluated by using multivariate general linear model after adjusting for age, gender, physical activity status, smoking status, alcohol consumption, BMI and total energy intake. Percent energy from dietary protein was dichotomised into 2 groups by using the median value of 14%/day (for all participants), for analysis.

*p<0.05 was considered significant.

Appendix 20: Facebook page dedicated to the dietary intervention study



Appendix 21: Differences in anthropometric parameters from baseline, at month 3 and month 6 between Hipcref diet group (n=65) and control diet group (n=63)

Variables	Treatment group	Baseline	Month 3	Month 6	Overall p value [a] (treatment group x time)	Within treatment group p value [b] (baseline and month 6)
Weight (kg)	Hipcref diet group	74.2 ± 1.5	72.0 ± 1.6*	70.8 ± 1.6*	**<0.001**ǂ	**<0.001**ǂ
	Control diet group	73.6 ± 2.4	74.7 ± 2.4*	74.9 ± 2.4*		**0.012**ǂ
BMI (kg/m2)	Hipcref diet group	29.6 ± 0.6	28.2 ± 0.6*	28.2 ± 0.6*	**<0.001**ǂ	**<0.001**ǂ
	Control diet group	29.4 ± 0.7	30.0 ± 0.7*	30.0 ± 0.7*		**0.007**ǂ
WC (cm)	Hipcref diet group	93.2 ± 1.4	88.9 ± 1.6*	87.0 ± 1.6*	**<0.001**ǂ	**<0.001**ǂ
	Control diet group	94.5 ± 1.7	95.6 ± 1.7	95.9 ± 1.7*		**0.028**ǂ
WHR	Hipcref diet group	0.91 ± 0.01	0.91 ± 0.01	0.90 ± 0.01	**0.001**ǂ	0.101
	Control diet group	0.92 ± 0.01	0.93 ± 0.01	0.94 ± 0.01*		**0.028**ǂ
Muscle mass (kg)	Hipcref diet group	23.8 ± 0.6	23.5 ± 0.6	23.5 ± 0.6	0.113	0.079
	Control diet group	23.0 ± 0.7	23.0 ± 0.7	23.0 ± 0.7		1.000
Fat mass (kg)	Hipcref diet group	30.8 ± 1.1	29.1 ± 1.1*	27.8 ± 1.2*	**<0.001**ǂ	**<0.001**ǂ
	Control diet group	31.5 ± 1.5	32.3 ± 1.5	32.5 ± 1.5*		**0.043**ǂ
Fat-free mass (kg)	Hipcref diet group	43.5 ± 0.9	42.7 ± 1.0*	42.9 ± 1.0	**0.010**ǂ	0.100
	Control diet group	42.1 ± 1.1	42.5 ± 1.2	42.5 ± 1.2		1.000
Percent body fat	Hipcref diet group	41.1 ± 0.9	39.5 ± 1.1*	38.8 ± 1.0*	**<0.001**ǂ	**<0.001**ǂ
	Control diet group	42.1 ± 0.8	42.8 ± 0.8	42.9 ± 0.8		0.228

Data were expressed as mean (±SE).

[a] A two-way mixed ANOVA was performed to assess the effect of treatment group x time on anthropometric parameters at month 3 and month 6 between Hipcref diet group and control diet group. Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

[b] Further, pairwise comparisons were performed to assess the difference at month 3 and month 6 compared to baseline values within the treatment group. Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

* = significant at $p<0.05$; differences from baseline at month 3, and the same at month 6.

ǂ $p<0.05$ was considered as significant.

Appendix 22: Differences in post-intervention differences in blood pressure and cardio-metabolic parameters at month 6 compared to baseline between the Hipcref diet group (n=65) and control diet group (n=63)

Variables	Treatment group	Baseline	Month 6	Changes	p value
Systolic blood pressure (mmHg)	Hipcref diet group	123.9 ± 1.8	118.6 ± 1.8	-5.3 ± 1.5	0.168
	Control diet group	121.5 ± 1.9	119.2 ± 1.9	-2.3 ± 1.0	
Diastolic blood pressure (mmHg)	Hipcref diet group	82.4 ± 1.4	78.1 ± 1.4	-4.3 ± 1.1	0.288
	Control diet group	79.4 ± 1.1	77.2 ± 1.2	-2.2 ± 0.9	
Pulse rate (bpm)	Hipcref diet group	77.2 ± 1.4	72.8 ± 1.6	-4.4 ± 1.4	0.624
	Control diet group	75.7 ± 1.4	72.3 ± 1.4	-3.4 ± 1.1	
Fasting glucose (mmol/L)	Hipcref diet group	5.2 ± 0.2	5.1 ± 0.2	-0.02 ± 0.2	0.448
	Control diet group	5.3 ± 0.3	5.4 ± 0.3	0.1 ± 0.2	
Fasting insulin (uU/mL)	Hipcref diet group	11.8 ± 1.5	8.3 ± 0.7	-3.4 ± 1.2	**<0.001***
	Control diet group	8.4 ± 0.8	12.8 ± 1.3	4.5 ± 0.8	
HOMA-IR	Hipcref diet group	3.0 ± 0.5	2.0 ± 0.2	-1.0 ± 0.4	**<0.001***
	Control diet group	2.0 ± 0.2	3.3 ± 0.4	1.3 ± 0.3	
Total cholesterol (mmol/L)	Hipcref diet group	5.5 ± 0.1	5.3 ± 0.1	-0.2 ± 0.1	0.461
	Control diet group	5.5 ± 0.1	5.4 ± 0.1	-0.1 ± 0.1	
Triglyceride (mmol/L)	Hipcref diet group	1.5 ± 0.1	1.5 ± 0.1	0.01 ± 0.1	0.919
	Control diet group	1.3 ± 0.1	1.4 ± 0.1	0.1 ± 0.1	
HDL cholesterol (mmol/L)	Hipcref diet group	1.5 ± 0.1	1.4 ± 0.1	-0.1 ± 0.1	0.624
	Control diet group	1.6 ± 0.1	1.4 ± 0.1	-0.1 ± 0.03	
LDL cholesterol (mmol/L)	Hipcref diet group	3.3 ± 0.1	3.2 ± 0.1	-0.1 ± 0.1	0.429
	Control diet group	3.4 ± 0.1	3.3 ± 0.1	-0.1 ± 0.1	
Total cholesterol/HDL cholesterol	Hipcref diet group	3.8 ± 0.1	4.0 ± 0.1	0.2 ± 0.1	0.933
	Control diet group	3.7 ± 0.1	4.0 ± 0.1	0.3 ± 0.1	
hsCRP (mg/L)	Hipcref diet group	6.1 ± 1.0	4.0 ± 0.6	-1.4 ± 0.6	**0.020***
	Control diet group	5.3 ± 1.0	5.7 ± 0.9	0.3 ± 0.4	

A two-way mixed ANOVA was performed to assess the effect of treatment group x time on blood pressure and blood biochemical parameters between the Hipcref diet group and control diet group.

Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

*$p<0.05$ was considered as significant.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment -insulin resistance; hsCRP, high-sensitivity C-reactive protein.

Appendix 23: Differences in post-intervention differences in dietary parameters at month 6 compared to baseline in Hipcref diet group (n=65) and control diet group (n=63)

Dietary parameters	Treatment group	Baseline	Month 6	Changes	p value
Total energy intake (kcal)	Hipcref diet group	2021 ± 47	1599 ± 31	-422 ± 38	**<0.001***
	Control diet group	1996 ± 38	1964 ± 36	-32 ± 18	
Protein intake (g)	Hipcref diet group	75.5 ± 3.0	108.0 ± 2.8	32.5 ± 3.4	**<0.001***
	Control diet group	74.1 ± 2.9	7.54 ± 2.2	1.3 ± 2.3	
Fat intake (g)	Hipcref diet group	84.7 ± 2.8	57.6 ± 1.8	-27.1 ± 2.7	**<0.001***
	Control diet group	80.8 ± 2.5	75.2 ± 2.4	-5.7 ± 1.9	
Carbohydrate intake (g)	Hipcref diet group	251.9 ± 8.7	175.2 ± 4.7	-76.8 ± 7.4	**<0.001***
	Control diet group	255.6 ± 7.3	265.8 ± 6.0	10.2 ± 4.7	
Percent energy from protein (%)	Hipcref diet group	14.8 ± 0.5	27.2 ± 0.6	12.4 ± 0.7	**<0.001***
	Control diet group	14.8 ± 0.5	15.3 ± 0.3	0.5 ± 0.5	
Percent energy from fat (%)	Hipcref diet group	37.4 ± 0.9	32.3 ± 0.7	-5.1 ± 0.9	**0.001***
	Control diet group	36.0 ± 0.9	37.5 ± 1.1	-1.9 ± 0.7	
Percent energy from carbohydrate (%)	Hipcref diet group	46.2 ± 1.1	43.3 ± 0.6	-2.9 ± 1.0	**0.007***
	Control diet group	47.9 ± 1.0	50.0 ± 1.4	5.6 ± 1.0	
SFA (% of TE)	Hipcref diet group	8.1 ± 0.5	8.2 ± 0.3	0.1 ± 0.5	0.070
	Control diet group	7.5 ± 0.5	9.0 ± 0.4	1.5 ± 0.5	
MUFA (% of TE)	Hipcref diet group	11.4 ± 0.6	10.6 ± 0.3	-0.8 ± 0.5	0.562
	Control diet group	11.0 ± 0.5	10.6 ± 0.4	-0.3 ± 0.5	
PUFA (% of TE)	Hipcref diet group	6.6 ± 0.4	8.1 ± 0.3	1.4 ± 0.4	**0.009***
	Control diet group	6.3 ± 0.4	6.5 ± 0.3	0.2 ± 0.4	
TFA (% TE)	Hipcref diet group	0.1 ± 0.1	0.1 ± 0.1	-	0.058
	Control diet group	0.1 ± 0.1	0.2 ± 0.1	-	
Energy-adjusted fibre intake (g)	Hipcref diet group	6.3 ± 0.4	13.5 ± 0.5	7.2 ± 0.5	**<0.001***
	Control diet group	5.5 ± 0.4	9.0 ± 0.5	3.5 ± 0.5	
Energy adjusted vitamin E intake (mg)	Hipcref diet group	5.7 ± 0.4	8.0 ± 0.2	2.3 ± 0.4	**<0.001***
	Control diet group	5.9 ± 0.4	6.4 ± 0.3	0.5 ± 0.4	

A two-way mixed ANOVA was performed to assess the effect of treatment group x time on dietary parameters between the Hipcref diet group and control diet group.

Covariates, age, gender, physical activity, smoking and alcohol consumption were adjusted for.

*$p<0.05$ was considered as significant.

- indicates no change.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; TFA, trans fatty acid, TE, total energy.