IRREGULARITY OF MEAL PATTERN AND METABOLIC HEALTH

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ABSTRACT

Background

The potential for an irregular, or chaotic, eating pattern which exhibits day to day variation has increased recently. Irregular eating may increase the risk of obesity and associated health consequences by possibly causing chrono-disruption because of the fluctuations in the timing of food intake disrupting entrainment of circadian patterns. This thesis 1. undertook a systematic review to identify the literature relating to meal pattern irregularity and obesity and associated negative health consequences, 2. developed a new methodology for characterising meal pattern irregularity and explored association with obesity and health outcomes (Study 1), 3. determined the potential impact of meal pattern irregularity on nitrogen balance (Study 2) and 4. investigated the feasibility of providing a hypo-energetic diet to participants with normal/overweight to explore day-to-day variation of energy expenditure, TEF, fasting blood glucose, fasting lipid profiles, fasting gut hormones, interstitial glucose concentrations and subjective appetite (Study 3).

Methods

1. A systematic search was undertaken using relevant databases for studies with adults and children in which between day variability in meal pattern was measured, and related to body weight, metabolic syndrome components and cognitive function. 2. Two methods for characterising meal pattern variability were proposed using NDNS data in study The first used a variety of different meal pattern properties to describe the degree of irregularity in meal patterns. The second method involved the derivation of two indices relating to the energy consumption and time of eating, each with two threshold systems. 3. Using cross-over design, a randomized controlled trial with two 7-day intervention periods, separated by a wash-out period of three weeks was conducted on 14 healthy female participants to investigate the effect of irregular meal pattern on nitrogen balance 4. Study 3 had a randomised, parallel design with 7 days (pre-standardisation period) during which participants consumed their estimated energy requirement. Participants then adopted one of two meal patterns for the 14-day intervention period when they consumed a hypoenergetic diet, followed by a further 3 days (post-standarisation period) consuming their estimated energy requirement.

Results

1. The systematic review showed that many different methods in the literature for characterizing between day variability in meal pattern tended not to utilise all the potential properties of the meal pattern. Moreover, between day variability in meal pattern was associated with obesity and health outcomes including higher weight, reduced thermogenic response to meals and poorer academic achievement. 2. The two novel methods for characterising meal pattern irregularity in Study 1 suggested that day-to-day variation appears to be associated with obesity, dyslipidemia and hyperglycemia. However, the nature of the association in Method 2 was not consistent between the two indices as the Irregular time index showed the opposite relation to BMI, HDL cholesterol and HbA1c. Although the effect size in each model of the linear regression were small between 0.6% and 15%, the associations were statically significant. 3. The findings from Study 2 have indicated that the effect of meal pattern irregularity on nitrogen balance over 7-day period is not significant (P=0.091). 4. Study 3 has demonstrated that the protocol to test the hypo-energetic diet based on regular or irregular meal pattern on energy expenditure, metabolism and circadian rhythm is feasible and required some amendments.

Conclusion

Following a regular meal pattern might be a potential additional goal for body weight management as it seems to improve energy expenditure and insulin sensitivity which may indicate a favourable effect on insulin resistance and the risk of cardiovascular diseases. However further randomised controlled studies are required to confirm these results and elucidate the magnitude of the effect, hence potential clinical significance.

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LIST OF ABBREVIATIONS

AHEAD	Action for Health in Diabetes
AOR	Adjusted odds ratio
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BDI	Beck Depression Inventory
BIA	Bioimpedance analysis
BMI	Body mass index
BMR	Basel metabolic rate
ºC	Degree Celsius
CASPAIN	Childhood and Adolescence Surveillance and Prevention of Adult Noncommunicable Disease
CCK	Cholecystokinin
CDC	Disease control and Prevention
CGM	Continuous glucose monitoring
CHD	Coronary heart disease
cm	Centimetre
COMA	Committee on Medical Aspects of Food Policy
CO2	Carbon dioxide
CL	Confidence level
CT	Computerised tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DLMO	Dim light melatonin onset
EAT	Eating Attitudes Test-
EDTA	Ethylene diamine tetra-acetic acid
EI	Energy intake
°F	Fahrenheit
FFM	Fat-free mass
FFQ	Food Frequency Questionnaire
g	Gram
GEM	Gas Exchange Measurement
GLP-1	Glucagon like peptide 1
h	Hour
HDL	High density lipoprotein
HNR	Heinz Nixdorf Recall Study
HTA	Human Tissue Act
HOMA-IR	Homeostatic model assessment for insulin resistance
iAUC	Incremental area under the curve
IPAQ	International Physical Activity Questionnaire
IV	Intravenous
kcal/day	Kilocalories per day
kg	Kilogram
KJ	Kilojoules
L	Litre
LDL	Low density lipoprotein
Max	Maximum

MET	Metabolic Equivalent of Task
Min	Minimum
min	Minute
ml	Millilitre
MRI	Magnetic resonance imaging
N	Nitrogen
NDNS	National Diet and Nutrition Survey
NB	Nitrogen balance
NCEP (ATP-III)	National Cholesterol Education Programme (Adult Treatment Panel III)
NAFLD	Non-alcoholic fatty liver disease
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung and Blood Institute
NSHD	National Survey of Health and Development
ONL	Obligatory nitrogen losses
OR	Odds ratio
OXM	Oxyntomodulin
O2	Oxygen
PA	Physical activity
PAL	Physical activity level
PAEE	Physical activity-induced energy expenditure
PCA	Principal Component Analysis
PPSOC	Portuguese Prevalence Study of Obesity in Childhood
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PYY	Peptide YY
QC	Quality control
REE	Resting energy expenditure
RMR	Resting metabolic rate
RR	Relative risk
SD	Standard deviation
SEM	Standard error of the mean
SEPAHAN	Study on the Epidemiology of Psychological, Alimentary Health and Nutrition
SCN	Suprachiasmatic nucleus
SNS	Sympathetic nervous system
SWA	SenseWear armband
TEE	Total energy expenditure
TEF	Thermic effect of food
TG	Triglyceride
TUN	Total urinary nitrogen
T2DM	Type 2 diabetes
UUN	Urinary urea nitrogen
VAS	Visual analogue scale
VCO2	Rate of carbon dioxide production
VO2	Rate of oxygen consumption
WC	Waist Circumference
WHO	World health organization
YSI	Yellow Springs™ glucose analyser

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

Appropriate nutrition is essential for human health and wellbeing across the lifespan and relates to both food choices and pattern of eating (World Health Organization (WHO), 2021). Recently, the potential for an irregular, or chaotic, eating pattern which exhibits day to day variation has increased due in part to the availability of ready prepared meals, greater availability of fast food outside the home and variation in work patterns (Hoglund *et al.*, 1998; Samuelson *et al.*, 2000). Concurrently, obesity and associated negative health consequences have increased (Bianchini *et al.*, 2002; Nguyen *et al.*, 2014; Han and Lean, 2016; Engin *et al.*, 2017). Irregular eating may increase the risk of obesity and associated health consequences (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022; Pot *et al.*, 2016 and Pot *et al.*, 2016), and hence is an important area for future study and is the focus of this thesis.

Recently there has been increasing interest in chrononutrition. Eating irregularly might cause chronodisruption because of the fluctuations in the timing of food intake disrupting entrainment of circadian patterns. (Garaulet and Gomez-Abellanand, 2014). This thesis will also consider methods of measuring circadian rhythm in the field and assess the practicality (feasibility) of undertaking a human feeding study that includes the measures required to explore potential interactions between chaotic eating and circadian patterns.

In the following literature review, as a background to the subsequent work, obesity, energy expenditure, energy intake, appetite, nitrogen balance and circadian rhythm are introduced.

1.2. Obesity

1.2.1. Definition and classification

Obesity is defined as excess body weight consisting of an abnormally high accumulation of body fat (Hill *et al.*, 2012). Obesity results from energy intake exceeding energy expenditure over an extended period (Wright and Aronne, 2012), which causes excess weight because of increased fat mass and fat cell size (Bray, 2004). Fat mass can be measured by one of several methods, including dual-energy X-ray absorptiometry (DEXA), computerised tomography (CT), magnetic resonance imaging (MRI), underwater weighing, bioimpedance analysis (BIA) and air displacement plethysmograph. In each of these methods the proportion of fat or non-fat mass can be measured to calculate the percentage of body fat (Duren *et al.*, 2008).

Given the complexity of the methods used to measure fat mass the body mass index (BMI) is often used to classify the degree of excess fat in the adult population (Webster-Gandy *et al.*,

2012), with different classifications associated with different degrees of risk to health (Aronne 2002). BMI is calculated as the weight in kilograms divided by the square of the height in metres (kg/m^2) (Nuttall, 2015). Originally, BMI was known as the Quetelet Index and was introduced by Lamber Adolphe Quetelet (Eknoyan, 2008). In 1835, Quetelet noted that in normal young adults, relating body mass to height squared, instead of just height reduced the influence of height on the index. Keys *et al.* (1972) established that this, the Quetelet Index, was highly associated with body fat. BMI has been associated with increased risk of disease and the WHO has adopted and published BMI ranges (Table 1.1) which classify underweight, healthy weight, overweight and obesity (WHO, 1995; Aronne 2002).

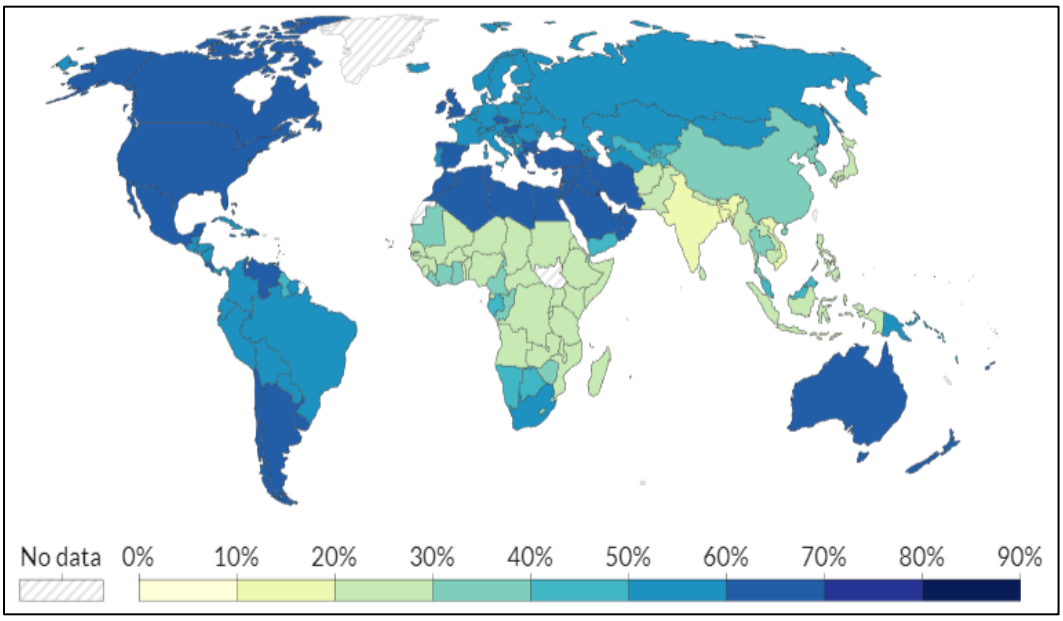
Table 1.1: WHO classification of overweight and obesity in adults (WHO, 1998)

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal weight	18.5 - 24.9
Overweight	25.0 - 29.9
Obese Class 1	30.0 - 34.9
Obese Class 2	35.0 - 39.9
Obese Class 3	> 40.0

1.2.2. Prevalence

According to the WHO (2021), globally, the prevalence of obesity nearly tripled between 1995 and 2016 (Figure 1.1). In 1995, there were about 200 million adults with obesity. Furthermore, the number had increased by the year 2000 to 300 million, with ongoing increases since. In 2016, more than 1.9 billion adults were overweight and more than 650 million were obese (WHO, 2021). It has been predicted that globally given the current trends, by 2050, 60% of males and 50% of females will be obese (Lobstein and Leach, 2007).

Figure 1.1 The prevalence of obesity worldwide (source: WHO, 2016)



Similar patterns of increase in obesity have occurred in the United States of America (USA) and Western Europe (Agha and Agha, 2017) to that seen globally. In the USA, comparing data collected during 1999–2000 with that collected during 2017–2020, the prevalence of obesity increased from 30.5% to 41.9%. The overall prevalence of obesity was similar for both sexes, but the prevalence of severe obesity was higher amongst women. In addition, the prevalence of obesity was 39.8% among young adults (aged 20 to 39 years), 44.3% among middle-aged adults (aged 40 to 59 years), and 41.5% among older adults aged 60 and older (Centres of Disease control and Prevention (CDC), 2022).

In the UK, the proportion of the adult population with obesity has increased since the 1980s. The percentage of obesity in relation to sex since 1991 has increased further by 65% in men, and 25% in women (Agha and Agha, 2017). The proportion of the population with obesity has increased over this period from 13.2% in 1993 to 26.9% in 2015 for men and from 16.4% to 26.8% for women in England (Public Health England, 2017). The most recent data available for obesity prevalence by sex revealed that it was 26% in men and 29% in women in 2018 (National health Service (NHS), 2020).

1.2.3. Risk factors and causes

There are many factors that are associated with an increased risk of developing obesity which occurs as a consequence of positive energy balance (Wright and Aronne, 2012). These factors include individual factors (such as sex, age, ethnicity, certain unhealthy dietary choices and physical inactivity), environmental factors (such as low socioeconomic status and availability

of energy dense fast food), family history, genetics (Walley *et al.*, 2006; Hurby and Hu, 2016;), and certain medical conditions including hypothyroidism, Cushing's syndrome, rare genetic disorders and conditions that reduce mobility. Similar factors have been shown to increase the risk of the maintenance of obesity once it has developed (CDC, 2020).

In general, females, weight for height, have a greater fat mass to fat-free mass (and total lean mass) ratio, which is in turn associated with a lower energy requirement and a lower energy intake for females than males (Wisniewski and Chernausek, 2009). This is explained by sex steroid hormones which are correlated with differences in body composition in children and young adults as well as females and males (Shah *et al.*, 2020). Males and females have a similar fat mass at birth. However, females enter puberty earlier and have a more rapid pubertal transition than males. Moreover, females also have three times higher plasms circulating concentrations of leptin hormone than males (Couillard *et al.*, 1997) and high levels of leptin are positively associated with the fat stores in the body and directly proportional to levels of adiposity (Jéquier, 2002; Wells, 2007). In Americans, a decline in body weight, body fat and BMI was found amongst the elderly, usually in the seventh and eighth decades (Institute of Medicine (IOM), 1995; Kuczmarski *et al.*, 1994; National Heart, Lung and Blood Institute (NHLBI), 1998). However, observational studies show a gradual increase in the average BMI and body fat from the age of 5 to 60 years (IOM, 1995). Similar trends have been shown in total body fat and percent body fat by others (Chumlea *et al.*, 2002; Siddarth, 2013). Middle-aged adults have the highest rates of overweight and obesity (IOM, 1995, Siddarth, 2013).

1.2.4. Consequences

1.2.4.1. Economic consequences

Worldwide, obesity has a negative impact on economic development. The impact of obesity on a country's prosperity can be seen in terms of absence from work and medical care costs including preventive, diagnostic, and treatment services (Agha and Agha 2017; CDC, 2020). In the US, being obese is associated with 36% greater annual healthcare costs compared with being of a healthy weight (Hammond and Levine, 2010). In the UK, it has been estimated that the NHS spent £6.1 billion on health problems related to overweight and obesity between 2014 and 2015. Furthermore, the overall cost of obesity to the wider society is estimated at £27 billion. The UK-wide NHS costs which will be attributable to overweight and obesity are predicted to reach £9.7 billion by 2050 and the wider costs to society is predicted to reach £49.9 billion per year (Public Health England, 2017).

1.2.4.2. Health consequences

People with obesity are at a higher risk than those with a healthy weight of developing health conditions and serious diseases such as hypertension, type 2 diabetes (T2DM), dyslipidemia (high LDL cholesterol, low HDL cholesterol, or high levels of triglycerides), cardiovascular disease (CVD), stroke, gallbladder disease, osteoarthritis (a breakdown of cartilage and bone within a joint), cancers, sleep apnea, breathing problems and mental illness (such as depression and anxiety) (Hruby *et al.*, 2016).

1.2.4.2.1. Metabolic syndrome

Metabolic syndrome refers to the combination of physiological, biochemical, clinical, and metabolic conditions that increases the risk of CVD and T2DM (Kaur, 2014). According to the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP-III), metabolic syndrome can be diagnosed when three or more of the following criteria are met: waist circumference ⩾102 cm in men and ⩾88 cm in women; HDL cholesterol >1.036 mmol l^{-1} (40 mg dl^{-1}) in men and >1.295 mmol l^{-1} (50 mg dl^{-1}) in women; triglycerides ⩾1.7 mmol l^{-1} (150 mg dl^{-1}) in men and women; systolic BP ⩾130mmHg or diastolic BP ⩾85mmHg in men and women; and fasting blood glucose (≥100 mg/dL) among men and women (Grundy *et al.*, 2005). According to the National Health and Nutrition Examination Survey (NHANES), the prevalence of the metabolic syndrome was 5% amongst participants of normal weight, 22% among those with overweight, and 60% among those with obesity (Park *et al.*, 2003).

Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic variations, elevated blood pressure, hypercoagulable state, and chronic stress are factors which are associated with the metabolic syndrome (Kaur, 2014). In the prevention of long-term risk for CVD, interventions such as weight management are recommended for those diagnosed with the metabolic syndrome, in order to reduce excessive central fat accumulation and to reduce other of the above negative consequences (Han and Lean, 2016).

1.2.4.2.2. Blood glucose and insulin resistance

Plasma glucose concentration reflects the balance between the rate of glucose entering the circulation and the rate of uptake into the tissues in the body. There are three sources of circulating glucose: intestinal absorption during the fed state, glycogenolysis, and gluconeogenesis (Maughan, 2009). Insulin is an anabolic hormone that is released in response to increased blood glucose and amino acids following ingestion of a meal (Nakrani *et al.*,

2021). In diabetes or pre-diabetes, the concentration of postprandial glucose rises to a greater extent than in healthy people as a result of a lack of insulin and of increased insulin resistance, poorly regulated hepatic glucose production, and abnormal gastric emptying after a meal (Aronoff *et al.*, 2004).

The measurement of blood glucose is generally obtained through measuring the venous plasma level. There is about 0.2-0.3 mmol/L difference between arterial and venous levels and in the postprandial state, venous blood glucose is lower than capillary blood glucose by 7% (Yang *et al.*, 2012). In an earlier study, the glucose concentration in blood which was taken from an antecubital vein was 4 mmol/l lower than that in arterialised venous blood 60 min after ingestion of glucose (Frayn *et al.* 1989). A higher level in the arterial blood is explained by the fact that glucose absorbed by the human body is sequentially conveyed into arteries and tissue cells via diffusion of glucose from the plasma to interstitial fluid (IF) hence reducing the level of glucose in veins (Liu *et al.*, 1992; Brouns *et al.*, 2005; Cengiz and Tamborlane, 2009). The concentrations of arterial and capillary blood glucose have been shown to be almost identical, although the distribution of the glucose to the systemic capillaries does not occur instantaneously (Liu *et al.*, 1992). Factors including the degree of glucose diffusion, the concentration differences in different tissues, blood flow, permeability of blood vessel to glucose, and acute variations in releasing insulin and glucagon, contribute to the difference in concentration and time-lag between glucose measured in the venous plasma and the interstitial fluid (Faerch *et al.*, 2021).

Insulin resistance is primarily an acquired condition related to excess body fat, especially visceral obesity, and can be considered as an impaired biologic response to insulin by the target tissues such as the liver, muscle, and adipose tissue. Insulin resistance impairs sensitivity to insulin mediated glucose disposal and initially leads to elevated beta-cell insulin production (hyperinsulinemia) (Wilcox, 2005). Progressively, insulin resistance can cause different metabolic consequences such as hyperglycemia, dyslipidemia, hypertension, elevated inflammatory markers and hyperuricemia. Metabolic syndrome, and non-alcoholic fatty liver disease (NAFLD) can occur as a result of insulin resistance. T2D occurs when insulin resistance is followed by impaired insulin secretion (Freeman and Pennings, 2022).

1.2.4.2.3. Dyslipidemia

Dyslipidemia is defined as an abnormal amount of lipids (cholesterol, triglycerides and/or phospholipids) in the plasma. The abnormalities in plasma cholesterol occur mainly in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein

cholesterol (HDL-C), and plasma triglycerides. It is characterized by elevations of total cholesterol levels greater than 6.2 mmol/L with increased levels of LDL cholesterol of greater than 4.1 mmol/L and a low level of HDL cholesterol less than 1.03 mmol/L, and triglyceride levels greater than 4.5 mmol/L (Kopin and Lowenstein, 2017). Dyslipidemia is considered as the biggest contributing factor resulting from obesity that results in the development of atherosclerosis and subsequent CVD (Wickramasinghe and Weaver, 2018).

Hypercholesterolemia is the most common disorder of dyslipidemia and the term used to refer to a high serum cholesterol level (Paththinige *et al.*, 2017). Hypertriglyceridemia occurs as a result of either increased TG production or decreased catabolism of TG-rich lipoproteins, and directly affects LDL and HDL cholesterol composition and metabolism. This gives TGs a crucial role in the progression of atherosclerosis (Badimon and Chiva-Blanch, 2019). It has been reported in large observational studies that there is a strong correlation between elevated levels of LDL-C, or decreased levels of HDL-C, and increasing risk for atherosclerotic coronary heart disease (CHD) (Kopin and Lowenstein, 2017). Higher levels of LDL-C can induce endothelial dysfunction, change the vascular smooth muscle cell phenotype, activate monocytes, and increase thrombogenicity (Badimon and Vilahur, 2012).

It was demonstrated that HDL cholesterol is cardioprotective, producing atherosclerotic plaque stabilization, improving endothelial dysfunction and limiting infarct size (Badimon and Vilahur, 2014). A low level of HDL-C (below 1.03 mmol/L in men) can increase the risk of atherosclerotic diseases and the reduction by 0.02-mmol/L might increase the coronary risk by 2%–3% (Kopin and Lowenstein, 2017).

1.2.5. Treatment and prevention of obesity

The treatment and prevention of obesity are based on reducing body weight (predominately excess fat) or maintaining a current healthy weight, respectively. To achieve these goals, behavioral changes should be made to modify eating patterns, including composition and quantity, and optimize physical activity habits. These combined changes should maintain the body either in negative energy balance (for weight loss) or in maintenance of energy balance (to prevent weight gain). Negative energy balance, when energy intake is lower than energy expenditure must occur over a sufficient period of time for the excess weight to be lost (Hill *et al.*, 2013; Hill *et al.*, 2012). Modifications of diet and activity have been shown to produce weight losses of up to 10% of initial weight over three to six months period (Jacob and Isaac, 2012).

To achieve behavioral modification in weight management therapies many components are considered important including self-monitoring, stimulus control, slower eating, goal setting, education, increasing exercise and social support (Jacob and Isaac, 2012). In terms of dietary modification, the key strategy for weight management that must be applied across dietary changes is to reduce energy intake. Reducing the amount and changing the proportions of the macronutrients (fat, carbohydrate and protein) consumed has been the basis for many weight loss diets such as the low fat, low carbohydrate and high protein diets (Smethers and Rolls, 2018). There is a controversy over whether a low fat or low carbohydrate diet is most effective for losing e weight, or whether a high protein diet which has increased satiating effects helps to sustain weight loss. However, all these diets can affect weight regulation positively (Kendall *et al.*, 1991; Shick *et al.*, 1998; Foster *et al.*, 2003; Sondike *et al.*, 2003; Brinkworth *et al.*, 2009; Johnstone, 2012).

1.3. Energy balance and weight regulation

As previously stated, energy balance is defined as the state achieved when energy intake equals energy expenditure. A positive energy balance occurs when energy intake exceeds energy expenditure, leading to weight gain. In contrast, in negative energy imbalance, the energy expenditure exceeds energy intake which leads to weight loss. The human body takes energy through the intake of food and drink, and expends energy predominantly through the basal metabolic rate, the thermic effect of food and physical activity (Hill *et al.*, 2013). Energy intake and energy expenditure can be altered through modifications in dietary intake, physical activity and sedentary behaviour to achieve energy balance.

Initially excess energy intake that is consumed in the form of carbohydrate and fat can be stored in the body as glycogen and triglyceride respectively. Carbohydrate is stored mainly as glycogen in skeletal muscle and liver. As glycogen is stored in association with water, the synthesis and catabolism of glycogen involve alterations in fluid balance. Further excess of carbohydrate is then converted to fatty acids for storage in the adipose cells (Maughan, 2008). Fat in the form of triglyceride is the largest source of energy storage and has no water bound to it (Hall *et al.*, 2012). Protein in food is mainly broken down into amino acids which are used to build, maintain, and repair body tissues, as well as to synthesize important enzymes and hormones. However, when there is a shortage of fats or carbohydrates, proteins can also yield energy. In some circumstances such as starvation and during latter stages of endurance exercise, skeletal muscle is broken down and used as fuel as a result of depletion of the glycogen reserves (Eberle, 2014).

When a negative energy balance occurs, the body will compensate by initially utilizing the energy in the liver glycogen store. After depletion of glycogen in liver, the deficit in energy is balanced by changes in the store of adipose tissue. Adipose tissue has a much higher energy density than the limited glycogen stores (Garrow, 1978). During weight loss, the catabolic state caused by energy deficit not only leads to the loss of fat mass but also causes undesirable loss of lean body mass, including skeletal muscle and some organs such as heart, liver, and kidney (Weiss *et al.*, 2017). The loss of lean body mass causes a reduction in energy expenditure (Drummen *et al.*, 2018). Furthermore, the reduction in skeletal muscle mass, cardiac mass and bone during weight loss lead to reduced muscle strength, altered muscle fibre type composition (Anthony, 2016), reduced maximal cardiac output, and increased fracture risk (Weiss *et al.*, 2017). Therefore, the aim in weight reduction is to decrease the excessive energy stores that are in the adipose tissue but maintain lean body mass to preserve function and energy expenditure.

The composition of weight loss is different depending on the duration of weight loss. At the beginning of a period of negative energy balance, the body can lose weight rapidly due to the loss of water as the glycogen stores are utilised. This is because the body uses the glycogen stores to maintain glucose supply to essential organs and tissues (such as brain, red blood cells and renal medulla) through glycogenolysis and to provide energy. Each gram of glycogen is stored in the liver along with 3 or 4 grams of water. After a period of time, the chemical composition of the weight loss is mostly triglyceride from adipose tissue beneath the skin and around the viscera. Approximately 80% of this adipose tissue is fat, roughly 2% is protein and the remainder is water (Garrow, 1978). The rate of the reduction in subcutaneous and visceral adipose tissue varied based on the amount of fat depots (Doucet *et al.*, 2002).

1.3.1. Energy expenditure

Total energy expenditure (TEE) can be defined in terms of the following three components: 1. basal metabolic rate, i.e. the energy costs of the processes essential for life and accounts for 60–80% of TEE, 2. Diet-induced thermogenesis or the thermic effect of food, i.e. the energy required to digest, absorb, and store food in the body which consists of ~10% of TEE and 3. physical activity-induced energy expenditure, i.e. the energy expended during physical activity which consists of 15–30% of TEE (Wouters and Baarends, 2006).

1.3.1.1. Basal metabolic rate (BMR)

BMR is the amount of energy that is expended in a resting condition to maintain the basic physiologic functions (e.g., respiration, heartbeat and muscle contraction) (Goran, 2000). The measurement of BMR must be undertaken in standard conditions of being awake but at rest, fasting, in thermoneutrality and whilst immobile. The measurement must be performed in a thermoneutral environment to avoid arousal resulting from heat production or heat loss for maintenance of body temperature (Westerterp, 2013); energy expenditure increases above basal levels during arousal. The difficulty of measuring metabolic rate under all of the standard conditions required for BMR, means that often resting metabolic rate (RMR) is measured as an approximation for BMR but under similar conditions. RMR tends to give a slightly higher energy expenditure (approximately 3%) than BMR because of arousal. Thus, due to the small difference, the terms basal and resting metabolic rate are often used interchangeably (Goran, 2000) although not correctly.

The average level of energy expenditure for BMR in an adult human is approximately 1 kcal/min. However, BMR can be varied due to many factors such as age (Molnar & Schutz, 1997), genetics (Bogardus *et al.* 1986), ethnicity (Foster *et al.*1997), nervous system activity (Poehlman *et al.* 1997) and body weight and composition (Jensen *et al.* 1988; Welle & Nair, 1990; Dionne *et al.* 1999; Weyer *et al.* 1999). It has been demonstrated that BMR is determined largely by fat-free mass (FFM), especially by the metabolically active organ mass. Most of the variance (53-88%) in BMR can be explained by fat-free mass (Nelson *et al.*, 1992). In addition to fat-free mass, fat mass is also a determinant of BMR, attributing 2% to 3% of the variability in BMR (Luhrmann *et al.*, 2001).

FFM consists of two components: 50% metabolically active (high metabolic rate organs/tissues such as skeletal muscle, brain, heart, liver and kidney) and 50% non-metabolically active (low metabolic rate tissues such as bone, inactive skeletal muscle and extracellular mass) (Muller *et al.*, 2002). As BMR is affected by body weight, body composition, age and sex, it was observed that older people and women had a lower BMR than younger people and men. This is because of differences based on age and sex in the amount of fat free mass and fat mass (Buchholz *et al.*, 2001).

1.3.1.2. Thermic effect of food (TEF)

TEF, is the additional energy expenditure postprandially above BMR that occurs in the short term (Tataranni *et al.*, 1995). It reflects the energy expended in absorbing, digesting, and

metabolising the food consumed (Hill *et al.*, 2013). It can be measured using indirect calorimetry through the assessment of the increase in oxygen consumption and carbon dioxide production postprandially. TEF consists of obligatory and facultative components. The obligatory component is the energy which is required for nutrient digestion, absorption, metabolism, transport, and storage during the postprandial phase. It is modulated by factors such as sympathetic nervous system (SNS) activity and glucose tolerance. The facultative component is the energy spent in excess of the obligatory requirements which occurs in several tissues especially in skeletal muscles (Astrup *et al.*, 1990). The increase in muscle SNS activity stimulates the energy expenditure in skeletal muscles which contributes to TEF of the whole-body (Asahara and Yamasaki, 2016).

TEF can be influenced by many factors such as age, energy content of a meal, meal size, meal composition, insulin resistance and physical activity (Reed and Hill, 1996). Although TEF is the smallest component of TEE, it can potentially play a role in the development of obesity. It was reported that subjects with obesity have a significantly lower TEF compared with people who are lean. The reduction of TEF in obese people could be explained by the degree of insulin resistance, which may be influenced by a low level of sympathetic activity (Jonee and Bray, 1997). It was demonstrated that insulin resistance was associated with a reduced rate of glucose uptake and storage and contributed to a reduced TEF (Bogardus *et al.*, 1985; Ravussin *et al.*, 1983).

A review by Raben *et al.* (2003) stated that protein has the highest thermogenic response (20–30%) followed by carbohydrate (5–10%). Fat seems to have little effect on TEF (0–3%). The thermic effect of alcohol (10-30%) is similar to the thermic effect of protein (Westerterp, 2012). The high thermogenic response of protein could be explained by the high costs of peptide bond synthesis, and costs of ureagenesis and gluconeogenesis. (Jonee and Bray, 1997).

1.3.1.3. Physical activity-induced energy expenditure (PAEE)

PAEE refers to the amount of energy expended due to physical activity (PA) which accounts for energy that is expended in addition to the BMR and TEF, including voluntary exercise, shivering, postural control, and voluntary movement (Hill *et al.*, 2013). Physical activity level (PAL) is the ratio of TEE to BMR. It provides an index for a 24-h period of the average relative excess energy expenditure which is related to PA (intensity × duration) (Schutz *et al.*, 2001). In general, PAL can be estimated from average values of 24-h TEE and BMR: PAL = TEE/BMR. Typical PAL values in free-living adults range from 1.40 to ~2.40 (Hills *et al.*, 2014).

The unit of physical activity intensity measurement is a MET which is defined as the amount of oxygen uptake during rest calculated per 1 kg mass of body weight and time. One MET equates to a rate of oxygen consumption of 3.5 ml O_2/kg/min (Jette *et al.*, 1990). Age, gender, and illness can have significant effects on this value. Based on the metabolic cost and difficulty of the activity, PA has been categorised to three levels of intensity, light, moderate, and vigorous (Gerrior *et al.*, 2006).

PAEE is the most variable component of TEE (Westerterp, 2013) and hence, PA has a crucial role in energy balance and the regulation of body weight. PA can increase the energy expenditure and the proportional contribution of fat oxidation to energy expenditure is greatest during low and moderately intense activities. The variations in frequency, duration and intensity of PA may generate considerable differences in TEE. In addition, an increase of physical activity with exercise training affects the body composition hence energy requirement and to some extent body weight (Baak, 1999) as the ratio of FFM to FM increases with training. A significant reduction in body weight was observed when PA was increased plus dietary restriction (Pi-Sunyer, 1998) and has been shown by many others (Ross *et al.*, 2000; Redman *et al.*, 2007; Donnelly *et al.*, 2013; Cox, 2017).

PAEE is determined by age, body size and body movement. The influence of body size on PAEE is corrected for by expressing PAEE per kg body mass or by expressing TEE as a multiple of BMR. The expression of TEE as a multiple of BMR is excluded when the relation between TEE and BMR has a non- zero intercept (Carpenter *et al.*, 1995). Therefore, TEE can be adjusted for the effect of body size in a linear regression analysis (Westerterp, 2013).

1.3.2. Energy intake (EI)

Energy intake is defined as the energy content of foods or beverages consumed and is provided by the major sources of dietary energy (macronutrients) carbohydrate (4 kcal/g, 17 kJ/g), protein (4kcal/g, 17 kJ/g), fat (9 kcal/g, 38 kJ/g) and alcohol (7 kcal/g, 29 J/g) (Goran, 2000). Carbohydrates and fats are completely oxidized in the body whilst protein is partially oxidized, with nitrogen being excreted as urea and other nitrogenous products. Total energy intake is determined by the number of ingestive (eating and drinking) events and the energy consumed per event (Mattes, 2014). According to an analysis of the NHANES data, it was suggested that the average daily energy intake increased by 168 kcal/day for men and 335 kcal/day for women from 1971 to 2000. With no regulation of energy balance (for example, by increased energy expenditure). This increase could explain a weight gain of 8.1 kg for men and 15.8 kg for women per year (Hill *et al.*, 2012).

A relationship between energy intake and physical activity has been found in several studies. It was observed that energy intake matched energy expenditure when a person was physically active (Mayer *et al.*, 1956). Nevertheless, when a person becomes less active, Blundell *et al.* (1999) found that energy intake does not adjust quickly and accurately to changes in energy expenditure, and this can lead to an increased tendency to gain weight. Similarly, a study by Stubbs *et al.* (2004) demonstrated that when PA was reduced from 1.8 × RMR to 1.4 × RMR over 7 days in health weight men, there was no compensatory reduction in energy intake. This caused a positive energy balance and consequently, an increase in weight.

A positive association between energy intake from carbonated beverages and body weight has been documented (DiMeglio and Mattes, 2000; James *et al.*, 2004). Daily energy intake was significantly greater when beverages were ingested compared with the solid food (Mourao *et al.*, 2007; Cassady *et al.*, 2012). Total daily energy intake was significantly higher by 12.4, 19 and 15% on days the beverage forms of the high-carbohydrate, -fat and -protein foods were consumed, respectively (Mourao *et al.*, 2007). Similarly, it was found that oral-liquid and gastric-liquid preloads elicited greater postprandial hunger and lower fullness sensations, more rapid gastric-emptying, attenuated insulin and glucagon-like peptide 1 release, and lower ghrelin suppression than did responses after oral-solid and gastric-solid preloads (all $P < 0.05$). Energy intake was greater on days when gastric-liquid preloads were consumed than when gastric solids were consumed (2311 ± 95 compared with 1897 ± 72 kcal, $P = 0.007$) (Cassady *et al.*, 2012). Beverages require less oral processing including rapid gastric-emptying and orocecal transit times) and evoke lower expected satiation values (ie, the degree to which an individual expects a particular food to be satiating), thus, beverages have a weaker effect on satiety than satiation (Cassady *et al.*, 2012).

1.3.2.1. Appetite

Appetite is the desire to eat and includes a range of parameters related to eating behaviour including sensations of hunger, drive to consume energy, and selection of specific nutrients and tastes (Rogers and Blundell, 1979). Appetite is the system that influences energy intake and is influenced by energy expenditure. In consideration of appetite control, three issues have been highlighted which are associated with obesity: the drive to eat, decisions about what to eat (food choice) and the suppression of eating to control the amount of food consumed (Gibbons *et al.*, 2019).

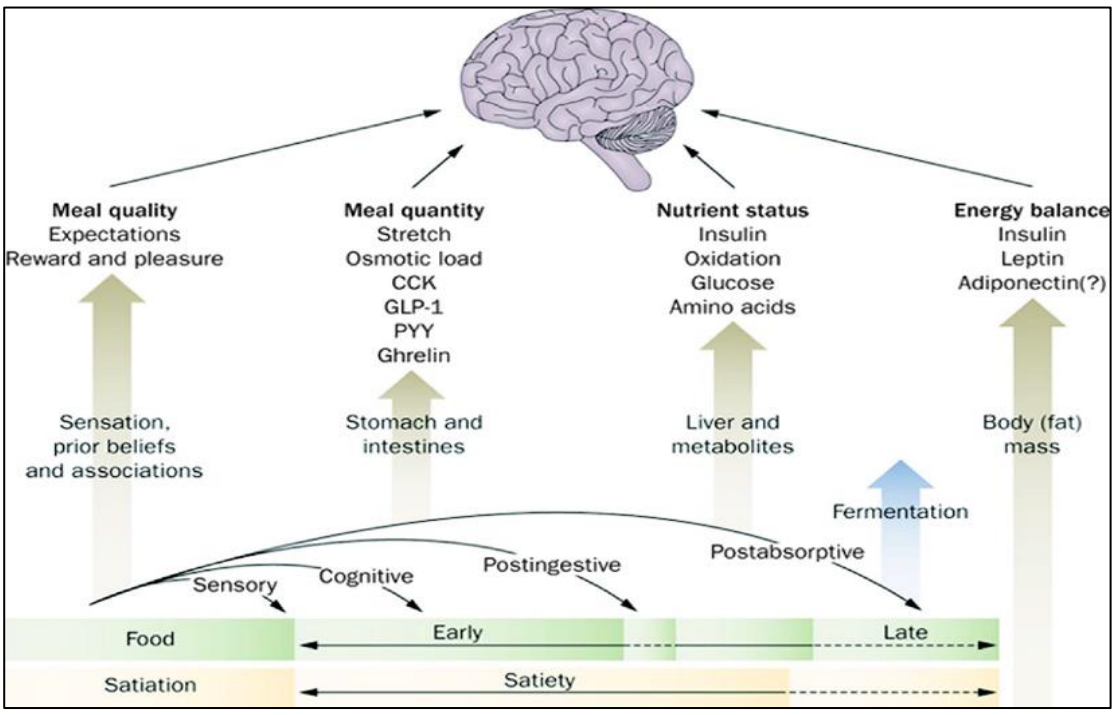
Hunger, satiation and satiety are major parts of a complex system of appetite control. Hunger refers to a subjective sensation that is related to the drive to consume food. The meaning of

hunger is the urge to eat arising from an energy deficit (Garrow, 1978). The postprandial return of hunger is inversely proportional to a decline in fullness arising from the digestion and assimilation of the meal. It was observed that hunger and fullness ratings were highly inversely correlated (Rogers and Brunstrom, 2016), and because of this the words hunger, and fullness were placed at opposite ends of the same rating scale. Satiation refers to the process that leads to the end of a bout of eating, which may be accompanied by a feeling of satisfaction. Satiety is the feeling of fullness that occurs after eating and potentially delays further energy intake (food consumption) until hunger returns (Benelam, 2009). Satiety is usually related to measures of duration until the next meal intake (Forde, 2018).

1.3.2.1.1. Satiety cascade

During food intake, the central nervous system receives and integrates a torrent of peripheral signals. John Blundell described (Figure 1.2) the complex series of interlinking process as the 'Satiety Cascade' (Blundell *et al.*, 1987). This provides a conceptual context for assessing the influence of food intake on satiation and satiety (Blundell *et al.*, 2010). A sequence of overlapping mediating processes initiated and controlled satiety (Blundell *et al.*, 1994). Satiation and satiety are impacted individually by the nature and timing of physiological processes.

Figure 1.2 The satiety cascade (Blundell *et al.*, 1987). This version of the satiety cascade includes the relationship between satiation and satiety (Source: Blundell, 2010)



Visual analogue scales (VAS) can be used to assess the extent of the different components of appetite. The change in appetite motivation VAS ratings of ≥15–25 mm on a 100-mm line scale can predict a significant difference in later energy intake (Sadoul *et al.*, 2014). Food intake inhibits appetite (Rogers and Brunstrom, 2016), however, self-reported appetite ratings alone do not reliably predict energy intake (Forde, 2017). However, eating behaviours which lead to a change in appetite may be targets for improving energy balance regulation and studies investigating variations in subjective appetite can signpost to the direction of further work in the manipulation of dietary intake and its measurement.

Appetite is regulated through a complex physiological system which includes numerous hormonal and neuronal signals (Figure 1.1). Short-term or episodic signals (gut hormones) including orexigenic (ghrelin) and anorexigenic peptides (for example, glucagon-like peptide-1 (GLP-1), pancreatic peptide (PP), peptide Tyrosine Tyrosine (PYY), oxyntomodulin (OXM) and cholecystokinin (CCK)), are released largely from the gastrointestinal tract into the blood stream and fluctuate periodically in relation to eating episodes. These signals help to regulate meal initiation and termination (Blundell *et al.*, 2008). Long-term or tonic signals, including leptin and adiponectin, are released from tissue stores, particularly adipose tissue, and oscillate comparatively with fluctuation only being noticed over longer time periods. This type of signals reflects the magnitude of stored body fat and may play a role in enhancing energy balance (Blundell *et al.*, 2008).

In general, episodic and tonic signals seem to have different roles in appetite regulation, however, both of them can also integrate with each other. For example, sensitivity to short-term signals can be influenced by signals that work in the long term, such as leptin (Horner *et al.*, 2020) and insulin (De Graaf *et al.*, 2004). This may provide a mechanism through which long-term energy requirements are interpreted into day-to-day food intake (Horner *et al.*, 2020). Hormones control food intake and energy expenditure in order to sustain energy homeostasis and thus maintain body weight and adiposity relatively constant (De Graaf *et al.*, 2004).

1.3.2.1.1.1. GLP-1

GLP-1 is secreted from L cells in the ileum and colon in response to the presence of nutrients (ie, carbohydrates and fat). GLP-1 leads to a delay in gastric emptying, stimulates insulin secretion, and inhibits glucagon release (Austin and Marks, 2009). This helps reduce blood glucose levels after carbohydrate consumption (De Graaf *et al.*, 2004). GLP-1 can be measured from blood plasma and its levels rise after a meal and fall in the fasted state (Orskov & Holst

1987). GLP-1 plays a role in the "ileal brake" mechanism (modifications of stomach and gut motility after food ingestion) that leads to moderation of the flow of nutrients from the stomach into the small intestine (De Graaf *et al.*, 2004).

In both people with healthy weight and with obesity, intravenous (IV) administration of GLP-1 in a dose-dependent manner reduces food intake (Verdich *et al.* 2001). However, the effect is small when infusions achieve post-prandial circulating levels (Verdich *et al.* 2001b). It was suggested that GLP-1 secretion is decreased in people with obesity (Holst *et al.* 1983, Ranganath *et al.* 1996, Naslund *et al.* 1999b) and weight loss normalizes the levels (Verdich *et al.* 2001b). When subcutaneous GLP-1 was given prior to each meal in people with obesity, their energy intake was reduced by 15% and a weight loss of 0·5 kg was seen over 5 days (Naslund 2003). The reduction in GLP-1 secretion appears consequently to be involved in the development of obesity and replacement may promote satiety (Wynne *et al.*, 2005).

1.3.2.1.1.2. PYY

It is a 36-amino-acid protein with neuropeptide Y and a pancreatic hormone-like structure, released from the gastrointestinal L-cells, particularly in the ileum, colon and rectum (Benelam, 2009 and Adamska *et al.*, 2014). Following food intake, PYY secretion is proportional to the amount of energy consumed at a meal (Wynne *et al.*, 2005). It is released into the circulation and rapidly peaks after 1-2 hours but remains raised for up to 6 h (Adrian *et al.* 1985). PYY concentrations are affected by meal composition; higher levels were found following fat consumption compared with carbohydrate or protein consumption (Lin & Chey 2003). The administration of PYY has been observed to decrease food intake both in rodents (Batterham *et al.* 2002; Adams *et al.* 2004; Chelikani *et al.* 2005) and in humans (Batterham *et al.* 2003; Degen *et al.* 2005).

PYY influences both people with healthy weight and those with obesity. Two hours after administration of PYY, energy intake at a buffet lunch was roughly diminished by 30% and during the 24 hours after intravenous infusion of exogenous PYY was significantly reduced. Fasting and postprandial PYY levels were lower in people with obesity than those who were lean despite the higher level of energy intake (Batterham *et al.* 2003), and BMI correlated negatively with PYY levels. This may indicate that a lack in PYY concentration could have contributed to the development or maintenance of obesity. PYY also reduces gastric secretions (Adrian *et al.* 1985), inhibits gastric emptying and increases transit time through the intestine (Savage *et al.* 1987). Thus, suggesting it may play a role in the mechanism of the ileal brake (Benelam, 2009).

1.3.2.1.1.3. Ghrelin

Ghrelin is a peptide hormone predominantly synthesised by oxyntic cells in the stomach but also from duodenum, ileum, caecum and colon (Date *et al.* 2000). In animals and humans, administration of ghrelin stimulates appetite and increases food intake (Tschöp *et al.* 2000; Wren *et al.* 2001). It was demonstrated that ghrelin rises before meals, suggesting a role in meal initiation, whereas anorexigenic peptides are produced in relation to food ingestion. In humans on a fixed feeding schedule, ghrelin levels are high during a period of fasting, fall after eating (Ariyasu *et al.* 2001, Cummings *et al.* 2001, Tschop *et al.* 2001) and are thought to be regulated by both energy intake and circulating nutritional signals (Tschop *et al.* 2000). Ghrelin concentrations decline in response to the ingestion of food or glucose, but not following ingestion of water, suggesting that gastric distension is not a stimulator (Tschop *et al.* 2000). Ghrelin concentrations decline quickly after each meal and return to pre-prandial concentrations before the initiation of the next meal (Cummings *et al.*, 2001).

The pre-prandial elevation of ghrelin levels and its reduction after meals suggests that ghrelin is a 'hunger' hormone responsible for meal initiation. Ghrelin contributes to an enhancement of appetite, increase in food intake and body weight and decrease in utilization of fat (Tschop *et al.*, 2000, Wren *et al.*, 2001). At equal loads, fat appears to be less effective than carbohydrate or protein at suppressing ghrelin (Monteleone *et al.*, 2003; Overduin *et al.*, 2005). Ghrelin may also alter energy balance by stimulating adipogenesis, inhibiting apoptosis, transitioning from fatty acid oxidation to glycolysis for energy expenditure, and inhibiting sympathetic nervous system activity (Austin and Marks, 2009). Ghrelin concentrations reflect body fat stores and nutritional status and are negatively correlated to adiposity; thus, individuals with obesity have lower levels than those who are lean (Shiiya *et al.*, 2002). This may be because levels of ghrelin have already been maximally suppressed due to excess fat storage (English *et al.* 2002).

1.3.2.1.1.4. Insulin

Insulin is a metabolic hormone and tonic signal (Horner *et al.*, 2020) which is released by β-cells in the pancreas. Insulin has also been implicated in the long-term regulation of energy balance and its levels dramatically rise in response to small increases in blood glucose concentrations after food intake (Polonsky *et al.* 1988). Insulin acts to control blood glucose levels. However, over the longer term, levels of plasma insulin are directly related to changes in adiposity, so that people with obesity have higher levels of insulin. In addition, insulin

concentration is determined by peripheral insulin sensitivity, and this is associated with the stores of total body fat and fat distribution (Porte *et al.* 2002).

In animal models, exogenous administration of insulin resulted in reduced food intake and weight (Woods *et al.* 1979; Ikeda *et al.* 1986), whereas inhibition of insulin's action results in an increase in energy intake and weight (McGowan *et al.* 1992). This suggests that insulin contributes to satiety (Benelam, 2009). In human studies, the effect of endogenous insulin on food intake and subjective satiety was investigated and suggested that insulin has a suppressive effect on appetite in those who are lean but less so in individuals with obesity. It has been found that the insulin concentration was negatively related to energy intake in lean men, but not in those with obesity (Speechly *et al.*, 2000 and Verdich *et al.*, 2001).

1.4. Protein and nitrogen balance

Nitrogen (N) is a fundamental component of amino acids, which are the molecular building blocks of protein. Nutritional demands for protein include growth and replacement of obligatory nitrogen losses (ONL) (Millward *et al.*, 1994). ONL reflect the nitrogen loss that is measured in participants consuming a protein-free diet for a relatively short period of time (Tessari, 2006). Amino acid nitrogen is needed for tissue protein synthesis and the production of several nitrogenous compounds involved in a range of functions (hormones, neurotransmitters, immune competence and peroxidative defences) (Tessari, 2006). Nitrogen balance provides a proxy for measuring protein balance as 98% of body nitrogen is found in body protein (Mason, 2015).

Dietary nitrogen is predominantly found in the food as amino acids in protein. The protein content in foods is estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor which, on average, is 6.25g of protein as equating to 1g of nitrogen. The factor of 6.25 is calculated based on the speculation that proteins contain 16% nitrogen. However, the non-protein nitrogen has been subtracted before multiplication by the 6.25 factor in some foods which contain a considerable amount of non-protein nitrogen in the form of urea, purines and pyrimidines (for example mushrooms) (Fujihara *et al.*, 1995). However, pure proteins differ in their nitrogen contents (Jones, 1941). This is because of the variations in their amino acid composition and differences the nitrogen content of amino acids which are high in arginyl, histidyl, glycyl and asparagyl residues and low in phenylalanyl and tyrosyl residues. The nitrogen content of proteins differs from 13% to 19%. This means that nitrogen conversion factors equate from 5.26 (1/0.19) to 7.69 (1/0.13) which are known as Johns factors. For

example, when using a conversion factor 6.25 in avocado protein which contains 13.4% nitrogen can result in a 15-20% error in the estimated protein content (Jones, 1941).

Therefore, to convert nitrogen to protein, the Jones factor for nitrogen content of the analysed food should be used when the specific factor is known whereas the general factor 6.25 should be used when the factor is not known. The use of the general factor for specific foods that are major sources of protein can result in an error in protein content that is relative to the specific factors and ranges from -2 percent to +9 percent. As protein contributes an average of 15 percent of energy, the use of the 6.25 factor should produce errors of no more than about 1 percent in estimations of energy content from protein in most diets (Food and Agriculture Organization, 2002).

Understanding of protein balance is fundamental to understand how lean mass can be preserved during weight loss in order to maintain function and reduce the reduction of energy expenditure. Protein balance is defined as the equilibrium between protein intake and anabolism, and protein catabolism and excretion of nitrogenous products (Breen and Phillips, 2011). Anabolic growth is achieved when rates of protein synthesis exceed degradation, producing a positive net protein balance. In contrast, a catabolic state is a result of a negative net protein balance (Anthony, 2016). A balance between protein synthesis (anabolism) and protein breakdown (catabolism) is required for good health and normal protein metabolism. More synthesis than breakdown indicates an anabolic state that builds lean tissues, more breakdown than synthesis indicates a catabolic state that utilizes lean tissues and leads to atrophy or loss of lean mass (Jeejeebhoy, 1986). Skeletal muscle cell mass is affected by protein turnover/degradation in all phases of life.

The human body is able to create a physiological adaptation to changes in protein intake. Adaptation in the metabolism of protein and amino acid in response to short-term changes in protein intake mostly occurs through alterations in whole-body protein turnover and amino acid oxidation, urea production and nitrogen excretion (Drummen *et al*, 2018). In response to a low or high protein intake, nitrogen excretion will decrease or increase as a consequence of changes in amino acid metabolism. Thus, the disposal of nitrogen through the urea cycle reflects the amount of protein intake and an adequate protein intake is needed to maintain fat-free mass (Drummen *et al*, 2018).

In the fed state, protein balance will be positive in response to a high protein intake which increases muscle protein synthesis, and negative with a low protein intake which reduces the muscle protein synthesis (Weiss *et al.*, 2017). It was shown that a shift from a spontaneous

protein intake of 0.9 g protein/kg/d to 1.4 g protein/kg/d over a 10-d period using dietary supplements in moderately malnourished, elderly participants was correlated with an increase in the fat-free mass and net protein synthesis, as measured in the post-absorptive and fed states (Tome and Bos, 2000). However, recent results have strongly suggested that there probably is limited anabolic stimulation by high protein diets in healthy young adults. Moreover, if the protein intake exceeds the body need, all excess is metabolized and the end-products are excreted, since the excess protein is not stored in the body (WHO, 1985). Excess protein would experience deamination, or release of amino groups from amino acids. In this way, nitrogen is released from the body and the remaining carbon bonds are converted to acetyl coenzyme A. Acetyl coenzyme A can then be synthesized into triglycerides through the process of lipogenesis or used as a substrate for Adenosine triphosphate (ATP) production. Therefore, the energy from excessive amounts of protein can indirectly be stored as fat (Febriani *et al.*, 2019) and/or be used as a fuel for energy expenditure which may displace the utilization of other sources of stored energy.

The utilization of dietary protein is affected by energy intake and energy balance. The basal energy requirements and the daily total amount of protein turned over are associated with the active tissue mass. It has been proposed that protein requirement, within a certain limit, is inversely related to energy intake, (i.e the more energy is consumed, the less protein is required). This could be explained by the use of protein as an energy source (further to its structural, regulatory and functional role) when energy from carbohydrate and fat do not meet requirements. Moreover, alternative energy substrates, such as carbohydrates, can stimulate the secretion of insulin, which in turn spares endogenous proteins (Young and Pellett, 1991). It has been suggested that an increase in the level of energy intake improves protein synthesis and diminishes amino acid oxidation. The influence of energy balance on Nitrogen balance (NB), a proxy for protein balance, may depend on the level of energy intake. Any change in energy intake below or above the individual's requirement appears to impact on N balance (1-2 mg of N retained per kcal) (Jequier, 1991). Garrow *et al* (1981) reported the effect of different protein contents and meal frequency, separately and combined, on N balance and found that higher protein concentrations are more protein-sparing, even at low energy intakes.

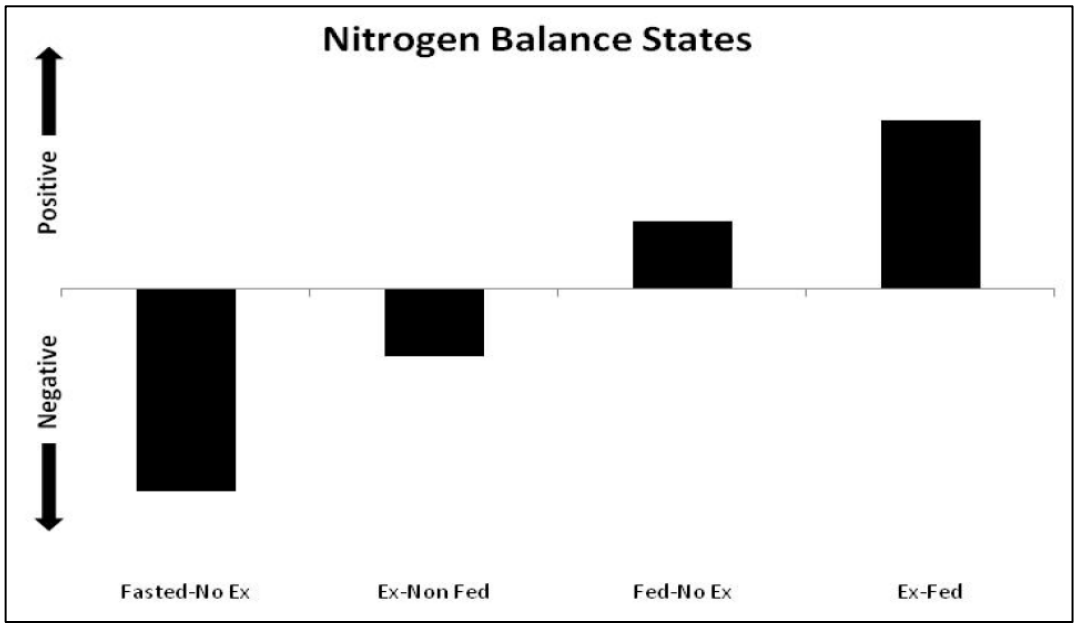
Using nitrogen balance, for example in the classic approach to determine the protein requirement of individuals (Millward, 1998; Millward, 2003), reflects the known relationship between protein and its constituent nitrogen. Measurement of whole-body nitrogen balance can thus act a proxy for measuring whole body protein balance. Zero nitrogen balance occurs when nitrogen intake equals nitrogen output (NB = 0) and is also referred to as nitrogen

equilibrium. NB is defined as the net difference between the total intake of nitrogen (from the diet) and total nitrogen losses (including the nitrogen in urine, faeces and miscellaneous losses) (Milner, 1993; Tome and Bos, 2000; Dickerson, 2005; Tessari, 2006). If more nitrogen is consumed as protein than is excreted then NB is considered to be positive, and the body nitrogen pool, hence protein pool, is increasing. If more nitrogen is being excreted than being consumed, NB is considered to be negative, as a consequence of net protein degradation potentially due to an inadequate intake of protein to meet obligatory loses, and potentially reflects loss of muscle mass (Sandri, 2013) and a decrease of body nitrogen pool.

Nitrogen losses can occur through different ways. They mainly arise from urinary losses in the form of urea, ammonia and creatinine but also in the form of faecal and miscellaneous losses (sweat, hair and nail) which are considered relatively stable and at low level (Calloway and Margen, 1971). The excretion of nitrogen cannot be reduced below the obligatory nitrogen losses. The losses have been estimated to be 36 mg/kg/day in the urine, 12 mg/kg/day in faeces and 8 mg/kg/day as miscellaneous nitrogen losses (sweat, sebum, desquamations, nails, hairs and saliva) (Bodwell *et al.*, 1979). Given the equivalence of 6.25 g of protein per gram of N, ONL consequently correspond on the whole to a protein amount of 0.35 g/kg/day. Apart from nitrogen intake, nitrogen excretion can be affected by many factors such as the nutritional circumstances (eg. starvation reduces urea and increases ammonia), renal function, the hydration state (Tessari, 2006), the level of catabolic stress (Loder *et al.* 1989), poor protein quality (Tome and Bos 2000) and physical activity (Poole *et al.*, 2010; Mori, 2014).

The acute effect of exercise on whole-body protein and amino acid metabolism, it was shown that a considerable exercise-induced increase in concentration of blood urea during the exercise returns to normal after exercising. This is when renal filtration, which drops during exercise, is restored to normal afterwards (Rennie *et al.*, 1980; Rennie *et al.*, 1981a). On an intake of 1 g protein/kg/day, participants who were in nitrogen balance in the rested state, the exercise produced an immediate negative N balance (Figure 1.3). Nevertheless, excess N loss over the following period then decreased by a 90% until zero balance was restored. The adaptation of N balance was integrated with an enhanced energetic efficiency, with reduced pulse and respiratory rates (Millward *et al.*, 1994).

Figure 1.3 Impact of feeding and exercise status on nitrogen balance. (Source: Poole *et al.*, 2010)



NB is also considered as an indicator of malnutrition and the catabolic stress level (Dickerson, 2005). A negative nitrogen balance may occur as a result of physical or emotional stress, starvation, consuming a very low energy diet, or a diet containing poor quality protein which is lacking in essential amino acids (Dickerson, 2005). A positive nitrogen balance represents anabolism and a net increase in total body protein, whereas a negative N balance represents net protein catabolism. For example, a negative N balance of 1 g/day represents a 6.25 g/day loss of body protein, which is equivalent to a 30 g/day loss of hydrated lean tissue. In addition, high protein intakes have been shown to result in a continuous, positive N balance of 1–3 g N/d, (Fisher *et al.*, 1967, Cheng *et al.*, 1978; Price *et al.*, 1994; Forslund *et al.*, 1999).

There are many formulae in the literature to calculate the NB based on human feeding studies in healthy populations and patients (Palmo *et al*, 1989; Milner *et al.*, 1993 and Dickerson *et al.*, 2005). A common method of estimating urinary nitrogen loss, a key component of total N loss, involves the estimation of total urinary nitrogen (TUN) based on measuring the urinary urea nitrogen (UUN). Numerous studies stated that the use of UUN in order to estimate TUN is acceptable in practical terms. This is because urea generally accounts for approximately for 80–90% of the total urinary nitrogen excretion and the remaining consisting of ammonia, uric acid, creatinine, purines, alpha amino nitrogen and other unidentified compounds (Palmo *et al*, 1989). Urea and ammonia are considered as the two major components of renal nitrogen

excretion (Weiner *et al.* 2015). Nitrogen excretion in the form of ammonia is approximately 10% of urea nitrogen excretion in normal conditions, but it can increase by 5- to 10-fold, enabling ammonia to have an important role in nitrogen balance. The excretion of ammonia differs considerably, dependent on protein intake, hypokalemia, glucocorticoid hormones (Weiner *et al.* 2015) and acid base status (Weiner and Verlander, 2017). Faecal nitrogen excretion is considered to be small in comparison to the urinary nitrogen losses. It accounts for no more than 2-5% of the total daily nitrogen excretion (Lee and Hartley, 1975).

UUN is more practical to measure in contrast to TUN which requires for example, the Kjeldahl method which is a costly and time-consuming process (Milner *et al.* 1993). Therefore, due to lower cost, accessibility and acceptable accuracy, UUN is widely used to calculate NB. Providing normal healthy subjects are included in nitrogen studies and a balanced energy diet is given, the use of UUN instead of TUN is reasonable. UUN should be measured and then TUN calculated using a correction formula. A classic equation is protein intake (g/d)/6.25 minus (UUN (g/d) + 4). The constant of "4" represents 2g of N derived from the urinary non-urea (uric acid, ammonia, creatinine, nitrates and amino acids) and 2g for N in faeces and integumentary (dermal) losses. The value for the four is estimated for average sized healthy adults with an adequate energy and protein intake (Mason, 2015). However, these values will vary depending on diet, body size and various clinical conditions such as thermal injury whilst the integumentary losses can be very substantial (Dickerson, 2005).

Given that the adequacy of the protein intake is important in determining nitrogen balance it should be carefully considered when designing human intervention studies in this area. Protein requirements for individuals, as previously stated, are expressed in terms of the amount of dietary protein needed to prevent a net loss of body protein as nitrogenous products. Hence, in healthy subjects with an intake of 1 g protein/kg per day, the amount of nitrogen lost in urine, faces and skin and miscellaneous sources will approximately equivalent to 0.85 g/kg, 0.1 g/kg and 0.03 g/kg respectively per day (Kondrup, 2008). With varying intakes, the loss in urine will vary while the losses in faces and skin will not vary substantially on an ordinary Western diet in a temperate climate.

In practice, N balance studies tend to be artificially positive because of overestimation of dietary nitrogen intake due to losses of food on cooking and eating in utensils and dishware and the difficulty in measurement of N loss from non-urinary nitrogen such as sweat, exfoliated skin, hair and nail growth, saliva and menses (Kopple, 1987) and underestimation of nitrogen losses due to incomplete urine collections and unmeasured outputs (Tessari, 2006). It is best

to wait at least 4 days after a substantial change in protein delivery before N balance is determined, because a labile N pool exists and this tends to dampen and retard changes that otherwise would be observed as a result of altered protein intake (Mason, 2015). For example, in healthy subjects, consuming diet with no protein for a prolonged period leads to a decrease in the loss of protein from 1 g/kg to 0.4 g/kg per day, as an adaptive response to inadequate intake (Kondrup, 2008).

Given the number of factors that can impact on nitrogen balance and that potentially impact on the validity of the equations for estimating TUN from UNN it is important to select participants carefully (eg. with a previously nutritionally adequate diet, of healthy body weight and in energy balance) and standardize conditions (eg. degree of stress and dietary composition) in order to minimise when designing studies investigating nitrogen balance in order to minimise the potential variation in the non-urinary urea nitrogen components which result in reduce potential bias. These factors will be taken into account in the study described in this thesis.

1.5. Circadian rhythm

A circadian rhythm is defined as physical, mental, and behavioural processes that follows a 24-hour cycle and which is self-sustaining if in a constant environment (Lopez-Minguez *et al.*, 2016). Diurnal rhythm is a circadian rhythm that is synchronized with the day/night cycle. The study of circadian rhythms is known as a chronobiology. Circadian rhythm is described as 'free running' when it is expressed in the absence of any 24-hour stimuli from the external environment. In these conditions the rhythm is not entrained by any cyclic change in the physical environment. Thereby, all diurnal rhythms that occur under natural conditions and continue to cycle under laboratory conditions free of any external time-giving cues from the physical environment (e.g., under constant light or constant darkness) are called circadian rhythms. Therefore, a diurnal rhythm should not be described as circadian until it has been demonstrated to occur under constant environmental conditions (Vitaterna *et al.*, 2001).

The diurnal system can be assessed at the home whilst subjects are free living and undertaking their usual daily activities 'masking effects' such as light, sleeping, physical activity and feeding are difficult to control in this environment. 'Masking', an immediate response to an external stimuli, may obscure an underlying clock- controlled pattern (Hiddinga *et al.*, 1997). Thus, in the laboratory setting, the use of specialized protocols such as constant routine and forced desynchrony (Czeisler *et al.*, 1999) have been designed to minimize the influence of

these factors and to obtain an accurate assessment of the underlying circadian timing (Wehrens *et al.*, 2017; Reid, 2019).

In order to achieve a better estimate of the endogenous rhythm, the masking effects have to be removed. In humans, this can be achieved by a protocol known as the "constant routine" (Mills *et al.*, 1978; Duffy and Dijk, 2002). This routine requires the subject to remain awake and sedentary or, preferably, supine, for a period of at least 24 h. During this time, the environment is kept constant with regard to light intensity, temperature, and humidity, and the subject is given identical meals at regular intervals. Such a protocol removes the masking influences at their source, and is generally considered to provide assessments of the phase of a circadian rhythm that are the "gold standard".

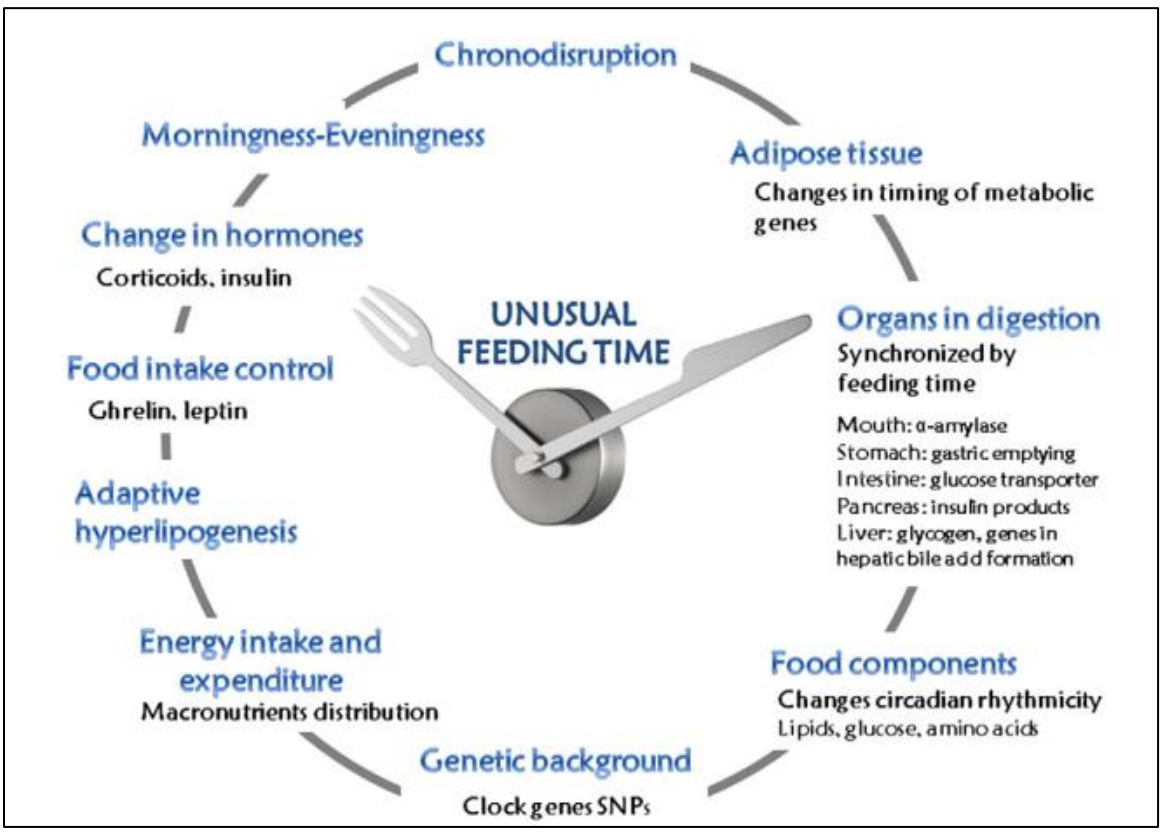
The circadian system can be divided into 2 parts: the central (master) clock which is located in the suprachiasmatic nucleus (SCN) of the brain, and peripheral clocks located in nearly every tissue and organ system such as the heart, the liver or the pancreas (Garaulet and Gómez-Abellan, 2014). Circadian rhythm is regulated by the central circadian clock which is entrained by light/dark cycles and other physiological factors (Garaulet and Gomez-Abellan, 2014). The central circadian pacemaker in the SCN keeps the peripheral clocks in synchrony to the central 24-hour circadian rhythm (Ruddick-Collins *et al.*, 2018). Peripheral clocks thus work together due to being synchronized by the central clock (Garaulet and Gomez-Abellan, 2014). Any changing during the day in this system potentially involve many hormones and may impact on physiological variables, reflecting the complexity of the impact of the circadian. Chronodisruption can occur when the peripheral clocks are desynchronized from the central clock.

The circadian system can be affected by many external synchronizers of the biological clocks such as feeding cues, changes from fasting to feeding (i.e., food timing), moving from rest to activity, light exposure and sleep time and duration. These stimuli are called Zeitgebers (Ekmekcioglu and Touitou, 2011). In humans, the rhythmic expression of metabolic pathways is attributed to the robust and coordinated expression of clock genes in many organs and tissues (Garaulet and Gomez-Abellan, 2014). Many physiologically and nutritionally relevant processes including glycolysis, gluconeogenesis and lipid metabolism have circadian rhythms which are synchronized by peripheral clocks. It was shown that many hormones oscillate on a circadian basis such as melatonin, cortisol, gonadal steroids, prolactin, growth hormone and thyroid hormone. The other nutrient-sensitive hormones such as insulin, ghrelin, leptin and

adiponectin also have daily oscillations and are synchronized by environmental stimuli (Gnocchi and Bruscalupi, 2017).

A meal pattern with minimal between day variation in pattern may help to maintain a stable internal temporal order in the circadian system. In the current 24-h society such a pattern is frequently abandoned due to for example shift work patterns, jet lag and the growing demands of study and even of leisure and pleasure in young people across the whole 24-hour period. Therefore, between day variability in meal pattern (Figure 1.4) may disturb this coordination and consequently may also alter the circadian rhythm of many hormones involved in metabolism (Garaulet and Gomez-Abellan, 2014).

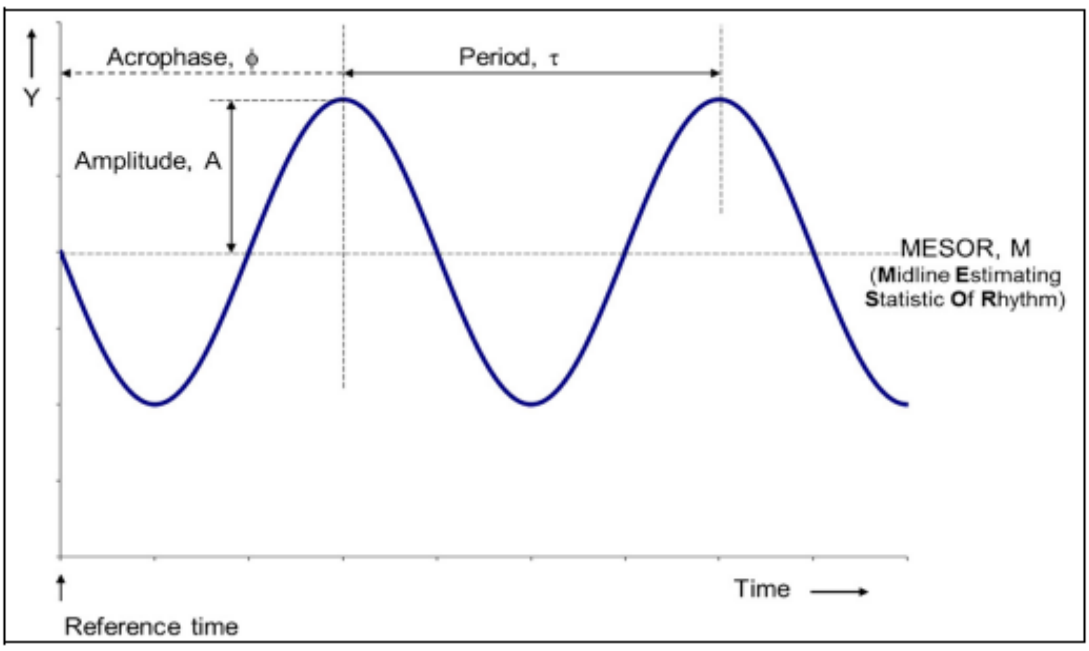
Figure 1.4 Consequences of an unusual mealtime (Source: Garaulet and Gomez-Abellan, 2014)



Several outputs (melatonin or cortisol levels, core body or wrist temperature, and rest-activity cycles) are used to as markers in the assessment of the circadian system by determining the phase, amplitude and period of circadian rhythms. The difference in the level between peak and trough values is the amplitude of the rhythm. The timing of a reference point in the cycle (e.g., the peak) relative to a fixed occasion (e.g., beginning of the night phase) is the phase.

The time interval between phase reference points (e.g., two peaks) is called the period. Other measures of circadian rhythms have been developed and applied to various populations through traditional cosine models (Reid, 2019). These measures include amplitude, one-half of the peak-trough variation of the 24 h rhythm; mesor, 24 h rhythm adjusted time mean level of activity; and the acrophase, the peak time of activity rhythm (Waterhouse *et al.*, 2005) (Figure 1.5).

Figure 1.5 Rhythm characteristics (Source: Cornlissen, 2014)



1.5.1. Markers of circadian systems

1.5.1.1. Melatonin

Melatonin is a hormone synthesized by the pineal gland and is controlled by the SCN and by ocular light exposure. Melatonin levels exhibit a circadian rhythm that peaks during the night in normally entrained individuals. Melatonin can be measured in saliva, blood or urine samples (Benloucif *et al.*, 2008). The rhythmic activity of the SCN regulates the melatonin secretion which is directly associated with day duration. In both nocturnal and diurnal animals, the release of melatonin reaches its peak in the middle of the night, between 24:00 and 03:00, inducing activity in the former and rest/sleep in the latter. Thus, melatonin can be considered as the central "relayer" which expresses information about light–dark cycles. Melatonin in mammals is also crucial in the control of reproductive and sleep behaviours (Gnocchi and Bruscalupi, 2017).

In both primates and humans, melatonin regulates adrenal glucocorticoid production, suppresses cortisol production, and entrains adrenal gland secretion rhythms (Torres-Farfan *et al.*, 2003). Many hormones such as gonadotropin-releasing hormone (GnRH), luteinising hormone (LH), and follicle-stimulating hormone (FSH) are produced under melatonin regulation (Pandi-Perumal *et al.*, 2006). As melatonin is also synthesized in peripheral tissues, such as the gastrointestinal tract, lymphocytes, bone marrow, the retina and skin, it may contribute to other physiological functions (Gnocchi and Bruscalupi, 2017) such as blood pressure regulation (Scheer *et al.*, 2004) and immune system modulation (Pandi-Perumal *et al.*, 2006) via paracrine signaling which is a system that allows cells to communicate with each other by releasing signaling molecules that bind to and activate surrounding cells (Handly *et al.*, 2015).

The phase of melatonin 24-hour rhythm can be defined as at the peak, the midline crossing point or offset of secretion. As melatonin is an output of the SCN, it improves the amplitude of SCN oscillation. It has been considered in obesity treatment because it has been demonstrated that there is a reduction in amplitude with obesity and occasionally a total loss of rhythmicity. Melatonin can adjust the phase of the SCN. Therefore, administration of melatonin during the early afternoon stimulates a phase advance whereas during the early morning it stimulates a phase-delay (Garaulet and Madrid, 2010).

"Dim light melatonin onset" (DLMO) is considered to be the most accurate marker of the body clock that can be used to appropriately time the administration of bright light or exogenous melatonin in order to obtain a desired phase shift (Burgess *et al.*, 2003). DLMO can be practically measured before going to sleep. A part of the rhythm can be assessed by taking only evening samples before sleep under controlled conditions to measure when DLMO melatonin synthesis begins (Wirz-Justice, 2007) (ie, the level starts to go up). However, when an entire 24-hour circadian rhythm is assessed, the melatonin rhythm is often not the choice marker for the body clock. This is because its measurement either requires frequent blood sampling through using an indwelling cannula (Benloucif *et al.*, 2008) (which restricts the circumstances and places in which it can be used) or requires a frequent collection of saliva or urine samples (Benloucif *et al.*, 2008 and Reid, 2019) (which causes major disturbance of sleep).

1.5.1.2. Body temperature

Body temperature in human remains steady within narrow ranges around 36.5–37.5 °C (or 97.7–99.5 °F). This is essential for health, as it allows organs and bodily functions and processes to work efficiently. If a person's body temperature strays too far from its narrow

range, hyperthermia or hypothermia can developed (Bicego *et al.*, 2007). The body core temperature is regulated by nerve cells in separate centres in the hypothalamus through the mechanisms of initiating the heat loss or gain when core temperature begins to rise or fall. The difference between the body core and skin temperature lets heat be exchanged to the environment through convection and radiation (Waterhouse *et al.*, 2005). The concept that the hypothalamus regulates body temperature at a "set point" reflects that a wide range of ambient temperatures only produces minor changes in core temperature (Edholm *et al.*, 1973) and exercise causes significant thermoregulatory responses. However, within these narrow ranges, a circadian pattern of core temperature is seen rapidly declining in the late evening and rapidly increasing in the morning (Waterhouse *et al.*, 2005). Circadian changes in core temperature that derive from the inputs of the body clock in the SCN are imposed on the thermoregulatory centres hence thermoregulatory responses (Refinetti and Menaker, 1991; Moore, 1995).

Circadian rhythms in core temperature are phased such that there is a peak of temperature at about 17:00 h, and a nadir at just after the mid-point of sleep about 05:00 h (Krauchi and Wirz-Justice, 1994). This rhythm results from several effects of the body clock, sleep, and physical and mental activity (Waterhouse *et al.*, 2005). The rhythm is only apparent and attributed to the body clock when sleep, physical and mental activities, and timing and composition of meals are all maintained and kept constant as possible according to a 'constant routine' (Hiddinga *et al.*, 1997). For example, if sleep is avoided, but all other factors are maintained constant, then the amplitude of a circadian rhythm of core temperature is reduced by about half. This reduction in amplitude reflects the influence of the sleep-wake cycle (and related differences in physical and mental activity) upon the circadian rhythm. Therefore, since the body clock and, therefore, the circadian rhythm of core temperature adjusts to alterations in the sleep-wake cycle or mealtime pattern for example, desynchrony between the temperature rhythm and the sleep-wake cycle or mealtime pattern can lead to many of the difficulties and chronodisruption associated with "jet lag" and "shift workers' malaise" (Waterhouse and DeCoursey, 2004).

The circadian rhythm of core temperature is achieved primarily by alterations of heat loss through changes in the cutaneous vascular tone of the forehead and distal limbs (Aschoff and Heise, 1972). These circadian variations in core temperature and heat loss are probably due to a rhythmic input from the hypothalamic thermoregulatory centres in the SCN and changing the thresholds for cutaneous vasodilatation and sweating (Refinetti and Menaker, 1991; Moore, 1995; Aizawa and Cabanac, 2002). The circadian rhythm of heat loss from the distal limbs, with rhythms of skin temperature and blood flow in these regions occur maximally in the late evening (when core temperature is rapidly declining) and minimally in the morning (when core

temperature is rapidly increasing). On the other hand, the cutaneous temperature of the trunk shows a phase that is closer to that of core temperature (Aschoff and Heise, 1972; Hildebrandt, 1974; Smolander *et al.*, 1993). Melatonin seems to contribute to these changes. There is an increase of its secretion in the evening, and this increase stimulates a fall in body temperature through cutaneous vasodilatation, while in the morning the opposite changes occur (Cagnacci, 1997).

As approximately two-thirds of RMR is involved in producing heat for homeothermy, 40–50% of total energy expenditure is used for the maintenance of a constant internal temperature in mammals (Landsberg, 2012). This percentage is supported by the fact that an increase in temperature of 1°C is correlated with a 10–13% increase in metabolic rate (Du, 1921). Thereby, the reduction in metabolic rate might be caused by the decrease in temperature. As part of an integrated metabolic response to starvation, a decrease in core temperature occurs which supports the proposal that regulation of temperature contributes to alterations in energy expenditure (Geiser, 1988; Inagaki *et al.*, 2007). These studies, furthermore, raise the probability that alterations in the central regulation of core temperature could be involved in the metabolic changes associated with obesity.

Wrist temperature can also be used to assess the circadian rhythm. However, this method is not a direct measure of core temperature measurement, thus, interpretation of the results for temperature profiles using skin temperature should made with caution as different factors, such as ambient temperature, location of the sensors and activity level, in addition to core temperature substantially affect the measurement. Interestingly, skin temperature during sleep is typically higher than core temperature which is lower during sleep (Reid, 2019). Core and wrist temperatures can be measured throughout the 24 h day in subjects who are free living (Waterhouse *et al.*, 2005). An ingested pill can be used for core temperature measurement, and a small button thermometer is used for peripheral temperature (Chapter 3 section 3.5.4.2)

Any change in exposure to the body clock synchronizers or physiological alteration of the normal pattern of circadian rhythm may affect the circadian function and cause physiological changes that lead to chronodisruption (Barraco *et al.*, 2017). This can contribute to the development of different illnesses, such as metabolic diseases (Farhud and Aryan, 2018), cancer, obesity, metabolic syndrome, cardiovascular diseases and depression (Garaulet and Gomez-Abellanand, 2014). Shift workers, as an example, show the impact of chrono-disruption on health. It has been illustrated that shift workers have an increased risk of chronic diseases such as cancer, CVD and the metabolic syndrome (Wang *et al.*, 2011).

1.6. Meal pattern irregularity and health outcomes

Many different aspects of meal pattern have been studied both in free living studies, and in highly controlled laboratory studies using a constant routine (Wehrens *et al.*, 2017). For example, an intervention study by Halsey *et al.*, (2012) showed that only female habitual breakfast eaters were found to significantly consume more energy (P = 0·005) especially later in the day during the no-breakfast condition. This could be explained by compensating the energy deficit in the morning. However, this is not inconsistent with Betts et al., (2016) results which found that both lean and obese adults consumed less energy during the morning when remaining in the fasted state compared with when consuming a breakfast. Moreover, the consumption of breakfast on a daily basis in lean adults correlated with an increase in physical activity thermogenesis, with limited evidence of any dietary compensation later in the day nor any change in resting metabolism (Betts et al., 2014). After multivariable adjustment, those who skipped breakfast had significantly higher odds ratio for obesity than breakfast eaters (Otaki et al., 2017 and Guinter *et al*., 2020). Furthermore, skipping breakfast was significantly linked to a decrease in daily potassium (P <0.001), dietary fibre intakes (P = 0.001) and subjective physical activity which was assessed by International Physical Activity Questionnaire (P = 0.035) (Otaki et al., 2017). However, blood metabolites indicative of cardiovascular and metabolic health were not significantly influenced by eating or skipping breakfast and neither was there any difference in adipose tissue glucose uptake (Betts et al., 2014).

Intermittent fasting has recently been considered. This is an eating pattern which is defined as a pattern which cycles between periods of food avoidance and eating, including alternate day fasting (the day's food is consumed over a 6-h period, allowing for 18 hours of fasting). The intermittent fasting has shown improving weight loss and reducing insulin resistance as well as favourably shifting the levels of leptin and adiponectin (Vasim *et al*., 2022). It has been found that early and mid-day time restricted feeding were effective in improving insulin sensitivity. Furthermore, early time restricted feeding has a positive effect on metabolic health, including improved fasting glucose and blood glucose and reduced total body mass and adiposity, as well as has other benefits such as improved β cell responsiveness, enhanced oxidative stress, boosted appetite, ameliorated inflammation and increased gut microbial diversity (Xie *et al*., 2022). In addition, using the constant routine in a study by Wehrens *et al.* (2017), it has been reported that timed meals regulate circadian rhythm by synchronizing peripheral clocks and may play a role in circadian rhythm disorders in people such as shift workers.

Interactions between exercising regularly and the association with eating pattern is another area of interest which was investigated in different studies. It was showed on weekends that boys and girls with higher or daily physical activity were more likely to consume breakfast after adjustment for socioeconomic status, percentage of body fat and total energy intake (Corder *et al*., 2014). Another study concluded that adhering to regular exercise has an impact on regulating food intake and gaols of improving health and controlling body shape (Joo *et al*., 2019). This could be explained by the findings that exercising reduced daily energy intake (Elder *et al*., 2007) and affected appetite by decreasing the feelings of hunger (Jokisch *et al*., 2012). Dietary pattern before and after exercise has been also studied. Exercise performance was enhanced if carbohydrate was consumed before exercise (Neufer *et al*., 1987). In addition, consumption of protein during and/or immediately after exercise can facilitate the skeletal muscle adaptive response to each exercise session by stimulating muscle protein synthesis and inhibiting protein breakdown resulting in more effective muscle reconditioning (Aguirre *et al*., 2013).

In the studies mentioned above (with the exception of intermittent fasting), between-day consistency has been assumed. A more limited amount of research has been undertaken on the impact of between-day variability or irregularity. An irregular meal pattern, defined as a meal pattern with between day variation, has been considered as a possible new risk factor for obesity and its consequences, specifically cardiovascular disease and metabolic syndromes (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022; Pot *et al.*, 2014 and Pot *et al.*, 2016). Desptite showing promise as a potential simple health message contributing to the to the reduction of obesity and its associated co-morbidities, the literature relating to such between day variation and obesity and its health consequences has not been systematically reviewed. A number of studies have attempted to characterize irregularity of meal pattern using a variety of different methods and definitions. Furthermore, the definition of eating incidents varied, and in some instances, was not defined at all. There is thus a need to undertake as systematic review of the area, identify previous methods used to characterise irregularity, and if indicated design a novel method of characterisation. This might then be applied to an existing dataset to explore associations between meal pattern variability and health outcomes. Recently, at a macronutrient level, the association between meal pattern irregularity and metabolism of carbohydrate and lipids was identified in several intervention studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022). However, no studies have investigated the impact of meal pattern irregularity on protein balance. Garrow *et al*, (1981),

however, have demonstrated that other meal pattern modification (frequent vs. infrequent) acutely affect nitrogen balance suggesting the impact of a variable meal patterns deserves consideration.

Furthermore, only one study (Demo *et al.*, 2015) has considered the impact of meal pattern variability on health outcomes when providing a hypo-energetic diet. A hypoenergetic diet would be relevant to exploiting potential benefits of meal pattern regularity when prescribing weight loss diets. An intervention study thus is required to investigate the effect meal pattern variability, whilst providing a hypo-energetic diet on energy expenditure, TEF, fasting blood glucose, fasting lipid profiles, fasting gut hormones, interstitial glucose concentrations, subjective appetite and circadian rhythm.

The impact of a non-constant lifestyle has been shown to be detrimental to the health of nightshift workers because of dysregulation of circadian patterns. Although a lesser disruption than shift working, day to day variation in meal pattern might have a similarly detrimental effect on circadian patterns. This might explain recent intervention study results showing negative effects with a variable meal pattern (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022). The first step in exploring the potential role of circadian dysregulation in relation to meal pattern variability, would be to investigate the feasibility of measuring the peripheral or core body temperature in human feeding studies considering the impact of day-to-day variation.

The overarching purpose of this thesis was to explore the association between meal pattern irregularity and health outcomes. The following specific aims were set in order to address gaps that had been identified in the knowledge (Table 1.2):

1. To undertake a systematic review to identify and critique previous methods for characterizing between day meal pattern variability in studies investigating the association between irregularity of eating and obesity, and its associated health outcomes in order to explore the relationship between inter- daily meal pattern variability and obesity, and its associated health outcomes (Chapter 2).

2. To identify novel methods of characterising the irregularity of meal pattern to capture meal pattern variability using a broad set of meal pattern properties informed by the current literature and applied these methods to the National Diet and Nutrition Survey (NDNS) database of dietary intake information in order to undertake an exploratory investigation into associations between day-to-day variability in meal pattern and health outcomes (Chapter 4).

3. To investigate the effect of irregular meal pattern, compared with a regular meal pattern, on N balance in healthy normal-weight females over an acute, 7- day period and to explore the repeatability of previous findings suggesting differences in appetite ratings when adhering to a regular and irregular meal pattern (Chapter 5).

4. To investigate the effect of providing a hypo-energetic diet to participants with normal/overweight based on regular or irregular meal pattern on energy expenditure, TEF, fasting blood glucose, fasting lipid profiles, fasting gut hormones, interstitial glucose concentrations, subjective appetite and circadian rhythm (Chapter 6). NB: this aim was subsequently modified due to restrictions posed by Covid 19 to test the feasibility of the protocol required to explore the proposed question. Thus, the fourth aim was to test the feasibility of undertaking an intervention study aimed to compare the impact of 14-day hypo energetic diet with regular and irregular eating (all food provided) on the TEF (the primary outcome measure), carbohydrate metabolism, lipid concentrations and subjective appetite while also measuring the diurnal rhythm of the core body temperature.

Table 1.2: The gap in knowledge and thesis aims

Gap in Knowledge	Thesis aims
A systematic review of methods for characterising meal pattern and exploring associations between meal pattern and health outcomes.	To undertake a systematic review to identify and critique previous methods for characterizing between day meal pattern variability in studies investigating the association between irregularity of eating and obesity, and its associated health outcomes in order to explore the relationship between inter- daily meal pattern variability and obesity, and its associated health outcomes (Chapter 2)
	To identify novel methods of characterising the irregularity of meal pattern to capture meal pattern variability using a broad set of meal pattern properties informed by the current literature and applied these methods to the National Diet and Nutrition Survey (NDNS) database of dietary intake information in order to undertake an exploratory investigation into associations between day-to-day variability in meal pattern and health outcomes (Chapter 4).
All studies in the literature investigated the effect of irregularity on carbohydrate and lipid metabolism. No previous intervention studies explore the effect of irregularity on protein or nitrogen balance	To investigate the effect of irregular meal pattern, compared with a regular meal pattern, on N balance in healthy normal-weight females over an acute, 7-day period and to explore the repeatability of previous findings suggesting differences in appetite ratings when adhering to a regular and irregular meal pattern (Chapter 5).
All previous intervention studies for irregularity used an iso-energetic diet and none of them explored the interaction between meal pattern irregularity and circadian rhythm.	To investigate the effect of providing a hypo-energetic diet to participants with normal/overweight based on regular or irregular meal pattern on energy expenditure, TEF, fasting blood glucose, fasting lipid profiles, fasting gut hormones, interstitial glucose concentrations, subjective appetite and to consider the interaction with circadian rhythm (Chapter 6). NB: due to Covid 19 this study was converted to a feasibility study.

CHAPTER 2: A SYSTEMATIC REVIEW OF ASSOCIATIONS BETWEEN DAY-TO-DAY VARIABILITY IN MEAL PATTERN AND BODY WEIGHT, COMPONENT OF THE METABOLIC SYNDROME AND COGNITIVE HEALTH

2.1 Introduction

The ongoing increase in overweight and obesity, resulting from sustained positive energy balance, has occurred worldwide over several decades. By 2016, 39% of the world population were living with overweight; and 13% with obesity (WHO, 2021). The excess weight, predominantly fat mass, is most detrimental to health when in the abdominal area (Paley, 2018) and is associated with insulin insensitivity, impaired glucose tolerance, dyslipidemia and hypertension. These conditions, collectively labelled the 'metabolic syndrome' (Engin, 2017), are associated with increased cerebro and cardiovascular diseases and Type 2 diabetes (Han and Lean (2016). Furthermore obesity, has been associated with an increased risk of cancers (Bianchini, 2002), poorer cognition and mental illness (Nguyen *et al.*, 2014; Cook *et al.*, 2017 and Van Vuuren *et al.*, 2019).

Traditionally, research demonstrating associations between diet and health has focused on the chemical composition of the diet, for example, the amount of carbohydrate consumed in 24 hours (McCarthy *et al.*, 2006). However, dietary intake can be considered in terms of the profile of an individual's consumption across the day. The time, type and amount of food, or drink, consumed are properties of this profile, described either using generic terms such as eating incident pattern or more commonly, meal pattern. Such measures as 'meal frequency', 'time of eating', or 'time when 50% of a dietary component has been consumed' have been used to describe properties of the 'meal pattern'. Typically, these measures have been expressed as the mean of several days, (eg. mean daily meal frequency) and the relation with health has been found to be equivocal (Ma *et al.*, 2003; Murakami and Livingstone, 2015). However, using a daily mean masks the potential impact of the between day variability in meal pattern; a high degree of between day variability being considered 'chaotic' or 'irregular' eating.

Recently, the potential for an individual to have an 'irregular' meal pattern has increased. This is due, in part, to greater availability of ready prepared meals, facilitating independence from established family meal-times; greater availability of 'fast-food' outside the home; and greater variation in work shift patterns. Concurrently, obesity and associated negative health consequences have increased (Dixon, 2010; Garaulet and Madrid, 2010; Hruby *et al.*, 2016). Recent observational and interventional studies, exploring associations between 'chaotic' consumption patterns and health, have identified that this may be an underexplored, novel risk factor for obesity and its consequences, namely components of the metabolic syndrome and risk of cardiovascular disease (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*,

2005; Alhussain *et al.*, 2014; Pot *et al.*, 2016 and Pot *et al.*, 2016). Energy intake regulation (Sjoberg *et al.*, 2003; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016), or energy expenditure, for example the thermogenic response to food (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016), may be affected, potentially independently of the nutritional composition of the diet (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016). However, results are inconsistent (Merten *et al.*, 2009; Shin *et al.*, 2009), potentially because of differences in the measures used to describe consumption patterns, and how measures of between day variability in pattern are derived.

The current systematic review was undertaken to explore the hypothesis that between day variability in meal pattern is associated with the risk of obesity and associated negative health consequences (including components of the metabolic syndrome, cognitive function and mental well-being). The aims were to 1. identify and critique previous methods for characterizing between day meal pattern variability in studies investigating the association between irregularity of eating and obesity, and its associated health outcomes in order to 2. explore the relationship between inter- daily meal pattern variability and obesity, and its associated health outcomes.

2.2 Method

The systematic review was registered with the International Prospective Register of Systematic Reviews PROSPERO (registration number CRD42019129890) and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the PRISMA 2009 checklist (Liberati *et al.*, 2009).

2.1.1. Search strategy

A systematic literature search was carried out for studies published at any time up to the end of August 2021 in which between day meal pattern variability had been measured and related to energy intake, energy expenditure, obesity, obesity related components of the metabolic syndrome, cognitive function and mental well-being in adults and children. The electronic databases used for the search were PUBMED including MEDLINE, Web of Science Core Collection, Cochrane library, EMBASE and Ovid platform including AMED (Allied and Complementary Medicine). Google scholar was accessed too.

The search comprised of the following key words used individually and in combination:

‘Regular’, ‘irregular’, ‘chaotic’, ‘meal pattern’, ‘eating’, ‘weight’, ‘obesity’, ‘BMI’, ‘thermogenesis’, ‘energy expenditure’, ‘appetite’, ‘energy intake’, ‘glucose’, ‘insulin’, ‘lipid profile’, ‘metabolic syndrome’, ‘psychological factor’, ‘cognition’, ‘cognitive function’.

The search was limited to articles written in English and Arabic.

2.1.2. Screening process

A. All study titles were screened (MA) and those clearly not eligible for inclusion (e.g. animal studies) were excluded. Abstracts were retrieved and reviewed if there was any ambiguity from the title (see B). B. Selected abstracts were then assessed, and where any ambiguity remained, following discussion with the co-authors (MAT and IAM), full texts were retrieved and reviewed (see C). C. Eligibility was then confirmed by two authors (MA and MAT). D. Reference lists of the included studies were checked for further potentially suitable articles and steps B- D were then followed again. The number of articles eliminated, and the justification was documented (Figure 1).

2.1.3. PICOS Criteria

2.1.3.1. Types of participants

Participants were adults or children in whom variability in between day variability in meal pattern had been measured. Studies on animals, pregnant or lactating women were excluded.

2.1.3.2. Types of Interventions

Study exposure variable was daily variability in at least one property of meal pattern.

Intervention studies that only examined eating a certain meal regularly vs. regularly skipping a specific meal (eg. breakfast) were excluded because although the two patterns were different, neither was variable between days.

2.1.3.3. Types of comparators

Studies with any comparator, or none were considered.

2.1.3.4. Types of outcomes

Study outcomes were indicators of obesity, energy intake and expenditure (contributors to obesity), or negative health outcomes that had been identified as associated with obesity prior to undertaking the review. These were the components of metabolic syndrome, cognition,

mental health and well-being. Cancer as a specific outcome was excluded, however this or any other outcome that was not identified *a priori* was noted in the results section.

2.1.3.5. Types of studies

Primary (interventional and observational) studies or observational studies in which data had undergone secondary analysis in relation to between day meal pattern variability, despite this not being the initial focus, were considered. Case studies, letters, reviews and conference abstracts were excluded.

2.1.4. Data extraction

Key information relating to participant characteristics, study design, sample size, age range, definition of an eating incident (eg. meal, snack or drink), method of characterizing the between day variability in meal pattern, intervention diet, length of intervention, outcomes, control of confounding factors, length of follow up and study main findings were extracted by one author (MA). A second author (MAT) then reviewed all the papers fully to confirm agreement with the data extraction made by MA. The format used for data extraction is shown in Tables 2.1 (observational studies) and 2.2 (intervention studies).

2.1.5. Quality assessment

A quality assessment of each included paper was then undertaken by one author (MA) to consider factors such as validity, precision and the risk of bias. The conclusions drawn were checked for agreement by the second author (MAT).

- The National Heart, Lung and Blood Institute (NIH) tool was applied to cross-sectional studies (NIH, 2014).

- The NEWCASTLE – OTTAWA scale (Wells *et al.*, 2009) was applied to case control and cohort studies.

- The Cochrane Collaboration tool was applied to randomized cross-over studies (Ding *et al.*, 2015).

- The JADAD scale was applied to randomized controlled studies (Jadad *et al.*, 1996).

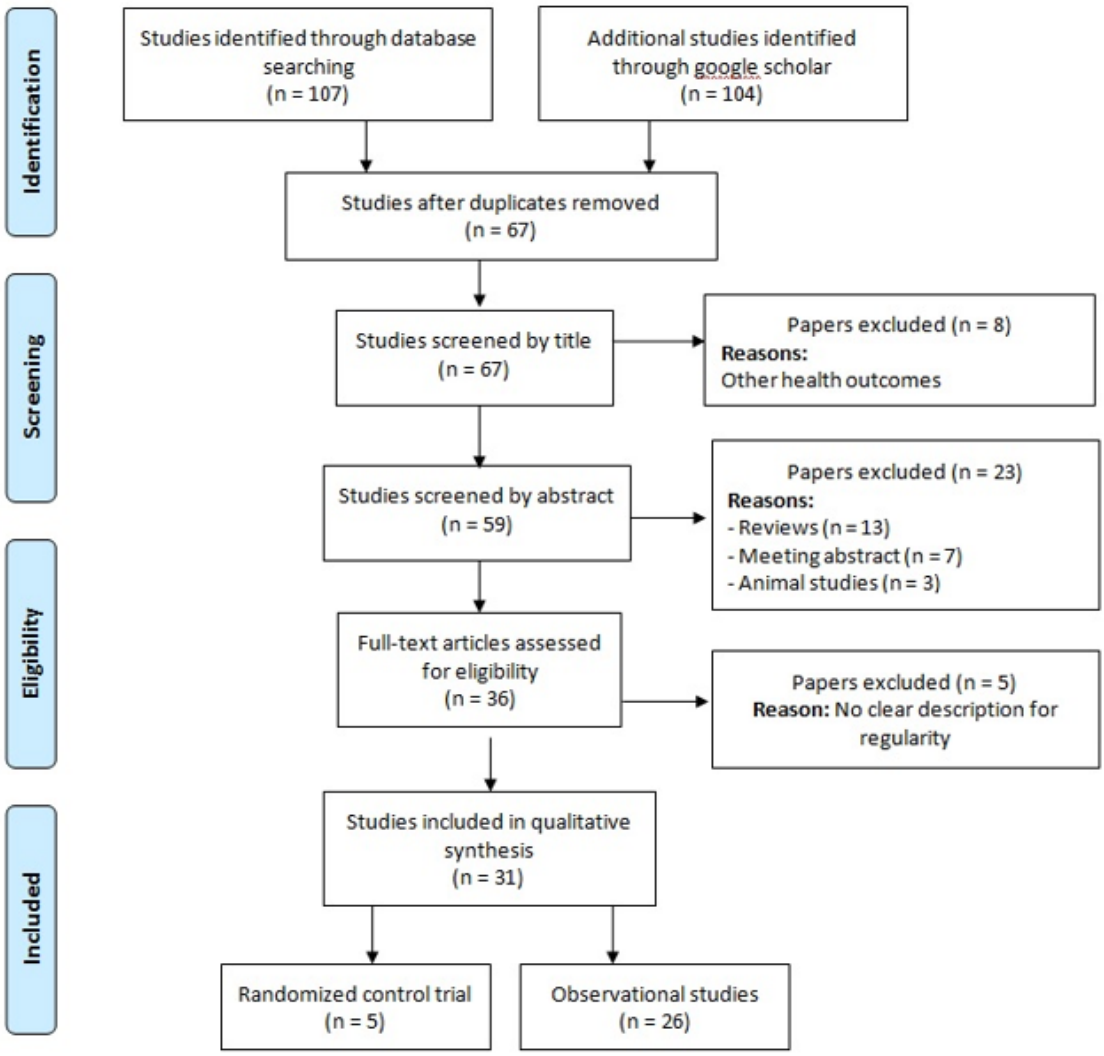
2.3. Results

2.3.1 Study selection

Figure 2.1 summarises the search strategy and flow of articles. Once duplicates had been removed, 69 articles were screened by title, producing 59 articles for abstract screening. This identified 36 articles for full text screening. 31 papers were eligible for inclusion in the review which included two papers which reported on different aspects of one study (Farshchi *et al.*, 2004a and Farshchi *et al.*, 2004b) and three papers reporting the same results from one study (Kagamimori et., 1999; Takahashi *et al.*, 1999; Sugimori *et al.*, 1999). Subsequent numbers relate to the papers as opposed to the number of distinct studies.

Studies were not suitable for pooling of their results, therefore a narrative analysis was presented, as opposed to a meta- analysis.

Figure 2.1 Flow chart for search strategy



2.3.2. Study characteristics

All papers were published in English but were conducted in: the UK (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Pot *et al.*, 2014; Pot *et al.*, 2016; Zimmerman *et al.*, 2018), the USA (Merten *et al.*, 2009; Mekary *et al.*, 2013; Demos *et al.*, 2015; Raynor *et al.*, 2008), Sweden (Sjoberg *et al.*, 2003; Sierra-Johnson *et al.*, 2008 ; Wennberg *et al.*, 2016), KSA (Al-Rethaiaa *et al.*, 2010), Jordan (Hamaideh *et al.*, 2010), Iran (Shafiee *et al.*, 2013; Saneei *et al.*, 2015), China (Ko *et al.*, 2007), Japan (Kagamimori et., 1999; Takahashi *et al.*, 1999; Sugimori *et al.*, 1999; Ibe *et al.*, 2016), Korea (Kim *et al.*, 2003; Shin *et al.*, 2009), Finland (Lehto *et al.*, 2011), Norway (Overby *et al.*, 2013; Stea and Torstveit, 2014; Stea *et al*, 2015), Germany (Muff *et al.*, 2011) and Portugal (Rodrigues *et al.*, 2017). Reference was made to the ethnicity of the participants in three studies (Pot *et al.*, 2014; Merten *et al.*, 2009; Wennberg *et al.*, 2016).

Twenty- six observational study reports were identified, consisting of three secondary data analysis (Pot *et al.*, 2014; Pot *et al.*, 2016; Zimmerman *et al.*, 2018), 19 cross sectional study reports (Kagamimori *et al.*, 1999; Sjoberg *et al.* 2003; Kim *et al.*, 2003, Ko *et al.*, 2007; Sierra-Johnson *et al.*, 2008 ; Merten *et al.*, 2009; Shin *et al.*, 2009; Al-Rethaiaa *et al.*, 2010; Hamaideh *et al.*, 2010; Muff *et al.*, 2011; Lehto *et al.*, 2011; Shafiee *et al.*, 2013; Overby *et al.*, 2013; Stea and Torstveit, 2014; Pot *et al.*, 2014; Saneei *et al.*, 2015; Wennberg *et al.*, 2016; Rodrigues *et al.*, 2017; Zimmerman *et al.*, 2018), five cohort study reports in which follow up was reported (Mekary *et al.*, 2013; Demos *et al.*, 2015; Stea *et al.*, 2015; Pot *et al.*, 2016; Ibe *et al.*, 2016) and two case- control study reports (Takahashi *et al.*, 1999; Sugimori *et al.*, 1999). Five randomized controlled trial reports (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Demos *et al.*, 2015) were identified.

2.3.3. Participant characteristics

Observational studies had a median of 1953 participants (range 351-46289) and with the exception of five studies (Sierra-Johnson *et al.*, 2008; Shin *et al.*, 2009; Pot *et al.*, 2014; Pot *et al.*, 2016; Wennberg *et al.*, 2016) in which the participants were recruited from the general population without any exclusions on health grounds. Of those that reported sex (n=26), one study (Mekary *et al.*, 2013) included female participants only, three studies (Shin *et al.*, 2009; Al-Rethaiaa *et al.*, 2010; Wennberg *et al.*, 2016) included only males, and 22 studies included males and females (Kagamimori et., 1999; Takahashi *et al.*, 1999; Sugimori *et al.*, 1999; Sjoberg *et al.*, 2003; Kim *et al.*, 2003; Ko *et al.*, 2007; Sierra-Johnson *et al.*, 2008 ; Raynor *et al.*, 2008; Merten *et al.*, 2009; Hamaideh *et al.*, 2010; Lehto *et al.*, 2011; Muff *et al.*, 2011;

Shafiee *et al.*, 2013; Overby *et al.*, 2013; Stea and Torstveit, 2014; Pot *et al.*, 2014; Saneei *et al.*, 2015; Stea *et al*, 2015; Pot *et al.*, 2016; Ibe *et al.*, 2016; Rodrigues *et al.*, 2017; Zimmerman *et al.*, 2018). Age was reported in all studies, where a mean was provided, it ranged from 14.6 – 58.7 years; in those studies which provided a range, it was from 3 – 60 years. Thirteen studies (Kagamimori et., 1999; Takahashi *et al.*, 1999; Sugimori *et al.*, 1999; Sjoberg *et al.*, 2003; Kim *et al*., 2003; Merten *et al*., 2009; Hamaideh *et al*., 2010; Lehto *et al*., 2011; Overby *et al*., 2013; Shafiee *et al*., 2013; Stea and Torstveit, 2014; Stea *et al*., 2015; Rodrigues *et al*., 2017) included only children, 12 studies (Sierra-Johnson *et al*., 2008; Shin *et al*., 2009; Al-Rethaiaa *et al*., 2010; Muff *et al*., 2011; Pot *et al.*, 2014; Saneei *et al*., 2015; Demos *et al*., 2015; Pot *et al*., 2016; Wennberg *et al*., 2016; Ibe *et al*., 2016; Ko *et al*., 2017; Zimmerman *et al*., 2018) included only adults, and one study included adolescents aged 16 and the follow up was in adulthoods aged 27 (Mekary *et al*., 2013).

Of those that reported BMI (n=15) the range was 17.5 to ≥30 kg/m^2. One study recruited participants who were overweight or obese (Wennberg *et al*., 2016) and the remainder did not have weight or any associated measure as an inclusion criterion.

The number of participants in the intervention studies ranged from 9-25 and all recruited healthy participants (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Demos *et al.*, 2015; Alhussain *et al.*, 2016). Of those that reported sex, four studies included female participants only, and one study included males and females (Demos *et al.*, 2015). The age ranged between 18 and 65 years. Two studies recruited participants who were overweight or obese (Farshchi *et al.*, 2005; Demos *et al.*, 2015). The mean (SD) baseline body mass index (BMI) ranged from 22.4 (2.4) kg/m^2 to 37.1 (4.8) kg/m^2. In total, the 30 participants included in intervention studies were required to follow a regular dietary intake pattern which was compared with an irregular dietary intake pattern (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) and 12 participants followed only regular eating due to a parallel design (Demos *et al.*, 2015). The duration of interventions ranged from 2 to 4 weeks.

2.3.4. Methods of characterizing meal pattern variability

2.3.4.1. Observational studies (Table 2.1)

Detailed consideration of methods:

- In two papers (Pot *et al.* 2014; Pot *et al.* 2016) secondary analysis was undertaken, using energy intake data recorded by participants in a diary in which they selected from predefined meal headings. The headings included 'breakfast', 'lunch', 'evening meal' and a range of other points in the day, including 'first thing' and 'late evening'. The latter were then collapsed into one meal category, 'between meals'. Energy intake variance was used as a proxy for the irregularity of each meal category, derived using the following steps: 1. The mean energy intake for the meal category over the 5 days was calculated. 2. For each day, the absolute difference between the individual energy intake on that day, for that meal category, and the mean energy intake, over the 5 days, for that meal category was calculated. 3. This was then divided by the mean energy intake, over the 5 days, for that meal category; and multiplied by 100. 4. The mean, over the 5 days of the resulting values, was calculated; giving the irregularity score for that meal category. A low score indicated a more consistent meal pattern for energy intake for that meal. This process was repeated for each meal. To derive an irregularity score for total daily energy intake, the process was repeated, using total daily energy intake on each of the 5 days.

- Secondary analysis of data using 30-minute time slots was undertaken by Zimmerman *et al.* (2018). Irregularity of eating was identified using 30-minute times slots when food might be consumed over a typical 7-day period, either by participant report (study 1) or by using timings reported in 7-day food diaries obtained from National Diet and Nutrition Survey (NDNS) year 2000 (study 2). An adjustment was then made for daily meal frequency (obtained by participant report, or from the food diary) by dividing this into the number of 30-minute slots when food might be eaten. A higher value reflected more chaotic eating. A similar process was conducted for snacks. In study 2, the time between adjacent eating

incidents was also calculated, and the degree of variability in this measure, across the 7 days was calculated.

- Frequency of consumption of a specific meal (eg, breakfast) in a week was used in eight cross-sectional studies (Sjoberg *et al.*, 2003; Raynor *et al.*, 2008; Marten *et al.*, 2009; Lehto *et al.*, 2010; Overby *et al.*, 2013; Shafiee *et al.*, 2013; Stea and Torstveit, 2014; Saneei *et al.*, 2016) and two cohort studies (Mekary *et al.*, 2013; Stea *et al.*, 2014); meal pattern was classified as regular if the frequency of each meal or breakfast is 7 days/week (Sjoberg *et al.*, 2003; Raynor *et al.*, 2008; Mekary *et al.*, 2013; Saneei *et al.*, 2016; Stea and Torstveit, 2014; Stea *et al.*, 2014;, 6 or more days/week (Overby *et al.*, 2013; Shafiee *et al.*, 2013), 5 days/weekdays (Lehto *et al.*, 2010) or at least 4 days/week (Merten *et al.*, 2009).

- Simple questions about the regularity of eating main meals (breakfast, lunch and dinner) or specific meals were asked in other studies with potential answers available being 'yes' or 'no' (Ko *et al.*, 2007; Hamaideh *et al.* 2010; Ibe *et al.*, 2016; Rodrigues *et al.*, 2017), adverbs of frequency (always, usually, often, sometimes and never) (Sierra-Johnson *et al.*, 2008 and Shin *et al.*, 2009), and 'regular' or 'irregular' (Sugimori *et al.*, 1999; Kim *et al.*, 2003; Al-Rethaiaa *et al.*, 2010; Muff *et al.*, 2011; Wennberg *et al.*, 2015) with no definition of irregularity.

- A simple question was included about whether mealtimes 'in general' were regular or irregular (Shin *et al,* 2009 and Takahashi *et al.*, 1999).

- Meal or snack intake was defined as irregular when the answer given was that the intake time was 'not fixed' (Kagamimori *et al.*, 1998)[j].

2.3.4.2. Intervention studies (Table 2.2)

- The participants habitual diet (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005) or food that was provided by the experimenter (Alhussain *et al.*, 2016), was consumed with either the same meal frequency everyday (6 meals/day) and at the same time points (regular) or with a varying meal frequency (3–9 meals/day) (irregular). With the irregular pattern the number of times of

eating was increased by adding additional points around the regular profile or reduced by combining times of eating and preserving one of the previous times of eating. Thus, the same total daily energy was distributed across the number of meals provided, with the same foods being consumed on each arm of the experiment. Both time and number of meals thus varied in the irregular meal pattern. However, the start and finish time was the same on both meal patterns, with the exception of one day (3 meals per day) when the final meal was at 1800h instead of 2100h. This occurred twice in the two-week intervention period.

- A pilot RCT by Demos *et al.* (2015), explored the impact of meal pattern variability on weight loss by comparing two groups. During a 4-week period of education, participants in the 'regular' intervention group were given a schedule for their eating episodes for each day. 'On-schedule' eating was defined as consuming a meal or snack within 15 min of the prescribed time and eating at all other time points was thus considered 'off schedule'. Those in the control group attended educational sessions on the prevalence and health consequences of obesity, the importance of fruit and vegetable intake, and common myths about diet and exercise. However, they were given no information about when to eat. An 18-week weight loss period followed, in which the regular diet group were required to follow the previously taught eating schedule.

2.3.5. Association between meal pattern variability, obesity and associated health consequences (Tables 2.1 and 2.2)

The outcomes considered were obesity or/ and weight (Kagamimori et., 1999; Takahashi *et al.*, 1999; Sugimori *et al.*, 1999; Sjoberg *et al.*, 2003; Ko *et al.*, 2007; Raynor *et al.*, 2008; Sierra-Johnson *et al.*, 2008 ; Merten *et al.*, 2009; Al-Rethaiaa *et al.*, 2010; Hamaideh *et al.*, 2010; Muff *et al.*, 2011; Lehto *et al.*, 2011; Stea *et al.*, 2015; Saneei *et al.*, 2015; Ibe *et al.*, 2016; Rodrigues *et al.*, 2017; Zimmerman *et al.*, 2018), the metabolic syndrome or any of its components (Sierra-Johnson *et al.*, 2008 ; Shin *et al.*, 2009; Shafiee *et al.*, 2013; Pot *et al.* 2014; Pot *et al.* 2016; Wennberg *et al.*, 2016) including type 2 diabetes (Muff *et al.*, 2011; Mekary *et al.*, 2013). Some studies assessed energy intake, energy expenditure, thermogenesis and appetite (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*,

2016), and cognitive function Kim *et al.*, 2003; Overby *et al.*, 2013; Stea and Torstveit, 2014).

2.3.5.1. Weight, BMI and obesity

Three cross-sectional studies (Sierra-Johnson *et al.*, 2008; Al-Rethaiaa *et al.*, 2010; Saneei *et al.*, 2015) aimed to investigate eating habits and obesity (defined by BMI) in adult participants. Subjects with 'irregular meal pattern' had a higher odds ratio for obesity (OR 1.29; 95 % CI 1.10–1.50), and abdominal obesity (OR 1.23: 95 % CI 1.09–1.38) (Saneei *et al.*, 2015), and higher mean BMI (27.4 kg/m^2 vs. 26.4 kg/m^2, P<0.0001) (Sierra-Johnson *et al.*, 2008) compared with those with a 'regular meal pattern 'in contrast to others who found no significant difference (Al-Rethaiaa *et al.*, 2010).
Irregular eating showed a statistically significant relationship (P<0.05) with obesity in men (Ibe *et al.*, 2016), and in both genders combined (Ko *et al.*, 2007) (OR 2.96, CI 1.22–7.17; OR: 1.20, 95% CI: 1.04, 1.39, respectively). Consuming regular breakfast (7 days/week) was associated with lower BMI (35.6±5.7 kg/m^2, P<0.0001) compared with consuming breakfast 3–6 days/week (BMI 36.7 ±6.3 kg/m^2) and 2 days/week (BMI 37.3 ±6.1 kg/m^2) (Raynor *et al.*, 2008).

Three RCT (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) examined the impact of irregular meal frequency and time of eating on weight in healthy women. There were no significant differences in participants' weight between the interventions. Moreover, the same finding was revealed in a pilot RCT by Demos *et al.* (2015) the intervention and control group after 4 weeks (p= 0.28) and 18 weeks (P=0.18).

Ten studies were conducted in children to explore the association between regular meal pattern and obesity. High BMI was significantly associated with irregular meal intakes (OR 1.03, 95% CI 1.01–1.06) (Muff *et al.*, 2011), irregularity of breakfast (P<0.001) and overall meal pattern (breakfast, lunch and dinner; P=0.002) (Lehto *et al.*, 2011) and irregular breakfast intake (P=0.006) in boys but not in girls (Sjoberg *et al.*, 2003). Adolescents who had a regular breakfast were at lower risk for overweight/obesity (OR=0.59; 95% Cl: 0.52 to 0.68; P<0.005) (Merten *et al.*, 2009) whereas skipping breakfast was associated with increased BMI (P ≤ 0.02) (Rodrigues *et al*., 2017). In contrast, others found eating breakfast

regularly was positively correlated with overweight and obesity (P≤0.001) (Hamaideh *et al.*, 2010). However, in two Japanese studies it was reported that there were no significant differences in irregular meal intake, and irregular mealtime, between obese and non-obese/control children (Kagamimori *et al*., 1999; Takahashi *et al.*, 1999). Children with obesity, however, had a significantly higher number of irregular snacks (P<0.001) compared with children who were healthy weight. In addition, it was noted in a study by Sugimori *et al.* (1999) that no significant relationship was seen between irregularity of meals and obesity, however, after adjusting for multiple confounders, irregular intake of snacks had a significant relationship with obesity in childhood. In addition, children who ate regular meals in 4th grade but not in 7th grade had higher odds (OR: 3.1; 95 % CI 1.1, 9.0) of being overweight in 7th grade compared to those who ate regular meals in 4th and 7th grade after adjusting for gender, maternal education and physical activity (Stea *et al.*, 2015).

2.3.5.2. Energy intake, appetite and gut hormones

Regular breakfast consumption is related to a 6-7% higher energy intake from breakfast (range given in the paper) and an 8% lower EI from in-between meals compared with irregular breakfast consumption. There was a difference in EI/BMR between boys with regular and irregular intakes (1.76 vs. 1.63, P=0.042), but there was not a significant difference in girls, (1.50 vs. 1.43; P=0.080) (Sjoberg *et al.*, 2003). On the other hand, differences were not significant following regular and irregular eating for a 14-d period in energy intake after a test meal or appetite before and after a test meal (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005). However, lower hunger and higher fullness ratings were seen pre-meal and post-meal during the regular intervention while subjects were free living for meals of the same energy content (Alhussain *et al.*, 2016). In addition, there was no difference in gut hormone response between the two interventions (Alhussain *et al.*, 2016).

2.3.5.3. Energy expenditure

Following irregular and regular eating for two weeks, differences in RMR were not significant (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) in healthy women. However, thermic effect of food (TEF) over the 3 h after a test meal was

significantly lower after the irregular meal intervention (0.4 kJ/min) (P=0.003) than after regular meal pattern (0.7 kJ/min) (Farshchi *et al.*, 2004b) and regular eating resulted in greater postprandial thermogenesis (120 KJ in 3h) (P<0.01) than irregular eating (90 KJ in 3h) (Farshchi *et al.*, 2005) in common with a further study when TEF after the regular intervention was 11.1±15.8 kcal (P<0.05) higher than after the irregular intervention (Alhussain *et al.*, 2016).

2.3.5.4. Metabolic syndrome

Three cross-sectional studies and three prospective cohort studies examined the relationship between irregularity of meal pattern and the metabolic syndrome including high fasting blood glucose, high blood pressure, low HDL-cholesterol, triglycerides and large waist circumference (Sierra-Johnson *et al.*, 2008; Shafiee *et al.*, 2013; Pot *et al.* 2014; Pot *et al.* 2016; Wennberg *et al.*, 2016). All these studies reported that irregular eating is associated with an increased risk of the metabolic syndrome whilst one cross-sectional study assessed the impact of irregularity of mealtime on metabolic syndrome and found no significant correlation between regular mealtime and risk of metabolic syndrome (Shin *et al.*, 2009).

The correlation between irregularity of dietary intake and type 2 diabetes was explored in two studies, firstly in relation to all meals (Muff *et al.*, 2011) and secondly in relation to breakfast (Mekary *et al.*, 3013). It was reported that the risk of type 2 diabetes was higher in individuals who had irregular breakfasts (0–6 times/week) than those who had regular daily breakfast consumption (RR: 1.28; 95% CI: 1.14,1.44). Eating meals regularly was also inversely related to insulin resistance (OR, 0.68 (95% CI, 0.48–0.97) (Sierra-Johnson *et al.*, 2008).

Three randomized cross-over studies investigated the effect of irregular meal frequency pattern on circulating insulin, glucose, lipids and uric acid in nine healthy lean women (Farshchi *et al.*, 2004a), ten healthy obese women (Farshchi *et al.*, 2005) and 11 normal-weight women (Alhussain *et al.*, 2016). The first study in lean women (BMI 23.7 (SD 7.4) kg/m^2) found that regularity of meal frequency did not impact on fasting circulating glucose and insulin but peak insulin and area under the curve responses to a test meal were higher

after the irregular meal pattern (0.91) compared with the regular meal pattern (0.77) (P=0.022). In the second study on obese women (BMI 37.1 (SD 4.8) kg/m^2), findings were similar to those seen in lean women, with no effect on fasting glucose and insulin, but peak insulin responses to a test meal were lower after the regular meal intervention than after the irregular meal intervention (P=0.005). The third study showed no significant differences in continuous glucose monitoring between 2 intervention periods in the 24-h mean, maximum, minimum, and incremental area under the curve (iAUC) for glucose concentrations. Nevertheless, meal pattern regularity was associated with a lower iAUC for glucose (P<0.05) after intake of a test drink over 3 h and, for some identical meals during the free-living period when interstitial glucose was monitored continuously.

No significant differences between interventions were found in fasting serum total cholesterol, LDL cholesterol, HDL cholesterol, or serum triglycerides (Alhussain *et al.*,2016). Nevertheless, the irregular meal pattern intervention was also associated with higher fasting total cholesterol (P<0.01) and LDL-cholesterol (P<0.05) (Farshchi *et al.*, 2004a) and regular eating was associated with lower fasting total (P<0.01) and LDL (P<0.02) cholesterol (Farshchi *et al.*, 2005). Moreover, these studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2005) showed no significant changes in the concentrations of plasma uric acid.

2.3.5.5. Cognitive function, psychological factors and wellbeing and academic performance

Three cross-sectional studies investigated the correlation between meal pattern regularity and academic performance (Kim *et al.*, 2003; Stea and Torstveit, 2014) and learning difficulties (Overby *et al.*, 2013) in adolescents. In academic performance, higher grade point average (GPA) was significantly associated with regularity of breakfast in boys Grade 5, 8, 11 and girls at Grade 8; lunch for boys at grade 5, 8 and 11 and girls for Grade 5; and at dinner for boys Grade 11 and girls Grade 8 and 11 (Coefficients ranging from 0.110-0.314 and p values from 0.000-0.043) (Kim *et al.*, 2003). Higher academic achievement in both girls and boys was positively correlated with a regularly consuming breakfast (AOR: 3.30 (2.45-4.45) and AOR: 1.76 (1.32-2.34), respectively), regularly consuming lunch (AOR: 1.44 (1.08-1.93) and AOR: 1.43 (1.09-1.89), respectively),

regularly consuming dinner (AOR: 1.44 (1.16-1.79)) and regular meal pattern in general (AOR: 1.50 (1.10–2.03)) (Stea and Torstveit, 2014). Regular consumption of breakfast was significantly related to decreased odds of both writing and reading difficulties (p=0.01) and mathematical difficulties (p≤0.001) (Overby *et al.*, 2013). In addition, consumption of lunch, dinner and supper regularly were correlated with decreased odds of mathematical difficulties (p≤0.001, p≤0.001 and p=007) respectively (Overby *et al.*, 2013).

Other health outcomes which were not identified *a priori* but became apparent whilst reviewing the selected papers were noted. A higher adherence to regular eating was associated with lower weekly binge frequency for individuals with recurrent binge eating who were undergoing cognitive behavioural guided self-help (Zendegui *et al.*, 2014). Increased regular consumption of evening meals over the course of treatment of Bulimia Nervosa was related to a significant decrease in the rate of binge eating (Ellison *et al.*, 2016). Irregular meal pattern was significantly related to greater odds of chronic uninvestigated dyspepsia (OR 1·42, 95% CI 1·12, 1·78), after controlling for potential confounders, compared with a regular meal pattern (Keshteli *et al.*, 2015). A case control study demonstrated that a poor lifestyle and poor dietary habits, such as physical inactivity, sleep disturbance, and irregular eating, increase the risk of irritable bowel syndrome (Guo *et al.*, 2015). A multinomial logistic regression analysis revealed that older age, current smoking, irregular meal pattern, lack of physical exercise, poor mental health, and chronic diseases or multi-morbidity were positively correlated with short sleep (Wang *et al.*, 2017). Breakfast regularity was associated with lifestyle related factors which were socioeconomic index, smoking and perception of body weight; irregularity of lunch and dinner only were associated with smoking and gender (Sjoberg *et al.*, 2003). Irregular eaters had significantly greater γ-glutamyl transferase concentrations (OR, 0.52 (95% CI, 0.33–83) than regular eaters (Sierra-Johnson *et al.*, 2008). Girls with an irregular breakfast and lunch intake were shorter and had earlier menarche (Sjoberg *et al.*, 2003).

Table 2.1: Characteristics and the results of observational studies

Study	Study design	Population and sample size	Duration	Definition of meal	Exposure	Method of characterise meal pattern regularity	Measurement tool for collecting intake regularity data	Health outcomes including energy intake and appetite	Adjustment for confounders	Main findings
Zimmerman *et al.* (2018) in UK	Secondary analysis of cross-sectional data Study 1: volunteer participants Study 2: NDNS data.	Study 1: n=115, (63 women, 51 men and 1 transgender) Mean age=32.9 years (±10.9) and mean BMI=28.4 kg/m^2 (±6.9) Study 2: n=1175, (557 men and 618 women) Mean age=41.9 (±12.0) and mean BMI=26.3 kg/m^2 (±5.1)	N/A	N/A	Irregularity in timing of eating	Study 1 and Study 2: Chaotic eating defined as the period of time over which the participant reported that they might consume food (estimated using 30-minute slots) (divided by the daily frequency of eating. Study 2: daily variation in between meal interval was considered. Drinks on their own were not considered intake occasions.	Study 1: self-administered questionnaire. Study 2: 7-day diaries from the NDNS.	BMI	Age, gender and dietary habits	In study 1 and 2, the variability in the timing of meals (β=−0.07, p=0.73), (β=0.16, p=0.27) or snacks (β=−0.10, p=0.75), (β=0.15, p=0.12) respectively is not associated with BMI

Rodrigues *et al.* (2017) in Portugal	Cross-sectional study (PPSOC)	10,258 school children (51% girls) aged 6–9 years	-	Breakfast	Breakfast consuming	Parents asked, 'Does your child eat breakfast regularly?' Answer was 'yes' or 'no'	Questionnaire	BMI and waist conferences	Sex, age, education level of the parents	Skipping breakfast was associated with more children having excess weight (p<0.01). A meal pattern identified by PCA which included ≤3 daily meals and skipping breakfast was associated with increased BMI z-score (β=0.04, (95% CI: 0.01, 0.07), P=0.03) and WC (β=0.20, (95% CI: 0.03, 0.38), P=0.03)
Pot *et al.* (2016) in UK	Secondary analysis of data from a Prospective cohort study (NSHD) with longitudinal comparison	At age 36: 1416 participants of whom 53% were female, and reviewed at age 43: (n=1505) and 53 years (n=1381) 1007 subjects included for all three time points	Results were compared between at ages 36, 43 and 53	All food and drinks consumed in pre-defined meal slots: breakfast, lunch, evening meal and between meals (first thing, mid-morning, tea, late evening and extras)	Regularity of energy intake and specific meals, and for total energy intake.	Scores of irregular intake of energy were calculated per subject as the variation in energy intake per meal time slot when compared with the 5-d mean energy intake of that meal time slot. Meal irregularity score based on 4 or more days (up to 5 days) food diary (A lower score = a more regular meal pattern of energy intake and a higher score = more irregular pattern of energy intake). A score for total energy intake regularity was also calculated.	5-d estimated diet diaries collected by research nurses	Metabolic syndrome or one of its components: HDL-Cholesterol, Triglycerides, BP, HbA1c, Waist circumference; BMI.	Sex, socioeconomic status, marital status, physical activity, smoking and energy intake	- Irregularity scores decreased significantly over the years (P<0·05) for breakfast, lunch and the daily total energy intake - Subjects at 36 years with a more irregular intake of energy at lunch (OR 1·42; 95 % CI 1·05, 1·91; P=0.02) and between meals (OR 1·35; 95 % CI 1·01, 1·82 P=0.03) had an increased risk for the metabolic syndrome **17 years later** (OR 1·42 for highest v. Lowest irregularity group, 95 % CI 1·05, 1·91) and with energy intake between meals Also, higher risk of increased BMI for breakfast (OR 1·51; 95% CI 1·18, 1·94; P<0·01) and evening meals (OR 1·52; 95% CI 1·19, 1·95; P<0·01 - For lunch, higher risk of increased WC (OR 1·58; 95 %

										CI 1·27, 1·96; P<0.01) and TAG levels (OR 1·33; 95 % CI 1·02, 1·72; P=0.03) For daily total (OR 1·40; 95% CI 1·13, 1·73; P<0·01 - Subjects at 43 years with a more irregular intake at breakfast had an increased risk of the metabolic syndrome **10 years later** (OR 1·53; 95 % CI 1·15, 2·04; P=0·01), as well as an increased BMI (OR 1·66; 95 % CI 1·31, 2·10; P<0.001), waist circumference (OR 1·53; 95% CI 1·23, 1·90; P<0.001) and diastolic BP (OR 1·42; 95% CI 1·13, 1·78, P<0.001). - For lunch, higher risk of increased BMI (OR 1·32; 95% CI 1·04, 1·67; P for trend 0·02) and diastolic BP (OR 1·27; 95% CI 1·01, 1·60; P=0·03
Ibe *et al*. (2016) in Japan	Cohort longitudinal study	2138 (1241men and 897 women) aged 18-30 yrs	2 years	N/A	Regularity of 3 meals	Eating 3 meals regularly was recorded as (yes, sometimes or no). The 'yes' answer was defined as a 'regular' and 'no' as an 'irregular' pattern	Self-report questionnaire	BMI	N/A	For men, eating three meals irregularly was significantly related to a 4% BMI gain (OR 1.60, 95% CI 1.11, 2.30), 6% BMI gain (OR 1.72, 95% CI 1.12, 2.63), 4 kg weight gain (OR 2.01, 95% CI 1.29, 3.13), 6 kg weight gain (OR 1.86, 95% CI 1.02, 3.37), and occurrence of obesity (BMI≧25) (OR 2.96, 95% CI 1.22, 7.17) For women, eating three meals irregularly was significantly related to a 4% BMI loss (OR

										1.99, 95% CI 1.01, 3.94), 6% BMI loss (OR 2.79, 95% CI 1.29, 6.03), 4 kg weight loss (OR 3.85, 95% CI 1.62, 9.12), 6 kg weight loss (OR 7.65, 95% CI 2.06, 28.46), and the occurrence of underweight (OR 3.95, 95% CI 1.32, 11.89)
Saneei *et al.* (2016) in Iran	Cross-sectional study using data from (SEPAHAN)	7958 adults with complete weight and height measures and subset of 6054 adults from whole sample with complete WC values	N/A	Main meals (Breakfast, lunch and dinner) and snacks	Regularity of eating meals	The regularity of taking meals and the frequency of only main meals (breakfast, lunch and dinner) per week were recorded. Latent class analysis was then applied, and two classes identified and labelled 'regular' and 'irregular'.	Regularity: Self-administered questionnaire Dietary intake: 106 item FFQ	Obesity by BMI	age, gender, total energy intake, physical, education, marital status and family size	Subjects with 'irregular meal pattern' had higher odds for overweight/obesity (OR 1.18: 95 % CI 1.08, 1.30), obesity (OR 1.29: 95 % CI 1.10–1.50), abdominal overweight/obesity (OR 1.23: 95 % CI 1.10–1.37), and abdominal obesity (OR 1.23: 95 % CI 1.09–1.38) compared with those with a 'regular meal pattern.' After excluding the effect of other classes, it was found that individuals with an 'irregular meal pattern' were 21, 24, and 22 % more likely to be overweight/obese, abdominally overweight/obese, and abdominally obese compared with those who had a 'regular meal pattern.'

Wennberg *et al.* (2015) in Sweden	Prospective, longitudinal cohort study (The Northern Swedish Cohort)	889 (439 male and 450 female) aged 16 years at recruitment	27 years	Breakfast, lunch and dinner	Regularity of eating meals	Regularity of breakfast, school lunch and dinner at age 16 years were recorded into a three-category variable: (i) all meals regularly, (ii) one meal irregularly and (iii) two or all meals irregularly	Questionnaire	**Metabolic syndrome measured at 43 years):** - Central obesity by waist circumference (cm) measurement - HDL-C (mmol/l) triglyceride (mmol/l), fasting blood glucose (mmol/l) by blood sample - Blood pressure (mmHg) by standard sphygmomanometer - Type 2 diabetes by self-reported	Sex, BMI, smoking, alcohol consumption, physical activity and consumption of sweets and pastries at age 16 years.	Irregular eating of meals at age 16 years was associated with higher prevalence of the metabolic syndrome at age 43 years (OR 1·74: 95 % CI 1·12, 2·71), but this was explained by concurrent BMI and unhealthy lifestyle at age 16 years.
Pot *et al.* (2014) in UK	Secondary analysis of cross-sectional data from a prospective cohort study (NSHD)	1768 participants. Data collected in 1999: male and female (approx. 53%) aged 53 years.	N/A	All food and drinks consumed in pre-defined meal slots: breakfast, lunch, evening meal and between meals (first thing, mid-morning, tea, late evening and extras)	Meal pattern regularity	Scores of irregular intake of energy were calculated per subject as the variation in energy intake per meal time slot when compared with the 5-d mean energy intake of that meal time slot. Meal irregularity score based on 4 or more days (up to 5 days) food diary (A lower score = a more regular meal pattern of energy intake and a higher score = more irregular pattern of energy intake). A score for	5-d estimated diet diaries were collected by research nurses	Metabolic syndrome or one of its components: HDL-Cholesterol, Triglycerides, BP, HbA1c, Waist circumference; BMI	Sex, socioeconomic status, marital status, physical activity, smoking and energy intake	- Irregularity scores of energy intake ranged from 0–160 and were highest for between meals - An increased risk of the metabolic syndrome was associated with more irregular energy intake during breakfast (OR for top v bottom tertile1.34 (0.99-1.81; P value for trend across 3 groups= 0.04) and between meals (OR for top v bottom tertile1.36 (1.01-1.85); P for trend across 3 groups 0.04) -Increased waist circumference was associated with irregular energy intake during breakfast (OR for top v bottom tertile1.90

						total energy intake regularity was also calculated. Participants were divided into tertiles for score.				(1.47; 2.45); p value for trend across 3 tertiles groups <0.01), evening meal (OR for top v bottom tertile1.36 (1.06; 1.75); p for trend across three groups 0.01), and daily total energy intake (OR for top v bottom tertile1.34 (1.04; 1.72); p value for trend 0.01). -Increased BMI was associated with a more irregular intake of energy at breakfast (OR for top v bottom tertile1.46 (1.14; 1.87), p for trend across 3 groups<0.01)
Stea *et al*. (2014) in Norway	Cohort longitudinal study	428 children (207 boys and 221 girls) primary school grades 4 in 2007 (9–10 years) and grade 7in 2010 (12–13 years)	3 years	Breakfast, lunch, dinner and evening meal	Meal pattern regularity	Subjects who consumed main meals fewer than 7 d/week were classified as breakfast, lunch, dinner and evening-meal skippers Subjects who ate these meals every day were classified as regular breakfast, lunch, dinner and evening meal eaters	Questionnaire	BMI	Gender, maternal education, physical activity and overweight	Those who ate regular meals in 4th grade but not in 7th grade had higher odds (OR: 3.1; 95 % CI 1.1, 9.0) of being overweight in 7th grade after adjusting for gender, maternal education and physical activity, but the odds ratio was not statistically significant after adjusting for overweight in 4th grade (OR 52.8: 95% CI 0.7, 11.6).

Stea and Torstveit (2014) in Norway	Cross-sectional study	2,432 adolescents (1258 girls and 1187 boys) aged 15–17 years	-	Breakfast, lunch, dinner and evening meal	Regularity of main meals	The frequency of consumption of each meal per week was recorded as the range from never to 7 days per week. Subjects who consumed all these meals every day were classified as regular breakfast, lunch, dinner, evening meal consumer	Self-reported questionnaire	Academic achievement	N/A	In both girls and boys, high academic achievement was associated with a regular consumption of breakfast (OR 3.30: 95% CI 2.454.45) and (OR: 1.76 (1.32, 2.34), respectively) and lunch (OR: 1.44 (1.08, 1.93) and OR: 1.43 (1.09, 1.89), respectively), and in boys, with a regular consumption of dinner (OR: 1.44 (1.16, 1.79)) and a regular meal pattern in general (OR: 1.50 (1.10, 2.03))
Overby *et al*. (2013) in Norway	Cross-sectional study	475 adolescents (236 boys and 239 girls) from grade 9 and 10. Mean age was 14.6 years	-	Breakfast, lunch, dinner and evening meal	Regular meals	Questions such as: "How often do you have breakfast each week?" also asked for lunch, dinner and evening meal. Response alternatives ranged from never or seldom to 7 days a week, dichotomized into having the meal five times a week or less and having the meal six times a week or more.	Questionnaire	Writing and reading difficulties and mathematical difficulties	Gender and overweight	Regular breakfast was significantly associated with decreased odds of both writing and reading difficulties (OR: 0.44 (0.2–0.8), $p = 0.01$) and mathematical difficulties (OR: 0.33 (0.2–0.6), $p \leq 0.001$) Eating lunch (OR: 0.40 (0.25–0.65), $p \leq 0.001$), dinner (OR: 0.09(0.04–0.23), $p \leq 0.001$), evening meal (OR: 0.51(0.31, 0.83), $p \leq 0.001$) regularly was also significantly associated with decreased odds for mathematical difficulties. Taking dinner regularly was associated with decreased odds of writing and reading difficulties (OR: 0.34 (0.15, 0.80), p= 0.013)

Shafiee *et al*., (2013) in Iran	Cross sectional study	5,604 students (2818 boys and 2786 girls) aged 10-18 years (CASPIAN) study	N/A	N/A	Regularity of eating breakfast	Three groups: ‘‘regular breakfast eater’’ (6-7days/week), ‘‘often breakfast eater’’ (3-5days/week), and ‘‘seldom breakfast eater’’ (0-2 days/week)	Likert scale questionnaire	Cardio-metabolic risk factors and BMI	Age, sex, family history of chronic disease, mother’s education, father’s education, physical activity, socioeconomic status and BMI	The risk of metabolic syndrome, general obesity and abdominal obesity were significantly increased in subjects who seldom ate breakfast (OR 1.96,95% CI (1.18, 3.27), OR 1.47, 95% CI (1.20, 1.82), OR 1.39, 95% CI (1.04, 1.86) respectively)
Mekary *et al*. (2013) in USA	Prospective cohort study (Nurses’ Health Study)	46,289 women aged (30-50) years	6 years	Breakfast	Regularity of breakfast consumption	Breakfast consumption per week (more than coffee or tea) as (Never, 1/week, 2/week, 3/week, 4/week, 5/week, 6/week, 7/week) (regular breakfast consumer if 7 times/week and irregular breakfast if 0-6 times/week)	Questionnaire	Type 2 diabetes by self-report based on American Diabetes Association criteria	Risk factors of T2D, including family history, alcohol intake, physical activity, menopausal status, smoking status, energy intake and BMI	Women who consumed breakfast irregularly (0–6 times/week) were at higher risk of T2D than were participants who consumed breakfast daily (RR: 1.28; 95% CI: 1.14, 1.44)

Muff *et al.*, (2011) in Germany	Cross-sectional data in HNR Study, a population-based prospective cohort study	(2,395 men and 2,419 women) aged 45–75 years	N/A	N/A	Regularity of meal intakes	'It is difficult to give detailed information as my meal intakes are often irregular'. Participants who answered 'yes' to this statement were categorized as having 'three irregular meal intakes. Those participants who denied the Statement was asked to mark all the meals they regularly consume on weekdays ('breakfast', 'second breakfast', 'lunch', 'snack in the afternoon', 'supper', 'snack in the evening') and were categorized as having (1) 'three regular meal intakes', (2) 'two regular meal intakes, one irregular', (3) 'one regular meal intake and two irregular'	Self-administered questionnaire	Diabetes and BMI	Socio-demographics, health conditions, health behaviours and work characteristics	An association between diabetes (OR 0.65, 95% CI 0.50, 0.84) and regular meal intakes. High BMI (OR 1.03,95% CI 1.01, 1.06) was related to irregular meal intakes
Al-Rethaiaa *et al.* (2010) in KSA	Cross-sectional study	357 male students aged 18-24 years	-	-	Regularity of meals	Question about taking meals regularly was answered as always regularly or irregularly	Self-reported questionnaire	Obesity using BMI	N/A	No significant correlation between meal regularity and obesity

Hamaideh *et al.* (2010) in Jordan	Cross-sectional study	824 subjects 14-17 years (no indication of distribution between boys and girls)	-	Breakfast	Regularity of breakfast	Eating breakfast regularly was recorded as (yes or no)	Self-reported questionnaire	Overweight and obesity	N/A	Eating breakfast regularly was positively correlated with overweight and obesity (R= 0.195, R^2= 0.038, β= 0.110, P≤0.001)
Lehto *et al.* (2010) in Finland	Cross-sectional study	604 school children (312 girls and 292 boys) aged 9–11 years	-	Breakfast, lunch and dinner daily during the school week	Regularity of meal pattern	Any meal normally eaten on 5 d during the school week was defined as a regular meal and all other alternatives as irregular meals	Questionnaire	Obesity using BMI	Diet, physical activity, screen time, sleep duration and the family's socio-economic status	- Irregular breakfast (β=1.28, (95% CI: 0.68, 1.88), P<0.001) and an irregular meal pattern (β=0.62, (95% CI: 0.16, 1.08), P=0.009) were associated with higher BMI - Regularity of school lunch, dinner or family dinner was not associated with BMI.
Merten *et al*. (2009) in USA	Data for a prospective descriptive study was derived from National Study of Adolescent Health	5,823 white and 1,965 African American adolescents aged from 11 to 18 years. 2nd data collection point: when12 to 19 years old (mean age 16.1 years) 3rd data collection point: when18 and 26 years (mean age 22.5 years)	-	Breakfast	Regularity of breakfast consumption	Breakfast consumption frequency during the week. Participants who consume breakfast at least 4 days per week or more were coded as having regular breakfast consumption. Participants that did not report eating breakfast at least four times a week were coded as irregular breakfast consumers.	In-home interview	Chronic obesity BMI 30 were considered obese	Community disadvantage, family poverty, parental presence in adolescence, race and sex	Regular breakfast consumption in adolescent significantly decreased the likelihood of chronic obesity adolescence and adult (recorded at 2nd and 3rdmeasurementpoint) (OR=0.59; 95% CI: 0.52, 0.68; P<0.001, OR=0.75: 95% CI: 68, 83: P<0.001) respectively

Shin *et al*. (2009) in Korea	Cross-sectional study	5337 men aged ≥ 30 years	N/A	N/A	Regularity of meal frequency and mealtime	Meal frequency as (3 times a day, 2 times a day or irregularly), breakfast frequency as (always, often or not at all) and mealtime regularity as (regularly, often or irregularly) were recorded	Self-administered questionnaire	ATP III NCEP for metabolic syndrome including BMI, high blood pressure (mmHg), waist circumference (cm), high triglyceride (mmol/l), low HDL-C (mmol/l) and high fasting glucose (mmol/l)	Age, family history of type 2 diabetes, smoking, and physical activity	No significant associations between meal frequency, breakfast frequency and meal time regularity, and the risk of metabolic syndrome
Raynor *et al*. (2008) in USA	Cross-sectional study (Look AHEAD survey)	5145 subjects (3,063 women and 2,082 men) aged 45–74 years with T2DM and BMI ≥ 25 kg/m2	-	Breakfast	Regularity of breakfast consumption	Participants reported the number of days per week they ate breakfast	Question about the number of days breakfast was consumed- not clear whether written or asked verbally. -	BMI	Age, sex, ethnicity, and socioeconomic status	Participants who consumed breakfast 7 days/week had a lower BMI (35.6±5.7kg/m^2, P<0.0001) than participants who consumed breakfast 3–6 days/week (BMI 36.7±6.3 kg/m^2) and ≤2 days/week (BMI 37.3±6.1 kg/m^2) In the multivariate model, a larger amount of overall intentional weight loss (B =0.01), self-weighing less than once per week (B=0.83), and more fast-food meals consumed per week (B=0.15) were associated (P<0.05) with higher BMI, whereas a greater number of days per week in which breakfast was consumed (B=-0.18) was associated (P<0.05) with lower BMI. R2=0.24 for the adjusted model. After adjustment, the durations of individual weight control practices were no longer related to BMI

Sierra-Johnson *et al*. (2008) in Sweden	Cross-sectional study (Health screening study)	3607 (1686 men and 1921 women) aged 60 years Those with known cardiovascular disease, diabetes, and/ or cancer were excluded.	N/A	Self-defined breakfast, lunch and dinner or evening meal	Regularity of eating meals	Eating of breakfast, lunch and dinner or evening meal each day were recorded as never, sometimes, usually, or always. Regular meal pattern if the answer was usually or always and irregular meal pattern the answer sometimes or never	Questionnaire	- Weight (Kg), BMI, waist and hip - Blood pressure (mmHg) - Lipid profile: HDL-C (mmol/l), LDL-C (mmol/l) and triglycerides (mmol/l) by fasting blood sample - Insulin resistance by HOMA-2 - Fasting glucose by blood sample - Adult Treatment Panel ATP-III for metabolic syndrome Food intake questionnaire re specific items	Sex, education, physical activity and smoking	- - Regular eaters (20%) fulfilled the criteria for the metabolic syndrome vs. irregular eaters (27%) (P<0.0001) - The adjusted OR for having the metabolic syndrome in subjects who were regular eaters was 0.58 (95% CI 0.41–0.83) Means of BMI, WC, triglyceride and HOMA-2were significantly lower (P<0.0001) in regular eaters (26.4,90.7, 1.28 and 1.26) respectively compared with irregular eaters (27.4,93.6, 1.41 and 1.46) respectively - Eating meals regularly was inversely related to insulin resistance (OR: 0.68, 95% CI, 0.48–0.97)
Ko *et al.* (2007) in China	Cross-sectional study	4841 subjects (2375 men and 2466 women) aged 17-83 years	-	N/A	Meal regularity	Regular meals everyday were recorded as (yes or no)	Questionnaire	**CVD risk factors:** Obesity, hypertension, Hypercholesterolaemia, smoking and DM	N/A	Irregular meals were significantly associated with obesity (OR: 1.20 (95% CI: 1.04, 1.39), P<0.05) and smoking (OR: 2.23 (95% CI: 1.82, 2.73), P<0.001)

Kim *et al*., (2003) in South Korea	Cross sectional study	6463 children (3401 boys and 3062 girls). (1,935 from grade 5 age, 10-11 y), (2,194 from grade 8, age 13-14 y), and (2,334 from grade 11, age 16-17) years.	N/A	N/A	Regularity of eating breakfast, lunch and dinner	Questions about the regularity of breakfast, lunch and dinner. The answer was 'yes' or 'no'.	Self-administered questionnaire	Academic performance	Cities and parent's education level	Academic performance (higher GPA) was significantly associated with regularity of breakfast in boys Grade 5 (coefficient = 0.177, P=0.000), in boys and girls Grade 8 (0.270, P=0.000, 0.278, P=0.000) and Grade 11 (0.156, P= 0.001; 0.142, P= 0.004), regularity of lunch in boys and girls Grade 5 (0.110,P=0.043; 0.259, P=0.000), Grade 8 (0.256, P=0.001 ; 0.176,P=0.024) and Grade 11 (0.240, P=0.000; 0.215, P=0.000) and regular dinner in girls Grade 8 (0.160, P=0.012) and in boys and girls Grade 11 (0.314, P=0.000; 0.294, P=0.000)
Sjoberg *et al*. (2003) in Sweden	Cross-sectional study	1245 subjects (611 boys and 634 girls) in grade 9, aged 15–16 years	N/A	The definition of main meals was based on the time of the intake during the day. All meals that were not defined as main meals were defined as in-between meals. Breakfast as the meal in the morning before school, lunch as the meal during lunch break at school and dinner as the main meal in the afternoon	Regularity of meal consumption	Regular breakfast intake (breakfast every day before school), irregular breakfast intake (omitted breakfast once a week or more), irregular breakfast and lunch intake (omitted both breakfast and school lunch at least once a week) and regular meal pattern (always breakfast, school, lunch and prepared dinner) were recorded	Questionnaire with 30-minute interview with a dietician.	BMI, food and energy intake by diet history.	BMI, ethnicity, smoking and energy intake	- For the boys, BMI was significantly higher (P=0.006) for participants with irregular breakfast intake but not for the girls - Subjects with regular breakfast had 6–7% higher energy intake from breakfast and 8% of lower EI from in-between meals compared with having irregular breakfast. - There was a difference in EI/BMR between boys with regular and irregular intakes: 1.76 vs1.63 (P=0.042), but not significant in girls: 1.50 vs 1.43 (P=0.080).

Takahashi *et al.* (1999) in Japan	Case control study (The Toyama Birth Cohort Study)	427 children who were obese and 854 children who were not obese (controls) aged 3 years (732 boys and 549 girls).	-	N/A	Irregular mealtimes and snacks intake	Question about mealtimes and snacking regularity were answered as regular or irregular	Questionnaire	Obesity using BMI	Birth weight, mother's BMI, father's BMI, snacks irregularity and limited playtime outdoors	- For irregular mealtimes, there was no significant difference between obese and control children. -Snacking irregularity significantly influenced the development of obese in3-year-old children (OR = 1.30, 95% CI 1.02–1.65, P< 0.05)
Sugimori *et al*. (1999) in Japan	Case control study	117 children who were obese and 234 children who were not obese (controls) aged 3 years (228 boys and 123 girls)	-	N/A	Irregularity of meals and snacks	Question about regularity of eating meals and snacks, the potential answer was regular or irregular	Questionnaire	Obesity using BMI	Birth weight, mother's BMI, father's BMI, sex, person other than the mother responsible for caring for the child, sleep duration, physical activity and eating snacks irregularly	No significant relationship between regularity of meals and obesity. After adjusting for confounders, irregular snack eating had a significant relationship with obesity in childhood (OR 1.56, 95 %CI 1.04-2.33, P=0.014)

Kagamimori *et al*. (1998) in Japan	Cross-sectional study from The Toyama Birth Cohort Study	8834 children aged 3 years	-	N/A	Irregular meals and snack intake	Irregular meal or snack intake when the answer given was that the intake time was not fixed	Questionnaire	Obesity using BMI	Gender, birth month, main caregiver, nursery school, mother's employment and living with extended family	There were no significant differences for regularity of meal intake but there was a significant difference for regularity of snack intake (P<0.001) between obese and non-obese children

BMI: Body Mass Index

NDNS: National Diet and Nutritional Survey

PPSOC: The Portuguese Prevalence Study of Obesity in Childhood

PCA: The Principal Component Analysis

WC: Waist Circumference

NSHD: National Survey of Health and Development

SEPAHAN: Study on the Epidemiology of Psychological, Alimentary Health and Nutrition

FFQ: Food Frequency Questionnaire

CASPIAN: Childhood and Adolescence Surveillance and Prevention of Adult Noncommunicable Disease

HNR: Heinz Nixdorf Recall Study (Schmermund *et al*. 2002; Stang *et al*. 2005).

AHEAD: Action for Health in Diabetes

Table 2.2: Characteristics and the results of intervention studies

Study	Study design	Population and sample size	Intervention and duration	Control	Overall study duration	Definition of meal	Method of characterize meal pattern regularity	Time of outcomes measurement	Outcomes	Main findings
Alhussain *et al.* (2016) in UK	RCT (Crossover)	11 normal-weight women (18–40) years	Regular meal pattern (6 meals/d) for 14 days vs. irregular meal pattern (3–9 meals/d) for 14 days	N/A	42 days	N/A	In first 14-d intervention period, the number of meals during the regular meal pattern was 6 meals/d (3 meals and 3 snacks). In second 14-d intervention period, the number of meals (including snacks) during the irregular meal pattern varied from 3 to 9 meals/d. The mean was 6 meals/d (i.e., 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 meals/d, respectively). The two 14-d intervention periods were separated by a washout period of 14 d. Participants followed their habitual diets during this washout period	During the intervention periods and before and after a test meal at the first and last days of the 2 intervention periods	**During the intervention period:** - Energy expenditure (ambulatory energy expenditure estimation (AEEE)) by SenseWear Armband device - Continuous interstitial glucose profiles by continuous glucose monitoring - Subjective appetite by paper-based visual Analog Scales **Before test meal:** - Total cholesterol (mmol/l), HDL-C (mmol/l), LDL-C (mmol/l), triacylglycerol (mmol/l) **Before and after test meal:** - Serum insulin, fasting blood glucose (mmol/l), and gut hormones (glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and ghrelin) **After test meal:** - Energy expenditure (REE) and (TEF) by indirect calorimetry - Subjective appetite by VAS	- Regularity was associated with a greater TEF ($P<0.05$). The TEF after the regular intervention was 11.1±15.8 kcal higher than after the irregular intervention. - Incontinuous glucose monitoring, no significant differences between the 2 intervention periods in the 24-h mean, maximum, minimum, and iAUC for glucose concentrations. However, regularity related to a lower incremental area under the curve (iAUC) for glucose after intake of the test drink (over 3 h) and, for some identical meals, during the 2 interventions (over 90 min) (day 7: after breakfast; day 9: after lunch and dinner) - No significant interactions for meal pattern in lipids, fasting blood glucose and insulin - No difference between interventions for the test-drink gut hormone response - A time effect was noted for fasting GLP-1, fasting PYY, PYY responses, and hunger-

										rating responses to the test drink (P=0.05) - Lower hunger and higher fullness ratings were seen premeal and postmeal during the regular period - No difference between interventions for energy intakes and subjective appetite rates after the ad libitum test meal across study visits
Demos *et al.* (2015) in USA	RCT Pilot	25 healthy subjects aged 21–65 years, with a BMI within 25–45 kg/m2	Eating meals regularly in a specific planned time	Subjects in control group received educational sessions only on topics other than meal pattern	4 weeks training followed by a 18 week weight loss period applying skills.	Three meals and two snacks in the intervention group	The intervention group had scheduled eating episodes for each day. 'On-schedule' eating was defined as consuming a meal or snack within 15 min of the planned time and eating at all other time points was thus considered 'off schedule'.	After 4 weeks of training followed by an18 week weight loss period	Weight by Tanita digital scales	No significant difference between intervention group and controls after 4 weeks (P= 0.28) and 18 weeks (P=0.18)
Farshchi *et al.* (2005) in UK	RCT (Crossover)	10 healthy obese women aged (32–47) years	Regular meal pattern (6 meals/d) for 14 days vs. irregular meal pattern (3–9 meals/d) for 14 days	N/A	42 days	Food or snack (solid or liquid) containing energy, with an interval of >1h between 2 eating occasions	In phase 1 (14 d), subjects' normal diet was consumed but at either 6 occasions/d (regular meal pattern) with regular intervals between meals or a chaotic meal plan (irregular meal pattern). In irregular meal pattern, the subjects were asked to observe a predetermined meal frequency consisting of 3–9 meals/d for 14 d, (7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 occasions/d on days 1 through 14), respectively, of the irregular meal pattern. After phase 1, subjects' habitual diet was followed for 14 d as a washout period. In phase 2 (14 d), cross over to the alternative meal pattern from phase 1 was done	After test meal at the first and last days of Phase 1 and 2	- Fasting blood glucose (mmol/l) by blood sample - Insulin resistance by (HOMA-IR) - Energy intake (EI) by 3-d food diary - Energy expenditure (RMR) by indirect calorimeter - Thermogenesis by trapezoidal method measuring the area under the curve of PPMR above the baseline RMR - Lipid profile: Total cholesterol (mmol/l), HDL-	- Blood glucose concentrations and serum insulin concentrations rose significantly in response to the test meal at all visits - Fasting glucose and insulin values were not affected by meal pattern, but peak insulin concentrations and AUC of insulin responses to the test meal were lower after the regular than after the irregular meal pattern (P<0.01 and 0.02, respectively)

								C (mmol/l), LDL-C (mmol/l), triacylglycerol (mmol/l) and uric acid (mmol/l) by blood sample	- No significant effect of either meal pattern on fasting RMR - Regular eating was associated with lower EI ($P < 0.01$), greater postprandial thermogenesis ($P < 0.01$), and lower fasting total ($P < 0.01$) and LDL ($P < 0.02$) cholesterol - Fasting values for all appetite rates and the profiles after the test meal did not differ significantly over the course of the experiment
Farshchi *et al.* (2004a) in UK	RCT (Crossover)	9 healthy lean women aged (18–42) years	Regular meal pattern (6 meals/d) for 14 days vs. irregular meal pattern (3–9 meals/d) for 14 days	N/A	42 days	Food or snack (solid or liquid) containing energy, with an interval of >1h between 2 eating occasions	In phase 1: (14 d), subjects' normal diet was consumed but either 6 occasions/d (regular meal pattern) with regular intervals between meals or a chaotic meal plan (irregular meal pattern). In irregular meal pattern, the subjects were asked to observe a predetermined meal frequency consisting of 3–9 meals/d for 14 d, (7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 occasions/d on days 1 through 14), respectively, of the irregular meal pattern. After phase 1, subjects' habitual diet was followed for 14 d as a washout period. In phase 2 (14 d), cross over to the alternative meal pattern from phase 1 was done	After test meal at the first and last days of Phase 1 and 2	- Fasting blood glucose (mmol/l) by blood sample - Insulin insensitivity by HOMA - Lipid profile: Total cholesterol (mmol/l), HDL-C (mmol/l), LDL-C (mmol/l), triacylglycerol (mmol/l) by blood sample - Uric acid (mg/l) by blood sample	- No significant differences in the peak values of blood glucose in each eating pattern - No significant changes in fasting serum insulin concentration. However, after the irregular meal pattern, the HOMA (0.9, $P=0.022$), peak insulin (428.4±150.0 pmol/l, $P=0.003$) and AUC (620 pmol/l.h, $P=0.001$) were greater than regular meal pattern (0.77), (332.4±107.4 pmol/l) and (500 pmol/l.h) respectively. - The irregular meal pattern was related to higher fasting total cholesterol (3.82) ($P <0.01$) and LDL-C (2.10) ($P <0.05$) compared to regular meal pattern (3.51 and 1.82) respectively. - No significant changes in plasma uric acid

Farshchi *et al.* (2004b) in UK	RCT (Crossover)	9 healthy lean women aged (18–42) years	Regular meal pattern (6 meals/d) vs. irregular meal pattern (3–9 meals/d)	N/A	42 days	Food or snack (solid or liquid) containing energy, with an interval of >1h between 2 eating occasions	In phase 1 (14 d), subjects' normal diet was consumed but either 6 occasions/d (regular meal pattern) with regular intervals between meals or a chaotic meal plan (irregular meal pattern). In irregular meal pattern, the subjects were asked to observe a predetermined meal frequency consisting of 3–9 meals/d for 14 d, (7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 occasions/d on days 1 through 14), respectively, of the irregular meal pattern. After phase 1, subjects' habitual diet was followed for 14 d as a washout period. In phase 2 (14 d), cross over to the alternative meal pattern from phase 1 was done	After test meal at the first and last days of Phase 1 and 2	- Weight (Kg) by balance scale - Body fat (%) by bioelectrical impedance analysis - Thermic effect of food (TEF) by trapezoidal method measuring the area under the curve of PPMR above the baseline RMR - Energy intake by 3-d food diary - Energy expenditure (RMR) by indirect calorimeter - Appetite by VAS questionnaires to assess subjective hunger, satiety, fullness and desire to eat	- There were no significant differences in weight and body fat - No significant differences in 3-day mean energy intake between the regular and irregular meal pattern. - No significant differences in subjective appetite before and after the test meal in all visits. - No significant differences in RMR over the experiment. - TEF mean over the 3 h after the test meal was significantly lower after the irregular meal pattern (0.4 kJ/min) (P=0.003) than after regular meal pattern (0.7 kJ/min)

2.3.6. Quality assessment

The results of the quality assessment, based on the study design, were presented in Tables 2.3, 2.4, 2.5 and 2.6. The rating scales used included options of good, fair or poor quality (observational studies), or low, high or an unclear risk of bias (intervention studies).

2.3.6.1. Observational Studies

2.3.6.1.1. Cross-sectional studies

The majority of studies (13 out of 19) were of good quality. Seven studies (Kim *et al.*, 2003; Marten *et al.*, 2009; Al-Rethaiaa *et al.*, 2010; Hamaideh *et al.*, 2010; Kagamimori *et al.*, 1998) had a fair quality and two studies (Ko *et al.*, 2007; Raynor *et al.*, 2008) had a poor quality (Table 2.3).

2.3.6.1.2. Cohort studies with follow up.

All studies were of good quality for selection, comparability and outcome except two studies (Wennberg *et al.*, 2015 and Stea *et al.*, 2015) which had a fair quality (Table 2.4).

2.3.6.1.3. Case control studies

All studies were of poor quality for selection, comparability, and exposure (Table 2.4). It was noted that no studies had attempted to identify and exclude participants who were considered to be at high risk of mis-reporting their dietary intake.

2.3.6.2. Intervention studies

2.3.6.2.1. RCT cross-over

The randomised cross-over studies were designed appropriately and were at a low risk of bias (Table 2.5). However, the selection of treatment order was unclear, in all but one study (Alhussain *et al.*, 2018) as the method of sequence generation was not reported. The risk of bias from carry-over effects was unclear, not being reported in any study. All data related to study outcomes and analytical results for both periods were provided for every stage in the studies so risk of bias in reporting the findings was low. Allocation concealment approaches were unclear because there was no description in any of the studies. Studies

were judged to have been blinded where possible; however, in many cases because of the dietary intervention, blinding of the participant and experimenter was not possible. All studies had a low risk of bias from incomplete outcome data or selective outcome reporting. No other risk factors for bias were identified.

2.3.6.2.2. Pilot RCT

According to Jadad scale, a study by Demo *et al.* (2015) was considered to be at a high risk of bias (Table 2.6). There was no clear explanation of randomization methods. This study was blind but not double blind. The number of participants who completed the trial was reported without mention of the causes of participant attrition.

Table 2.3: Quality assessment for cross-sectional studies

Criteria	Zimmerman *et al.* (2018)	Rodrigues *et al.* (2017)	Saneei *et al.* (2016)	Pot *et al.* (2014)	Stea and Torstveit (2014)	Overby *et al.* (2013)	Shafiee *et al.*, (2013)	Muff *et al.*, (2011)	Al-Rethaiaa *et al.* (2010)	Hamaideh *et al.* (2010)	Lehto *et al.* (2010)	Merten *et al.* (2009)	Shin *et al.* (2009)	Raynor *et al.* (2008)	Sierra-Johnson *et al.* (2008)	Ko *et al.* (2007)	Kim *et al.*, (2003)	Sjoberg *et al.* (2003)	Kagamimori *et al.* (1998)
	Yes, no or other (CD, cannot determine; NA, not applicable; NR, not reported)																		
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NR	Yes	Yes	NR	Yes	Yes	NR	Yes	Yes	Yes	Yes	NR	No	No	Yes	No	Yes	Yes	Yes
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	NR	No	No	NR	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
6. For the analyses in this paper, were the exposure(s) of	Yes	NR	NR	Yes	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

interest measured prior to the outcome(s) being measured?																			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	NA	NA	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes	No	Yes	No	No	Yes	No
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

implemented consistently across all study participants?																			
12. Were the outcome assessors blinded to the exposure status of participants?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Quality Rating (Good, fair or poor)	Good	Good	Good	Good	Good	Good	Good	Good	Fair	Fair	Good	Fair	Good	Poor	Good	Poor	Fair	Good	Fair

Table 2.4: Quality assessment for case control and cohort (with follow up) studies

Category	Statement	Takahashi *et al.* (1999)	Sugimori *et al.* (1999)	Pot *et al.*, (2016)	Ibe *et al.* (2016)	Wennberg etal.(2015)	Stea *et al.* (2014)	Mekary *et al.* (2013)
	Case control studies							
Selection	1. Is the case definition adequate?							
	2. Representativeness of the cases							
	3. Selection of Controls	*	*					
	4. Definition of Controls	*	*					
Comparability	1. Comparability of cases and controls on the basis of the design or analysis	*	*					
		*	*					
Exposure	1. Ascertainment of exposure							
	2. Same method of ascertainment for cases and controls	*	*					
	3. Non-response rate							
	Cohort studies							
Selection	1. Representativeness of the exposed cohort			*	*	*	*	*
	2. Selection of the non-exposed cohort			*	*	*	*	*
	3. Ascertainment of exposure			*				
	4. Demonstration that outcome of interest was not present at start of study			*	*			*
Comparability	1. Comparability of cohorts on the basis of the design or analysis			*	*	*	*	*
				*	*	*	*	*
Outcome	1. Assessment of outcome			*	*	*	*	*
	2. Was follow-up long enough for outcomes to occur?			*	*	*	*	*
	3. Adequacy of follow up of cohorts			*		*	*	
Total	A maximum of one star for each numbered item within the Selection and Exposure or Outcome categories. A maximum of two stars can be given for Comparability	5 *	5 *	9 *	7 *	7 *	7 *	7 *

Table 2.5: Quality assessment for cross-over RCT

Item	Description	Alhussain *et al.* (2016)	Farshchi *et al.* (2005)	Farshchi *et al.* (2004)
		Scoring: (low, unclear or high) risk of bias		
1. Appropriate cross-over design	Three points are considered: (1) the condition of the patients should be chronic and stable; (2) the intervention should not provide permanent change, but rather temporary relief; (3) the effect of the first intervention should not last into the second treatment period.	Low	Low	Low
2. Randomized treatment order	The order of receiving treatments should be randomized adequately.	Low	Unclear	Unclear
3. Carry-over effect	The authors should evaluate the carry-over effect and provide relevant information clearly.	Low	Low	Low
4. Unbiased data	That only first-period data are available is considered a risk of bias.	Low	Low	Low
5. Allocation concealment	The study should apply appropriate approaches to ensure the allocation sequence is concealed	Low	Low	Low
6. Blinding	The study should apply a proper blinding method to prevent performance and detection bias. Those involved in blinding (participants, doctors, measurers, or analysts) depends on the particularity of the studies.	Low	Low	Low
7. Incomplete outcome data	The authors should provide relevant information about the completeness of outcome data, including the level of incompleteness, reasons, and analytic method to tackle these data shortcomings, etc.	Low	Low	Low
8. Selective outcome reporting	The authors should report all the outcomes fully. Selective reporting of part of outcomes or data for an outcome or subsets of the data or analyses using the same data and etc. should be avoided.	Low	Low	Low
9. Other bias	Any other potential risk of bias that may affect the quality of cross-over studies.	Low	Low	Low

Table 2.6: Quality assessment for the pilot RCT study

Study	Randomization	Description of randomization	Blinding	Description of blinding	Withdrawals and dropouts	Total Jadad score
Demo *et al.* (2015)	1	-1	0	-1	1	0

2.4 Discussion

Emerging evidence suggests that between day variability in meal pattern (also known as an 'irregular' or 'chaotic' eating or meal pattern) may be a novel risk factor for obesity and associated co-morbidities. This review aimed to critique previous methods used to characterise between day meal pattern variability and establish existing evidence of associations between such variability and obesity; and associated health outcomes. The search identified 31 papers which met the study inclusion criteria, representing a broad subset of the general population. A wide variety of methods had been used to characterize between day meal pattern variability in observational studies. This resulted in heterogeneity in the properties of meal pattern used to compare variability with the outcome measures of interest. However, the majority of studies (23/31), to some extent supported the hypothesis that between day meal pattern variability increases the risk of obesity, and/or showed associated negative health consequences for one or more of the other health outcomes of interest; including components of the metabolic syndrome and learning difficulties.

The approach used to characterize between day meal pattern variability in observational studies ranged from simple questions eliciting a self- report of perceived irregularity, to more complex procedures designed to quantify irregularity as a numerical index. However, many assumed that the rich complexity of the dietary intake profile (infinite potential times of dietary intake and range to quantities consumed) can be collapsed down to a limited number of pre- defined meal descriptors which were disassociated to a lesser or greater extent from the time of consumption. Such descriptors reflected the perception of social

norms held by the authors, for when dietary intake would occur, rather than reflecting the available detail of the recorded intake profiles. Examples of descriptors included 'main' meals (breakfast, midday meal and evening meal), with, or without provision to capture 'snacks'. Imposing such a simplistic and rigid construct limits valid differentiation between meal patterns, such as between regular breakfast consumers with, or without, variability caused by different work and non-work breakfast times. Furthermore, categorizing all snacks together without a temporal anchor (Pot *et al.*, 2014; Pot *et al.*, 2016), further reduces discrimination. Questions such as 'Was breakfast consumed regularly' only having the response options 'yes' or 'no' meant that a 'regular' pattern of never consuming breakfast would elicit the response 'no' in common with inconsistent breakfast consumption (irregular). Irregularity was often not defined, relying on individual perception or was inconsistently defined. Merten *et al.* (2009) for example used the definition of regular consumers as having breakfast at least 4 days per week or more; in contrast to Mekary *et al.* (2013) who characterized regularity as breakfast 7 times/week. In addition, the lack of clear definition of meals, snacks and eating incidents compromises interpretation.

In all studies using self-recorded food intake records, misreporting may occur. Employing weighed food intake records or involving professional, trained staff improves accuracy (Black and Wilson, 1995, Okura *et al.*, 2004 and Short *et al.*, 2009). None of the observational studies had attempted to identify and exclude participants who had mis-reported their food intake, by for example, applying cut- offs that identify those for whom the intake is unphysiological relative to their estimated energy expenditure (Black *et al.*, 2000). It is of note that those who live with obesity underreport to a greater extent than those who are lean (Prentice *et al.* 1986; Poppitt *et al.*, 1998). Furthermore, those with an irregular meal pattern, may under report to a greater extent than those with a regular meal pattern (Klingberg *et al.*, 2008). The latter two factors may result in an under estimation of the difference in intake between irregular eaters and regular eaters.

Intervention studies allowed a more focused approach. Four studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) distilled out specific dimensions of irregularity for consideration, by controlling aspects such as the start and

end time of eating and the dietary composition (across days and between interventions). A template meal pattern was constructed using a number of pre specified time points (regular eating) and then certain time points were omitted (the intake being given at a remaining time point) or added to achieve the required variability in frequency (irregular).

In conclusion, many different methods had been used to characterize variability in meal pattern which impacts on achieving the second aim of this review, namely, to explore the hypothesis that there is an association between day meal pattern variability and obesity; and its co-morbidities. However, the majority of studies showed some degree of support for the hypothesis. Although, one study found a positive association between eating breakfast regularly and overweight and obesity (Hamaideh *et al.*, 2010). However, this may have been because Jordanian breakfasts are uncharacteristically high in energy and fat, and the breakfast considered was a second breakfast, consumed at school. In other studies, no associations were found (Kim *et al.*, 2012). Only a small number of intervention studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Demos *et al.* 2015; Alhussain *et al.*, 2016) were identified, which limits our confidence in attributing causation. However, in these studies, a reduction in between day variability in meal pattern was associated with potential advantages, such as suppressed appetite, beneficial fat and glucose metabolism and an increased thermogenic response to a test meal (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016). Compatible results have been found in a further intervention study published after the inclusion date of the systematic review (Alhussain *et al.*, 2022). Given the range of effect sizes seen across all studies, further work is required, ideally with larger intervention studies in patient groups, to confirm not just statistically significance, but also clinical significance.

Evidence that both energy expenditure and energy intake may be implicated as mechanisms, is of interest. The negative association between meal pattern irregularity and glucose metabolism, with insulin resistance seen in some studies, may partially explain the blunted postprandial thermogenesis (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) following consumption of a test meal. Whilst the energy expenditure differences are relatively small, taken over sufficient time, the more positive energy balance, (in the absence of a reduced energy intake) could result in weight gain.

With respect to energy intake, dietary choices with greater between day meal pattern variability, may predispose to a more energy dense diet and associated higher risk of positive energy balance. Regular eaters had on average a significantly higher intake of fruits, vegetables, bony fish, fatty fish, and wine than those who were irregular eaters (Sierra-Johnson *et al.*, 2008). An irregular intake of breakfast resulted in a lower total intake of protein, calcium, fibre and zinc in girls and of iron and vitamin C. Adolescent males and females with irregular breakfast intake had a markedly higher percentage of energy from sucrose and higher intake of alcohol ($P<0.001$). Interestingly, irregular consumption of main meals was related to other lifestyle factors (eg smoking) and to poorer food choices and nutrient intakes (Sjoberg *et al.*, 2003). Several studies identified irregularity as being associated with more between meal snacks, and irregularity in consumption of between meal snacks as being associated with greater body weight. As snacks tend to be more energy dense than meals (Barrington and Beresford, 2019), predisposing to obesity, this may explain the association between irregularity and obesity, with a higher consumption of sugar sweetened beverages, associated with greater obesity, potentially being included as snacks (Saneei *et al.*, 2015).

Variability in between day meal pattern was associated with an increasing risk of other components of the metabolic syndrome either via the impact on fat mass, as would be expected with increased BMI (Meigs *et al.*, 2006), or independently of increased BMI. In intervention studies, where the diets fed were iso-energetic, deleterious effects were seen in carbohydrate metabolism and fat metabolism with no difference in fat mass over the two-week intervention period. Differences were seen in fasting lipids (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) which can lead to a deleterious effect on cardiovascular risk factors (Miller, 2009). Differences in dietary selection, depending on the variability of the meal pattern, when free living may explain differences in cardiovascular risk. Further, longer term intervention studies are indicated to explore these effects.

Studying the interaction between between-day meal pattern variability and circadian or chrono-biological rhythms may provide further insights into the mechanisms behind the results seen. Circadian rhythms, controlled by the biological central clock located in the

suprachiasmatic nucleus (SCN) (Lowrey; Takahashi, 2004) are entrained by light/dark cycles and enable internal co- ordination, with the SCN viewed as the central clock. Circadian rhythms, by definition, persist without external stimuli. However, they can be entrained by external stimuli resulting in eventual alignment of the circadian rhythm with the external stimuli. For example, eventual alignment to the new light dark cycle in a different time zone. Peripheral clocks enable isolated tissue to exhibit a circadian rhythm independently of the SCN but are aligned to the cycles maintained by the SCN. Many physiologically and nutritionally relevant processes including glycolysis, gluconeogenesis and lipid metabolism have circadian rhythms driven by peripheral clocks as do hormones such as insulin, glucagon, adiponectin, leptin and gastric acid secretion (Lowrey; Takahashi, 2004). Consequently, many postprandial metabolic processes are influenced by the time-of-day, including gastric emptying, intestinal absorption, glucose tolerance and postprandial energy expenditure or thermic effect of food (TEF) (Ruddick-Collins *et al.*, 2018). Chrono-disruption occurs following an alteration in the circadian rhythm stimulated by external stimuli. Shift workers, for example, exhibit an increased risk of chronic diseases such as cancer, CVD and the metabolic syndrome (Wang *et al.*, 2011). To a lesser extent, between day variability in meal pattern might cause sufficient chrono-disruption, by failing to entrain the peripheral clocks, to disrupt potentially beneficial physiological anticipation of dietary intakes at specific time points in the day.

A strength of this review is the adherence to Cochrane guidelines, PRISMA and the use of a range of quality assessment tools. However, the relatively small number of included studies and the small sample size in the majority of studies are limitations. The studies included in this review are heterogeneous with respect to the study population, methods and level of control which prohibited undertaking a meta-analysis.

The majority of the studies included were at low risk of bias and had good quality. Most studies were cross sectional which is considered to be the lowest level of the aetiology hierarchy as well as limiting the potential to attribute causal relationships (McNair and Lewis, 2012). Data collection for each participant is usually recorded at one time point. Cohort studies measured irregularity only at baseline and did not re assess irregularity when following up outcomes which limits confidence in attributing causality.

2.5 Conclusion

This systematic review investigated the association between inter- daily meal pattern variability and obesity; and associated health consequences. Many different methods for characterizing between day variability in meal pattern were identified which tended not to utilise all the potential properties of the meal pattern. Future work would usefully identify more comprehensive methods of capturing variability and ideally be used consistently across all studies. The limited number of intervention studies suggest causality between meal pattern irregularity and factors that may be indicative of poorer health, in the longer term. Longer studies are required, in general and clinical populations, in order to fully establish if adopting a less meal pattern is a low cost lifestyle intervention that would be beneficial to health.

CHAPTER 3: GENERAL METHODS

In this chapter, Study 2 refers to the effect of irregular meal pattern on nitrogen balance study, which is reported in Chapter 5, Study 3 refers to the feasibility study of RCT which is reported in Chapter 6.

3.1 Ethics and trial registration

All studies were run in the David Greenfield Human Physiology Unit, School of Life Sciences, Queen's Medical Centre, the University of Nottingham. All lab visits took place within the unit. The studies were approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (see individual study chapters for reference numbers). All studies involving participants were registered with clinicaltrials.gov (see individual study chapters for registration numbers)

3.2 Participants and recruitment

Participants were recruited from the student and staff populations of the University of Nottingham through poster and email advertisements and/or from the general public by an advertisement on a local website (callforparticipants.com). Irrespective of the method of advertising, key inclusion criteria for age, BMI and health were stated. Potential participants expressed an interest in participating via email. The study protocol was explained by providing a copy of the relevant participant information sheet by return email (Study 2: Appendix 1 and Study 3: Appendix 2). Participants who expressed that they remained interested in participating, were then invited to attend a screening visit to ensure they met with the full inclusion criteria of the study.

3.2.1. Inclusion criteria:

- Female (Study 2), and male and female (Study 3).

- Age between 18 and 40y (study 2) 18 and 45y (study 3).

- BMI between 18.5 and 25 kg/m^2 (study 2) and 18.5 and 30 kg/m^2 (study 3).

- Healthy with no self-reported history of serious medical conditions

assessed by questionnaire (Study 2: Appendix 1 and Study 3: Appendix 2).

- Not taking any medically prescribed medication other than the oral contraceptive pill.

- Non-smokers.

- Non-high-alcohol consumers (<14units a week to be included).

- Not pregnant or lactating.

- With regular menstruation or taking the oral contraceptive pill.

- Not currently dieting/seeking to lose weight.

- Weight was stable during the previous 3 months (self-reported weight change less than ±2 kg).

- No significant clinical symptoms of depression (score ≤10 using the Beck Depression Inventory).

- No evidence of eating disorder (score ≤20 on Eating Attitudes Test-26).

- No self-reported allergy or intolerance to any foods that would be provided during each study.

3.3 Randomization and blinding

A technician in the laboratory, who was not part of the research team, performed the randomization using online software (randomization.com) for the sequence of interventions in both Study 2 and Study 3. No blinding could be done due to the nature of the intervention. In Study 3, as the study design was parallel, age, sex and BMI would be matched in the full study between the intervention groups. This was not done in the feasibility study for practical reasons.

3.4 Screening

In the screening visit, which lasted approximately an hour, the study protocol was explained verbally to participants. It was emphasised that participants should only take part if they were willing to follow all the study instructions and consume the study menu. Written consent was obtained using the standard University of Nottingham consent form (Study 2: Appendix 1 and Study 3: Appendix 2). Then weight, and height were measured following standard procedures (CDC, 2007) (Section 3.4.1) in order to confirm participants' self-reported weight and height, hence their suitability for the study. Screening questionnaires were completed to assess medical health, sleep duration, physical

activity levels, eating behaviour and depression (Section 3.4.3). A blood sample (8 ml) (random in Study 2 and fasting in Study 3) was obtained by an experienced research technician via venipuncture for a routine blood investigation (Section 3.4.4). Eligible subjects were then required to complete a 3-day food diary (2 weekdays and 1 weekend) (Section 3.4.5) and asked to return the diary before beginning the study. Participants were made aware that they could withdraw from the study at any time without being required to provide any reasons.

3.4.1. Weight and height measurements

Body weight was measured using an electronic scale to the nearest 0.1 kg (Seca, Germany) whilst participants were wearing light clothing with no shoes. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Germany) according to the NHANES Anthropometry Procedures Manual as defined by the CDC (CDC, 2007). Body Mass Index (BMI) was calculated based on weight and height using the standard equation (Keys *et al.*, 1972):

BMI (kg/m^2) = Body mass (kg) / Height (m^2).

3.4.2. Body composition

Estimation of the participant's body composition was obtained by skinfold thickness measurement (Durnin and Womersley, 1974), which measures subcutaneous body fat and therefore, indicates body composition. The measurement was made while the participant stood in a relaxed condition at four sites, at the triceps, biceps, subscapular and suprailiac on the right side of the body using the pincer type callipers (John Bull British Indicators). At the selected sites, the calliper was used, where a fold of skin and subcutaneous fat were grasped firmly with the thumb and forefingers, pulling it away from the muscle tissue underneath. The jaws of the calliper were applied at a constant pressure, at right angles to the pinch and about 1cm below. A reading was taken within 2 seconds of applying the force. The measurements were made three times and then an average of the three readings for each site was determined.

- Biceps: a vertical fold on the front of the upper arm, at the midpoint of the muscle, directly above the centre of the cubital fossa, opposite the site of the triceps skinfold, with arm hanging vertically.
- Triceps: at the back of the upper arm, at a point equidistant between the tip of acromion and olecranon, with the arm hanging vertically.
- Subscapular: below and laterally to the tip of the inferior angle of the scapula, with the shoulder and arm relaxed.
- Suprailiac: Over iliac crest in mid-axillary line of the body, parallel to the cleavage lines of the skin.

The calculation of body fat % includes 4 skinfold sites measurement, triceps, biceps, subscapular and supra-iliac, and substituting the log of their sum into one of the following Durnin and Womersley equations to estimate body density (Table 3.1), based on the participant's age and sex (Durnin and Womersley, 1974). The density value can then be converted to percent body fat using Siri equations.

Table 3.1: Durnin and Womersley equations

Age (years)	Equations for men	Equations for women
17-19	D = 1.1620 - (0.0630 × L)	D = 1.1549 - (0.0678 × L)
20-29	D = 1.1631 - (0.0632 × L)	D = 1.1599 - (0.0717 × L)
30-39	D = 1.1422 - (0.0544 × L)	D = 1.1423 - (0.0632 × L)
40 -49	D = 1.1620 - (0.0700 × L)	D = 1.1333 - (0.0612 × L)
> 50	D = 1.1715 - (0.0779 × L)	D = 1.1339 - (0.0645 × L)

D: Density

The density result was placed into the Siri equation (Siri, 1956) to estimate % body fat:

% Body Fat = 495 / Body Density – 450 (White subjects)

% Body Fat = 437.4 / Body Density – 392.8 (Black subjects)

The same calliper was used by the same investigator throughout all study visits.

3.4.3. Questionnaires

Participants were required to complete the following questionnaires during the screening visit:

3.4.3.1. Beck Depression Inventory (BDI)

The BDI (Appendix 1) is a self-rating scale designed to assess the degree of depression (Beck *et al.*, 1961). It is considered as the most used tool for assessing depressive symptoms in the world (Richter *et al.*, 1998). The BDI consists of 21 items and each item refers to a symptom of depression and the respondent rates each item by using a four-point scale ranging from zero to three. Some items have more than one statement marked with the same score which are labeled as a, and b (for example, 2a and 2b).

The result of this questionnaire was found based on the total scoring. Total score of 0-9 was considered minimal depression, 10-18 mild depression, 19-29 moderate depression, and 30-63 severe depression. Participants who scored ≥ 10 on the BDI were excluded from taking part to avoid the potentially confounding impact of depression on appetite and body weight (Paykel, 1977, Weissenburger *et al.*, 1986).

3.4.3.2. Eating Attitudes Test-26 (EAT-26)

The EAT-26 (Appendix 1) is a self-report tool for measuring eating behaviour and attitude commonly observed in patients with anorexia nervosa and was established originally by Garner and Garfinkel (1979). It has become the most widely used instrument in different cultures and was shown to be effective in detecting eating disorders in the general population (Garner *et al.*, 1982, Nasser, 1997, Garfinkel and Newman, 2001, Pereira *et al.*, 2008).

The original Eating Attitudes Test-40 consisted of 40 items (Garner and Garfinkel, 1979). A response is given for each item on a 6-point forced choice likert scale. A score of 3 is presented for extreme eating disordered responses, a score of 2 for the adjacent, less extreme responses and a score of 1 for the next alternative. The other three remaining alternative answers are presented as no score. An overall score of higher than 30 is an indicator of an eating disorder.

The 26-item version (EAT-26) used in Study 1 and Study 2 was developed by performing a factor analysis of EAT-40 (Garner *et al.*, 1982) in order to enable an easier and faster instrument. 14 items were removed, as they did not load considerably on the three factors obtained which were: dieting (relating to avoidance of fattening foods and the preoccupation with being thinner), bulimia and food preoccupation (relating to thoughts about food and indications of bulimia), and oral control (relating to self-control of eating and perceived pressure from others to gain weight). EAT-26 is well associated with EAT-40 (r=0.98) and proved sufficient validity and reliability (Garner *et al.*, 1982). The score was given using the same scale as the original 40-item, but with a score of 20 as the cut-off point.

3.4.3.3. International Physical Activity Questionnaire (IPAQ)

The IPAQ (Appendix 1) is a self-reported instrument to measure physical activity (PA) levels over the previous 7 days. IPAQ has become one of the most commonly used physical activity questionnaires (Van Poppel *et al.*, 2010). It has been used and validated for adults 18 to 65 years old in diverse settings (Craig *et al.*, 2003). IPAQ identifies the level of physical activity by reporting three types of physical activity which are vigorous-intensity, moderate-intensity and walking. An overall score of physical activity is the sum of the frequency (days) and duration (min) of each type of activity. Three levels of physical activity are proposed as the final classification: low, moderate and high. The purpose of using the IPAQ in the screening visit was to estimate the energy requirement given to each participant (Section 3.6.1, estimation of energy requirement).

3.4.4. Routine blood tests

During the screening visit, a random blood sample (8-10 ml) in Study 2 and fasting blood sample in Study 3 were taken from participants by an experienced research technician from the cubital vein. Samples were analyzed for full blood count, urea and electrolytes in all studies with the addition of blood glucose in Study 3. This was to confirm that the participant was healthy. The blood samples for the full blood count, urea and electrolytes were analysed by the Clinical Chemistry and Haematology Laboratories in the Queen's Medical Centre, University of Nottingham. Blood glucose was measured immediately

using a Yellow Spring Instrument analyzer device (YSI). Abnormal results were reviewed by a qualified doctor who confirmed the suitability of the participant to take part in the study.

3.4.5. Food diaries

Participants were given a 3-day food diary (2 weekdays and 1 weekend), which included a record of the time of eating (Appendix 1). The purpose of using food diaries was to characterize their habitual dietary intake which they were required to adhere to whilst recording. They were given a digital scale (Salter Aquatronic Electronic Digital Kitchen Scales, Salter housewares LTD, Tonbridge, UK) and verbal and written instructions for weighing and recording all food and drink consumed over the 3 days. Participants were asked to return the diaries before starting the study intervention.

Data from the records were entered and analysed using a food composition program available in (Nutritics.com). Daily energy intake and macronutrients composition were calculated from each day for each participant and the results were shown as the averages of the 3 days.

3.5 Main study protocols

Study 2 had a randomised crossover design with two 7-day intervention periods, separated by a wash-out period of 3 weeks. Participants consumed their habitual diet during the wash-out period, which was included to avoid interaction between the two interventions. The protocol of Study 2 is shown in Figure 3.1.

Study 3 had a randomised, parallel design with 7 days as a pre-standardisation period during which participants consumed their estimated energy requirement as 6 meals per day. Participants then adopted one of two meal patterns for the 14-day intervention period when they consumed a hypoenergetic diet, followed by a further 3 days (post-standarisation period) consuming their estimated energy requirement as 6 meals per day. The protocol for Study 3 is outlined in Figure 3.2. Participants were required to follow a sleep- light protocol during the pre-standardisation period, intervention period and post standardisation period in Study 3.

Participants were otherwise free living but required to consume food provided by the investigator during the intervention periods. The meal pattern intervention period consisted of consuming:

1. a regular meal pattern (consuming the same number of meals every day), and/or

2. an irregular meal pattern (consuming a different number of meals every day resulting in a mean of 6 meals per day over a 7-day period.

Participants in both Studies 2 and 3 attended the laboratory for screening and then for returning the 3-day food diary. Then in Study 2, participants attended the laboratory every day during the intervention period (7 days), except on the weekend, in order to deliver their 24-h urine sample from the previous day, for both intervention periods. In Study 3 participants attended the laboratory for 6 visits. This included pre and post intervention period visits.

In order to avoid the potential impact on outcome measures of the stage in the menstrual cycle (Solomon *et al.*, 1982, Dye and Blundell, 1997, Davidsen *et al.*, 2007, McNeil and Doucet, 2012), the start of each intervention was identical for each participant in terms of menstrual period which was immediately after finishing the period in Study 2 and (days 1-7) in Study 3.

Figure 3.1: Study 2 protocol

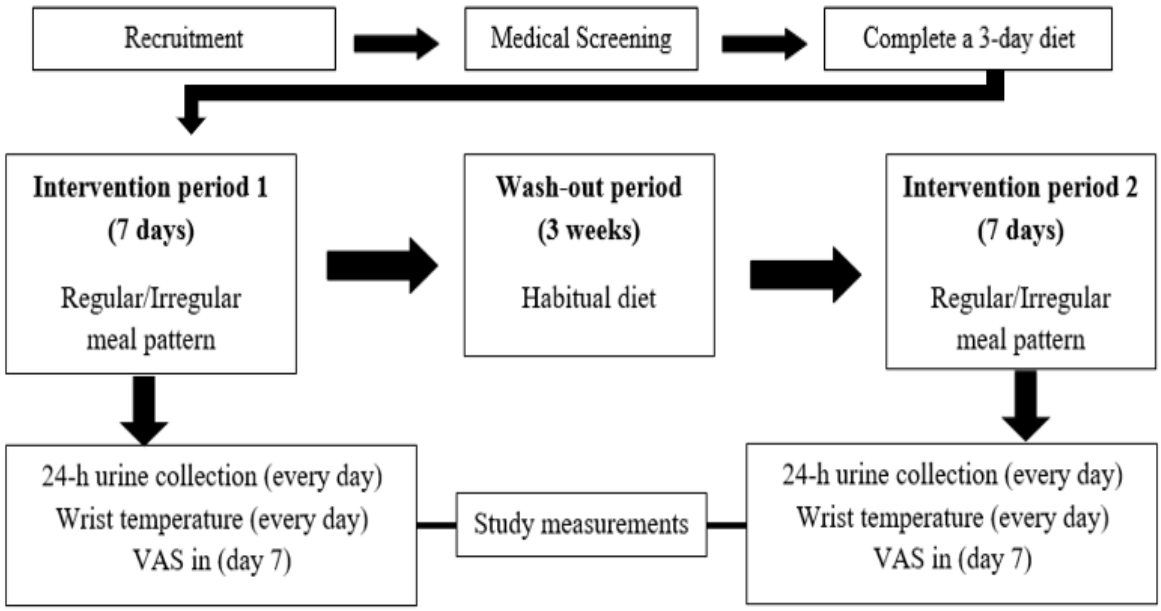


Figure 3.2: Study 3 protocol

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	Standardization period							Pre	Intervention period														Post	Post intervention period		
	-7	-6	-5	-4	-3	-2	-1	Lab day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Lab day	+1	+2	+3
R	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
IR	6	6	6	6	6	6	6	6	7	4	9	3	5	8	6	5	9	8	3	4	7	6	6	6	6	6
TEF								√															√			
Fasting blood sample for lipids glucose insulin Gut Hormones								√															√			
CGM for glucose	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Core temperature					√	√	√																	√	√	√
Wrist temperature	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Appetite ratings							√	√							√							√	√	√		
Sleep diary	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

3.5.1. Dietary intervention periods

Participants were required to follow either two meal patterns in crossover design for a week (Study 2) or one meal pattern in parallel design for 2 weeks (Study 3). Following the design of a previous study in the University of Nottingham (Alhussain, 2016), these meal patterns consisted of consuming 1) a regular meal pattern of 6 daily meals, or 2) an irregular one consisting of consuming a different number of meals every day (between 3 and 9). It was arranged in such a way as to achieve an average figure of 6 meals/day during the intervention period for 7-day (i.e. 7, 4, 9, 3, 5, 8, 6 meals per day) and for 14-day (i.e. 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, 6 meals per day).

To avoid the potentially confounding effect of time of eating, participants were asked to eat the meals at specific times between 08:00 and 21:00 (Table 3.2), during both interventions. The only deviation from this instruction was that when they had 3 meals/day, during the irregular period, their last meal was at 18:00 instead of 21:00 in order to allow participants to eat with their families. The meals time were the same as in the previous studies (Alhussain *et al*, 2016).

Table 3.2: Study meals time

	Number of eating occasion (daily meals)						
	3	**4**	**5**	**6**	**7**	**8**	**9**
Time*	08:00	08:00	08:00	08:00	08:00	08:00	08:00
			11:00	11:00	11:00	11:00	09:30
							11:00
	12:30	12:30	12:30	12:30	12:30	12:30	12:30
						14:00	14:00
				15:30	15:30	15:30	15:30
	18:00	18:00	18:00	18:00	18:00	18:00	18:00
					19:30	19:30	19:30
		21:00	21:00	21:00	21:00	21:00	21:00

*Time is based on a 24 h clock.

Each of the intervention periods had identical foods. All foods to be consumed were provided free of charge and were supplied in a 4-day cycle of menus consisting of a variety of food items commonly consumed in the British diet. An example of one day menu (1900

kcal) is shown in Table 3.3. Participants were assigned the menus that was closest to their energy requirement in the particular phase of the study (± 100 kcal). The macronutrient composition of the diet, as a percentage of total energy for the day, was approximately 50% carbohydrates, 35 % fat and 15 % protein. These macronutrient percentages were based on the Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy (COMA) (Department of Health, 1991). The menu was designed to supply energy amounts estimated to meet participants' energy requirement for weight maintenance in Study 2 and to provide 800 kcal/day less than estimated energy requirements in Study 3. Energy requirements were based on the Henry equation (2005) multiplied by an activity factor which was determined through an activity questionnaire completed during screening (Section 3.4.3.3).

The food that was provided was weighed, packaged and labelled by the experimenter before being given to the participants. All participants declared an intention to consume all the food supplied. However, they were asked to record any left-over food in the compliance diary provided (Appendix 1). Participants were strictly instructed not to consume any other food or drink (except water) outside the study menus. Moreover, they were also instructed to avoid drinking alcohol, limit the coffee or tea to two cups per day (without sugar and milk) during the intervention periods. They were advised to maintain their normal physical activity and not participate in any new physical activity/exercise program other than that reported at the beginning of the study in the screening visit.

Table 3.3: Example of a 1900 kcal menu

Meal No	Meal Time	Meal	Energy /kcal	CHO	Pro	Fat	Na /mg
1	8.00	Whole milk 100ml+ 2 cubes sugar Hovis Wholemeal Bread 2 slices Butter 10g 2 rich tea biscuits	372	53	13	34	478.3
2	11.00	Muller Corner Yogurt with Strawberry 1 pot Kellogg's Frosties Bar 1 pack	271	58	12	30	150.1
3	12.30	Sainsbury's macaroni cheese ¾ pack (250g) 1 medium Apple	435	55	11	34	452.1
4	15.30	Petits Filous Raspberry & Strawberry Fromage Frais 1 pot 85g Oatcakes 3 pieces	219	54	15	31	76.0
5	18.00	Uncle Ben's Vegetable Rice ¾ pack (120g) + Tuna can Orange Juice (150m)l	446	50	26	24	457.0
6	21.00	Whole Milk 100ml 3 rich tea biscuits	160	44	14	42	121.7
Total Daily			**1903**	**52**	**15**	**33**	**1735.2**

3.5.2. Estimation of energy requirement for the intervention and standardization periods

The energy requirement is defined as the amount of food energy necessary to balance energy expenditure to maintain body size, body composition and a level of required physical activity consistent with good health (Macdonald, 2000). For each participant, an individual energy requirement was estimated based on their estimated BMR and multiplied by a Physical Activity Level reflecting their level of activity, as established by the IPAQ questionnaire.

3.5.2.1. Estimated basal metabolic rate

BMR (kcal/day) was calculated in both studies using the Henry equations including body weight and height (Henry, 2005) for women and men aged 18-30 and 30-60 years. Based on the participant's age and sex, the option of the equation was determined:

Female:

18-30 years: BMR = 10.4 x weight (kg) + 615 x height (m) + (-282)

30-60 years: BMR = 8.18 x weight (kg) + 502 x height (m) + (-11.6)

Male:

18-30 years: BMR = 14.4 x weight (kg) + 313 x height (m) + (113)

30-60 years: BMR = 11.4 x weight (kg) + 541 x height (m) + (-137)

3.5.2.2. Physical activity level

IPAQ was used to estimate the level of participant's physical activity as (low = non active, moderate = moderately active and high = active) (Section 3.4.3.3). The COMA on Energy and Protein Requirements suggest the following activity levels based on intensity of occupational work (Table 3.4) (Department of Health, 1991).

Table 3.4: Physical activity levels based on sex

	Light	**Moderate**	**Heavy**
Men	1.55	1.78	2.10
Women	1.56	1.64	1.82

3.5.3. Measurements made during the intervention periods

3.5.3.1. Ambulatory energy expenditure estimation

To enable a comparison of activity between interventions, a BodyMedia SenseWear™ Armband (SWA), (Model MF-SW, BodyMedia Inc, Pittsburgh, PA, USA) was worn to

assess energy expenditure and physical activity pattern in free living subjects. The device was placed on the back of the upper left arm (the tricep muscle), halfway between the acromion process of the scapula and the elbow. The Armband logo was required to face upward towards the shoulder and the silver sensors, on the underside of the Armband, were to be in contact with skin. Participants were instructed to wear the device continuously, including while sleeping and to remove it only for brief periods for bathing, showering or swimming (Appendix 1).

3.5.3.1.1. SWA function

The SWA is a device that can be used to quantify energy expenditure and physical activity in free-living subjects. The device is portable and designed to be worn on the upper arm over the triceps. It works by internal sensors which include an accelerometer, a thermal flow sensor, a galvanic sensor that records skin response, a skin temperature sensor, and an air temperature sensor which continuously records and analyses the physiological parameters (Santos-Lozano *et al.,* 2017). The accelerometer in the armband has three axes and uses a microelectromechanical sensor that measures movement.

3.5.3.1.2 Analysis of SWA data

The data were downloaded using BodyMedia SenseWear® 7.0 software which was created by the manufacturer. Energy expenditure (kcal/min) and physical activity level as metabolic equivalence (METs) were calculated using an algorithm that combines acceleration, heat flow, other parameters, and the subject's demographic characteristics (age, gender, height and weight.

3.5.3.2. Nitrogen balance

In order to measure the N balance, 24-h urine collection for each day of intervention periods was undertaken. Each participant was given bottles and a 'shewee' to aid collection. Written instructions on how to collect the urine over the period of 24-h urine collection were given (Appendix 1). The first urine sample of the first day of the intervention week was discarded and then all urine after that was to be collected until the first-time urine after

waking up on the next day of the intervention week. Thus, the first urine sample from 2nd day should be collected in the previous day bottle.

3.5.3.2.1. Urinary urea

Participants were asked to visit the laboratory on each weekday of the intervention period to deliver their 24-hour urine samples. At weekends they were instructed to keep the urine samples in a cold place to avoid degradation. Once the participant had delivered the sample to the lab, the volume of the 24-h sample was measured and a 1 ml aliquot was taken after mixing well, then, the aliquot was stored in a -70 °C (HTA accredited) freezer (Berkel and Boer, 2010). The urea was analysed then by an experienced biochemist, Sally Cordon, using the HORIBA, (France) machine at the end of each intervention week.

3.5.3.2.2. HORIBA (Urea analysis)

HORIBA is a programmable spectrophotometer which is used to measure the amount of chemicals in a solution by measuring the strength of light absorption as a light beam passes through the sample. The fundamental theory is that light is absorbed or emitted over a certain wavelength spectrum by each compound (Albert *et al.*, 2012).

3.5.3.2.3. Nitrogen loss, intake and balance calculations

After receiving the results of urea concentration (mmol/l), the nitrogen output was calculated by the following steps:

The molecular weight of urea CO $(NH_2)_2$ = 60.06 (g/mol) (Woods, 1970) and nitrogen N_2 = 28.014 (g/mol) (Barker, 1870).

The mass percentage of nitrogen in urea = 28.014/60.06 = 46.64%

1. Urea was converted from mmol/l to g/l using this equation:

(Urea (mmol/l) /1000) × 60.06

2. Urea g/24-h was found using this equation:

24-h urine volume/1000) × urea g/l

3. The nitrogen g/24-h from the urea was calculated using this equation:

Urea g/24-h × 0.4664

Nitrogen balance was calculated for each participant in each study day based on the nitrogen input from food, output in urinary urea and other estimated nitrogen losses using the equation:

N balance = N intake (Protein intake/6.25)- N output + 4

"4" represents N derived from the urinary non-urea, feces and dermal losses (Dickerson, 2005).

3.5.3.3. Appetite

Participants were provided with a set of VAS forms, in which to record subjective appetite before and after each meal in both intervention periods when 6 meals/day were consumed on day 7 in study 2 and on day 7 and day 14 in study 3. The VAS form was used to measure subjective appetite ratings with words anchored at each end of a 100-mm horizontal line that reflected the most positive and the most negative rating for a question.

3.5.3.3.1. VAS

The VAS consists of 5 questions which were in the form 'How (rating) do you feel?' and the ratings were 'hungry', 'satisfied', 'full', and 'much of thinking to eat' (Flint *et al.*, 2000) (Appendix 1). Participants were required to make a vertical mark through the horizontal line providing a score between 0 to 100 mm expressing their current feeling.

3.5.3.3.2. Analysis of VAS data

Quantification of each subjective appetite rating (hunger, satiety, fullness and prospective food consumption) was done by measuring the closest distance (mm) where the placed mark intersected the horizontal line using a plastic ruler.

3.5.3.4. Wrist temperature

The peripheral wrist temperature was assessed continuously during the intervention period in Study 2 and Study 3 by a wireless (thermochron iButton®, UK) temperature logger (model DS1922L). The iButton® was programmed to sample every 10 min with 0.0625 ◦C resolution and attached to a double-sided cotton sport wrist band using Velcro®, with

the sensor face of the iButton being placed over the inside of the wrist, on the radial artery of the non-dominant hand (Figure 3.3). Participants were instructed to wear it continuously, including whilst sleeping and to remove it only for brief periods for bathing, showering or swimming and record the time of removing and wearing the wrist band using a paper record (Appendix 1).

3.5.3.4.1. Thermochron iButton® temperature logger

The iButton® device (DS1922L) is a rugged, self-sufficient system that is designed to measure temperature and record the result in a protected memory section. The recording is done at a defined rate. A total of 8192 8-bit readings or 4096 16-bit readings, taken at equidistant intervals ranging from 1s to 273hr, can be stored. Additionally, 512 bytes of Static random-access memory (SRAM) store application specific information and 64 bytes store calibration data. A period of data collection can be programmed to begin immediately, after a defined delay, or after a temperature alarm. DS1922L provides digitised readings of temperature, with a determined resolution, set at the time of programming the device, of either 0.5 ◦C or 0.0625 ◦C. Access to the memory and control functions can be password protected. The DS1922L is configured and communicates with a host-computing device through a serial 1-Wire® protocol, which requires a single data lead and a ground return (Maxim Integrated, 2015).

Figure 3.3: Thermochron iButton® temperature logger and wrist band

3.5.3.4.2. Analysis of the data for wrist temperature

The wrist temperature data for the last day of each intervention (day 7) in Study 2 (day 14) in Study 3 (when the number of meals was 6 on both interventions) was downloaded from the iButton in excel sheets and cleaned by removing the values which were not related to the wrist temperature as participants took off the wrist band when showering or swimming. The range of wrist temperature was selected between a 32 and 37 °C based on previous study (Sarabia *et al.*, 2008). The mean value of 10 min wrist temperatures over an hour was calculated.

Wrist temperatures were analysed using cosine curve fitting via Prism software. After applying cosinor analysis for a 24-h period, the comparison of fits including null hypothesis, alternative hypothesis and P value was found. Then, best fit values, standard error and 95% CL of Mesor, Amplitude and Achrophase were calculated for each participant.

3.5.3.4.3. Sleep-wake routine

Participants were requested to follow a fixed sleep-wake (and light exposure) routine (Wehrens *et al.*, 2017) pre instructions to minimise the variation in their sleep-wake and light exposure times. They were asked to fill out a sleep diary form (Appendix 2) in Study 3. This was to control the masking effects of sleep and light during the diurnal rhythm measurement. They were instructed to:

1. Go to bed between 22:45 and 23:15 and get out of bed between 6:45 and 7:15 without deviation from their agreed in-bed and get-up times by more than 10 minutes each night and remain in the dark, trying to sleep, without distraction (such as light emitting devices).
2. Take a nap (if they choose to do so) within a 4 h window in the afternoon (centred 12 hours away from the midpoint of their night-time sleep). Naps scheduled this way are thought not to phase shift circadian rhythms.
3. Obtain 15 min exposure to outdoor natural light (without wearing sunglasses) within 90 min of waking up to strengthen the synchronisation of the circadian rhythms.

4. Consume less than 100 mg caffeine within the first 3 hours after waking and up to 4 units of alcohol per day.

3.5.4. Measurements during pre and post standardization periods in Study 3

3.5.4.1. Wrist temperature

The peripheral wrist temperature was assessed continuously during the whole study period including pre and post standardization periods in Study 3 by a wireless (thermochron iButton®, UK) temperature logger DS1922L (Section 3.5.3.4).

3.5.4.2. Core body temperature

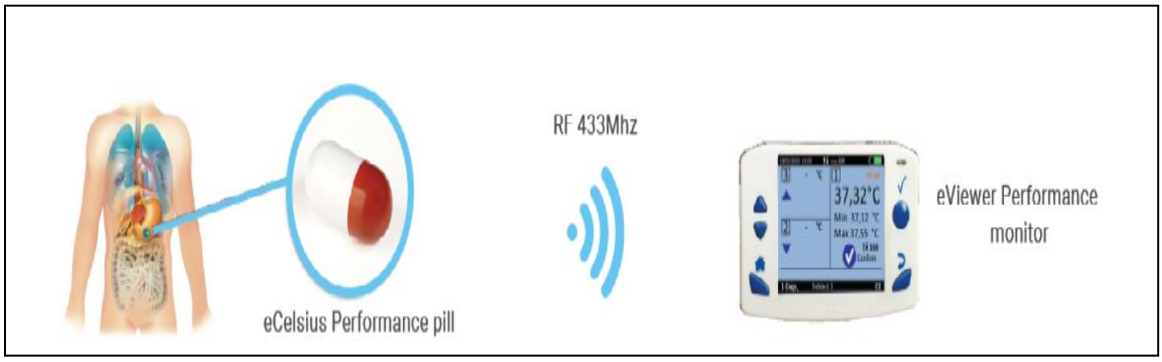
The core temperature was assessed before and after the intervention period in Study 2 by an ingestible telemetry e-Celsius Performance pill and associated e-Viewer Performance monitor (BodyCap, France). The e-Celsius Performance pill was configured by e-Performance Manager software v1.3.2. and activated by e-Celsius Performance activator. After activation, the pill was ingested with water. Every 30 seconds, the e-Celsius pill wirelessly transmits internal temperature measurements to an external monitor, called the e-Viewer Performance monitor. The pill usually takes one or two days to leave the gastro-intestinal system, depending on individual transit time. The participant was instructed to keep the monitor beside them at all the time to be in the communication range of the pill. At all times the participants wore an information alert bracelet stating that they had ingested an MRI- incompatible electronic device and should not undergo an MRI investigation (Appendix 2).

3.5.4.2.1. e-Celsius Performance pill and e-Viewer Performance monitor

e-Celsius Performance from BodyCap is a device designed for continuous core temperature monitoring. The ingestible pill continuously monitors and records core body temperature and has an internal memory embedded, enabling guaranteed continuous recording in any environment without restricting movement. The pill is delivered individually packaged. Once activated, it communicates in real time, and continuously transmits the core temperature data and needs from 15 to 30 mins after ingestion until capturing the core body temperature. The single-use e-Celsius® Performance pill is removed naturally on defaecation, allowing it to monitor the core temperature over a period corresponding to the

gastrointestinal transit time (24-48 hours on average). The temperature accuracy is ±0.2 °C (±0.36 °F), the temperature resolution is 0.01C (0.03202 °F) and the temperature range is from 25 °C – 45 °C (77-113 °F). The e-Viewer® Performance monitor receives and records the data transmitted by the capsule (e-Celsius® Performance, 2020). The communication distance between the capsule and the monitor ranges from 1 to 3m (depending on environment and subject). The pill also has its own memory. In case of a communication disruption between the monitor and the pill, data continue to be captured by the capsule and automatic synchronization is resumed once communication re-established. The same monitor can manage up to three capsules simultaneously in order to manage several subjects in parallel or for extended use and store 150000 items of data per pill. The pill is not reused (Figure 3.4).

Figure 3.4: e-Celsius Performance pill and e-Viewer Performance monitor



3.5.4.2.2. Analysis of the data for core temperature

The core temperature data for pre- and post- intervention standardisation periods in Study 3 from the e-Celsius pill was downloaded in excel sheets. Data of a complete 24h period starting from 8:00 am on the day after pill ingestion was selected to be used for analysis in order to standardise the measured period for all participants as each participant has a different pill ingestion time. Core temperatures were analysed with the same method of wrist temperature analysis using cosine curve fitting via Prism software. After applying cosinor analysis for a 24-h period, the comparison of fits including null hypothesis, alternative hypothesis and P value were found. Then, best fit values, standard error and 95% CL of Mesor, Amplitude and Achrophase were calculated for each participant.

3.5.4.2.3. Sleep-wake routine

Participants were instructed to follow fixed sleep-wake (and light exposure) routine and fill out a sleep diary form in study 3 to minimize the variation in their sleep-wake time and light exposure time (section 3.5.3.4.3).

3.5.4.3. Interstitial glucose

The freestyle libre 2 (Abbott, UK) Continuous Glucose Monitoring (CGM) (Figure 3.5) was used to continuously monitor glucose in interstitial fluid for the whole of study 2 period (7-day standardisation period, pre and post intervention lab visits, 14-day intervention period, 3-day post intervention standardisation period). The FreeStyle Libre 2 sensor was placed before starting the study on to the back of the upper arm with a simple, disposable device called an applicator. With the Freestyle Libre there is no need to take any finger prick glucose reading to calibrate the CGM. The participant was instructed to scan the sensor once every eight hours by the reader, this is because the sensor holds the data for eight hours at a time and to keep the reader dry, clean and always charged. A 24 h contact number was written for each participant for any queries or if problems arose

3.5.4.3.1. CGM

Freestyle Libre2 CGM (Figure 3.5) works through a chemical glucose oxidase mechanism for glucose measurement and uses subcutaneous, wired enzyme glucose sensing technology to automatically detect glucose levels in interstitial fluid (Heller and Feldman, 2010; Blum, 2018). A disposable sensor, which can be placed for 14 days, incorporating glucose oxidase enzyme is used to convert interstitial glucose into electronic signals and sends the electronic signals continuously to a portable monitor which has a memory for storage. Glucose was automatically measured every minute, and readings were recorded and stored at 15-minute intervals (Hirsch *et al.*, 2019).

The Libre2 Reader should be scanned near the sensor when a glucose reading is needed and for every 8 hours, as the sensor holds eight hours of data at a time. The reader device can display the glucose information, including current glucose, a trend graph, and a trend arrow. The arrow indicates the direction and velocity of the patient's current glucose level. This technology is factory-calibrated, and patients do not have to calibrate with blood

sample glucose meter readings (Blum, 2018). Recording data can be uploaded into (Libre View) software after signing up, which is easy, free and accessible online, so no special hardware is needed. CGM normal range is shown in Table 3.5 (Hirsch *et al.*, 2019).

Table 3.5: Glucose range in Freestyle Libre2 CGM

Reading	Indication
3.9 – 10.0 mmol/L	Normal
10.1 – 13.9 mmol/L	High
> 13.9 mmol/L	Very high
3.0 – 3.8 mmol/L	Low
< 3.0 mmol/L	Very low

Figure 3.5: Freestyle Libre2 CGM

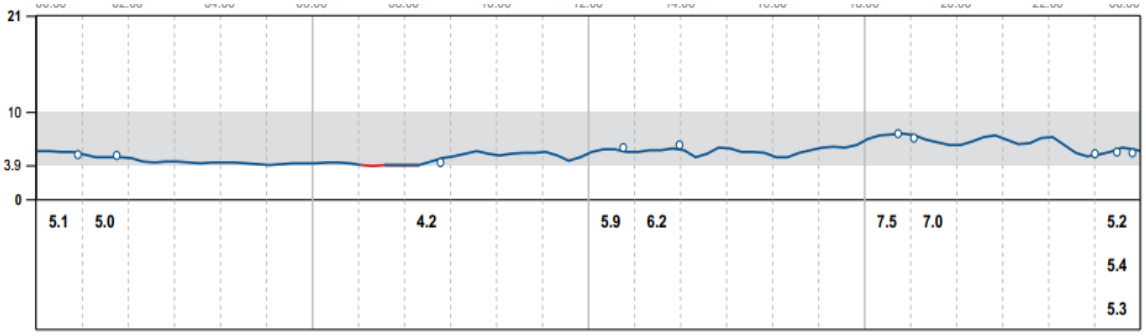
3.5.4.3.2. Analysis of CGM data

Data from CGM in Study 3 over the study period were downloaded and glucose profiles were evaluated based on the results collected on the day before the pre-lab visit, day after the post-lab visit and during the intervention period on day 7, 8 and 9 (Figure 3.6). The mean, maximum, minimum and Incremental Area Under the Curve (iAUC) were calculated per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00). Postprandial iAUC for 90 min was also calculated following each meal in all selected days

(which were day -1, day 7, day 8, day 9 and day +1) in regular and irregular interventions with the use of the trapezoidal method as following steps (Brouns *et al.*, 2005):

1. Subtract the baseline value (preprandial glucose period) from each value of 15-min periods of postprandial glucose
2. Sum the postprandial glucose values for the 2 periods (starting with the preprandial period (baseline and the next 15-min period of postprandial glucose).
3. Divide the result by 2.
4. Multiply the result by the measurement period which was 15 min
5. Sum iAUC for all postprandial periods.

Figure 3.6: Example of 24 h of CGM data



3.5.4.4. Subjective appetite

Participants were provided with a set of VAS forms to record subjective appetite before and after each meal in both intervention periods when 6 meals/day were consumed in (day -1) pre and (day +1) post standardisation periods (Section 3.5.3.3).

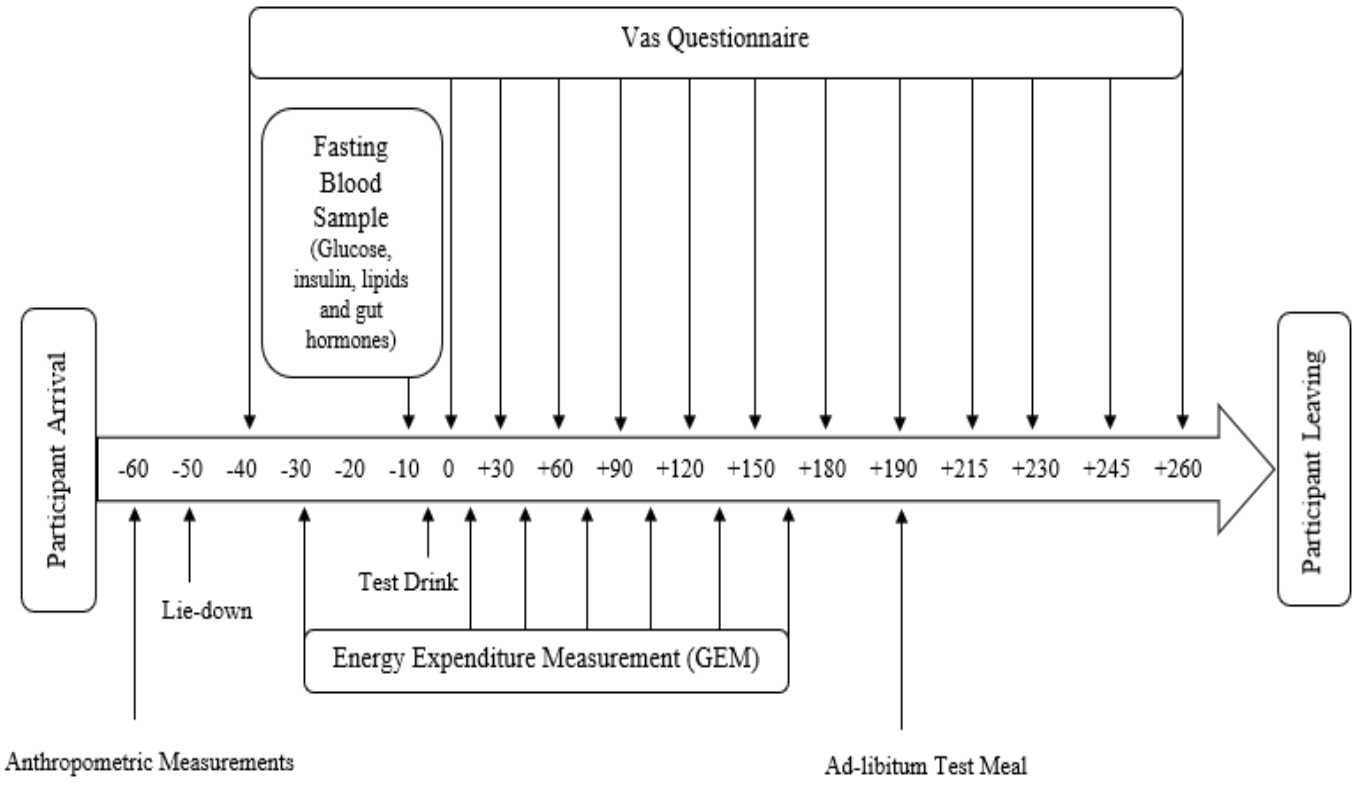
3.5.5. Pre and post intervention laboratory visits protocol in Study 3

The purpose of the pre and post intervention laboratory visits in Study 3, which lasted up to 5 h, was to assess the effect of regular and irregular meal patterns on metabolic variables, subjective appetite and ad-libitum food intake. The protocol is summarised in Figure 3.7. Participants were instructed to attend each laboratory visit at 8:00 am and to fast for a

minimum of 12 h overnight, before the laboratory visit. They also were required to avoid any exercise other than walking related to carrying out their daily normal activities for 48 h before the visit.

Immediately after arrival, weight and the other anthropometry measurements were taken (Section 3.5.5.1). Participants then rested in a semi-supine position in bed for 20 min in a temperature-controlled (23-24 °C) room. REE was measured for 20 min (Section 3.5.5.2). After a fasting blood sample was taken, a test drink (Section 3.5.5.4) was given. Then, TEF was measured for a further 3 h. An ad-libitum test meal was then consumed as a lunch (Section 3.5.5.5). Subjective appetite ratings (Section 3.5.3.3) were assessed during fasting, 3 h following the test drink and 1 h following the ad-libitum test meal.

Figure 3.7: Laboratory day visit protocol



3.5.5.1. Anthropometric measurements

3.5.5.1.1. Weight

Each participant's weight was taken in the fasted state wearing similar light clothes on each visit (details in Section 3.4.1). The same scale was used throughout all study visits.

3.5.5.1.2. Height

Height was taken during the first visit (details in Section 3.4.1).

3.5.5.1.3. BMI

BMI was calculated using the standard equation (details in Section 3.4.1).

3.5.5.1.4. Circumference measurements

Waist and hip circumferences were measured to the nearest 0.1 cm in a horizontal plane, a parallel level to the floor using a non-elastic measuring tape wrapped snugly, but not to the point that the tape was constricting. Waist circumference was taken while the participant was standing with feet about 25–30 cm apart and at a point midway between the lower margin of the last rib and the top of the iliac crest (WHO, 2008). Hip circumference was taken at the point yielding the maximum circumference over the buttocks of the participant. (WHO, 2008). Both measurements were made by the same investigator throughout all study visits to limit any variability. The waist to hip ratio was calculated by dividing waist circumference by hip circumference (WHO, 2008).

3.5.5.1.5 Body composition

The participant's body composition was estimated by skinfold thickness measurement (Durnin and Womersley, 1974), which measures subcutaneous body fat and therefore, indicates body composition (details in Section 3.4.2).

3.5.5.2. Energy expenditure measurement

Energy expenditure was measured using an Open Circuit Indirect Calorimeter (Gas Exchange Measurement (GEM system; Europa Scientific Ltd, UK)). After taking the anthropometric measurements, the participant was asked to rest semi-supine on a bed and relax for 20 min. Then, a ventilated hood was placed over the head of the participant head, with the surrounding pliable plastic sheet secured in place (Figure 3.8). Air for respiration

passes through the holes in the hood, whilst expired and room air in the hood were extracted through a tube connecting the hood to the indirect calorimetry machine. REE was measured in the fasted state for 20 min. TEF was then measured for periods of 20 min at 30 min intervals during the 3 h after test drink consumption. The participant was instructed to relax and breathe normally during the measurements. Sleeping, reading, or listening to music was not allowed while the measurement was performed. However, the participant was allowed to read in the intervals between the measurements while resting on the bed.

Figure 3.8: Energy expenditure measurement

3.5.5.2.1. Indirect calorimetry

The GEM is an open circuit indirect calorimeter designed to estimate energy expenditure and substrate turnover through measuring respiratory gas exchange and respiratory quotient (RQ) through O_2 and CO_2 concentrations of inspired and expired air. This is considered to be the most convenient way for measuring energy expenditure in human

studies at rest (Fellows and Macdonald, 1985). Flow rate is continually measured to use in combination with the gas analysis.

An air flow through hood, with a mass flow meter, mixing chamber and a vacuum pump, was used to draw room air over the participant's face at a rate of 50-60 l/min. The machine was connected to a computer, and data from the mass flow meter and gas analysers were used to calculate the volume of O_2 and CO_2 using the software provided by the manufacturer. The respiratory gas exchange data were then converted to energy expenditure by using the Weir equation (Weir, 1949):

Energy expenditure (kJ/min) = rate of airflow in a ventilated hood (STPD l/min) x (% O_2 in inspired air - % O_2 in expired air) x 0.2087

The principle of the Weir equation is based on measurement of the airflow rate in a ventilated hood and the changes in percentage O_2 concentrations between inspired and expired air. STPD is the standard temperature and pressure of dry air. Protein utilisation is not required to be measured in this equation. 0.2087 is a factor based on protein reflecting approximately 12.5% of total energy intake. The error in energy expenditure measurement is less than 0.5 % for 0-25 % of protein intake from total energy.

3.5.5.2.2. Indirect calorimetry validation and calibration

Before starting the study, a validation run using alcohol was done to ensure valid values were being obtained using the indirect calorimeter. A known amount of alcohol (ethanol) was burnt under a special hood for each 20 min run. This procedure was repeated monthly, and the results indicated that the calorimeter was operating appropriately as it was measuring accurately in respect of the alcohol respiratory exchange ratio. These were close to the expected value of 0.667 and the recovery percentage of the respiratory quotient was 98 %, indicating that the measurement and calculation were at an acceptable level, as recovery percentages should be 100% ±5% (Li *et al.*, 2019). Prior to the validation run and on the study days, the GEM was turned on for half an hour prior to use, to warm up. Then calibration of the gas analysers was done using two cylinders of pressurised gas of known composition (oxygen (O_2) and carbon dioxide (CO_2)). This calibration was repeated between each 15-minute measurement over the study day. To ensure that the calibration

had been effectively undertaken, the values from calibrations must fit within these ranges shown in Table 3.6 (GEM, UK):

Table 3.6: Ranges of values from calibration

	O_2	CO_2
ZERO	-0.990 to +0.123	-0.020 to +0.020
SPAN	+19.587 to +21.110	+0.962 to +1.041
INSPIRED	+20.800 to +21.270	+0.047 to +0.139

3.5.5.2.3. Analysis of energy expenditure data from indirect calorimetry

All data for energy expenditure resulting from indirect calorimetry were downloaded in an excel spreadsheet for each 20-min measurement period before and 3-h after consuming the test drink. The data for each 20-min measurement were cleaned by removing the data for the first 5-min, to allow stabilizing of the energy expenditure. Then the remainder of the data for 15-min were averaged for each period (fasting, after 30 min, after 60 min, after 90 min, after 120 min, after 150 min and after 180 mins). iAUC of TEF (Figure 3.9) were calculated for each period and for 3-h with the use of the trapezoidal method and the following steps (Brouns *et al.*, 2005) (an example of the calculation in Table 3.7):

1. Subtract the baseline value (fasting energy expenditure period) from each value of energy expenditure in all periods.
2. Sum the energy expenditure values for the 2 periods (starting with the fasting period (baseline and the 30 min period).
3. Divide the result by 2.
4. Multiply the result by the measurement period which was 15 min.
5. Sum iAUC for all measurement periods.

Figure 3.9: Fasting and iAUC of energy expenditure (source: Brouns *et al.*, 2005)

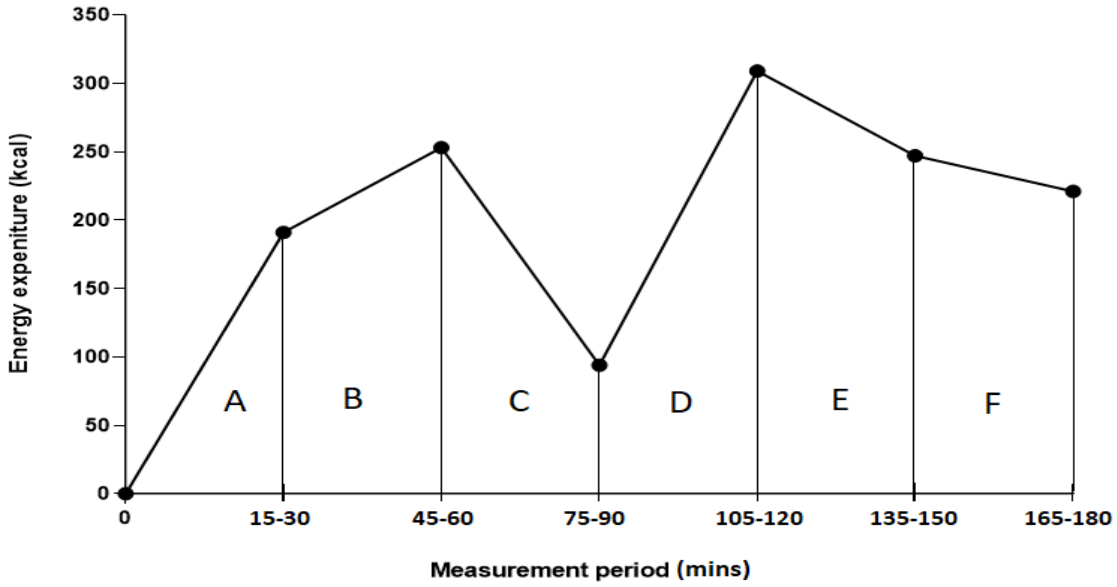


Table 3.7: An example for iAUC calculation of energy expenditure

	Measurement periods	Energy expenditure (kcal/day)	Baseline subtraction	iAUC (kcal)	iAUC (kcal) over 3h
	Fasting (0-15)	1170=78	0	0	
A	After 30 min (15-30)	1361=90.7	1360-1170=191	(0+191)/2 ×15=1432	
B	After 60 min (45-60)	1423=94.8	1423-1170=253	(191+253)/2× 15 =3330	
C	After 90 min (75-90)	1264=84.3	1264-1170=94	(253+94)/2 ×15 =2602.5	18067
D	After 120 min (105-120)	1479=98.6	1479-1170=309	(94+309)/2 ×15 =3022.5	
E	After 150 min (135-150)	1417=94.4	1417-1170=247	(309+247)/2 ×15 =4170	
F	After 180 min (165-180)	1391=92.7	1391-1170=221	(247+221)/2 ×15 =3510	

Incremental AUC = 18067/15 = 1204.4/60 = 20.1 kcal/min

3.5.5.3. Blood sampling and analysis

A 9.5 ml blood sample was taken in the fasted state just before ingestion of the test drink to assess the mean of fasting blood serum total, HDL, LDL-cholesterol, triacylglycerol, glucose, insulin, plasma Glucagon Like Peptide-1 (GLP-1), Peptide YY (PYY) and ghrelin. 5 ml was dispensed in a serum-separating (SS) tube to assess the mean of the fasting blood serum total, HDL, LDL cholesterol, triacylglycerol (TAGs). 0.5 ml was dispensed into microcentrifuge tube for glucose. 4 ml blood sample was distributed and dispensed into, (3 ml) Ethylenediamine Tetraacetic Acid (EDTA) tubes for GLP-1, PYY and ghrelin, and (1 ml) SS tube for insulin (allowed to clot for 30 min at room temperature before centrifugation). EDTA tubes containing either 20 µl dipeptidyl peptidase IV (DPP-IV) inhibitor (Millipore, Billerica, MA, USA) for GLP-1 measurement or 50 µl aprotinin (Nordic Pharma, Reading, UK) for PYY and ghrelin measurements (Figure 3.10). All samples were centrifuged (5702 R, Eppendorf, Germany) for 10 min at 3000 r.p.m at 4 ºC (Figure 3.11). The supernatant was aliquoted into plastic labelled tubes and kept at -80 ºC until further analysis.

Figure 3.10: Blood sample collection follow chart

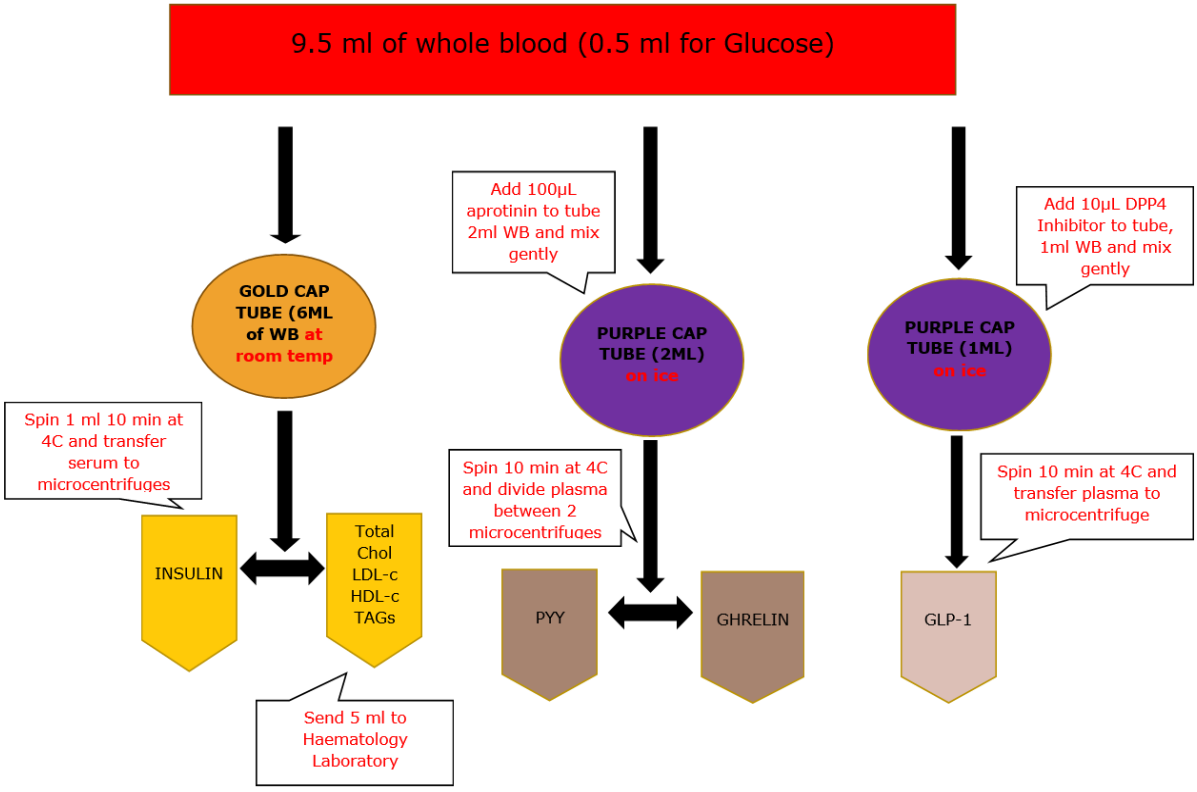
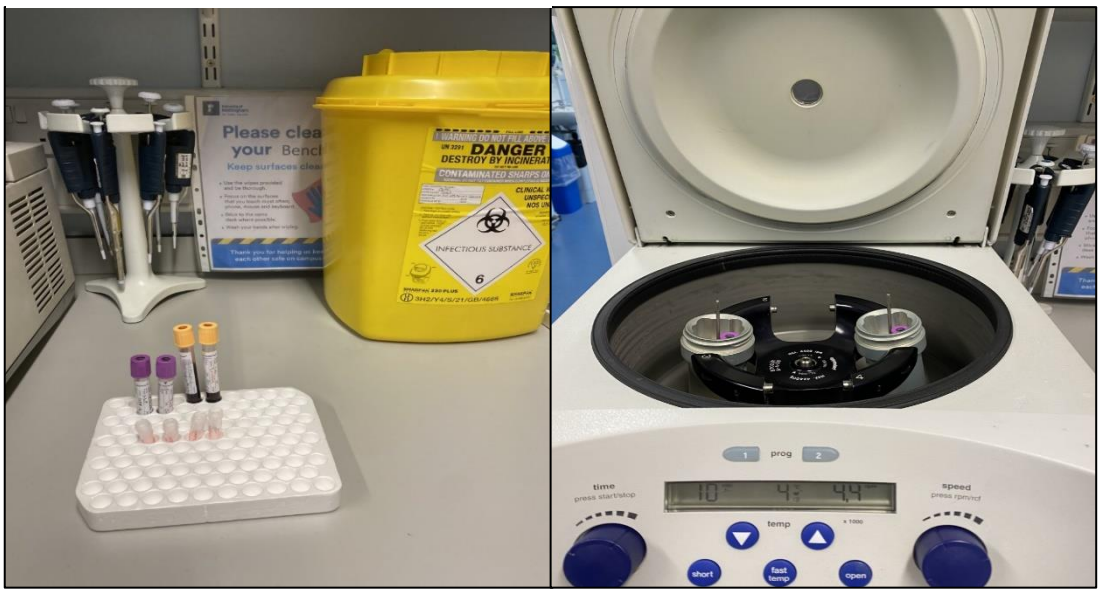


Figure 3.11: Blood sample processing



3.5.5.3.1. Lipids

Serum total, HDL, LDL-Cholesterol and triacylglycerol levels were analysed by the Clinical Chemistry and Haematology Laboratories in the Queen's Medical Centre, University of Nottingham.

3.5.5.3.2. Glucose

Blood glucose concentration was measured immediately using a Yellow Spring Instrument (YSI) 2300 STAT PLUS Glucose and Lactate Analyzer device (Yellow Springs, USA). This is a laboratory instrument designed to use in clinical care and sports medicine application to measure glucose in whole blood, serum or plasma. After calibration, a small amount of blood was placed, and the result was then displayed after 65 seconds. The glucose concentration working range (mmol/L) of the YSI is 0–27.8 mmol/L.

3.5.5.3.2.1. YSI 2300 STAT PLUS

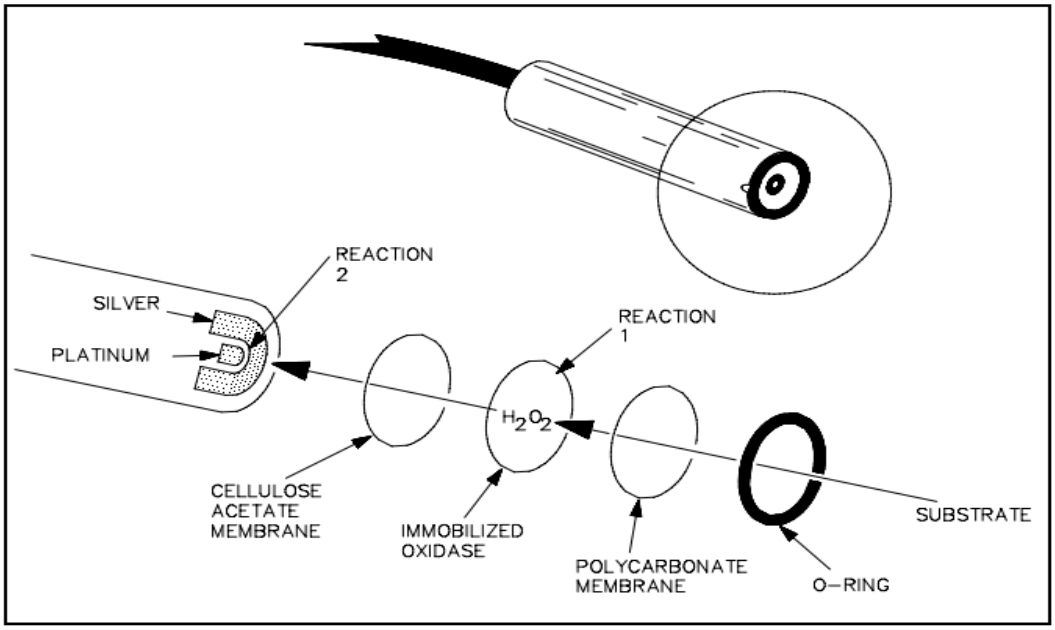
The YSI STAT 2300 is a device for the quantitative measurement of glucose concentrations, based on the use of immobilised glucose oxidase and a membrane covering a hydrogen peroxide sensor. After an initial calibration, 25 microliter of plasma is injected into a reaction chamber (Chua and Tan, (1978).

Each probe is fitted with a three-layer membrane containing immobilised enzyme in the middle layer. Figure 3.12 shows an exploded view of the membrane and it's linked to face of the probe (User's Manual for YSI 2300 STAT Plus™ Glucose and Lactate Analyzer, 2009). The face of the probe, covered by the membrane, is situated in a buffer-filled sample chamber into which a sample is injected. Some of the substrate diffuses through the membrane. When it contacts the immobilized oxidase enzyme, it is oxidised and then produces hydrogen peroxide. Using glucose as an example (Reaction 1). The hydrogen peroxide (H_2O_2) is, in turn, reacted and oxidized at the platinum anode, creating electrons (Reaction 2). A dynamic equilibrium is reached when the rate of H_2O_2 production and the rate at which H_2O_2 leaves the immobilised enzyme layer are constant and is shown by a steady state response. The electron flow is linearly proportional to the steady state H_2O_2 concentration and, therefore, to the concentration of the substrate.

REACTION 1 (glucose): β-D-glucose + O_2 $\xrightarrow{\text{Gox}}$ Glucono-δ-lactone + H_2O_2

REACTION 2: H_2O_2 $\xrightarrow{\text{Pt anode}}$ $2H^+ + O_2 + 2e^-$

Figure 3.12: Sensor Probe and Enzyme Membrane



The platinum electrode is held at an anodic potential and is capable of oxidising several substances other than H_2O_2. To avoid these reducing agents from contributing to sensor, the membrane includes an inner layer, consisting of a very thin cellulose acetate film. This cellulose acetate film crosses H_2O_2 but prevents chemical compounds with molecular weights above approximately 200. The film also preserves the platinum surface from proteins, detergents, and other substances that could foul it. Nevertheless, the cellulose acetate film can be diffused by different compounds such as hydrogen sulfide, low molecular weight mercaptans, hydrazines, hydroxylamines, anilines and phenols (User's

Manual for YSI 2300 STAT Plus™ Glucose and Lactate Analyzer, 2009). At 45 seconds, a digital result for glucose is displayed.

3.5.5.3.3. Insulin

Serum samples were defrosted, and serum insulin concentrations were measured by a commercially available Invitrogen ELISA kit KAQ1251 (Waltham, MA, USA). The low Quality control (QC) of assay performance was 10.9% and the high QC was 9.5% (Mean 10.2%). All samples were assayed in one assay to avoid inter-assay variation by Sally Cordon (SC).

3.5.5.3.4. GLP-1

Plasma samples, from whole blood collected in EDTA coated tubes containing DPP-IV inhibitor, were defrosted. GLP-1 concentrations were then measured using a Merck ELISA kit EGLP-35K (Millipore Corporation, Billerica, MA, USA). The low QC of assay performance was 15.3% and the high QC was 7.0% (Mean 11.2%). All samples were assayed in one assay to avoid inter-assay variation by SC.

3.5.5.3.5. PYY

Plasma samples, from whole blood collected in EDTA coated tubes containing aprotinin, were defrosted. PYY concentrations were then measured with a Merck ELISA kit EZHPYY66K (Millipore Corporation, Billerica, MA, USA). The low QC pf assay performance was 4.0% and the high QC was 6.6% (Mean 5.3%). All samples were assayed in one assay to avoid inter-assay variation by SC.

3.5.5.3.6. Ghrelin

Plasma samples, from whole blood collected in EDTA coated tube containing aprotinin, were defrosted. Plasma total ghrelin concentrations were then measured with an Invitrogen ELISA kit BMS2192 (Waltham, MA, USA). The low QC of assay performance was 10.3% and the high QC was 7.7% (Mean 9%). All samples were assayed in one assay to avoid inter-assay variation by SC.

3.5.5.4. The test drink

The test drink (Ensure® Compact, Abbott, UK) vanilla flavour milkshake was given in a glass as a breakfast at room temperature (19 ºC). The participant was instructed to drink it over a period of 5 min. The milkshake test drink consisted of 48 % of energy as carbohydrate, 35 % as fat, and 17 % as protein (Table 3.8). The amount of test drink provided for the participants with a BMI less than 25kg/m^2 was 10 kcal/kg actual body weight. For those participants with a BMI above 25kg/m^2, the weight they would be if they were to have a BMI of 22.5 kg/m^2 was calculated using their measured height. The resulting weight was then used to calculate the feed, based on 10 kcal/kg. An adult healthy participant needs to have about 35 kcal/kg body weight in order to achieve a stable body weight (Scientific Advisory Committee on Nutrition, 2011). The test drink provided approximately one third of this value as a breakfast to stimulate a relatively large metabolic response which would return to the baseline 3 h postprandially.

Table 3.8: Dietary composition of the test drink

Ensure® Compact	CHO	Protein	Fat
125 ml (300 kcal)	36 g	13 g	12 g
% Energy	47.9%	17%	35.1%

3.5.5.5. Ad-libitum test meal

A pasta-based test meal providing 167 kcal/100 g with 13, 34 and 53 % energy provided by protein, fat and carbohydrate, respectively was offered at lunchtime to assess ad-libitum food intake. This meal had a homogeneous nature, so energy intake could be assessed from the weight of food consumed. The meal was prepared using increments of 125 g of dry pasta (Sainsbury's, London, UK) cooked in 800ml of boiling water- (microwaved on full power for 7 minutes, mixed and then microwaved for a further 6 minutes). The cooked pasta was then drained in a colander and rinsed with cold water and then mixed with 40 g of cheddar cheese (Sainsbury's) that has been pre-portioned and frozen in a -20°C freezer until required, 15 g of olive oil (Sainsbury's), and 170 g of tomato and basil pasta sauce

(Loyd Grossman tomato and basil pasta sauce, UK). All the prepared increments were then mixed together and chilled until required. A portion was then heated in the microwave for 3 min and the temperature was checked before being offered to the participants. The temperature must be ≥82°C (Food Standard Agency, 2018). The participant was served portions of ~500 g (cooked weight) and asked to consume as much as they wanted until they felt 'comfortably full'. The plate of pasta was continually topped up when it was about ¾ empty (Figure 2.4). This was to ensure that the participant always has ample hot food available and was not cued to stop eating by having emptied the plate. Any left-over was removed and the energy intake was calculated based on the weight of food consumed. Duration and speed (g/min) of eating were also calculated.

3.5.5.6. Subjective appetite

The VAS was provided n on single A4 paper sheets. The participant was instructed to complete the VAS just before, after and then every 30 min after consumption of the test drink for 3h. Further VAS were completed before and immediately after consuming the test meal, and then at 15, 30, 45 and 60 min. Each VAS was taken from the participant before the next one was provided to avoid impacting previous VAS scores on the next set of scores. During this period of time (after test meal), the participant was asked to stay in the laboratory, but was free to read (section 3.5.3.3).

3.6 Statistical analyses

SPSS software (version 28 for windows; SPSS) was used for data entry and analysis in Study 2. All data are presented as means ± standard deviation (SD), unless otherwise stated. Data were tested for normality with the Kolmogorov-Smirnov test to inform whether parametric or non-parametric analysis should be used. Paired samples T test (2-tailed) analysis was performed to compare data between the regular and irregular interventions for: the mean of 7-day consumed intake of energy and macronutrients, physical activity level, the mean of pre-meal and the mean of post-meal for each appetite rates (huger, satiety, fullness and PFC) obtained during the intervention periods. The same test analysis was also undertaken to compare habitual diet with each of the consumed regular and irregular diets (not corrected for multiple testing).

Having ensured that there were no violations with respect to undertaking an ANOVA including testing for outliers, variance and sphericity, firstly a two-way repeated measured ANOVA (Factor 1: meal pattern, two levels: regular and irregular; Factor 2: Visit, two levels: Pre and post) was undertaken to test the impact of meal pattern on body weight, and BMI.

Secondly a two-way repeated measures analysis of variance (ANOVA) test (Factor 1: meal pattern, two levels: regular and irregular; Factor 2: Day, seven levels) was undertaken to test the impact of meal pattern on nitrogen balance across the 7 days.

Where an interaction was found, simple main effects were compared. If there was no interaction, then main effects were compared. The Bonferroni correction was selected to correct for multiple comparisons when post hoc tests were undertaken. Differences were considered significant if $p<0.05$.

In this thesis a feasibility study is reported with respect to Study 3. The eventual fully powered study would be expected to use the following statistical methods. SPSS software (version 21 for Windows; SPSS) will be used for data entry and analyses. All data will be presented as means ± SDs unless otherwise stated. Data will be tested for normality with the use of the Kolmogorov-Smirnov test to inform whether a parametric or nonparametric analysis should be used. Values for the iAUC of the TEF, appetite ratings, and continuous glucose will be calculated with the use of differences from baseline. Values greater than baseline values will be considered as positive, and values less than baseline values will be considered as negative. The area above or below baseline will be calculated with the use of the trapezoid rule. Comparisons of baseline data at the pre-study energy intake, ambulatory energy expenditure estimation, VAS, and continuous glucose monitoring will be made with the use of Paired samples T test (2-tailed).

Two-way repeated-measure ANOVAs (factor 1: meal pattern, two interventions, regular and irregular meal pattern; factor 2: visit, before and after each 14-d intervention) will be undertaken to investigate the effect of the 14-d meal-pattern interventions on dependent variables (e.g., weight, the iAUC for the TEF, energy intake of ad libitum pasta, subjective appetite and mean of mesor and amplitude of peripheral wrist and core temperature). When an interaction is identified, simple main effects will be explored with the use of pairwise

comparisons. When no interaction is found but significant main effects are identified, pairwise comparisons will be made for the effect of the meal pattern or visit. Differences will be considered significant at $P < 0.05$ for all statistical tests.

3.7. Stability of urea in urine study

The 24-h urine sample was used to measure the urea nitrogen. Although each period of sample collection finished at 0800h by adding the last urine sample, the samples sometimes were not brought to the laboratory for freezing at -80, prior to analysis, until the late afternoon. Moreover, when collected over the weekend, samples might be brought to the laboratory for analysis 24-72 hours after collection. To circumvent the problem of failure to refrigerate the sample when stored for longer periods, laboratories add preservatives such as hydrochloric acid (HCl) before collection, to the collection vessel, aiming to reduce bacterial action or chemical decomposition (Berkel *et al.*, 2010). A disadvantage of the use of preservatives, however, is the possibility of interference with analytical methods (Ercan *et al*, 2015) and potential safety issues for the participants if they were to be splashed with the reagent. In the intervention study 2 described in this thesis (Chapter 5), the 24-h urine was collected without HCl preservative. When freezing and analysis are delayed, keeping the sample in an uncontrolled temperature environment without preservative may reduce the quality of the results if there is any decomposition. Therefore, as Berkel *et al.* (2010) only demonstrated stability up to 72 hours a methodological study was designed and undertaken to assess the effect of storing the urine at room temperature for up to 80h (as potentially required on this protocol) on the stability of urinary urea nitrogen. Ethical approval was covered by that obtained from study 2 (189-1812).

3.7.1. Method

A fresh, 20 ml urine sample, was collected from six healthy (self- report) volunteers at 9:00 in the morning and kept at room temperature. 5 aliquots (1ml each) were taken from each sample during the first 24 hours after the urine was excreted. The first aliquot was taken immediately after collection and then one aliquot every two hours until 5:00 pm. Then, 2 aliquots per day (9:00 am and 5:00 pm) were taken from the original sample for the following three days. Each aliquot was stored immediately after it had been taken from the

original sample in a -20C freezer. All of the 11 aliquots for the 4 days were analysed together for urinary urea nitrogen (section 3.5.3.2.1). The pH of each aliquot was measured before urea analysis.

One of the 6 participants was asked to provide another fresh 20 ml urine sample on a different occasion and the same study protocol was repeated, in order to test the consistency of the result within a participant. The participant was instructed to have the same food on both occasions on the day prior to collecting the sample.

3.7.1.1. Statistical analyses

SPSS software (version 28 for windows; SPSS) was used for data entry and analysis. Data were tested for normality with the Kolmogorov-Smirnov test to inform whether parametric or non-parametric analysis should be used. One-way repeated measure ANOVA (Factor 1: time, 11 levels) was conducted to assess the change of urea over the time. Differences were considered significant at $P < 0.05$ for all statistical tests.

3.7.2. Results

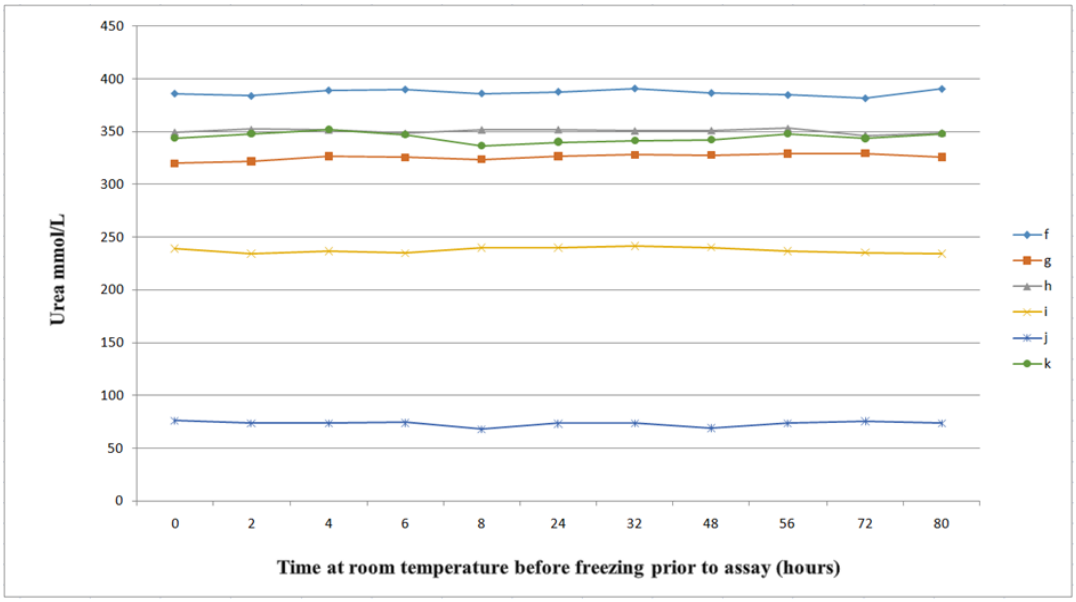
The results for the urinary urea as shown in table (3.9) and figure (3.13). There was not a significantly effect of time of exposure to room temperature across the samples. ($P= 0.45$). The mean of coefficient of variation within individual across measurements of the time points was 1.34 ± 0.90 %, range: 0.57 – 3.25 %. Moreover, the mean coefficient of variation (CV) between individual (Interindividual) was 41.3 ± 0.38 % and repeated variability within individual (Intraindividual) was 49.7 ± 1.1 %.

Table 3.9: Urea in different timescales

Urea values for each participant over the time. There was no change in urea (one-way repeated measure ANOVA, P=0.45).

Figure 3.13: Urinary urea values for each participant plotted against length of time at room temperature

Sample												80
f	Urea (mmol/L)	386.07	383.83	389.01	389.83	386.2	387.75	390.72	386.6	384.8	381.7	390.37
	pH	8	8	8	8	8	8	8	8	8	8	8
f2 same subject	Urea (mmol/L)	183.56	184.04	185.13	180.31	185.1	191.26	186.35	187.37	186.4	187.71	186.43
	pH	7	7	7	7	7	7	7	7	7	7	7
g	Urea (mmol/L)	320.1	322.06	326.84	325.74	323.9	326.92	328.33	327.86	329.3	329.56	325.96
	pH	7	7	7	7	7	7	7	7	7	7	7
h	Urea (mmol/L)	349.43	352.07	351.75	348.46	351.8	351.95	350.71	351.08	353.1	346.43	348.89
	pH	6	6	6	6	6	6	6	6	6	6	6
i	Urea (mmol/L)	239.07	234.42	236.81	234.89	240.1	240.01	241.76	240.46	236.9	235.6	234.41
	pH	6	6	6	6	6	6	6	6	6	6	6
j	Urea (mmol/L)	76.16	73.85	74.02	74.3	68.38	73.47	73.87	69.34	73.98	75.76	73.94
	pH	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
k	Urea (mmol/L)	344.16	348.23	351.94	347.31	336.9	340.31	341.43	342.44	347.8	343.68	348.06
	pH	7	7	7	7	7	7	7	7	7	7	7



There was no change in urea (one-way repeated measure ANOVA, P=0.45)

3.7.4 Discussion and conclusion

Keeping the urine samples at room temperature for a prolonged period did not produce noticeable differences in urinary urea content. The results of urea show that there was an analyte stability and no sign of degradation in urea, even when the pH reaches 8, and without preservative. Others have suggested that degradation of nitrogenous urine analytes is possibly responsible for the observed increase in urinary pH over time. Increased temperature is directly associated with increases in urine pH (Cook *et al.*, 2007). The urinary urea stability noted in this study is consistent with findings by Berkel and Boer, (2010). They observed that urea concentration is stable without preservative for up to 72 hours irrespective of storage temperature. Moreover, a recent study demonstrated that urea is 24h stable before analysis with or without HCl preservative (Feres *et al.*, 2011).

The within individual CV showed consistency from day to day. Nevertheless, the between individual CV is large, indicating substantial differences between participants. This could be explained by the variations in the urine volumes, body weights and dietary protein of the participants which may have contributed to the between person differences in urea concentrations.

In conclusion, this small study describes the experimental evaluation of room temperature on urea stability up to 80 hours. The findings reveal that there is no significant effect of prolonged storage at room temperature on urea concentration, which has important implications for practical work with 24-h urine collection. In Study 2, participants were asked to store samples that could not be delivered immediately to the lab in an unheated part of the house, which would be expected to reduce degradation even further as the study was undertaken in winter months.

CHAPTER 4: DEVELOPMENT OF NOVEL METHODS FOR CHARACTERISING THE DAY-TO-DAY VARIABILITY OF MEAL PATTERN AND EXPLORATION OF ASSOCIATION WITH HEALTH

4.1. Introduction

There is emerging evidence that variability in day-to-day meal patterns may be a new risk factor for major challenges to health such as obesity, metabolic disorders and cardiovascular diseases (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Pot *et al.*, 2014; Alhussain *et al.*, 2016; Pot *et al.*, 2016). In the systematic review presented in Chapter 2, however, existing methods of characterising the degree of variability in eating patterns were found to have limitations, such as assuming a particular underlying meal pattern (e.g. breakfast, lunch and dinner) (Sierra-Johnson *et al.*, 2008; Wennberg *et al.*, 2015), which may have complicated the results and the validity of the conclusions. Effective characterisation of day-to-day variability is essential for the meaningful exploration of associations between meal pattern irregularity and health, and this was identified as an important area to consider in this thesis.

Traditionally, when studying meal pattern and health, an individual's meal pattern has been characterised by calculating a mean value for a particular meal pattern property over several days of intake (such as the mean of meal frequency over 7 days) (Murakami and Livingstone, 2015; Ma *et al.*, 2003). However, using the mean over several days in this way does not address the impact of an individual's level of day-to-day variation in meal pattern on health. In Chapter 2, it was noted that while several studies have aimed to investigate the relationship between day-to-day variability of meal pattern and health outcomes, different methods had been used to characterise the degree of variability. These included capturing irregularity of eating by using simple questions about participants' self-perception of between-day meal pattern regularity in general, or of specific meals via a self-reported questionnaire (Saneei *et al.*, 2016). Other studies focussed on the identification of specific meal pattern properties from food intake records, often focusing on the frequency of consumption of specific meals (such as the frequency of breakfast consumption (Merten *et al.*, 2009)). Some researchers focussed on a range of computational methods that were applied to pre-existing dietary intake data to create a single new variable representing meal pattern variability (Pot *et al.*, 2014; Pot *et al.*, 2016). Meanwhile, the definition of meals or eating incident was not consistent in many studies (Lehto *et al.*, 2010; Sierra-Johnson *et al.*, 2008), and in some instances it was not defined

at all (Muff *et al.*, 2011; Shin *et al.*, 2009; Ko *et al.* 2007; Kim *et al.*, 2003; Sjoberg *et al.*, 2003). Most computational methods used to characterise meal pattern variability imposed or assumed pre-selected meal categories such as breakfast, lunch and dinner, focussing only on these main meals and ignoring snacks and other eating occasions which may have contributed to irregularity. The use of such broad categories not only imposed prior assumptions about eating habits, but also disassociated intake data from actual 'clock time', thus failing to use the full richness of data when discriminating between different meal patterns on different days. It was also found that there are sociolinguistic differences in the naming of meals (Rodríguez-González, 1999; Jankowski and Tagliamonte, 2019) which may lead to confusion amongst participants and researchers, leading to inconsistency in results.

One potential novel method of characterising day-to-day meal pattern variability would involve using a broader set of meal pattern properties than have previously been used. Collectively these would describe an individual's meal pattern on a particular day, (eg. time of first meal of the day, time when 50% of energy has been consumed and daily meal frequency. Measures of variability in each property could then be related to health outcomes to identify significant associations. Alternatively, characterisation could involve the derivation of a single index of variability based on energy intake (or intake of another dietary component such as protein) at multiple time points across the day, but without reliance on imposed meal descriptors such as breakfast and lunch. The first method would enable a comparison to be made of the importance of different sources of variability, for example variations in the timing of the first meal of the day could have a greater impact on health than variations in the number of meals. The second method would provide a more integrated approach to the use of intake timings and quantitative intake data without the imposition of social expectations of meals.

4.1.1. National Diet and Nutritional Survey (NDNS)

The NDNS rolling programme is an ongoing continuous cross-sectional survey designed to assess the dietary intake, energy and nutrient intake values, nutritional status and health outcomes of the general population aged 1.5 years and over living in private households in the UK (Food Standards Agency, 2019). The programme was set up in 1992 and includes

surveys of pre-school children, young people and older people that were carried out in the 1990s. The rolling NDNS sample population is characterised by a range of socio-demographic characteristics including age, socio-economic classification, housing tenure, education and qualifications. The survey sample is drawn from the Postcode Address File, through which participants are randomly selected from across the UK, and data are obtained through a series of visit from an interviewer and a nurse.

During these visits, participants are provided with a 4-day prospective food diary record which they are asked to complete, while the nurse obtains a blood sample which is analysed for fasting total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, glucose and glycated haemoglobin (HbA1c). Measurements of anthropometry and blood pressure (BP) are also obtained. The resulting database includes details of time of food and drink consumption, the type and amount of food consumed and health outcomes. It is therefore suitable for developing methods for characterising variability in day-to-day food intake and for exploring associations with health outcomes. The NDNS database from 1-9 years (2008/2009 - 2016/2017) was used as the basis for undertaking the work in this chapter.

We can conclude that day-to-day variability in meal pattern may be associated with health outcomes, but current methods of characterising day-to-day variability have limitations, and novel methods of characterisation are required before undertaking further work in this area. The overall aim of the work described in this chapter is to propose novel characterisation methods exploring day-to-day variability in meal pattern and undertake exploratory work to consider associations between variability and health outcomes using pre-existing data from the NDNS programme (Public Health England, 2019).

The first objective was to identify novel methods of characterising the irregularity of meal pattern to capture meal pattern variability using a broad set of meal pattern properties informed by the current literature and by deriving a single variability index. These methods were then applied to the NDNS database of dietary intake information in order to describe the degree of day-to-day variability in meal pattern. The second objective was to undertake an exploratory investigation into associations between day-to-day variability in meal pattern and health outcomes BMI, systolic BP, diastolic BP, fasting total cholesterol, LDH and HDL cholesterol, fasting glucose and HbA1c level using the characterisations of day-

to-day meal pattern variability derived in the first objective. The definition of meals as 'eating incidents' would be considered when required.

The hypothesis to be tested via the second objective was that that greater day-to-day variations in meal pattern would be associated with higher BMI and poorer health outcomes (higher systolic BP, higher diastolic BP, higher total cholesterol, higher LDL-cholesterol, lower HDL-cholesterol, higher fasting blood glucose and higher HbA1c) compared to a lower level of day- to-day variations in meal pattern.

4.2. Methods

4.2.1. Characterizing of day-to-day variability in meal pattern

4.2.1.1. Method 1: Day-to-day variation in specific meal pattern properties

This method for characterizing the irregularity of meal pattern was developed with reference to the previous literature, and in collaboration with Dr. Daniel Abasolo (Senior Lecturer in Biomedical Engineering, School of Mechanical Engineering Sciences, Faculty of Engineering and Physical Sciences, Surrey University). Dr Abasolo assisted us in the calculation of Shannon Entropy (see point 4.2.1.8).

Firstly, a set of properties were identified which described different aspects of the profile of dietary intake on a particular day based on the previous literature relating to meal pattern and health. A measure of variability across the number of days of dietary intake (4 days) was then proposed (eg. SD in the variable across the days, or range between maximum value and minimum values). Four days was selected because this was available from the NDNS data, but a longer period could be considered if the methods were applied to a different data set with more days of available data The properties identified and measures of variability were as follows:

- Energy intake on each day. Measure of variability: SD of the mean for 4 days.
- Meal frequency on each day- the 24-hour period was divided into 48, 30-minute time slots. If any energy was consumed within a time slot, then the 30 minutes time slot was considered to count as an 'eating incident' and contributed a count of one to the 'meal frequency' of that day. This was irrespective of the amount of energy

consumed. Measure of the variability: SD of the mean for 4 days; the difference between the maximum and minimum of meal frequency over 4 days.

- The time of the first meal. Measure of variability: SD of the mean for 4 days.
- The time of the last meal. Measure of variability: SD of the mean for 4 days.
- The time when 50% of energy had been consumed. Measure of variability: SD of the mean for 4 days.
- The interval between the first and last meal. Measure of variability: SD of the mean for 4 days; difference between the longest and shortest interval between the first and last meal over 4 days.
- The interval between the first meal and when 50% of energy had been consumed. Measure of variability: SD of the mean for 4 days; difference between longest and shortest interval time between the first meal and when 50% of energy has been consumed over 4 days.
- Shannon Entropy (SE) of each day. Measure of variability: SD of the mean for the 4 days.

Shannon entropy is a concept addressing randomness and predictability, with greater entropy associated with greater chaos (and greater unpredictability) within a data set (Abasolo *et al.*, 2006). SE was calculated, by Dr. Daniel Abasolo, for each day of food intake reported. Firstly each 24 hours period was divided into 48, 30-minute bins. The amount of energy consumed within each bin was then calculated. This was then compared with the expected energy intake per bin (ie. the average amount of energy per bin if food intake has been spread evenly across the 30-minute slots over the day) using the following equation hence deriving the SE entropy for that day.

$$H(f) = -\frac{1}{\ln(N)} \sum_{i=1}^{N} p_i \ln(p_i),$$

H= Shannon entropy (SE)

N= the number of bins

Pi= the amount of energy consumed in bin i.

The degree of variability between the 4 days reported by a participant was then represented by a calculation of standard deviation across the 4 days.

4.2.1.2. Method 2: Irregularity index method

This method for characterizing the irregularity of meal pattern was proposed and developed with reference to the previous literature, and in collaboration with Prof Richard Emes (Associate Pro-Vice Chancellor in Faculty of Medicine and Health Sciences, Chair of Bioinformatics in School of Veterinary Medicine and Science and Director of Bioinformatics in Advanced Data Analysis Centre in University of Nottingham). The following three steps of the method were undertaken by Prof Richard Emes using the NDNS data. The final step (Step 4) was then undertaken by the researcher to create two novel irregularity indices for each member of the survey.

Step 1

Each 24-hour day period (starting at 00:01) was divided into 48 consecutive 'bins', of a preselected, equal length (30 minutes) of time (total number of bins across the four days was thus 4 x 48 bins= 192). The total energy consumed within each bin, for a specific food intake day record, was then calculated, for that individual at that time slot on that specific day. If two eating incidents occurred, for example, 25 minutes apart, within the same bin then they would both contribute to the bin total at that time slot. This process was repeated for each bin, over the full day, for the number of days for which food intake records were available for that individual. A 4-day food intake record characterised using 30-minute bins would thus result in energy intake totals for 192 bins (4 days, each with 48 bins).

Step 2

A median was then calculated for the energy intake at a particular time slot using the number of days food intake records available for a participant. This median was then used as a comparator (Step 3) against which the actual intake within each bin, at a particular

time, on each day was compared. Where no energy had been consumed within a bin, on a specific day, a '0' was attributed for that day when calculating the median.

Step 3 Flagging of time slots as 'irregular'

2a. Flagging method A: The fold change from the median was calculated for each bin, at each time slot on a specific day, by dividing the total energy intake in a bin on a specific day, by the median energy intake for that time slot calculated using all available days for that individual, at that time slot (as described in step 2). The value would be 1 if the energy consumed on a particular day in a bin was identical to the median for that time slot, across all days. If the energy in a bin divided by the median energy for time bin was greater, or less than the selected threshold it was classified as an "irregular bin" according to the following thresholds. The thresholds applied to enable three versions of the method were i. ≤ 0.5 or ≥ 2, ii. ≤ 0.4 or ≥ 2.5 and iii. ≤ 0.3 or ≥ 3. An irregular bin with threshold i, thus would have an energy intake that was either greater than or equal to double the median, or less than or equal to half the median for that time slot. Where one or more bins at a particular time slot were considered 'irregular', then that time slot was flagged as irregular for that individual.

2b. Flagging method B: The difference between the actual intake and the median for the four bins at that time slot was calculated by subtracting the median energy intake for that time slot (calculated using data from all available days for that individual at that time slot- as described in step 2) from the actual energy intake for that bin at that time, on a specific day. The value would be zero if an identical amount of energy had been consumed on a particular day, as the median for that time slot. If the absolute (+ or -) difference between the energy in a time bin and the median energy for time slot in the day was greater than a preselected threshold, then that bin was classified as an "irregular bin". The thresholds for irregularity that were applied were that the value in relation to the median was i, $\leq$ or $\geq$ the median by 100kcals, ii, $\leq$ or $\geq$ than the median by 200kcals and iii, $\leq$ or $\geq$ the median by 300kcals. Where one or more bins at a particular time slot were considered irregular, then that time slot was flagged as 'irregular' for that individual.

Step 4 Calculation of an 'Irregularity indices'

The following two overall irregularity indices were then calculated for each participant (Table 4.1):

i. The number of flagged irregular time slots as a ratio of the total number of time slots in a 24-hour period* = **`Irregular time index'**. *48 time slots in a 24-hour period.

ii. The sum of the energy consumed on each day that was in an irregular bin, divided by the sum of the energy consumed on the four days on all occasions, expressed as a ratio = **'Irregular energy index'.**

Table 4.1: An example of the derivation of the irregular time index for an individual

	Bin 15 7:00	Bin 16 7:30	Bin 19 8:30	Bin 20 9:00	Bin 21 9:30	Bin 27 12:30	Bin 31 14:30	Bin 37 17:30	Bin 40 19:00
Day 1	√	√	0 kcal	0 kcal	√	√	×	0 kcal	0 kcal
Day 2	√	√	0 kcal	√	0 kcal	√	×	0 kcal	×
Day 3	0 kcal	√	×	0 kcal	×	√	√	√	0 kcal
Day 4	×	√	0 kcal	√	×	√	×	√	×
Time slot	Irregular	Regular	Irregular	*	Irregular	Regular	Irregular	*	Irregular

Key- √ represents a 'regular bin', x represents an 'irregular bin'

* This time slot would depend on the intake and therefore the median. I If the threshold for flagging as irregular is 100kcal and the intake was for example 100kcal on two days and 0 on other two days. The median would be 50kcal. Then both 0 and 100kcal are within the threshold difference from the 50kcal median. The time slot would be classed as regular as no day was more irregular than the threshold but, if the intake was 400kcal on two days. The median would be 200kcal. All four days are then greater than the threshold different from the median, The time slot would be classed as irregular.

The total number of bins above the threshold = (16x√) + (9 x ×) = 25

Regular bins = number of (√) = 16 and irregular bins = number of (×) = 9.

If we assume that the days with 0 kcal were regular bins. Then, there are 5 time slots where there was at least one irregular bin hence considered irregular time slots. Therefore the 'Irregular time index' is 5/48= 0.1

If an individual had one irregular time slot and a total energy intake over the 4 days of 7750 kcals then if for the irregular time slot

Day 1 intake 50 Kcal -- classed as irregular

Day 2 intake 400 Kcal -- classed as irregular

Day 3 intake 200 Kcal -- classed as regular

Day 4 intake 210 Kcal -- classed as regular

Total irregular intake = 450 Kcal

The energy intake index would be: Total Irregular intake (Kcal) / (Total Regular intake (Kcal) + Total Irregular intake (Kcal) = 450/7750=0.06

4.2.1.3. Applying Methods 1 and 2 to the NDNS dataset

The NDNS database (Food Standards Agency, 2019) for year 1-4 (2008/2009 – 2011/2012), year 5-6 (2012/2013 – 2013/2014) and year 7-9 (2014/2015 - 2016/2017) were obtained by downloading from UK Data Services website in November 2019, and the two methods described above (**4.2.1.1 Method 1: Day-to-day variation in specific meal pattern properties; 4.2.1.2 Method 2: Irregularity index method)** were applied to those participants who met the following inclusion criteria: male or female, aged 18 years or over, four days of food intake data available including weekends and weekdays.

4.2.1.4. Specific parameters used for application of the NDNS dataset using method 2

The values chosen for the characterisation were:

1. Bin length=30 mins

2. Minimum energy intake (food or drink) to be considered as an "event'' ≥ 0

3. When using flagging method 2a, the thresholds compared were ≤ 0.5 or ≥ 2, ≤ 0.4 or ≥ 2.5 and ≤ 0.3 or ≥ 3.

4 When using flagging method 2b, the thresholds compared were $\leq$ or $\geq$ 100kcals, $\leq$ or $\geq$ 200kcals and $\leq$ or $\geq$ 300kcals.

4.2.2. An exploratory investigation of the relationship between meal pattern irregularity, and health parameters was undertaken using data characterised for irregularity using methods 1, 2a and 2b.

4.2.1.1. Identification of the population to be studied

Using the previously described population from the NDNS database subgroups were identified who had health outcome data for the areas of interest (age 18 and above). Not all subgroups had the same number of participants, depending on the availability of data for each health outcome.

4.2.1.2. Data manipulation and statistical analysis

Appropriate syntax was developed in R (v3.5.0) analysis software and used in conjunction with R studio (1.1.453) in order to calculate the meal pattern properties in Method 1 (Advised by Statistician: Michael Wellington (PhD Student in Agricultural Science, Australian National University) and the Irregularity indices in Method 2a and 2b (Advised by: Associate Pro-Vice Chancellor in Faculty of Medicine and Health Sciences, Chair of Bioinformatics in School of Veterinary Medicine and Science and Director of Bioinformatics in Advanced Data Analysis Centre in University of Nottingham). Further Statistical analysis was carried out using Statistical Package software SPSS (version 28).

4.2.2.3. Statistical analysis undertaken to explore associations between the irregularity characteristics using Methods 1, 2a and 2b and health outcomes.

The means and standard deviations for all meal pattern properties were determined. A multiple linear regression analysis was undertaken using the variables derived from the novel methods proposed to characterise meal pattern variability to establish if there were correlations between Method 1 (meal pattern variability for all properties) or Method 2a and 2b ('irregular bin index' and 'irregular energy index') and health outcomes. The variables indicating meal pattern property variability were entered as the independent variables (predictors) and each health outcomes (BMI, systolic BP, diastolic BP, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, glucose and HbA1c) was entered as the dependent variable in the different models. Age and gender were controlled for and included as predictors in each regression analyses. Regression analysis model assumptions (independence of observations, linear relationship between (a) the dependent variable and each of the independent variables, and (b) the dependent variable and the independent variables collectively, homoscedasticity of residuals, multicollinearity, significant outliers, high leverage points or highly influential points and the normality distribution of residuals) were tested. $P<0.05$ was considered to be statistically significant (Laerd Statistics, 2020).

4.3. Results

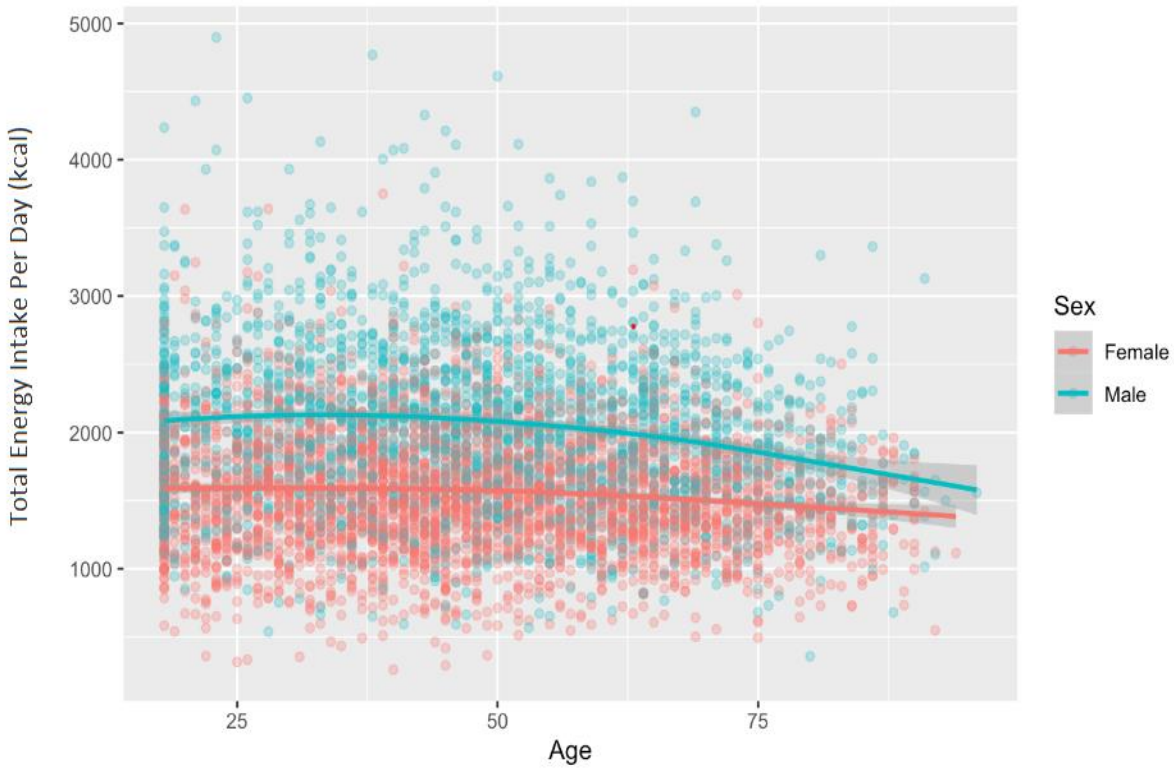
4.3.1. Characterizing of day-to-day variability in meal pattern

Two methods for characterising meal pattern variability were proposed (with the derivation of two indices in Method 2) and applied to the NDNS dataset for 5902 participants who met the inclusion criteria. The characteristics of the population are shown in Table 4.2. The distribution of total energy intake per 24hr is shown in Figure 4.1. Fifty-eight percent of the sample were female. The mean age was 50±17 years (Male: 48±15 years and female: 47±16 years). Table 4.3 shows day to day meal pattern variability for the properties considered in Method 1 and Table 4.4 shows the day-to-day meal pattern variability described using the `irregular time index' and `irregular energy index` described in Method 2. Participants with a higher value have a more irregular meal pattern when compared with participants with a lower value.

Table 4.2: Descriptive characteristics of the study sample

	Sample no.	**Mean (SD)**
Age (Years)	5902	50±17
Young adults (18-39 years)	2095	28±6
Middle-aged adults (40-59 years)	2117	49.5±5
Older adults (≥ 60 years)	1690	70±7
Male (42%)	2459	-
Female (58%)	3443	-
Weight (kg)	5902	77±17

Figure 4.1: The distribution of total energy intake/day based on sex and age



4.3.1.1. Method 1

Table 4.3 shows that for all meal pattern parameters, older adults show significantly less variability than middle-aged and young adults (P=0.02). Table 4.4 shows that for all meal pattern parameters, apart from SD of last mealtime and SD of 50% of energy consumed, women show significantly less variability than men.

Table 4.3: Difference in meal pattern variability based on age group

	Young adults	Middle-aged adults	Older adults
	Mean (SD)	Mean (SD)	Mean (SD)
no.	2095	2117	1960
SD of energy intake per 24hrs*	475±284	427±258	331±189
SD of meal frequency per 24hrs*	1.5±1	1.4±1	1.1±0.7
Max – min in meal frequency per 24hrs	3.2±2.4	2.8±1.8	2.3±1.1
SD of first mealtime*	1.6±1.5	1.2±1.3	0.8±1.2
SD of last mealtime*	1.1±0.69	0.95±0.68	0.81±0.65
SD of time for 50% of energy consumed*	2.6±1.6	2.5±1.6	2.2±1.6
SD of (first meal-50% of energy) intervals*	3.03±1.9	2.8±1.7	2.5±1.8
Max – min in (first-50% of energy) intervals	6.1±4.1	5.8±3.1	5.2±2.9
SD of (first-last meal) intervals*	2.04±1.5	1.69±1.3	1.28±1.1
Max – min in of (first-last meal) intervals	3.9±3.4	3.6±3.2	3.2±3
SD of Shannon entropy*	0.06±0.03	0.05±0.03	0.047±0.02

All values are means ± SDs. There was a statistically significant difference in mean of meal pattern variability for each meal pattern property between all groups as determined by one-way ANOVA (P=0.02). A Tukey post hoc test revealed that the mean value of meal pattern variability in older adults was significantly less than in middle-aged and older adults *P < .05.

Table 4.4: Difference in meal pattern variability based on sex*

Meal pattern	**Male**		**Female**		**P-value**
	no.	Mean	no.	Mean	
SD of energy intake	2459	477±288	3443	366±216	<0.001
SD of meal frequency		1.4±1		1.2±0.8	<0.001
Max – min in frequency		3.1±2.4		2.7±1.8	<0.001
SD of first mealtime		1.3±1.5		1.2±1.3	<0.001
SD of last mealtime		0.98±0.72		0.95±0.72	NS
SD of 50% of energy time		2.4±1.66		2.3±1.61	NS
SD of (first meal-50% of energy) intervals		1.8±1.5		1.6±1.3	<0.01
Max – min in (first-50% of energy) intervals		6.2±4.1		5.9±3.8	<0.01
SD of (first-last meal) intervals		1.8±1.5		1.6±1.3	<0.001
Max – min in of (first-last meal) intervals		3.9±3.4		3.6±3	<0.001
SD of Shannon entropy		0.049±0.04		0.047±0.03	0.004

*T-test was used to find the difference between male and female.

4.3.1.2 Method 2

Table 4.5 and 4.6 show the variability by age and sex for the irregular time index and irregular energy index using the range of thresholds for classifying irregularity using fold difference to the median and absolute energy differences from median. Older adults show significantly less variability in both indices than middle-aged and young adults (P value <0.05). Women show significantly less variability in irregular time index and irregular energy index than men.

Table 4.5: Difference in meal pattern based on age group

	Irregular time index			Irregular energy index		
	Young adults	Middle-aged adults	Older adults	Young adults	Middle-aged adults	Older adults
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
no.	2095	2117	1960	2095	2117	1960
2-fold change threshold	0.35±0.13	0.34±0.11	0.32±0.10*	89±0.26	86±0.18	82±0.31*
2.5-fold change threshold	0.33±0.12	0.31±0.12	0.30±0.11*	67±0.1	64±0.3	61±0.3*
3-fold change threshold	0.33±0.15	0.32±0.10	0.30±0.12*	59±0.6	58±1.2	56±0.3*
100kcal difference from median	0.24±0.20	0.23±0.4	0.22±0.10*	87±1.5	85±1.3	81±1.2*
200kcal difference from median	0.19±0.10	0.16±0.78	0.14±0.32*	77±0.19	70±0.42	66±0.52*
300kcal difference from median	0.15±0.34	0.13±0.06	0.11±0.5*	60±0.8	57±1.0	53±0.9*

All values are means ± SDs. There was a statistically significant difference in mean of meal pattern variability for both indices between all groups as determined by one-way ANOVA (P=0.03). A Tukey post hoc test revealed that the mean value of meal pattern variability in older adults was significantly less than in middle-aged and older adults *P < .05.

Table 4.6: Difference in meal pattern based on sex*

Meal pattern	Irregular time index			P-value	Irregular energy index			P-value
	no.	Male	Female		no.	Male	Female	
2-fold change threshold		0.34±0.15	0.33±0.13	<0.001		0.86±0.36	0.84±0.34	<0.001
2.5-fold change threshold		0.33±0.15	0.32±0.13	<0.001		0.65±0.33	0.63±0.30	<0.001
3-fold change threshold		0.31±0.15	0.30±0.13	<0.001		0.59±0.33	0.58±0.32	<0.001
	2459				3443			
100kcal difference from median		0.23±0.8	0.22±0.6	<0.001		0.88±0.16	0.82±0.15	<0.001
200kcal difference from median		0.18±0.10	0.16±0.06	<0.001		0.73±0.26	0.66±0.25	<0.001
300kcal difference from median		0.14±0.06	0.12±0.06	<0.001		0.61±0.26	0.54±0.24	<0.001

*T-test was used to find the difference between male and female.

4.3.2. An exploratory investigation of the relationship between meal pattern irregularity, and health parameters using the results of the characterisation of irregularity methods

Table 4.7 shows the sample size and health outcome data for the population characterized for meal pattern variability using Methods 1, 2a and 2b. The difference in sample size for each variable reflects the available data for that variable.

Table 4.7: Sample size and health outcome data for the population

	Sample no.	Mean (SD)
BMI	5519	27.6±5.3
Systolic BP	4406	128±18.5
Diastolic BP	4406	74.5±11.7
Cholesterol	2792	3.7±2
Triglyceride	2368	2.4±1.1
HDL-cholesterol	2825	3.4±2.1
LDL-cholesterol	2926	4.1±1.5
Glucose	2932	5.1±1.1
HbA1c	2429	4.2±2

4.3.2.1. Method 1

R^2 for each overall multiple linear regression model using the predictors identified in Method 1 and listed health outcomes was between 0.6 and 11% with an adjusted R^2 of between 0.3 and 11%. For each predictor in each overall regression model, those independent variables with statistically significance, predicted health outcomes (the dependant variables), ($p < .0005$). Table 4.8 shows the coefficient results from applying a multiple linear regression with each health outcome. All assumptions which were tested to fit a multiple regression analysis had been met in each regression model.

Table 4.8: Linear regression analysis result for each health outcome using models adjusted for age and sex

	SD of energy intake	SD of frequency	Max and min frequency difference	SD of first mealtime	SD of last mealtime	SD of 50% of energy time	SD of (first-last meal) intervals	SD of (first meal-50% of en ergy) intervals	Max – min of (first-last meal) intervals	Max – min in (first-50% of energy) intervals	SD for Shannon entropy
						B					
BMI	0.001*	0.57	-0.27	0.13	0.04	0.002	-0.13	-0.08	0.06	0.007	4.88*
Systolic	0.0003	-3.17	1.24	0.059	0.96	-0.306	1.76	-0.24	-0.863	0.165	1.725
Diastolic	-0.0003	-1.461	0.584	-0.133	0.377	-0.18	1.415	-0.777	-0.586	0.388	1.95
Chol	0.00002	-0.241	0.129	-0.013	0.017	-0.005	-0.294*	0.029	1.131*	-0.009	0.71
Trig	0.00007	0.111	-0.04	0.024	-0.028	0.003	-0.224**	0.005	0.104**	-0.007	0.102
HDL	-0.00001	0.002	0.009	0.006	0.013	-0.015	0.005	0.026	-0.013	-0.004	-0.25
LDL	0.00002	-0.284	0.134	-0.033	0.027	0.006	-0.2	-0.018	0.097	0.01	0.69
Glucose	0.00003	-0.214	0.081	0.0004	-0.038	0.034*	-0.165	0.061	0.09*	-0.039	0.891*
HbA1c	0.00008*	-0.083	0.021	0.019	0.006	0.021*	-0.041	-0.004	0.017	-0.01	-0.23

B = unstandardized regression coefficient; *P < .05, **P < .01, ***P < .001

4.3.2.2. Method 2

R2 for each overall multiple linear regression model for each listed health outcomes was between 0.6 and 15% with an adjusted R2 of between 0.5 and 15%. In addition, in each overall regression model, those independent variables with statistically significance, predicted health outcomes (the dependant variables), ($p < .0005$). From Tables 4.9 to 4.14, the coefficient results of applying a multiple linear regression with each health outcome were presented. All assumptions which were tested to fit a multiple regression analysis had been met in each regression model. Table 4.15 shows a summary of the results of associations between methods of variability in meal pattern and health outcomes.

Table 4.9: Linear regression analysis for a 2-fold change

	Irregular time index	**Irregular energy index**
	B	B
BMI	-1.07*	1.241 ***
Systolic BP	-5.72	1.41
Diastolic BP	0.85	1.61*
Cholesterol	0.66***	0.12
Triglyceride	0.07	0.03
HDL-cholesterol	0.24 ***	-0.07 *
LDL-cholesterol	0.37*	0.20 *
Glucose	-.01**	0.12 *
HbA1c	-0.06	0.12**

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .001.

Table 4.10: Linear regression analysis for a 2.5-fold change model adjusted for age and sex

	Irregular time index	Irregular energy index
	B	B
BMI	-1.58*	1.69 ***
Systolic BP	-4.92	1.10
Diastolic BP	1.35	1.26*
Cholesterol	0.73***	0.02
Triglyceride	0.09	0.04
HDL-cholesterol	0.25 ***	-0.11**
LDL-cholesterol	0.24*	0.12
Glucose	-0.17	0.18**
HbA1c	-0.33**	0.07

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .001.

Table 4.11: Linear regression analysis for 3-fold change

	Irregular time index	Irregular energy index
	B	B
BMI	-1.47*	1.58 ***
Systolic BP	-5.03	1.28
Diastolic BP	1.50	1.25*
Cholesterol	0.74***	-0.01
Triglyceride	0.1	0.03
HDL-cholesterol	0.24 ***	-0.09**
LDL-cholesterol	0.43*	0.07
Glucose	-0.16	0.15**
HbA1c	-0.32**	0.07

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .001.

Table 4.12: Linear regression analysis for abs 100 kcal difference

	Irregular time index	Irregular energy index
	B	B
BMI	-3.91***	3.57***
Systolic BP	-4.85	1.75
Diastolic BP	-1.88	2.10
Cholesterol	0.29	0.17
Triglyceride	-0.08	0.03
HDL-cholesterol	0.54***	-0.16***
LDL-cholesterol	-0.15	0.31**
Glucose	-0.52**	0.28***
HbA1c	-0.43 **	0.18*

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .001.

Table 4.13: Linear regression analysis for abs 200 kcal difference

	Irregular time index	Irregular energy index
	B	B
BMI	-5.96***	3.76***
Systolic BP	-4.67	1.43
Diastolic BP	-3.38	1.67
Cholesterol	-0.16	0.15
Triglyceride	-0.53	0.10
HDL-cholesterol	0.54***	-0.14**
LDL-cholesterol	-0.41	0.26 *
Glucose	-0.63**	0.29***
HbA1c	-0.59 **	0.21**

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .001.

Table 4.14: Linear regression analysis for abs 300 kcal difference

	Irregular time index	Irregular energy index
	B	B
BMI	-6.96***	3.85***
Systolic BP	-10.78	3.32
Diastolic BP	-5.16	2.50
Cholesterol	0.04	0.02
Triglyceride	-0.51	0.15
HDL-cholesterol	0.59***	-0.20***
LDL-cholesterol	-0.27	0.14
Glucose	-0.58*	0.32***
HbA1c	-0.59*	0.24**

B = unstandardized regression coefficient, *P < .05, **P < .01, ***P < .0.001.

Table 4.15: Summary of the results of associations between methods of variability in meal pattern and health outcomes

	Method 1	Method 2a (fold change) and 2b (difference) for Irregular time index						Method 2a (fold change) and 2b (difference) for Irregular energy index					
		2-fold	100 kcal	2.5-fold	200 kcal	3-fold	300 kcal	2-fold	100 kcal	2.5-fold	200 kcal	3-fold	300 kcal
BMI	SD of daily energy intake, SD for Shannon Entropy	-	-	-	-	-	-	+	+	+	+	+	+
Systolic BP													
Diastolic BP								+		+		+	
Cholesterol	**SD of (first-last meal) intervals- Negative, Max – min of (first-last meal) intervals- positive**	+		+		+							
Triglyceride	**SD of (first-last meal) intervals- Negative, Max – min of (first-last meal) intervals- positive**												
HDL-cholesterol		+	+	+	+	+	+	-	-	-	-	-	-
LDL-cholesterol		+		+		+		+	+		+		
Glucose	**SD of 50% of energy time, Max – min of (first-last meal) intervals, SD for Shannon entropy**	-	-		-		-	+	+	+	+	+	+
HbA1c	**SD of energy intake, SD of 50% of energy time**		-	-	-	-	-	+	+		+		+

(+) Positive and (-) Negative association

4.4. Discussion

4.4.1. Characterizing of day-to-day variability in meal pattern

The study therefore set out firstly to identify novel methods of characterising meal pattern irregularity and then apply them to the NDNS database of dietary intake information to allocate measures of meal pattern variability to each participant. Secondly, the study explored the associations between day-to-day meal pattern variability and the health outcomes using the NDNS database. Two methods were proposed and applied to the NDNS data. The first used a variety of different meal pattern properties to describe the degree of irregularity in meal patterns. This contrasted with previous studies that considered variability in the consumption of, for example, one or more meal categories, such as breakfast (Merten *et al.*, 2009; Hamaideh *et al.*, 2010; Mekary *et al.*, 2013; Shafiee *et al.*, 2013; Rodrigues *et al.*, 2017). By using existing meal pattern records, the novel method described in this chapter did not have to rely on participants making a value judgement about the degree of variability in their meal pattern, as the degree of variability for each property was numerically quantified based on food intake records.

The second method involved the derivation of two indices, each with two threshold systems. The first index gave a measure of the number of 30-min time slots labelled as 'irregular' because for at least one out of the four days, a 'bin' was identified, in comparison with the median, as being irregular for that particular time slot for energy consumption. Selecting a 30-minute time slot was based on data from previous studies (Farshchi *et al.*, 2005; Alhussain *et al.*, 2016), which had reported the length of time taken to consume a test meal of pasta and was considered sufficiently short to allow the research to discern between different patterns. The second index was derived by quantifying the amount of energy consumed in all eating incidents ('bins') that fell outside a stated threshold from the median for each time point in the day, and was expressed as a ratio of total energy intake over the four-day. Energy intake data was thus used that retained the context of the time when it was consumed. Different thresholds were used when characterising

whether the energy consumed on a particular day at a particular time slot ('bin') was considered irregular in relation to the median. Associations between the degree of meal pattern variability and various health outcomes were then considered, and will be discussed later in more detail with reference to the different thresholds as appropriate. Other methods have used broad, socially grounded terms such as 'dinner' (Lehto *et al.*, 2010; Wennberg *et al.*, 2015), or a categories such as 'between meal snacks' (Pot *et al.*, 2014) which disassociate energy intake from the 'clock time' of consumption.

4.4.1.1. Degree of day-to-day variability in meal pattern with age and sex

Applying these methods to the NDNS data provided some interesting contrasts based on participant characteristics. For example, using Methods 1 and 2, we found that older adults had a lower level of meal pattern variability than younger groups for all meal pattern properties. This could be explained by differences in lifestyle. It was found that older adults had a better overall health-promoting lifestyle and were more committed to eating with family (Lin *et al.*, 2002) than younger adults (Walker *et al.*, 1988). Younger adults tend to follow modern lifestyles which encourage moving away from regular eating and eating more meals outside the home (Sierra-Johnson *et al.*, 2008), or skipping meals entirely (Howarth *et al.*, 2007). It will be interesting to observe whether the current younger age groups adopt a less variable meal pattern as they age, or whether they preserve their current more variable patterns of eating. Based on the outcomes of the systematic review in Chapter 2 and the findings subsequently discussed, the latter scenario may result in poorer health outcomes than the current group of older people. These observations may also explain why a higher proportion of younger people are living with overweight and obesity compared with the earlier experiences of the current older age group. The current meal patterns of older adults may reflect lifelong habits which younger adults will not have experienced as they age.

Women were found to demonstrate less variability for the majority of meal pattern properties considered compared with men. Other studies have concluded that males

and younger adults tend to eat outside the home while females are more committed to eating with family (Lin *et al.*, 2002), which results in women maintaining a more regular eating pattern. Two observational studies were identified in the systematic review (Chapter 2) which supported the findings that males who eat irregularly have a higher BMI than females (Sjoberg *et al.*, 2003; Ibe *et al.*, 2016)

4.4.2. Associations between day-to-day variability in meal pattern and the health outcomes

The summary of the results of all associations between day-to-day variability in meal pattern and health outcomes is shown in Table 4.15. The proportion of variance in the dependent variable that is explained by the independent variable in both methods was low (between 0.6% and 15%) which is considered a small effect size according to Cohen (1988). However, the statistical significance of the independent variables in predicting each health outcome indicated that changes in the independent variables are linked with shifts in the dependent variable, although they do not explain a great deal of the variability in the dependent variable. This may explain the small numbers of **slope coefficients** in each model of the linear regression, which means that the amount of variation explained by the irregularity of meal pattern is very small. However, if the coefficient of each independent variable is significantly statistically different to 0 (zero), we can conclude that a linear relationship in the population was present, and that a one unit increase in each independent variable is associated with a measurable increase or decrease in the dependent variable (the coefficient). For example, the results of Method 1 showed that an increase in SD of energy intake of 1 kcal is associated with an increase in BMI of 0.001 kg/m^2 (the coefficient for SD of energy intake is .001). Another example in Method 2 using the irregular energy index (2-fold threshold) showed that an increase in the irregular energy intake index of 1 kcal is associated with an increase in BMI of 1.25 kg/m^2 (the coefficient for irregular energy intake index is 1.25). This warrants further investigation.

4.4.2.1. Energy intake and BMI

Comparing male and female data, men showed higher levels of energy consumption than women, as would be expected because men typically have more lean body mass, and therefore a higher energy requirement (Mahan and Escott-Stump, 2004). The significant positive association between daily energy intake variation (adjusted for age and sex) and BMI seen in Method 1 is consistent with results in studies by Pot *et al.* (2014) and Pot *et al.* (2016). Moreover, the Shannon entropy showed a similar association with BMI, as an increase in the variability of the Shannon entropy by 1 unit is associated with an increase in BMI by 4.88 kg/m^2.

Using Method 2, the irregular energy index showed a consistent positive association with BMI across all thresholds, which meant that an increase by 1 kcal in the variability of energy intake is associated with an increase in BMI by the value of the coefficient of the irregular energy intake index. However, the degree of irregularity of meal patterns using Method 2 based on the Irregular times index was significantly negatively associated with BMI, which was an unexpected result, possibly due to considering a time slot to be irregular if just one of the bins at that time was considered irregular, resulting in a loss of discernment. Meanwhile, the index did not take intake into account the amount of energy that was consumed under irregular conditions (in contrast to the Irregular energy index). This discrepancy requires further investigation.

4.4.2.2. Blood pressure

No significant associations between systolic or diastolic blood pressure and the degree of variability for all meal pattern properties were found using Method 1. However, for the Irregular energy index (all fold change thresholds) in Method 2a, significant positive associations with diastolic blood pressure were observed. A previous study (Odegaard *et al.*, 2013) found that eating breakfast regularly is strongly associated with a reduced risk of hypertension, and this result was supported later in a study by Pot *et al.* (2016) which showed that subjects at 43 years old with a more irregular intake at breakfast had an increased risk of raised diastolic blood pressure (OR 1·42; 95 % CI 1·13, 1·78) ten years later.

4.4.2.3. Lipids profile

In Method 1, an increase in the SD of the interval time between first and last meal and the difference between the longest and shortest intervals of first and last meal were negatively and positively associated with cholesterol and triglyceride, respectively. Furthermore, according to Method 2, increased irregularity of meal pattern based on irregular time index was positively associated with HDL-cholesterol (all thresholds) and with LDL-cholesterol in Method 2a (2-, 2.5- and 3-fold change thresholds). However, increased irregularity of meal pattern based on irregular energy index (all thresholds) was negatively linked to HDL-cholesterol but positively linked to LDL-cholesterol (2-fold change and the absolute 100 and 200 kcal thresholds). An increase in energy irregularity was associated with a decrease in HDL-cholesterol by one unit and an increase in LDL-cholesterol by one unit. The association between lipid profile and meal pattern irregularity accord with the findings of previous studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2005). These findings suggest that a regular meal pattern tends to optimise lipid metabolism. There is sufficient evidence of a relationship between a change in circulation of lipid (dyslipidemia) and an increased risk of cardiovascular disease (Isomaa *et al.*, 2001; Lakka *et al.*, 2002; Perez *et al.*, 2007). However, the negative correlation between Irregular time index and HDL-cholesterol (at all thresholds) was an unexpected result. It is important to note the observation was not that there was no significant association, that there was a significant negative association, suggesting further investigation is warranted.

4.4.2.4. Fasting glucose

Variability in meal patterns in Method 1, based on the SD of 50% of energy time, difference between the longest and shortest interval between the first and last meal and the SD of Shannon entropy, and in Method 2 based on the irregular energy index were associated with increased fasting glucose. Moreover, based on the SD of energy intake and the SD of 50% of energy time in Method 1 and the irregular energy index in Method 2, increased irregularity in meal pattern was significantly linked to increased HbA1c, reflecting potentially higher levels of postprandial

plasma glucose (Haddadinezhad and Ghazaleh, 2010; Srihardyastutie and Soeatmadji, 2014). This is also consistent with the results of studies by Alhussain *et al.* (2016), who found that a greater postprandial glucose response to the test meal was observed after the irregular meal pattern than after the regular meal pattern. Regulating and keeping the energy intake and the time of eating meals constant could minimize any fluctuations in fasting glucose concentration. Higher HbA1c levels usually occur in individuals who already have insulin resistance, which could be explained by an increase in weight (National Institute of Diabetes and Digestive and Kidney Diseases, 2009). Blood glucose level is considered to be an important factor in predicting mortality, metabolic complications and cardiovascular risk (Lorenzo *et al.*, 2010). In contrast to the irregular energy index used in Method 2, the irregular time index was negatively correlated to fasting glucose and HbA1c. Further studies might give a different weighting to time slots depending on whether 1, 2, 3 or all 4 bins fell above the Irregularity threshold in order for them to be considered irregular.

4.4.3. Strengths of the study

The NDNS database that was used to help develop the methods used in this study was considered to be an exclusive source of high-quality data on types and quantities of foods consumed in the UK. NDNS data includes all age ranges, which improves the validity of results and supports the generalizability of the study to other populations. Meanwhile, comparing the data by gender and age for energy intake showed higher levels of energy consumption for men and younger adults, as would be expected, thus supporting the validity of the data (figure 4.2). This is due to males typically having more lean body mass than women, and a loss of lean body mass being seen with age (Mahan and Escott-Stump, 2004). However, the potential impact of misreporting was not taken into account in the analysis. It could be expected that those who are living with greater overweight or obesity, might underreport to a greater extent (Prentice *et al.*, 1986; Klingberg *et al.*, 2008). Moreover, the wide variability of the NDNS data appeared to match the wide range of age. All previous studies that characterised the regularity of meal patterns included only a small age range (young, middle-aged or older adults). Method 1

involved Shannon entropy, a novel method that was used to estimate the degree of repeatability of short evolving patterns throughout the complete data series (Marin *et al.*, 2011). In our data, the method gave us a non-negative finite value, where high values suggest higher variability and irregularity in the recorded eating pattern. The association between day-to-day variability and circadian rhythm has been suggested in this thesis as well as in previous literature.

4.4.4. Limitations of the study

The two methods used to apply thresholds (Methods 2a and 2b) when deriving the Irregular energy index and Irregular time index when classifying the bins as 'irregular' were arbitrary, as there was no previous relevant literature to draw on. As previously mentioned, discernment may have been lost by flagging a time slot as 'irregular' irrespective of whether thresholds were exceeded on one or more days. In future work, a more discerning approach might be to weight timeslots according to the number of bins at a particular time slot that showed at least one day bin that was considered 'irregular'. Meanwhile, genetic and socioeconomic factors, levels of physical activity and other health conditions such as hypothyroidism and post-menopausal issues, which were not considered in this study, could also contribute to increased weight.

The irregularity of meal patterns in the current study reflects the variation in time and energy consumption in meals or eating incidents over the 4 days. It has been speculated that consuming irregular levels of energy at irregular times influences the internal body clock – the circadian rhythms – causing chrono-disruption which may contribute to the risk of obesity and metabolic disease (Pot *et al.*, 2016). This may explain some of the findings in this chapter.

4.5. Conclusion

The study was able to characterise day-to-day variability in meal pattern using two new methods. Having applied these methods to an existing dietary intake database, associations were found suggesting that day to day irregularity may be associated with negative health outcomes. However, some results showed very weak

associations, and in some cases contrasted with the previous literature, suggesting that further exploration – ideally via intervention studies – is required to establish the clinical impact of day-to-day variation in meal pattern on health.

CHAPTER 5: THE EFFECT OF IRREGULAR MEAL PATTERN ON NITROGEN BALANCE: A RANDOMIZED CONTROLLED TRIAL IN HEALTHY NORMAL WEIGHT WOMEN

5.1. Introduction

Recently, irregularity of meal pattern has been considered as a possible new risk factor for obesity and its associated consequences, specifically cardiovascular disease and metabolic syndrome (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Pot *et al.*, 2016 and Pot *et al.*, 2016). The systematic review in Chapter 2 highlighted that whilst the effect of an irregular meal pattern on carbohydrate and lipid metabolism has been considered in a limited number of studies (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) the impact on protein metabolism has not been addressed. This is despite a relationship between meal pattern, specifically frequency, and nitrogen loss, an indicator of protein metabolism (see below), being identified by Garrow *et al.* (1981). Garrow *et al* (1981) demonstrated that not only dietary protein content, but also meal pattern, may modify protein metabolism. In a series of studies, they demonstrated, using a hypoenergetic diet, that not only increasing protein intake but also increasing meal frequency resulted in a less negative nitrogen balance over a 1-week period. Identifying whether day to day variability in meal pattern impacts on protein metabolism in the short term, when following a diet that meets energy requirements, was identified as an important new area to consider and was addressed in the present chapter using nitrogen balance as a marker for protein balance.

Nitrogen is a fundamental component of amino acids, which are the molecular building blocks of protein. Nitrogen balance is viewed as a marker of protein balance as 98% of body nitrogen is found in protein (Mason, 2015). Thus, nitrogen loss is an integrated measurement of catabolism/oxidation of all amino acids and thus of protein loss. NB refers to the net difference between the intake of nitrogen (input) and its excretion (output). NB has been considered as an indicator of the catabolic stress level (Dickerson, 2005) and reflects the net change in the nitrogen pool in the body.

Preserving metabolically active tissue, such as protein rich muscle, is considered beneficial to weight loss as basal metabolic rate, hence energy expenditure, is

maintained. Should irregular eating be associated with a loss of lean tissue, indicated by more negative nitrogen balance, this might contribute to a lower energy expenditure which may contribute to the apparent associations between excess weight and irregular eating identified in the systematic review (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022; Pot *et al.*, 2016 and Pot *et al.*, 2016).

It was thus hypothesized that in an acute study over 7 days, irregular eating would result in greater nitrogen loss than with regular eating on isoenergetic diets that met estimated energy requirements. Accordingly, the primary aim of this study was to investigate the effect of irregular meal pattern, compared with a regular meal pattern, on N balance in healthy normal-weight females over an acute, 7- day period.

The second aim of the study was to explore the repeatability of previous findings suggesting differences in appetite ratings when adhering to a regular and irregular meal pattern. Specifically, Alhussain *et al.* (2016) found that higher post-meal ratings for hunger and lower post-meal ratings for fullness (P= 0.01) were observed during an irregular, compared with regular, intervention period on day 7 of the intervention. Therefore, the secondary aim of this study was to compare appetite ratings across the 7 days of the interventions.

Finally, the third aim of the study was to assess the feasibility of measuring diurnal peripheral wrist temperature in the participants enrolled in the study, in anticipation of undertaking future studies investigating the effect of irregularity of meal pattern on diurnal rhythm.

5.2. Methods

The study was approved by the University of Nottingham, Faculty of Medicine and Health Sciences Research Ethics Committee (189-1812). The study was registered at ClinicalTrials.gov with the identification number: NCT05226650.

5.2.1. Participants

Participants with healthy normal weight (BMI 18.5-25 kg/m^2) females aged 18-40 years were recruited. Inclusion criteria were explained in detail in Chapter 3, Section 3.2.1.

5.2.2. Sample size

There were no similar studies in the literature considering meal pattern irregularity and nitrogen balance on which to base sample size calculation. However, a previous study by Garrow *et al.* (1981) compared nitrogen loss between different meal frequencies (1 meal per day with 5 meals per day) over a one-week period whilst providing an 800kcal/d diet. Using data from the first week of their intervention, (so comparable with our 7-day intervention), in order to detect the 3.02g/ d difference in balance that they saw, 7 participants were required, based on an assumption that the within- participant standard deviation for the response variable was 1.36g/kg. The probability is 92% that the study will detect a treatment difference at a two-sided 0.05 significance level if the true difference between treatments is 3.02g/kg. In this study, 14 participants were recruited, to allow for dropouts and as it was not known whether providing a diet that met energy requirements might result in smaller difference in nitrogen balance between the two interventions.

5.2.3. Screening

All potential participants attended a screening visit to establish that they met the inclusion criteria for the study and to confirm the general health of participants. Eligible participants were asked to complete a weighed 3-d food diary. The procedures of the screening were explained in detail in Chapter 3, Section 3.4.

5.2.4. Study design

The study design was described in detail in Chapter 3 and outlined in Figure 3.1. In summary, the study followed a randomized crossover design with two (7-day) intervention periods separated by a wash-out period of 21 days. In period 1,

participants followed either a regular (6 meals/day) or an irregular meal pattern (3-9 meals/day). In period 2, participants followed the alternative meal pattern to that followed in period 1. In order to control for menstrual cycle, which is known to influence food intake (Dye and Blundell, 1997, Brennan *et al.*, 2009, Tucci *et al.*, 2010, McNeil and Doucet, 2012) and nitrogen balance in women (Bisdee *et al.*, 1989), interventions were started towards the end of the first week of the menstrual cycle on both occasions.

5.2.5. Dietary intervention

In the two intervention periods, identical foods were provided to a participant in amounts designed to meet their energy requirements (Chapter 3, Section 3.5.2). In order to avoid monotony and boredom during the intervention period, the food was supplied in a 4-day cycle of menus. Menus were designed for 1900 kcal/day, 2100 kcal/day and 2200 kcal/day to meet the different estimated energy requirements of participants. Participants were assigned the menus with the energy content closest to their energy requirement (±100 kcal). The procedures for dietary intervention were explained in detail in Chapter 3, Section 3.5.1.

5.2.6. Measurements at the baseline

Before starting each intervention, body weight was taken, and BMI was calculated. The procedure of measuring weight and calculation of BMI were explained in detail in Chapter 3, Section 3.4.1.

5.2.7. Measurements during each day of the intervention week

1. Participants collected a 24-hour urine sample each day. Nitrogen balance was calculated based on the urinary urea nitrogen using daily 24-h urine samples. All details were described in Chapter 3, Section 3.5.3.2.

2. A booklet was provided to record the subjective appetite (hunger, fullness and desire to eat) which included VAS before and after each single meal on day 7 during both intervention periods when 6 meals were consumed on both interventions. The protocol was explained in Chapter 3, Section 3.5.3.3.

3. A Body Media Sensewear armband was worn to estimate physical activity level to ensure that each participant had a similar level of physical activity in both intervention periods. All details were described in Chapter 3, Section 3.5.3.1.

4. Diurnal peripheral wrist temperature was recorded by participants wearing a wireless iButton data logger as explained in detail in Chapter 3. The results of this feasibility study are not reported in this chapter but in Chapter 6, Section 3.5.3.4.

5.2.8. Statistical analysis

Statistical analysis was carried out using SPSS software (version 28). All data were found to be normally distributed using the Shapiro-Wilk's test and expressed as mean (SD or SEM). The details of the statistical methods were explained in Chapter 3, Section 3.6.

5.3. Results

5.3.1. Characteristics of the participants

Fourteen participants completed both interventions and were included in the analysis. The flow of participants through the recruitment process is shown in Figure 5.1. Table 5.1 shows the participant characteristics at baseline for weight, height, BMI, body fat percentage, physical activity, pre-study dietary intake and estimated energy requirement.

Figure 5.1: The recruitment process

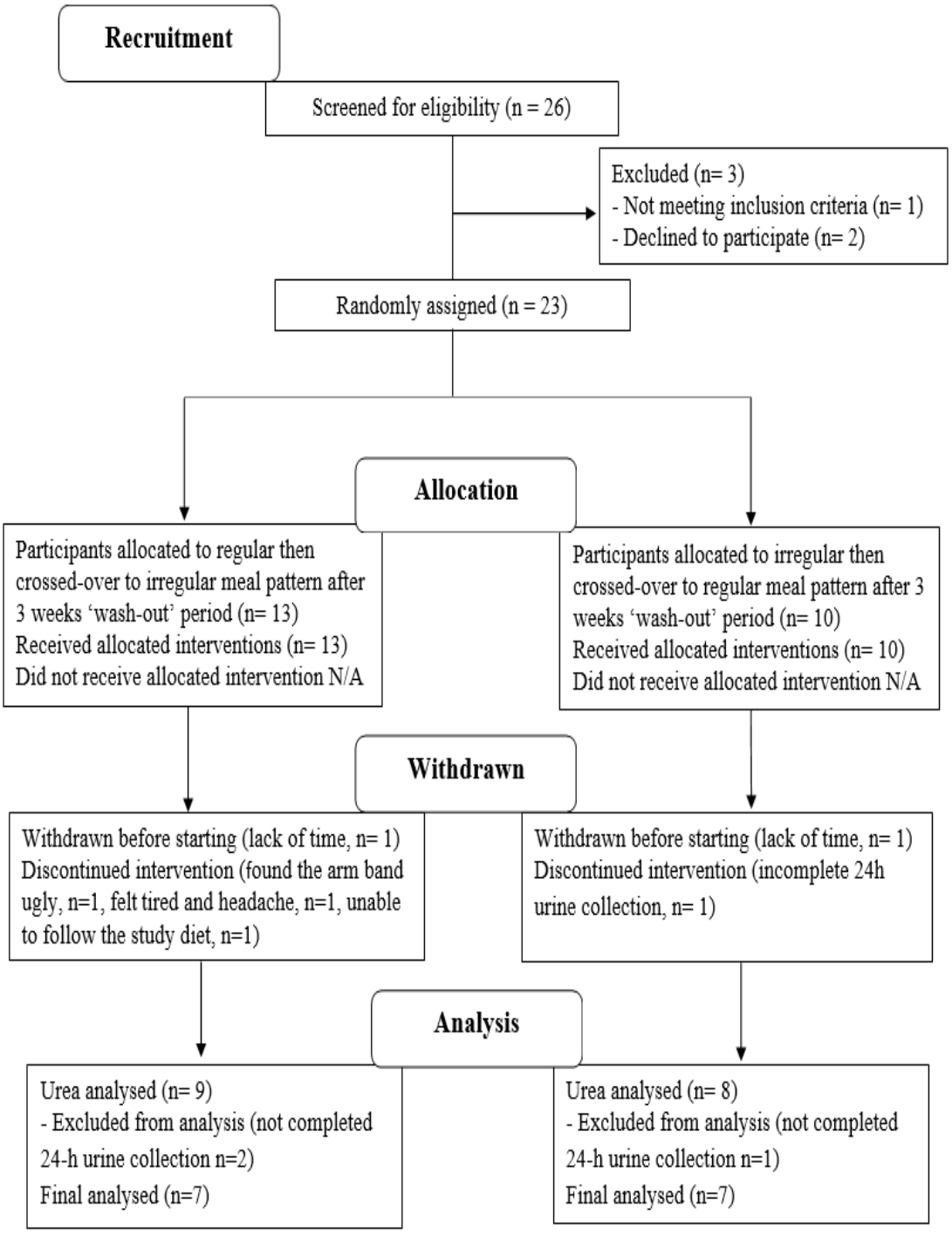


Table 5.1: Participants characteristics*

Participants characteristics	
Age (years)	26.1 ± 4.9
Weight (kg)	53.1 ± 6.6
Height (cm)	161 ± 6.04
BMI (kg/m^2)	20.4 ± 1.5
Body fat (%)	22.5 ± 3.2
Physical activity level	Moderate
Pre-study dietary intake (kcal/day)	2049 ± 102**
Estimated energy requirement (kcal/day)	2035 ± 105
Prescribed intervention diet (kcal/day)	2053± 83

*All values are means ± SDs. n = 14.
**There was no significant difference between pre-study dietary intake and estimated energy requirement.

5.3.2. Dietary intake

Table 5.1 shows the mean daily energy intake before the start of the study based on self-reported 3-day food records (2 weekdays and 1 weekend) and the estimated energy requirement in the prescribed intervention diet to maintain body weight. There was no significant difference between these two values for the participants. The dietary compliance records completed by participants during the two intervention periods showed that for energy intake, 93 ± 0.02% and 94 ± 0.01% of the energy prescribed was consumed in the regular and irregular intervention periods, respectively. The reported consumed energy intake values during the regular and irregular interventions were not significantly different for energy (Regular: 1932 ± 192 v. Irregular 1961 ± 144 kcal/day). The composition of consumed foods also did not differ significantly between the two intervention periods (Regular: 52 ± 0.9% carbohydrate, 15 ± 0.4% protein and 33 ± 0.8% fat in regular v Irregular: 53 ± 0.3% carbohydrate, 15 ± 0.5% protein and 32 ± 0.7% fat).

However, there was a significant difference between the estimated energy requirement of the prescribed diet (2049± 102 kcal/day) and the energy actually consumed in each intervention (Regular: 1932± 192 kcal/day) (paired T-test,

p=0.008), and (Irregular: 1961± 144 kcal/day). For the three macronutrients as a proportion of total energy, pre study daily self-reported percentage of total energy from carbohydrate was significantly higher and percentage of total energy from protein was significantly lower than in the diet that was consumed diet (carbohydrate: 60 ± 4.1% vs 52 ± 0.9%) (protein: 10 ± 0.7% vs 15 ± 0.8 %) (paired T-test, $p < 0.01$). For percentage of total energy from fat, self-reported diet was similar to the consumed diet (32.5 ± 0.7% vs 34 ± 0.7%). The mean of absolute protein intake was 50.9 ± 1.6 g/day on the habitual diet which provided 1.04g/kg/day of protein and 71.9± 2.1 g/day on consumed diets which provided 1.35g/kg/day of protein. Therefore, the habitual diet and the diet consumed during the interventions met the dietary (Department of Health, 1991).

Based on the self-reported pre- study food diary for three days (2 weekdays and 1 weekend), participants' habitual diet was characterised visually (investigator's qualitative analysis) as a chaotic or irregular meal pattern compared with the prescribed regular study meal pattern. This was done by looking at each point when food was consumed and considering how frequently a meal was consumed by the participants at that time on each of the three days when they were recording their habitual intake. It was noted also that their eating tended to be more similar to the prescribed menu during weekdays compared with the weekends. The first meal of the day was the only meal that was eaten on a regular basis between a narrow time slot (6:00 and 7:00 am). Although there tended to be consumption of a subsequent meal around the middle of the day it fell between a three-hour window. (12:00 - 3:00 pm). A third meal tended to be eaten later in the day, but the time varied broadly from (8:00 - 11:00 pm).

5.3.3. Physical activity

The compliance data for wearing the Sensewear, showed that it was worn 99 ± 1.4% and 99 ± 1.0% of the regular and irregular intervention periods respectively. There was no significant difference in the mean physical activity level during the regular and irregular intervention periods (Regular: 1.62 ± 0.17 v Irregular: 1.64 ± 0.23 times).

5.3.4. Weight and BMI

Table 2 shows participants body weight and body mass index before and after each intervention. There was neither a significant interaction effect of meal pattern interventions, nor a main effect of meal pattern or visit.

Table 5.2: Weight and BMI of participants over the study

	Regular meal pattern		**Irregular meal pattern**	
	Before	After	Before	After
Body weight (kg)	53.5 ± 5.9	53.2 ± 5.4	53.4 ± 5.7	53.6 ± 5.6
BMI (kg/m^2)	21 ± 2.21	20 ± 2.01	20 ± 2.12	21 ± 2.02

All values are means ± SDs. n = 14. There was no significant interaction or main effect of the visit on weight and BMI (2-way repeated measure ANOVA). Post, postintervention; Pre, pre-intervention.

5.3.5. Nitrogen balance

Table 5.3 and Figures 5.2, 5.3 and 5.4 show the mean and standard deviation, across the participants, for estimated dietary nitrogen intake, loss and balance for the irregular and regular meal patterns on each of days 1 to 7 for the two interventions. The mean nitrogen balance was positive on all days.

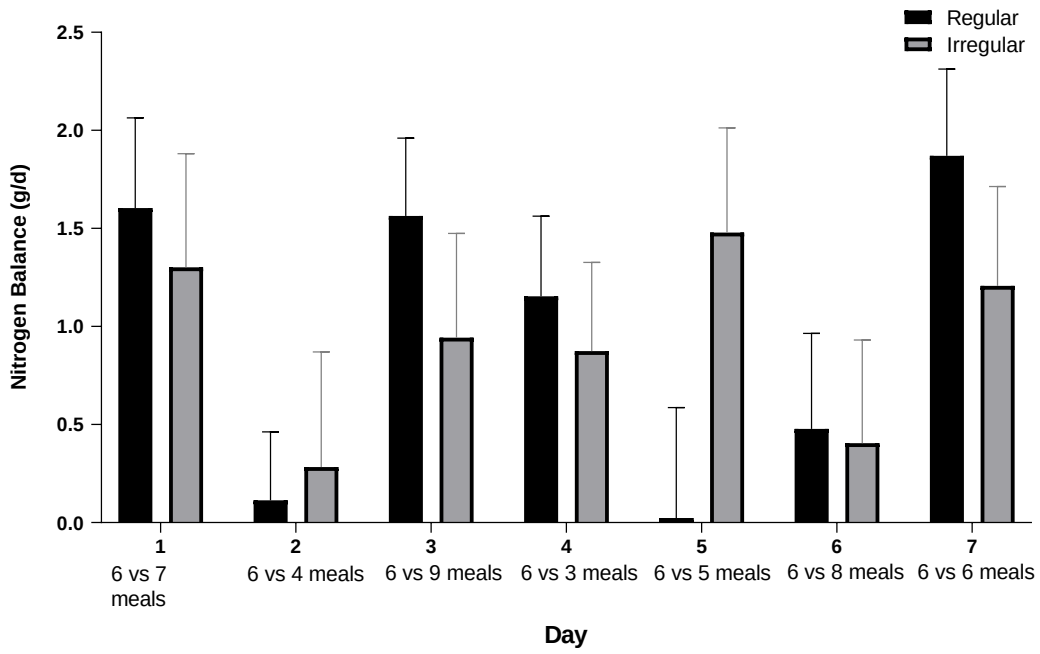
A two-way repeated measure ANOVA was run to determine the effect of regular and irregular meal pattern over 7 days on nitrogen balance. The nitrogen balance data was normally distributed ($p > .05$) as assessed by Shapiro-Wilk's test of normality. Moreover, mild outliers were detected when visually inspecting the box plots and they were not excluded from the analysis. There was no significant interaction effect of the meal pattern regularity by 7-day measurements on nitrogen balance $F (6, 78) = 1.90$, $P=.091$, partial $\eta 2 = .128$ which is a small size effect (Cohen, 1992). No main effect of treatment was seen $P=0.88$. The main effect of days showed that there was a statistically significant difference in nitrogen balance between day points, $F (6, 78) = 3.55$, $p= .004$. Post hoc T – test comparisons with Bonferroni correction of pairs of days, irrespective of treatment, showed no significant differences.

Table 5.3: Nitrogen intake, output and balance*

	Nitrogen intake (g/day)		Nitrogen output (g/day)		Nitrogen balance (g/day)	
	Regular meal pattern	Irregular meal pattern	Regular meal pattern	Irregular meal pattern	Regular meal pattern	Irregular meal pattern
Day 1	11.61± 0.82	11.80 ± 1.04	10.01 ± 1.92	10.50 ± 2.19	1.60 ± 1.7	1.30 ± 2.1
Day 2	10.39 ± 1.40	10.59 ± 1.01	10.28 ± 1.62	10.30 ± 2.12	0.11 ± 1.3	0.28 ± 2.1
Day 3	11.49 ± 1.38	11.87 ± 1.23	9.92 ± 1.64	10.93 ± 1.81	1.56 ± 1.4	0.94 ± 1.9
Day 4	11.56 ± 1.18	11.53 ± 1.51	10.40 ± 1.83	10.65 ± 1.77	1.15 ± 1.5	0.87 ± 1.6
Day 5	11.34 ± 1.40	11.73 ± 0.88	11.31 ± 2.12	10.25± 2.08	0.02 ± 2.1	1.48 ± 1.9
Day 6	10.22 ± 1.39	10.70 ± 1.09	9.74 ± 2.33	10.30 ± 2.16	0.48 ± 1.8	0.41 ± 1.9
Day 7	11.50 ± 1.17	11.87 ± 0.88	9.59 ± 1.59	10.67 ± 1.80	1.87 ± 1.6	1.21 ± 1.8
Mean	11.15 ± 0.59	11.44 ± 0.55	10.17 ± 0.57	10.51 ± 0.25	0.92 ± 0.44	0.97 ± 0.76

*All values are means ± SDs. n = 14. No significant difference between regular and irregular meal patterns was found (2-way repeated measure ANOVA, P=0.091).

Figure 5.2: Nitrogen balance over the study for each meal pattern



Mean ± SEM for the N balance over the study period in 14 healthy women after regular and irregular meal pattern. There was no significant meal pattern interaction effect between regular and irregular meal-pattern periods (2-way repeated measure ANOVA, P=0.091). No main effect of treatment. However, there was a statistically significant main effect of day in nitrogen balance (p= .004). Post hoc T – test comparisons between days, with Bonferroni correction for multiple comparisons showed no significant differences.

Figure 5.3: Nitrogen loss over the study period for each meal pattern

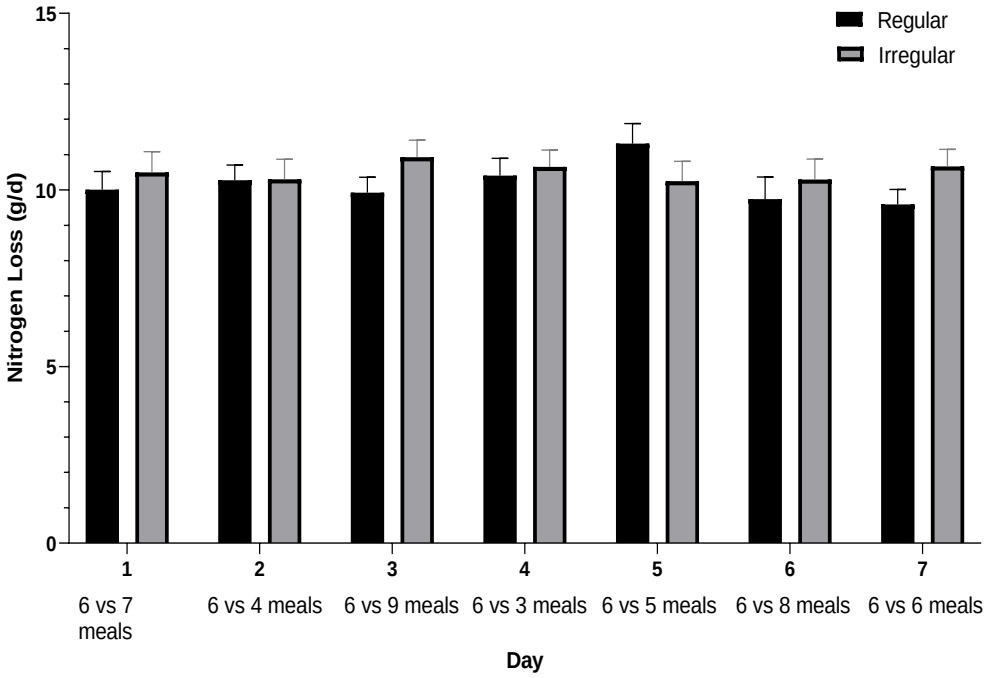
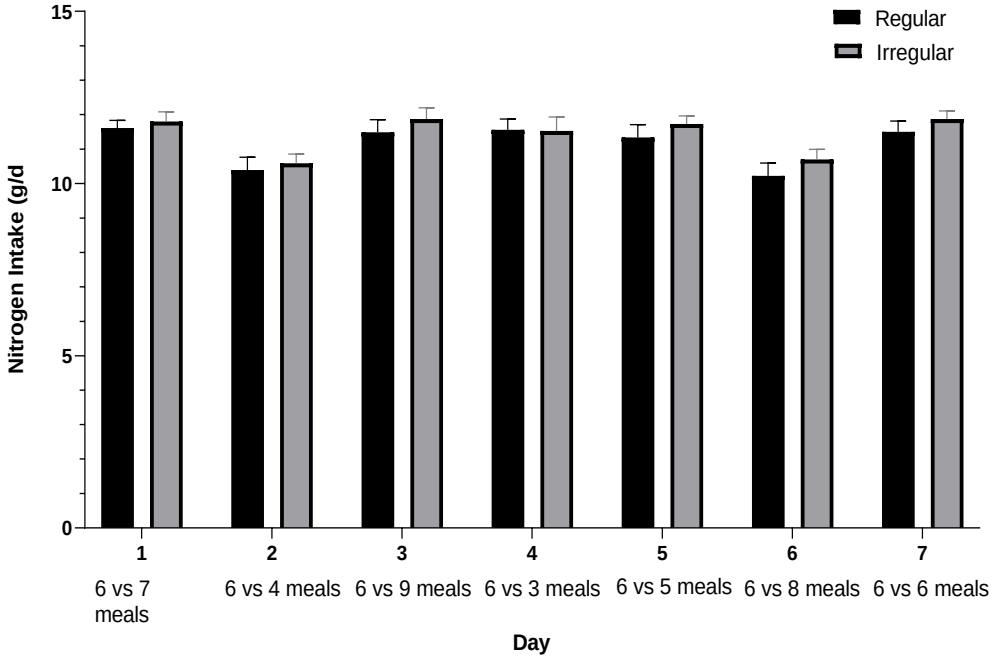


Figure 5.4: Nitrogen intake over the study period for each meal pattern



5.3.6. Subjective appetite ratings

Participants completed ratings before and after the consumption of each meal during the last day (day 7) in both regular and irregular intervention periods. Six meals per day had been consumed with each intervention. Comparing the daily mean pre meal values for the two intervention and comparing the daily mean post meal values for the two interventions (Table 5.4) demonstrated no statistically significant differences in all appetite ratings (hunger, satiety, fullness, and prospective food consumption (PFC)). The comparison of mean differences between subjective appetite ratings before and after the meal on day 7 of regular and irregular meal patterns was shown in Table 5.5.

Table 5.4: Comparison of appetite ratings (all day points combined) on day 7 of regular and irregular meal patterns*

mm	Regular meal pattern		Irregular meal pattern	
	Pre-meals	**Post-meals**	**Pre-meals**	**Post-meals**
Hunger	49.5± 19.5	19.1± 14.3	49.9± 16.6	19.3± 12.5
Satiety	43.5± 17.3	74.5± 14.3	40.7± 14.4	73.5± 11.4
Fullness	42.1± 18.5	74± 12.7	41.4± 15.8	73±12.8
PFC	54.1± 17.8	23.9± 15.1	53.1± 15.9	26.3± 15.9

*All values are means ± SDs. n = 14. No significant differences between regular and irregular meal pattern in all appetite ratings of pre or post meal (paired T-test).

Table 5.5: Mean differences between subjective appetite ratings before and after the meal on day 7 of regular and irregular meal patterns*

	Regular meal pattern	Irregular meal pattern
Hunger	-30.1 ± 15.8	-30.8 ± 14.6
Satiety	31.5 ± 17.6	33.5 ± 13.5
Fullness	32.9 ± 21.4	32.2 ± 12.5
PFC	-30.7± 15.05	-27.4 ± 11.9

* All values are means ± SDs. n = 14. No significant differences between regular and irregular meal pattern in all appetite ratings of pre or post meal (paired T-test).

5.3.7. Diurnal peripheral wrist temperature

The result for the test of feasibility of obtaining peripheral wrist temperature using iButton was presented in Chapter 6 section 6.

5.4. Discussion

The primary aim of this study was to investigate the acute effect of an irregular meal pattern, compared with a regular meal pattern, on nitrogen balance in healthy normal-weight females over a 7- day period in whom energy and protein requirements had been met. The difference in nitrogen balance between the two interventions was not significantly different. The secondary aim of the study was to assess the effect of regular and irregular interventions on subjective appetite rating across the 7-day period. There were no significant differences in pre- and post- meal values between interventions in all appetite ratings. Thirdly the feasibility of using an ibutton was assessed. The results were reported in Chapter 6.

It is not thought that the impact of irregular meal pattern on nitrogen balance has been studied before. However, others have considered the impact of other meal pattern differences. It thus appears that whilst Garrow *et al* (1981) demonstrated that with a hypoenergetic diet, a higher meal frequency may result in less negative nitrogen balance than a lower meal frequency, greater day to day variation in meal pattern does not have a marked effect on nitrogen balance over a 7-day period. A tendency was seen (P=0.091) for an intervention by time interaction, across the 7 days with the irregular meal pattern compared with the regular pattern (not significant). Visual observation showed that nitrogen balance was more positive with a regular meal pattern than an irregular one, on 5 of the 7 days. It would be of interest to extend the study to 10 – 14 days as there is some debate as to how long adaptation in nitrogen balance takes to occur (Munro and Allinson, 1964; Price *et al.*, 1994; Young, 1999).

The diets in this study were designed to meet estimated energy and protein requirements and the participants previously had an adequate dietary intake of

energy and protein, had been in good health, had not recently modified their level of activity and had not experienced any recent physical stress, trauma or immobilization (Schonheyder *et al.*, 1954; Dickerson, 2005) from which they might have been recovering. It would thus have been expected that they would be in nitrogen equilibrium prior to the study. It was noted that during the study nitrogen balance for both interventions was positive meaning that nitrogen intake was greater than nitrogen output (Mason, 2015).

The protein content in the consumed diet was higher compared with the participant's habitual pre-study diet. This might have induced a more positive energy balance, although evidence to support this is conflicting. Their habitual diet met their protein requirements given that the Reference Nutrient Intake (RNI) of protein for adults is 0.75g protein per kg body weight per day (Lonnie *et al.*, 2018); this equates to 45g/day for women of average body weight (60kg). However, it is interesting to note that the habitual reported percentage of total energy from protein was approximately 10% which is lower than the recommended 15% of total energy from protein and equated to an average for the group of 50g per day which is less than the current estimate for protein intake for this age group of 62g/day (Public Health England (2019).

Over the longer term, the most conspicuous adjustment to an increase in protein intake is an increase in amino acid oxidation and in subsequent nitrogen excretion, mainly as urea and especially pronounced in the fed state (Price *et al.*, 1994). However, it may have been that there was insufficient time for adaptation in the urea cycle response to this difference over a week (Drummen *et al.*, 2018). It was showed that the nitrogen balance took about 10 days to get into a new equilibrium when intake was increased or decreased. There was a mobile pool of soluble protein which increased or decreased its total protein content when intake changed (Millward *et al.*, 1991). An increase in the protein intake, assuming to above obligatory losses, induces a series of adaptive processes (Tom and Bos, 2000; Drummen *et al.*, 2018). Acutely, high protein intake stimulates protein synthesis and turnover, and causes a small suppression of protein degradation (Tang and Phillips, 2009; Gilbert *et al.*, 2011). In addition, a net 24-hour positive NB of 2 to

4 g is a sign of optimal level for anabolism (Hammer *et al.*, 2017). However, a positive nitrogen balance following high intake of protein (Garlick *et al.*, 1999; Price *et al.*, 1994; Westerterp-Plantenga *et al.*, 2004) does not always contribute to an increase in protein anabolism. It has been strongly suggested that there is limited anabolic stimulation by high protein diets in healthy young adults (Tome and Bos, 2000) who have not been immobilsed as the body's capacity to increase amino acid anabolism through an increase in lean body mass is limited (Millward, 2007).

The study diet contains only high-quality protein (animal sources) which typically possess a high biological value and provide all essential amino acids. The quantity of amino acids entering the metabolic pool from the catabolism of tissue proteins is appreciable in relation to that from food and it is the amino acid balance of the resultant mixture that determines the rate of protein anabolism. The high-quality protein may reduce the nitrogen loss unlike poor quality protein which is correlated with an increase in nitrogen output due to the inefficient utilization of indispensable amino acids (Tome and Bos, 2000). Were this different to the participants' habitual diet which included more low-quality protein, then there may have been a period of adaptation to the different protein quality. For example, if a mixture of amino acids lacking one of the essential amino acids (lysine) was consumed, the tissue proteins which have a relatively high proportion of lysine will compensate diets low in this amino acid (Miller and Payne, 1964), thus, the nitrogen equilibrium could not be maintained and a negative nitrogen balance was accrued (Bender, 2012).

In contrast to the potential effects of an increased protein intake, and improved biological protein quality, urinary nitrogen excretion may have been increased by compliance with the diet that was incomplete in both interventions, being 93% and 94% of energy given in the regular and irregular meal pattern respectively. The participants might thus have been expected to be in in negative energy balance providing energy expenditure had not been underestimated. However, their weight was stable across the study, suggesting that there was no major difference between energy intake and expenditure. Had this not been the case then providing their nitrogen intake was sufficient to meet their obligatory losses of nitrogen (as

measured in urine), any dietary intake above obligatory losses may have been used as an energy source potentially resulting in further urinary excretion of urea (Bodwell *et al.*, 1979).

A large change in body weight would not have been expected in either intervention because of the short duration of the intervention as noted by others using the same dietary protocol in earlier studies in obese females (Alhussain *et al.*, 2022 and 2016 and Farshchi *et al.*, 2005). Interestingly however it was noted that that numerically weight was decreased after the regular intervention and increased after irregular intervention as in the previous studies (Alhussain *et al.*, 2022; Alhussain *et al.*, 2016 and Farshchi *et al.*, 2005). This is compatible with the irregular meal pattern showing an association with a lower TEF (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022) which might contribute to the development of obesity over the long term.

With respect to the measurement of urinary nitrogen, positive balance may have been as a result of an underestimation of N excretion as only urinary urea was measured. This is approx. 85% of total urinary N under normal conditions and the fecal N loss plus loss of skin protein mean that the total excretion is likely to be about 15% higher than estimated N loss from urea (Lee, 1969 and Palmo *et al,* 1989). However, a factor of 4 g/d was added to the equation for NB calculation (2g of N derived from urinary non-urea and 2g for N in faeces and integumentary (dermal) losses (Dickerson, 2005).

Another contributor to the apparent positive nitrogen balance may have been incomplete urine collection, resulting in an underestimate of the nitrogen losses. Various methods have been proposed for assessing the completeness of a urine collection which might have been included such as use of a PABA tablet (Para-aminobenzoic acid) (Sharma *et al.*, 2014). PABA tablets which can be taken at evenly spaced intervals throughout the urine collection period. The last tablet should be taken at least 8 hours before the final sample of urine collection on the second day. This time is required for PABA clearance through the kidneys to ensure

that all PABA is excreted by the time the participant collects their final urine sample (Cox *et al.*, 2018).

There may also have been an overestimation of the nitrogen in the diet as the nitrogen was calculated based on the protein content, which was obtained from the nutrition facts label in each food item, using the nitrogen-to-protein conversion factor (6.25g) of protein as equating to 1g of nitrogen. It was thus assumed that protein contains 16% nitrogen whereas not all proteins have this amount of nitrogen (Jones, 1941). Jones (1941) reported that the nitrogen content of avocado protein is 13.4% and amandin (an almond protein) is 19.3%. Consequently, using a 6.25 conversion factor can lead to a 15–20% error in the protein content (Mariotti *et al.*, 2008). Furthermore, collagen contains 18% nitrogen, thus, the nitrogen-to-protein ratio in meat products will be varied as a function of the collagen level (Benedict, 1987).

A significant main effect of time was seen although post hoc analysis did not identify any significant differences between days. Visual observation suggested that there did not appear to be a linear progressive change across the 7 days of the study which might have suggested adaptation. On the other hand, it was noted that less positive nitrogen balance was seen on days 2 and 5 which corresponded with a recorded lower intake of protein on those days. However, any interpretation of these results should be done with cautions as N intake and output are more likely to balance over a few days, so a 7-day average is much more meaningful than within day comparisons. In future studies, further strategies should be applied to ensure that the intake of protein on each day is the same across the intervention period.

The present study failed to demonstrate any significant differences in pre- and post-meal VAS ratings between the two intervention periods. This result is in agreement with a study conducted in obese women (Alhussain *et al.*, 2022) and in contrast with a previous study conducted in healthy women (Alhussain *et al.*, 2016). This latter difference was possibly due to a lower energy intake with respect to the consumed diet compared with the prescribed diet, according to the dietary compliance records, in this study. Alhussain *et al*, (2016) reported 98-100%

compliance with the dietary prescription in contrast to 93-94% in this study. The participants in the current study had similar characteristics to those in the previous study with respect to age, BMI, and body fat; however, their ethnicity may have been different, which possibly resulted in differences in response to the meal pattern.

5.4.1. Strengths of the study

A major strength of this study is the careful attention that was paid to ensuring that other factors that influence nitrogen balance were controlled in order to prevent them confounding the results. Providing normal healthy subjects are included and a balanced energy diet is given as in this study then the use of UUN instead of TUN is acceptable (Milner *et al.* 1993).

Careful attention was paid to when female participants undertook the study with respect to their menstrual cycle as menstrual cycle stage has been shown to influence nitrogen balance in women (Bisdee *et al.*, 1989). Participants commenced both study interventions at the same time point of their menstrual cycle which was as soon as their menstrual period had finished which was between day 8 and 14. Positive N balances have been observed around ovulation while negative balances were recorded just before the onset of menstruation (luteal phase) (Egun and Atinmo, 1993). This could be explained by decreasing amino acid levels and elevated nitrogen utilization in the luteal phase (Draper *et al.*, 2018).

During the study, physical activity was monitored by the SWA device, and it was demonstrated that participants had complied with the request to undertake similar levels of activity during the intervention periods. This was important as physical activity is a factor which affects nitrogen balance (Rand *et al.*, 2003). It has been demonstrated that acutely during exercise, protein oxidation increases as a result of metabolizing proteins by the skeletal muscle in response to motor stimuli in healthy adults (Gontzea *et al.* 1974; Chesley *et al.*, 1992; Lemon 1994). In contrast, over a longer time period exercise develops muscle mass (Poole *et al.*, 2010).

The aim of this study was to test the impact of meal pattern irregularity whilst meeting energy requirements. Whilst there had been good agreement between the pre study reported energy intake, and the estimated energy requirement less than 100% compliance meant that participants consumed less than they had been prescribed. However, maintenance of a stable body weight across the study indicates that they were in fact in energy balance. Energy balance is important because it affects the utilization of dietary protein. An increase in the level of energy intake beyond requirements leads to N retention by improving protein synthesis and diminish amino acid oxidation, whereas a decrease in the energy of the diet leads to N loss from the body (Ministry of Health, 1964). This is because in prolonged negative energy balance, the body utilizes lean tissues by protein breakdown to use it as a source of energy (Jeejeebhoy, 1986; Tessari, 2006). Nevertheless, higher dietary protein in the diet even when energy intake is low for short period, which was the case in the Garrow *et al.* study, may have a significant N-sparing effect (Garrow *et al*, 1981). Any change in energy intake below or above the individual's requirement it seems impacts on N balance (1-2 mg of N retained per kcal) (Jequier, 1991).

5.4.2. Limitations of the study

Potential limitations of the study have already been discussed above. In conclusion, it is suggested that a longer study should be undertaken, to explore further the finding of a tendency for the treatment effect. Ideally the study would be undertaken in a metabolic unit in order to fully control the study conditions and ensure complete collection of urine. Ideally it would be preceded by a period of standardization to the protein intake that would be provided during the study.

5.5. Conclusion

The findings from the present study have indicated that the effect of meal pattern irregularity on nitrogen balance over 7-day period is not significant. With regard to subjective appetite, there were no changes in all appetite ratings with consuming a regular or irregular meal pattern which was inconsistent with previous studies. It would be of interest in future works to study overweight and obese individuals in the same protocol with restricted nitrogen content to make the findings applicable to a broader population and to conduct longer term studies.

CHAPTER 6: A FEASIBILITY STUDY OF A RANDOMIZED CONTROLLED TRIAL CONSIDERING THE EFFECT OF A HYPO-ENERGETIC DIET WITH AN IRREGULAR MEAL PATTERN ON ENERGY EXPENDITURE, METABOLISM AND DIURNAL RHYTHM IN NORMAL WEIGHT AND OVERWEIGHT ADULTS

6.1. Introduction

Given that obesity, which is defined as excess body weight consisting of an abnormally high accumulation of adipose tissue, is considered to be an epidemic (Hill *et al.*, 2012), it is vital to identify novel factors that may contribute to its cause. In the systematic review described in chapter 2, irregular meal patterns were identified as a potential novel contributor to obesity and its comorbidities. Obesity results from energy intake exceeding expenditure over an extended period (Wright and Aronne, 2012) and has been associated with impaired metabolic function and poor health (Singla *et al.*, 2010). However, the majority of studies identified in Chapter 2 were designed to explore association, rather than causation.

A limited number of intervention studies were found which compared the impact of an irregular and regular meal pattern on health-related factors in normal weight and obese participants (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022). Only one study, that of Demos *et al.* (2015), looked at weight reduction in response to eating meals regularly at a specific planned time without considering metabolic parameters. In response to a test drink, for example, the thermic effect of food (TEF) in normal-weight and obese women was significantly greater ($P<0.05$) following a regular meal pattern than an irregular meal pattern (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016). Irregular meal patterns also produce a degree of insulin resistance, higher fasting concentrations of total cholesterol and LDL cholesterol (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2005) and higher glucose responses (Alhussain *et al.*, 2016).

To date, intervention studies exploring meal pattern irregularity and metabolic consequences have ensured that the energy requirements of the participants were met. It is not known whether consuming a regular meal pattern whilst reducing energy intake to below energy requirements results in greater health benefits than introducing the same energy deficit while following an irregular meal pattern. Exploring this factor would be relevant to improving dietary health interventions

designed to reduce overweight and obesity and their consequences (Kendall *et al.*, 1991; Shick *et al.*, 1998; Foster *et al.*, 2003; Sondike *et al.*, 2003; Brinkworth *et al.*, 2009; Johnstone, 2012). It has been hypothesised that adhering to a regular meal pattern whilst consuming a diet with an energy deficit would result in more beneficial health related measures than following an identical diet that is consumed under an irregular meal pattern. However, the feasibility of undertaking such a study, in which participants are required to comply with a reduced energy intake relative to their requirements with contrasting degrees of meal pattern regularity, is unknown, and therefore warrants testing before undertaking a full study.

The level of meal pattern irregularity identified in Chapter 2 might cause chronodisruption because the changes in timing of food intake may also disrupt the chrono-biological or circadian rhythm of hormones such as insulin, glucagon, adiponectin and leptin as well as gastric acid secretion (Garaulet and Gomez-Abellanand, 2014). It has been postulated that chrono-disruption may contribute to the apparent negative health consequences seen to result from an irregular meal pattern (Pot *et al*, 2014; Pot *et al.*, 2016). Extreme meal pattern irregularity, as seen in those working night shifts, results in chronodisruption and negative health consequences similar to those affected by the less extreme meal pattern irregularity reported in the systematic review (Chapter 2) (eg. insulin resistance (Sierra-Johnson *et al.*, 2008)). Core body temperature has a circadian pattern which can be changed by factors that cause chronodisruption, such as shift pattern work (Hiddinga *et al.*, 1997). However, the impact of more moderate irregularity on core body temperature has not been considered, as noted in Chapter 2. We can therefore postulate that following an irregular meal pattern may result in differences in core body temperature pattern throughout the day when compared with a regular meal pattern, and that this will be sustained beyond the period of intervention.

In order to study the impact of meal pattern irregularity on health outcomes while providing a hypoenergetic diet, and to consider the potential for chronodisruption, it was necessary to expand previously tested intervention study protocols (Farshchi *et al.*, 2005; Alhussain *et al.*, 2016). The gold standard method to test the impact of dietary interventions on circadian patterns is to study the impact of an intervention

under a ‘constant routine protocol’ (Duffy and Jink, 2002) in a residential metabolic unit (Weherns *et al.*, 2017) in order to avoid the effect of masking factors. Masking factors refer to environmental influences as well as factors within the body which modulate its overt rhythm (Minors and Waterhouse 1989). However, a more practical alternative is to undertake a free-living study in which masking factors, which impact on circadian patterns, are standardised and kept as constant as possible (Sarabia *et al.*, 2008).

Firstly, a standardisation week is required before the interventions begin, during which both groups receive the same diet with the same meal pattern in order to minimise the impact of their habitual diet. During this period and throughout the study other key factors, such as time of going to bed (Barrett *et al.* 1993) and exposure to the light (Badia *et al.* 1991; Dijk *et al.* 1991), should also be standardised. Participants are then exposed to the respective dietary interventions, before measurements of the outcomes of interest are made during a second standardised period. This allows any lasting impact of the intervention on diurnal patterns to be measured under standard conditions. The term diurnal is used to reflect the fact that strictly constant routine protocol conditions such as sleep time and exposure to light have not been achieved. Core body temperature can be measured using a swallowed pill sized device which transmits body temperature to a receiver. However, the feasibility of applying the extended protocol, including using swallowed devices for measuring core body temperatures in free living participants, has not been tested before.

This chapter describes a feasibility study prior to undertaking a full study. The aim of this study is to test the feasibility of undertaking an intervention study aimed to compare the impact of 14-day hypo energetic diet with regular and irregular eating (all food provided) on the TEF (the primary outcome measure), carbohydrate metabolism, lipid concentrations and subjective appetite while also measuring the diurnal rhythm of the core body temperature. Measurements of peripheral wrist temperature were also made for comparison. Healthy and overweight participants were studied.

6.2. Method

The study was approved by the University of Nottingham's Faculty of Medicine and Health Sciences Research Ethics Committee (473-2001). The study was registered at ClinicalTrials.gov with the identification number: NCT05569837.

6.2.1. Participants

Participants with healthy normal weight and overweight (BMI 18.5-30 kg/m^2) female and male aged 18-45 years were recruited. Inclusion criteria were explained in detail in Chapter 3, Section 3.2.1.

6.2.2. Design

A feasibility study of a randomised controlled (non-blinded) trial pattern was undertaken following a parallel design, hypo-energetic diet with two meal pattern interventions which were regular (6 meals per day) and irregular (3 to 9 meals per day). The design of the study protocol was explained in detail in Chapter 3 Section 3.5 and is outlined in Figure 6.1. The study lasted for 26 days for each participant (free living) once they had been screened and enrolled in the study. Participants started with a 7-day pre-standardisation period during which they consumed a diet that met their energy requirements according to their regular meal pattern. After that, they attended the first lab day visit. They were then randomized to receive a hypoenergetic diet for 14 days, either following the regular meal pattern or the irregular meal pattern. They then attended a further lab visit which was followed by a further 3-day post-standardisation period during their energy requirements were met by following a regular meal pattern. For female participants, Day 1 (the start of the intervention) was timed to fall during the early phase of the menstrual cycle (days 1-7).

Due to the nature of the intervention being used, no attempt was made to blind either the researchers or the participants. The research could not be blinded due to the necessity to provide the food in appropriate portions and at appropriate times.

Figure 6.1: Study protocol

Study duration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Study period	Pre-standardisation Period							Pre Lab Day	Intervention period														Post Lab Day	Post-standardisation Period		
Study day	-7	-6	-5	-4	-3	-2	-1		1	2	3	4	5	6	7	8	9	10	11	12	13	14		+1	+2	+3
Regular	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Irregular	6	6	6	6	6	6	6	6	7	4	9	3	5	8	6	5	9	8	3	4	7	6	6	6	6	6
TEF								√															√			
Fasting blood sample*								√															√			
CGM	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Core temperature					√	√	√																	√	√	√
Wrist temperature	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Appetite							√	√							√							√	√	√		
Sleep diary	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

√: measurement was undertaken on this day

* Fasting blood sample for glucose, insulin, lipids and gut hormones

6.2.3. Sample size for feasibility study and full study

The sample size for the feasibility study (N=4) was based on an estimation of the number of participants that would provide a view of the general acceptability of the activities required, the available resources and the available time. With respect to the primary outcome measure for the subsequent main study, a previous study on irregular eating in lean and obese women had shown significant differences between and within treatment changes in thermogenic response and insulin area under the curve response to the test drink, total- and LDL cholesterol in a sample size of 10 participants. Considering the primary outcome measure – the thermogenic response to food – given that in contrast to our previous studies which had a crossover design, this study would be a parallel design and the sample size would be increased. Based on previous data, it was estimated using G*Power (v 3.1) software (Faul *et al*, 2007) that the sample size should be increased to 28 participants, with 14 participants in each group in order to detect a 13.9 kcal difference in TEF over the 3-hour period with 80% power at a significance level of 0.05.

6.2.4. Screening visit

Participants who expressed interest were contacted by email and given information about the study. If they believed that they met the inclusion criteria, they were invited to a screening visit. The details of screening process were explained in Chapter 3, Section 3.4). In brief, participants completed questionnaires, anthropometric measurements were taken and a blood sample was obtained. Once accepted into the study, participants were provided with a 3-day food diary and instructions and were asked to return the diary to the lab, where it was checked for legibility and completeness of detail to enable analysis.

6.2.5. Randomisation

After the screening visit, participants were randomised to either the regular or irregular meal pattern group. The randomisation scheme was performed with the use of the Second Generator Plan from online tool (randomization.com) before the study began. Participants were assigned to the randomisation scheme in order of

recruitment. The study investigator generated the randomisation scheme, enrolled the participants and assigned them to the appropriate intervention. Each participant was randomly allocated to one of the two interventions:

1. Regular meal pattern: 6 meals every day for two weeks.
2. Irregular meal pattern: consuming a different number of meals every day (between 3 and 9) for two weeks.

Participants were contacted to arrange the dates for lab visits and informed which group they had been randomised to after the screening visit.

6.2.6. Pre study visits

Participants were requested to attend the lab in order to return the 3-day food diary. The day before the start of the study, participants also attended the lab for the first CGM sensor to be inserted and to be given all the food for their 7-day pre-standardisation period.

6.2.7. Standardization periods and intervention period visits

Table 6.1 shows all the visits that were made during the study period. From first study day (-1 to day -7) participants consumed the food that they had been given for the 7-day pre-standardisation period. After this period had ended, they attended the lab to swallow the e-Celsius Performance pill on day (-3). After that, they attended the pre-laboratory day, where they were provided with their food for the following 7 days according to the intervention that

they had been allocated. On day (5) of the first week of the intervention period, they returned for the second CGM sensor to be inserted and to collect further food for the rest of the intervention period. They then attended for the post-laboratory day, and at the end of the visit, participants swallowed the e-Celsius Performance pill and collected the food for the final 3-day post-standardisation period. On the last day (+3) of the post-standardisation period, they returned to the lab to drop off

all devices including the CGM scanner, e-Celsius Performance monitor, wrist and SWA bands, VAS forms and menus with the compliance form.

Table 6.1: Visits during the study period

Activity	Visits*							
	1	2	3	4	5	6	7	8
Informed consent to be signed	✖							
Sociodemographic characteristics/Biometrics	✖							
General health questionnaire	✖							
Beck depression inventory assessment	✖							
Eating attitude test (EAT-26)	✖							
Physical activity questionnaire	✖							
Blood screening tests	✖							
Training and issuing of food diaries	✖							
Returning 3-day food diary		✖						
Wrist band for iButton			✖					
Insertion of CGM new sensor			✖			✖		
Providing menu and food for standardization period			✖				✖	
Swallowing pill for core temperature				✖			✖	
Weight measurement					✖		✖	
Test drink					✖		✖	
Energy expenditure measurement					✖		✖	
Blood samples for glucose, insulin, lipids and gut hormones					✖		✖	
Providing food for intervention period					✖	✖		
Subjective appetite assessment					✖		✖	
Test meal					✖		✖	
Sensewear armband placement					✖		✖	
Collect the study devices and paperwork								✖

*1: Screening visit, 2 and 3: Pre study visits, 4: Swallowing e-Celsius pill visit, 5: Pre-lab day visit, 6: Insertion of CGM sensor visit, 7: Post-lab day visit and 8: Last visit

6.2.8. Dietary provision

In the 7-day pre-standardisation period, participants followed a regular meal pattern of 6 daily meals. In the 14-day intervention period, one of the intervention meal patterns consisted of consuming a regular meal pattern of 6 daily meals. The second intervention pattern is an irregular one consisting of consuming a different number of meals every day (between 3 and 9). After the intervention period, participants followed a regular meal pattern of 6 daily meals for 3 days during the post-standardisation period. The number of meals for each study period is shown in figure 6.1. All foods to be consumed during the study were provided free of charge.

Participants were given foods that are commonly consumed in the British diet. Identical foods were provided in amounts designed to meet the energy requirements of the participants during the pre- and post- standardisation periods and to reduce participants' energy intake by 800 kcal per day relative to their estimated energy requirements during the intervention period. The food was supplied in a 4-day cycle of menus in order to avoid monotony and boredom during the 26-day period. Menus were designed for 1900 kcal/day, 2150 kcal/day, 2250 kcal/day and 2900 kcal/day to meet the different estimated energy requirements of the participants for standardisation periods and 1100 kcal/day, 1300 kcal/day, 1400 kcal/day and 2100 kcal/day to meet the different estimated energy requirements of participants during the intervention periods after subtracting the daily 800 kcal. Details of how the participants' energy requirements are described in Chapter 3 (Section 3.5.2).

6.2.9. Measurements during pre and post standardisation periods

- Peripheral wrist temperature was measured through a wireless iButton data logger as described in Chapter 3, Section 3.5.3.4.

- Core temperature was measured on days (-1 to -3) and (+1 to +3) using an ingestible telemetry pill and an associated monitor (e-Celsius Performance pill and e-Viewer Performance monitor e-Celcius™ BodyCap) as described in Chapter 3, Section 3.5.4.2.

- Subjective appetite was assessed by recording each subjective appetite rate using VAS before and after each meal on day (-1) and day (+1), when 6 meals were consumed, as described in Chapter 3, Section 3.5.3.3.

- Continuous interstitial glucose was measured using the CGM FreeStyle Libre System as explained in Chapter 3, Section 3.5.4.3

- A constant sleeping, waking and light exposure routine was followed and monitored by a sleep diary form as described in Chapter 3, Section 3.5.3.4.3.

6.2.10. Measurements during intervention period

- Energy expenditure and physical activity patterns were measured continuously using SWA as explained in Chapter 3, Section 3.5.3.1.

- Continuous interstitial glucose was measured using the CGM FreeStyle Libre System as detailed in Chapter 3, Section 3.5.4.3

- Subjective appetite was assessed using VAS before and after each meal on days 7 and 14 during intervention period, when 6 meals were consumed on both interventions, as described in Chapter 3, Section 3.5.3.3.

- Peripheral wrist temperature was measured using the iButton data logger, as explained in more detail in Chapter 3, Section 3.5.3.4.

- A constant sleep-wake (and light exposure) routine was followed and monitored by a sleep diary form as described in Chapter 3, Section 3.5.3.4.3

6.2.11. Laboratory-visit protocol and procedures

The protocol for the laboratory visits was explained in detail in Chapter 3, Section 3.5.5 and outlined in Figure 2.3. Participants were required to attend the David Greenfield Human Physiology Lab following an overnight fast at 8am on the day between day -1 and day 1 and on the day between day 14 and day +1. During these visits, anthropometric measurements, REE, TEF (following the test drink consumption), fasting lipids, glucose, insulin and appetite hormones and subjective

appetite were determined. A pasta meal was offered ad-libitum 3 hours and 15 minutes after the test meal had been given.

6.2.11.1. Anthropometric measurements

Immediately after arrival, anthropometric variables including weight, height, waist circumference, hip circumference, waist to hip ratio and skinfold-thickness were measured. Procedures for these anthropometric measurements were described in Chapter 3, Section 3.5.5.1.

6.2.11.2. Energy-expenditure measurement

REE and TEF were measured using an indirect calorimetry system during the laboratory visit. The protocol of energy expenditure measurement was explained in Chapter 3, Section 3.5.5.2.

6.2.11.3. Blood sampling

Fasting blood samples were taken immediately before ingestion of the test drink, as explained in Chapter 3, section 3.5.5.3, to measure serum total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, blood glucose, serum insulin, plasma glucagon-like peptide 1(GLP-1), plasma peptide YY (PYY), and plasma ghrelin.

6.2.11.4. Test-drink consumption

The standardised test drink (a vanilla flavoured milkshake) was served at room temperature in an open glass as a breakfast. The test drink was consumed over a period of 5 minutes and provided energy based on the participant's healthy body weight. The calculation and composition of the milkshake were explained in Chapter 3, Section 3.5.5.4.

6.2.11.5. Ad libitum test meal

A pasta-based test meal was served at lunchtime to assess ad libitum food intake. The composition and recipe for the pasta were explained in Chapter 3, Section 3.5.5.5.

6.2.11.6. Subjective Appetite assessment

Participants were asked to complete the VAS during the lab visit before and 3 hours after the test drink as well as before and 1 hour after the test meal to assess subjective appetite. The details of this process were explained in Chapter 3, Section 3.5.3.3.

6.2.11.7. Continuous interstitial glucose measurement

Continuous interstitial glucose measurement was undertaken using the CGM FreeStyle Libre System during the laboratory visits as explained in Chapter 3, Section 3.5.4.3.

6.2.12. Exit discussion with each participant

Once each participant had completed the study, they were invited to express their views on the protocol. Firstly, they were asked a general questions such as 'How would you describe your experience of following the protocol?', 'Tell me about how you found attending the number of visits required?', 'How did you find the diet that we provided you with?', 'What are your thoughts on the type of food that was given to you?', 'How did you find taking the pill?', 'Did you experience any problems with the pill?', 'What was you experience of the sensory that we applied to your arm' and 'Tell us about your sleep with the sensor and the armband on your arm'. This was then followed by further questions to explore their responses in more detail as appropriate. For example, if they stated that they found the protocol hard they would be asked to tell us more about why they found it hard.

6.2.13. Data collection

Data such as anthropometric measurements, blood results and energy requirement and intake from the screening and lab visits was documented using a Case Report Form (CRF) (Appendix 2). Data relating to energy expenditure from GEM and SWA, continuous glucose and wrist and core temperatures were collected from each device and downloaded into an Excel sheet. All data were stored confidentially at the University of Nottingham.

6.2.14. Statistical Analysis

All data are reported as mean ± SD, unless otherwise stated, for the feasibility study. The details of the statistical method for the full study planned after the feasibility study were explained in Chapter 3, Section 3.6.

6.3. Results

6.3.1. Feasibility of recruitment

Participant recruitment took place between November 2021 and December 2021. One participant had seen a poster asking for participants, and seven others had heard about the study via word of mouth. These eight participants expressed an initial interest in the study by email and were sent further information. Six participants responded to the email and were invited for screening. One participant revealed at the screening that she had hypothyroidism and did not therefore meet the inclusion criteria. The total period taken to identify the five participants who met the criteria was two months, and the percentage of those screened who were eligible for inclusion was 80%. One participant did not take part in the study despite being eligible because of the time constraints involved in completing the research. We were therefore left with four participants aged between 25 and 35 years, with BMIs ranging from 19.7 to 30 kg/m^2 (Table 6.1). These participants completed the study (two in each intervention arm), so the rate of study completion was 100% (Figure 6.1). Informal discussion with the student population revealed that they felt that the protocol was too demanding.

6.3.2. Feasibility of applying the study procedures and collection of outcome measures

The overall research protocol was successfully followed by all participants, with all visits occurring when scheduled. Participants had no difficulty in completing the food intake diary. Venepuncture was successfully undertaken at screening and at the lab visits, allowing participant eligibility and metabolic response to the interventions to be established. Measurement by all devices (SWA, CCG, i-button and e-Celsius, GEM) was initiated according to the protocol, and the devices were

tolerated by the participants. The continuous glucose monitoring using FreeStyle Libre was applicable for free living throughout the study and the changing of the sensor, which only lasts 14 days, was done for all participants to cover the full study period. Swallowing the e-Celsius pill to measure core temperature was achieved without difficulties or adverse effects. During the lab day visits, it was observed that staying awake under the GEM hood to measure REE was challenging for all participants as they were not allowed to do any activity, even reading or listening to the radio. Meanwhile, GEM indirect calorimetry sometimes had to be calibrated on more than one occasion due to unexpected results. VAS forms were completed at the required time points.

6.3.3. Feasibility and acceptability of the of the dietary intervention and pasta meal

The study intervention was successfully achieved in terms of providing a hypo-energetic diet with regular or irregular meal patterns. Participants collected their food as scheduled. The requirement to come to the lab at the weekend for some food collection visits caused some difficulty as it was out of working hours, which meant that a co-worker had to be identified who would attend along with the investigator. No difficulties were reported with following preparation and cooking instructions.

The dietary compliance records completed by participants during all study periods showed that for energy intake, 99 ± 0.02% and 100 ± 0.01% of the energy prescribed in pre-standardisation period and 100 ± 1 % and 100 ± 0.01% of the energy prescribed in intervention period were consumed by participants who subsequently followed the regular and irregular meal patterns, respectively,

The pasta meal was served as per protocol and was judged to be acceptable by the participants. The amount of pasta that had been prepared was sufficient for all participants.

6.3.4. Feasibility of data collection procedures

Data for most measurement variables was collected successfully. However, 20% of the peripheral wrist temperature data was missing due to the instability of the wrist band during participants' movement, which influenced the peripheral wrist temperature and resulted in a recorded temperature that was outside the normal range (Chapter 3, Section 3.5.3.4.2).

With regard to measurement of core temperature, forgetting to recharge the e-Viewer monitor led to a loss of 30% of data for one participant. Moreover, the rechargeable battery in all e-Veiwer monitors were failing by the end of the study. It was observed that the pill needed between 15 and 30 mins after ingestion to start capturing the core body temperature. The duration of the collected core temperature data from e-Viewer monitor varied between 24 and 72 hours among participants, depending on the transit time of the pill to pass naturally through the body.

6.3.5. Feasibility of resources and ability to manage and implement the study

Adequate lab space and full resources were available at the site where the study was undertaken. During the lab day, the investigator had all necessary resources available and was able to undertake all necessary tasks, including measuring energy expenditure, processing the blood samples, offering the test drink and the ad libitum meal during the visit. An additional research assistant was responsible for collecting the fasting blood samples. However, there were differences in time as to when the test meal was offered to the participants as there was no set time between finishing TEF measurement and serving the pasta, and the researcher had to measure the TEF as well as preparing and serving the meal.

During the standardisation and intervention periods, the food order was sometimes received incomplete, and this was exacerbated by food shortages attributed to Covid 19 impacting the supermarket supply. As some food items were not delivered due to being out of stock, food items from the menu were replaced when necessary

by others which matched the energy and macronutrients content. This problem affected the scheduled time for participants attending to collect the study food.

All necessary software was available for processing data and exploratory analysis.

6.3.6. Feasibility of participant responses to intervention

As all participants followed the study protocol and intervention, the exploratory results used to measure the outcomes will be described in the following section. Where existing data was available against which we could check the likely physiological validity of the results obtained, a comparison was made, and fasting blood samples, range of blood glucose values seen using CGM and core body temperature measurements were obtained). All data fell within the expected limits.

6.3.6.1. Characteristics of the participants

Table 6.1 shows information relating to the participants who were recruited and completed the feasibility study. Three females were recruited (one of healthy weight and two who were overweight) and one male (who was overweight). Twenty-eight participants will be recruited in the full study. The flow of participants through the recruitment process is shown in Figure 6.1. Table 6.2 shows that all participant characteristics at baseline for weight, height, BMI, waist and hip ratio, body fat percentage, physical activity and dietary intake met the necessary inclusion criteria.

Figure 6.2: The flow of participants through the study

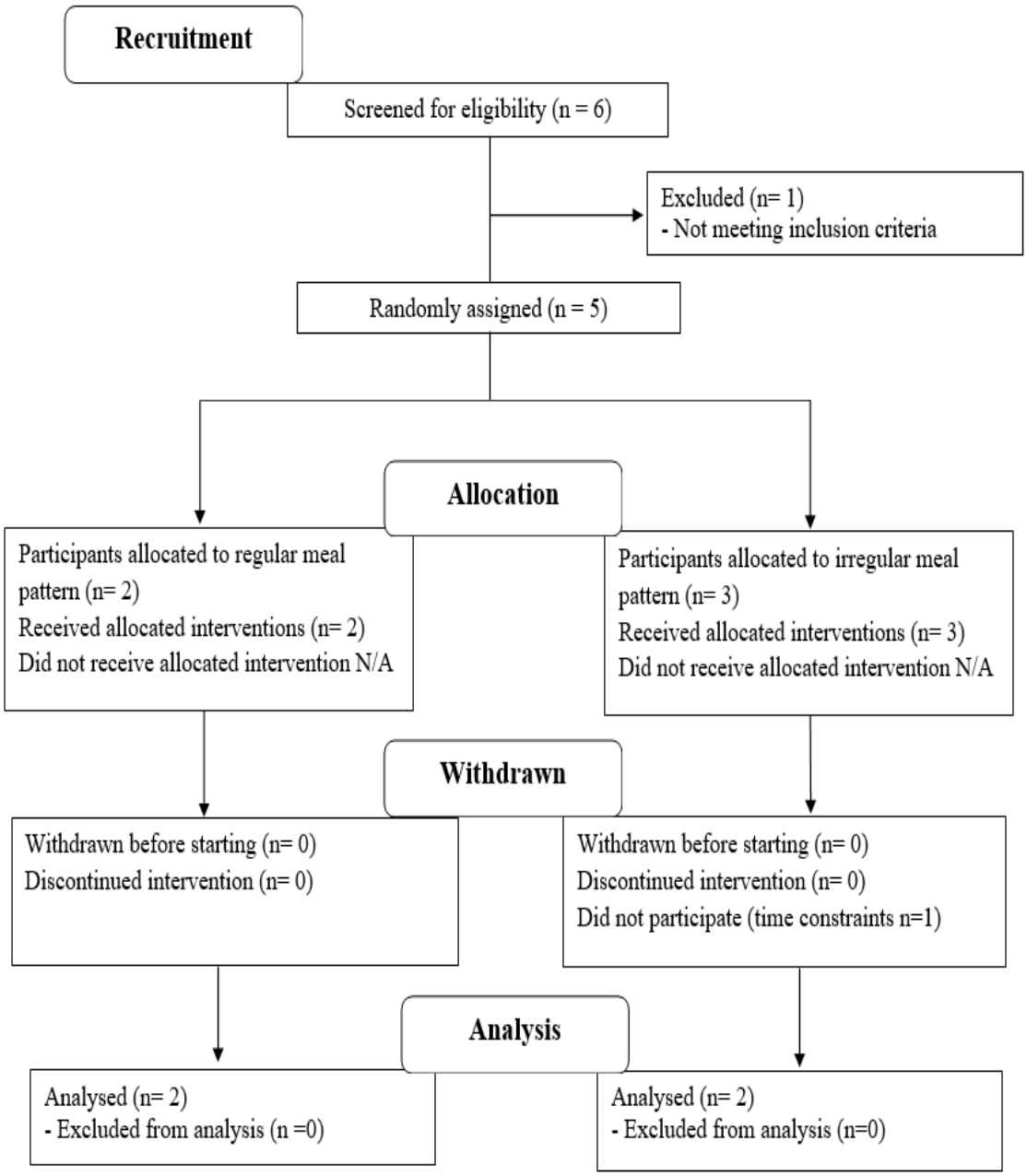


Table 6.2: Characteristics of participants at screening

Participants ID	P1	P2	P3	P4	Mean ± SD
Age (years)	35	32	25	35	31.8± 4.7
Gender	Female	Male	Female	Female	-
Weight (kg)	63.6	84.9	46.2	71.3	66.5± 16.1
Height (cm)	158.5	168	153	156	158.9± 6.4
BMI (kg/m^2)	25.4	30	19.7	29.2	26.1± 5
Waist-hip ratio	0.75	0.98	0.70	0.79	0.80± 0.1
body fat (%)	40.5	42.1	22.2	40.9	36.4± 9.5
Physical activity	Moderate	Moderate	Moderate	Moderate	-
Pre study dietary intake (kcal/day)	2154	2900 2100	1900 1100	2277 1400	2307± 424
Estimated energy requirement (kcal/day)	1300				1600± 875

6.3.6.2. Anthropometric measurements

Changes in weight, BMI, waist-hip ratio and body fat percentage before and after each intervention are shown in Tables 6.3 and 6.4. Numerically, all participants had lost weight after the 14-day intervention, and with the exception of P3 showed a numerical reduction in percentage body fat.

Table 6.3: Anthropometric measurements over the intervention period for each participant

	Regular meal pattern									Irregular meal pattern		
Participant ID	**P1**			**P2**			**P3**			**P4**		
	Pre	Post	Diff*	Pre	Post	Diff*	Pre	Post	Diff*	Pre	Post	Diff*
Body weight (kg)	62.1	61.4	1.1	85	83.6	1.4	45.5	45	0.5	69.9	68.4	1.5
BMI (kg/m^2)	24.8	24.5	0.3	30	29.6	0.4	19.4	19.2	0.2	28.7	28.1	0.6
Waist-hip ratio	0.75	0.73	0.02	0.98	0.97	0.01	0.70	0.68	0.02	0.79	0.79	0
Body fat (%)	40.5	40.1	0.4	42.1	41	1.1	22.2	22.5	0.3	40.9	40.5	0.4

* Difference between pre and post for each participant

Table 6.4: The differences in anthropometric measurements between regular and irregular meal pattern*

	Regular meal pattern	Irregular meal pattern
Body weight loss (kg)	1.25 ± 0.2	1 ± 0.7
BMI loss (kg/m^2)	0.35 ± 0.07	0.4 ± 0.2
Waist-hip ratio loss	0.015 ± 0.007	0.01 ± 0.01
Body fat loss (%)	0.75 ± 0.4	0.35 ± 0.07

*All values are means ± SDs. n = 4

6.3.6.3. Energy intake and expenditure

6.3.6.4. Free-living energy intake and expenditure during intervention period

Mean energy intake and expenditure (estimated using SenseWare data) during the 14 days of the intervention are shown in Table 6.5.

Table 6.5: Energy intake and expenditure during intervention period (free living)

	Regular meal pattern			Irregular meal pattern		
Participant ID	**P1**	**P2**	**Mean ± SD**	**P3**	**P4**	**Mean ± SD**
Prescribed energy intake (kcal/day)	1300	2100	1700 ± 565	1100	1400	1250 ± 212
Consumed energy intake (kcal/day)	1290	2050	1670 ± 537	1100	1350	1225 ±176
Energy expenditure (kcal/day)	1859	3398	2628 ± 1088	1600	2500	2050 ± 636
Physical activity (MET)	1.6	1.5	1.55 ± 0.1	1.5	1.5	1.5 ± 0

6.3.6.5. Energy expenditure during the laboratory visits (indirect calorimetry data)

Figures 6.3 and 6.4 show the basal metabolic rates and TEF response to the test drink for each meal pattern which were calculated using the incremental area under the curve (iAUC) as shown in Chapter 3, Figure 3.9 and Table 3.5.

For the participants who adhered to a regular diet, the mean TEF numerically increased after the intervention period (before: 21.5±12.1; after: 28.8±7.8), whereas

for the participants who consumed an irregular diet during the intervention there was a decrease in TEF (before: 20.2±10.5; after: 16.3±6.9 kcal/min).

Figure 6.3: Total energy expenditure before and after intervention

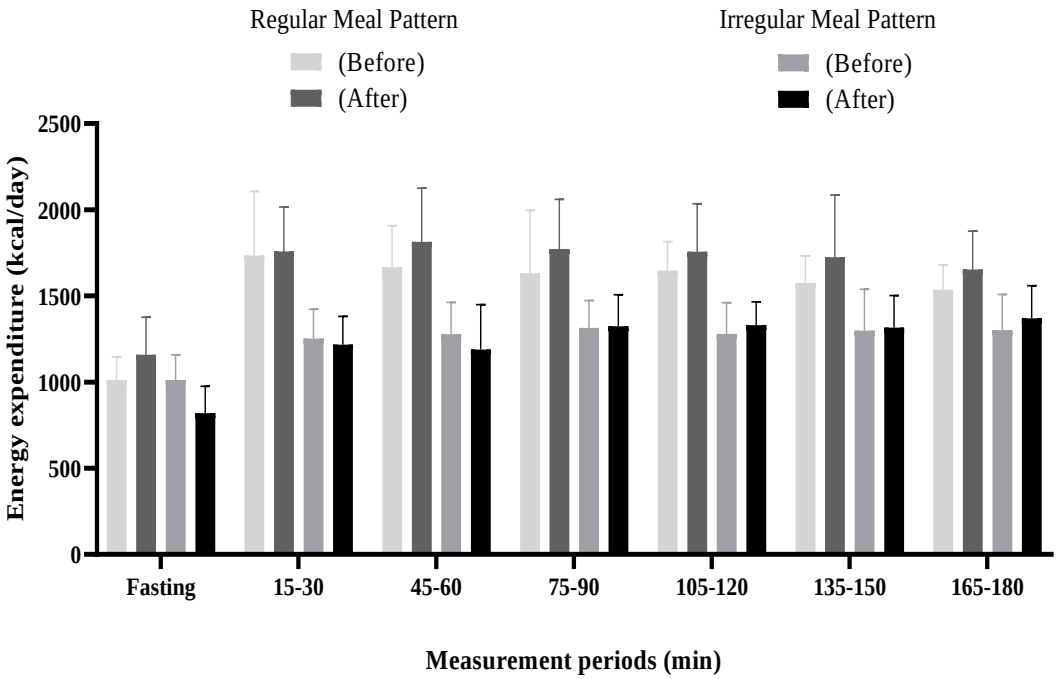
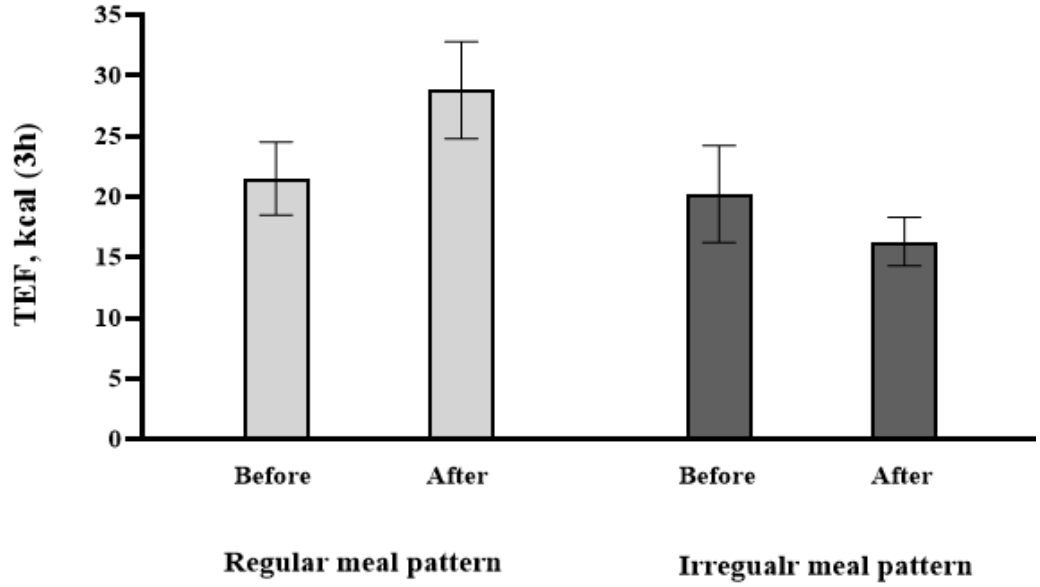


Figure 6.4: TEF over three hours in response to the liquid test drink before and after each intervention period



6.3.6.6. Energy intake of the ad libitum test meal

Energy intakes, the duration of eating and speed of consumption of the ad libitum test meal before and after intervention are shown in Tables 6.6 and 6.7.

Table 6.6: Energy intake of the ad libitum test meal for each participant at the lab visit before and after the intervention

	Regular meal pattern				**Irregular meal pattern**			
Participant ID	**P1**		**P2**		**P3**		**P4**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Energy intake (kcal)	772	710	876	821	543	598	786	810
Duration of eating (min)	9.5	7.5	11.3	10.8	12.4	13.2	8.4	8.9
Speed of eating (g/min)	50.3	46.8	49.6	48.9	45.1	48.3	49.7	51.5

Table 6.7: Energy intake of the ad libitum test meal for each meal pattern

	Regular meal pattern		**Irregular meal pattern**	
	Pre	Post	Pre	Post
Energy intake (kcal)	824 ± 73	765.5 ± 78	664.5 ± 171	704 ± 149
Duration of eating (min)	10.4 ± 1.2	9.15 ± 2.3	10.4 ± 2.8	11.05 ± 3
Speed of eating (g/min)	49.95 ± 0.4	47.85 ± 1.4	47.4 ± 3.2	49.9 ± 2.2

*All values are means ± SDs. n = 4

6.3.6.7. Free-living continuous glucose monitoring

For all participants, the mean, maximum, minimum and iAUC were calculated for each meal pattern on the day before the pre intervention lab visit (6 meals were consumed during both intervention periods), the day after the post intervention lab visit (6 meals were consumed during both intervention periods), and 3 days into the intervention period (day 7 (6 meals were consumed during both intervention periods). On day 8, 6 and 5 meals were consumed in the regular and irregular periods, respectively, and on day 9, 6 and 9 meals were consumed in the regular and irregular periods, respectively (Table 6.8).

Table 6.8: Analyses of continuous glucose monitoring data during the intervention periods

	Regular meal pattern					Irregular meal pattern				
Study day No.	Day -1	Day 7	Day 8	day 9	Day +1	Day -1	Day 7	Day 8	Day 9	Day +1
No. of meals/day	6 meals	6 meals	6 meals	6 meals	6 meals	6 meals	6 meals	5 meals	9 meals	6 meals
Glucose (mmol/L)										
Mean, h										
24 h	5.2±0.4	5.3±0.5	5.3±0.4	5.4±0.6	5.1±0.4	5.3±0.3	5.4±0.4	5.2±0.5	5.5±0.3	5.4±1.1
Day	5.5±0.5	5.1±0.7	5.1±0.5	5.5±0.4	5.4±0.5	5.2±0.9	5.5±0.4	5.3±0.5	5.6±0.3	5.2±0.6
Night	4.9±0.2	4.9±0.3	5.2±0.6	5.1±0.9	4.9±1.0	5.0±1.1	4.2±0.6	5.0±0.2	5.2±0.5	5.3±0.8
Maximum, h										
24 h	7±1.1	6.1±0.8	5.6±0.6	6.2±1.0	6.2±0.9	6.8±	5.6±1.2	5.3±0.7	6.8±0.81.4	7.2±1.2
Day	7±1.1	6.1±0.8	5.6±0.6	6.2±1.0	6.2±0.9	6.8±	5.6±1.3	5.2±0.6	4.2±0.3	7.2±1.2
Night	5.3±0.9	3.3±0.3	4±0.2	4.3±0.7	4.6±0.4	5.2±	5.1±1.0	5.3±0.2	6.8±0.2	6.2±1.1
Minimum, h										
24 h	4.3±1.2	2.9±0.7	2.9±0.2	3.1±1.1	2.9±0.7	4.4±1.2	3±0.5	2.2±1.2	2.8±0.9	4.7±1.1
Day	4.3±1.2	2.9±0.7	2.9±0.2	3.1±1.1	2.9±0.7	4.4±1.2	3±0.5	2.8±0.3	3.1±1.1	4.7±1.2
Night	4.2±1.1	3.1±1.0	3.4±0.6	3.4±0.8	3±1.3	4.4±1.2	3.5±0.8	2.2±0.4	2.8±1.0	4.8±1.3
iAUC*, h										
24 h	478±208	528±198	496±392	599±229	432±378	386±98	450±231	520±126	743±365	845±387
Day	397±166	455±283	376±122	523±234	348±196	226±89	357±163	487±245	689±325	765±355
Night	-89±54	-85±65	-74±25	-130±90	-145±78	-68±48	-65±73	-70.5±82	-126.4±64	-134±94

Mean ± SD, n = 4. Day hours were from 0700 to 2359. Night hours were from 2400 to 0659. iAUC, incremental AUC, * (Postprandial iAUC for 90 min was analysed after each meal for each day and the baseline was the glucose level before eating the meal).

6.3.6.8. Fasting blood parameters

Tables 6.9 and 6.10 show the results of fasting levels of glucose, insulin, lipids (total cholesterol, LDL, HDL and triglycerides), and gut hormones in each group before and after the meal pattern interventions.

Table 6.9: Fasting blood measurements for each participant

	Regular meal pattern				**Irregular meal pattern**			
Participant ID	**P1**		**P2**		**P3**		**P4**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Total cholesterol (mmol/L)	4.5	4.1	5.7	5.3	3.2	3.3	4	3.8
LDL (mmol/L)	2.9	2.6	4	3.7	1.3	1.6	2.5	2.3
HDL (mmol/L)	1.2	1.2	1.2	1.2	1.5	1.5	1.2	1.2
Triglycerides (mmol/L)	0.62	0.59	1.13	1.01	0.85	0.55	0.72	0.71
Glucose (mmol/L)	4.04	4.09	3.87	3.45	3.05	2.97	4.28	4.47
Insulin (mIU/L)	5.47	6.94	21.50	24.92	2.34	5.66	7.65	6.76
PYY (pg/mL)	80.56	77.65	62.78	89.21	58.20	87.78	52.01	52.01
GLP-1 (pmol/L)	0.23	0.35	0.78	1.57	7.84	8.71	0.13	0.05
Greline (pg/mL)	3437.1	3755.4	997.8	743.7	4807.8	3918.9	3558.5	4319.2

Table 6.10: Fasting blood measurements for each intervention*

	Regular meal pattern		**Irregular meal pattern**	
	Pre	Post	Pre	Post
Total cholesterol (mmol/L)	5.1 ± 0.8	4.7 ± 0.8	3.6 ± 0.6	3.55 ± 0.4
LDL (mmol/L)	3.45 ± 0.8	3.15 ± 0.8	1.90 ± 0.8	1.95 ± 0.5
HDL (mmol/L)	1.2 ± 0	1.2 ± 0	1.35 ± 0.2	1.35 ± 0.2
Triglycerides (mmol/L)	0.87 ± 0.4	0.8 ± 0.3	0.78 ± 0.8	0.6 ± 0.1
Glucose (mmol/L)	3.95 ± 0.1	3.77 ± 0.5	3.66 ± 0.9	3.72 ± 1.1
Insulin (mIU/L)	13.4 ± 11.3	15.9 ± 12.7	4.9 ± 3.8	6.2 ± 0.8
PYY (pg/mL)	71.67 ± 12.6	83.43 ± 8.2	55.10 ± 4.4	69.89 ± 25.3
GLP-1 (pmol/L)	0.51 ± 0.4	0.96 ± 0.9	3.99 ± 5.5	4.38 ± 6.1
Greline (pg/mL)	2217.45 ± 1724.8	2249.55 ± 2129.6	4183.15±883.4	4119.05 ± 283.1

*All values are means ± SDs. n = 4

6.3.6.9. Subjective appetite

6.3.6.9.1. Response to the test drink and the ad libitum test meal

The responses in fasting as well as after the test drink and the ad libitum meal for all subjective rates are shown in Table 6.11 for all participants in pre and post lab visits.

Table 6.11: Fasting and iAUC for subjective appetite ratings following the test drink and the ad-libitum meal

		Regular meal pattern				Irregular meal pattern			
	Participant ID	**P1**		**P2**		**P3**		**P4**	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Hunger (mm)	Fasting (mm)	55	61	53	62	51	66	53	64
	Test drink (mm over 1h)	-1541	-4498	-2533	-4526	-2214	-4525	-1954	-4387
	Ad-libitum meal (mm Over 1h)	-2957	-2884	-3172	-3002	-2906	-2977	-3071	3102
Satiety (mm)	Fasting (mm)	20	25	24	27	20	19	22	20
	Test drink (mm over 1h)	5491	5843	5408	4939	5950	5866	5817	5740
	Ad-libitum meal (mm over 1h)	3199	3695	3389	3562	3196	3773	3297	3461
Fullness (mm)	Fasting (mm)	17	24	17	26	17	17	18	17
	Test drink (mm over 1h)	5732	6382	6530	6072	5974	5945	5842	5793
	Ad-libitum meal (mm over 1h)	3265	3479	3356	3712	3416	3983	3964	3895
PFC (mm)	Fasting (mm)	60	61	63	63	58	64	62	66
	Test drink (mm over 1h)	-3274	-3521	-3060	-3454	-2897	-3934	-3107	-3826
	Ad-libitum meal (mm over 1h)	-3423	-3517	-3107	-3164	-2672	-3020	-2950	-3193

6.3.6.9.2. Responses to the meal patterns during the intervention

Comparisons of appetite ratings (all day points combined) on days 7 and 14 (6 meals per day) of regular and irregular meal patterns were shown in Tables 6.12 and 6.13.

Table 6.12: Comparison of appetite ratings on day 7 of the intervention period

	Regular meal pattern				Irregular meal pattern			
Participant ID	**P1**		**P2**		**P3**		**P4**	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hunger (mm)	48	11	49	17	46	25	44	29
Satiety (mm)	40	79	42	75	41	72	46	78
Fullness (mm)	42	78	42	78	43	75	45	74
PFC (mm)	51	25	56	22	52	27	57	24

Table 6.13: Comparison of appetite ratings on day 14 of the intervention period

	Regular meal pattern				Irregular meal pattern			
Participant ID	**P1**		**P2**		**P3**		**P4**	
	Pre	Post	Pre	Post	Pre	post	Pre	Post
Hunger (mm)	47	13	46	15	48	22	45	25
Satiety (mm)	42	74	43	77	40	74	44	76
Fullness (mm)	40	80	41	79	40	73	43	77
PFC (mm)	56	24	55	20	54	29	56	28

6.3.6.10. Wrist peripheral and core temperatures

Cosinor curve fitting was used to analyse the rhythm of core temperatures for only three of the participants, as the fourth participant had missing data due to a battery failure in the e-Viewer monitor. Table 6.14 and figures 6.5 to 6.8 show the results of the cosinor analysis for wrist and core temperatures.

Table 6.14: An example of cosinor analysis outcomes for rhythm characteristics of wrist and core temperature

Rhythm characteristics	Best-fit values	95% CI
Mesor	37.11	37.06 to 37.16
Amplitude	-0.3347	-0.4093 to -0.2600
Achrophase	3.977	3.127 to 4.837

Figure 6.5: An example of diurnal rhythm of peripheral wrist temperature before and after regular meal pattern intervention

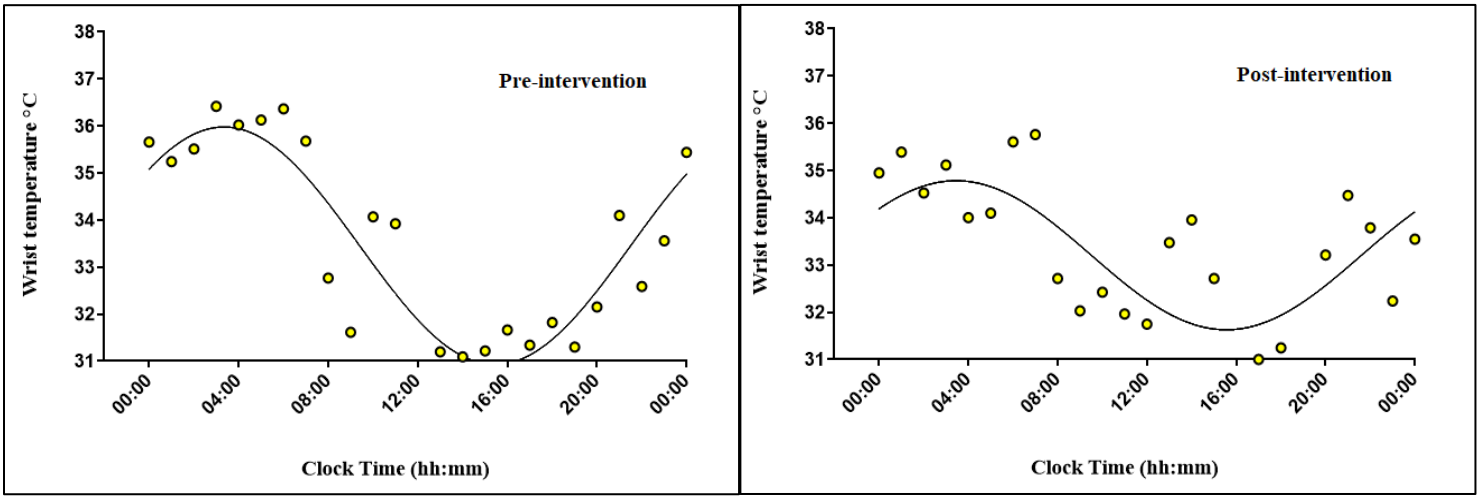


Figure 6.6: An example of diurnal rhythm of peripheral wrist temperature before and after irregular meal pattern intervention

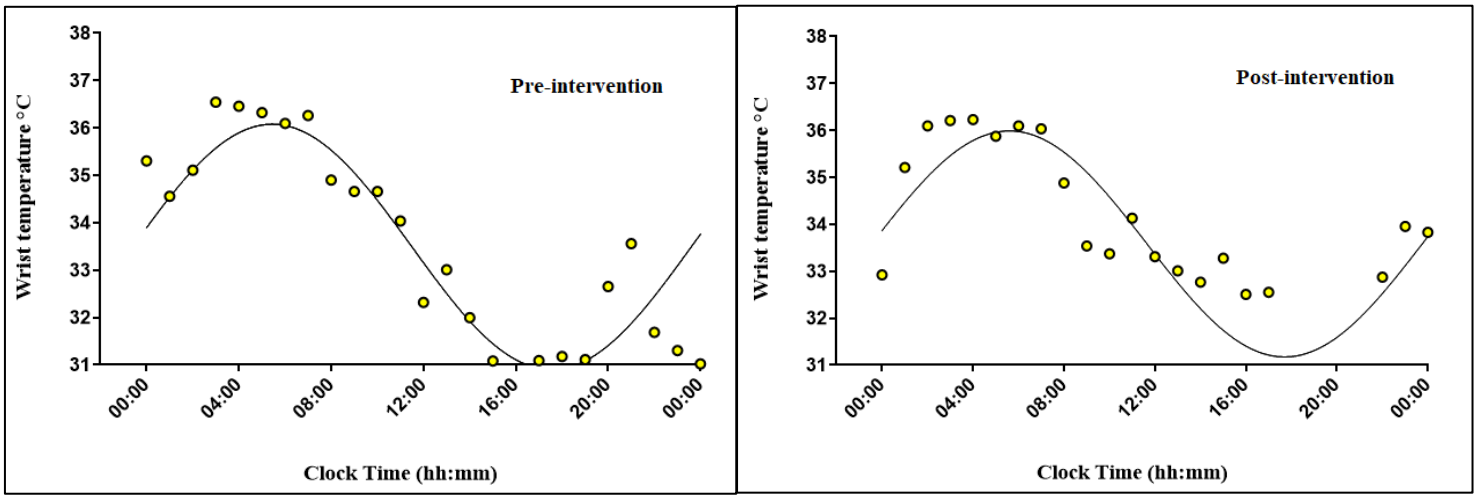


Figure 6.7: An example of diurnal rhythm of core temperature before and after regular meal pattern intervention

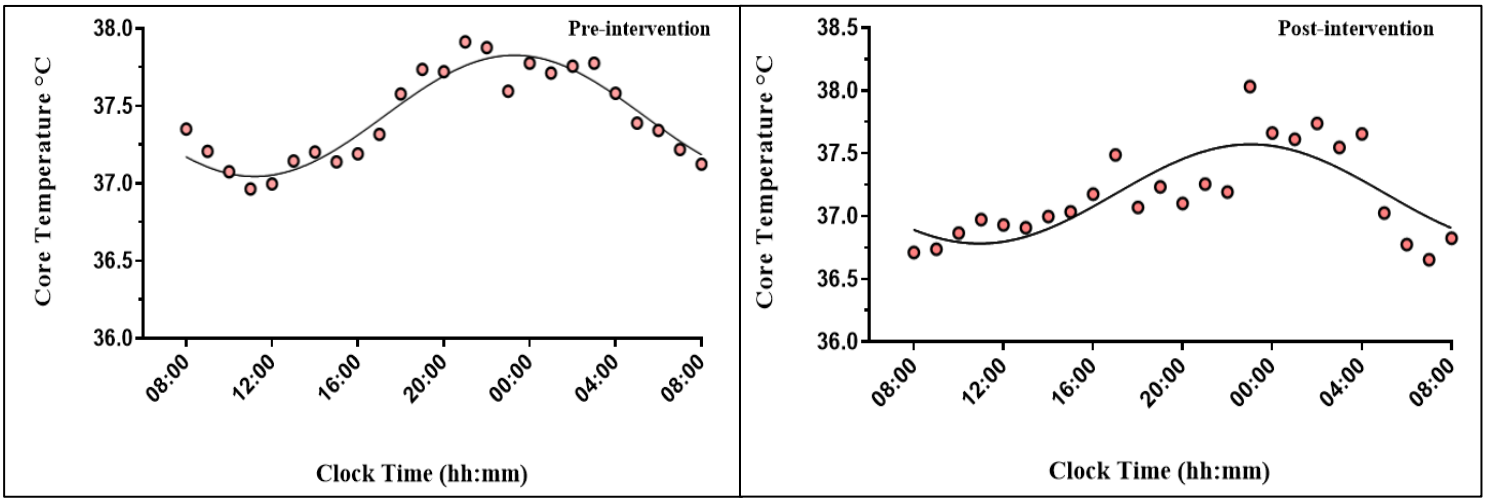
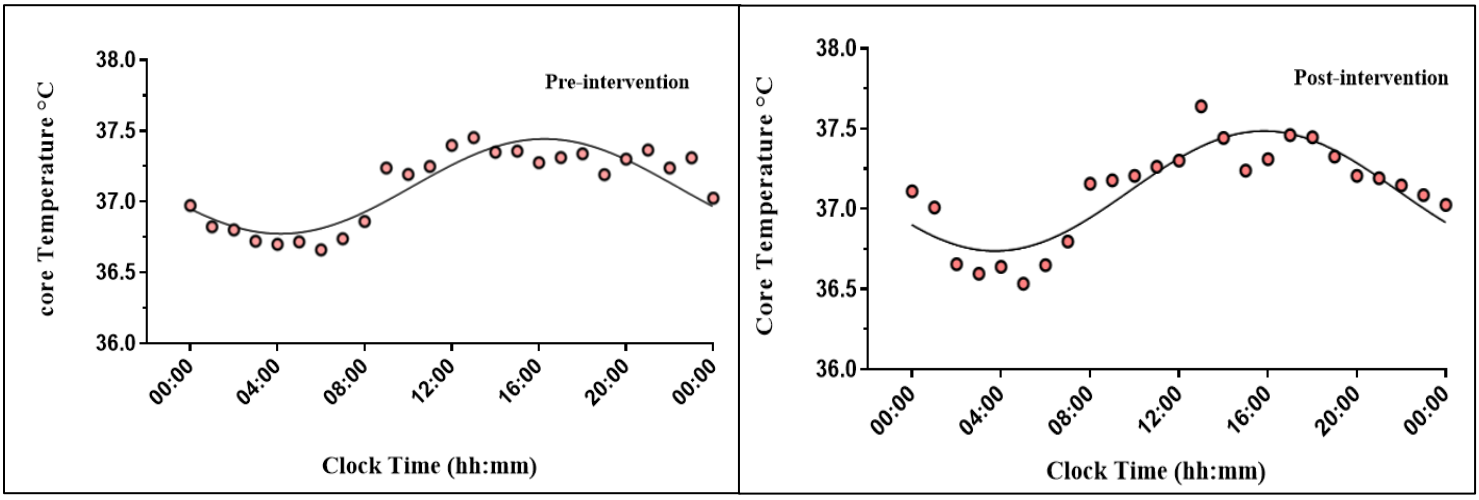


Figure 6.8: An example of diurnal rhythm of core temperature before and after irregular meal pattern intervention



6.4. Discussion

This was the first study to test the feasibility of conducting a randomised controlled trial to compare the impact of 14-day hypo-energetic diet with regular and irregular meal pattern on the TEF, carbohydrate metabolism, lipid concentrations and subjective appetite whilst also measuring diurnal rhythm. The results of this feasibility study will be used to decide whether to progress to a full-scale trial, and if so, what methodological issues, if any, need to be addressed and changed.

In terms of the feasibility of recruitment, the number of poster responses for potential participation was low, and we can conclude that the study poster, which was only placed in university areas, was not a sufficiently adequate method for advertising. The study advertisement should be distributed more widely through different platforms such as websites, social media and public areas to improve the outcome. In addition, the age range of the participants (25-35 years) in the study was small, limiting confidence in how feasible the protocol would be when used across the broader inclusion age range of 18-45 years. Estimating the required sample size for feasibility studies is also an important aspect, although there is no consensus for the required sample size. There is a wide variation in sample size recommendations varying from 10 to 75 per group (Billingham *et al.*, 2013). Sample size can be based on the main aims of the study and the precision of feasibility parameters and clinical outcomes. These may inform the main trial sample size, particularly the standard deviation, but also the event rate and effect size, and to a lesser degree the clinical scale evaluation (Lewis *et al.*, 2021). In this case the sample size was decided pragmatically on what was possible within the time available.

The study protocol, including the hypo-energetic diet, the regular or irregular meal pattern and the diurnal rhythm assessment was feasible. However, the study involved eight visits, which was an issue for busy participants, suggesting that the 3-day food diary could be returned at the same visit as the first CGM sensor is inserted. This means that the study comprises seven visits, including one pre study visit. It would also have been helpful to have included a focus group with potential

participants, rather than speaking to each participant individually, in order to scope their views on the protocol and how it might be made more acceptable. However, when this work was completed Covid 19 regulations did not permit this.

The estimated energy requirement for participants in the pre-standardisation period was similar to the energy intake they reported during the pre-study habitual diet period. Given that the participants' weight were stable, this suggests that the energy intake in the habitual diet was not under- or over-reported. In the intervention period, the dietary compliance records showed that compliance was good, and based on the amount of weight lost – which ranged between 0.5 and 1.5 kg during this period in both meal patterns – the results were compatible with the 800kcal energy deficit.

Applying the study procedures was not problematic and proved to be safe as no adverse effects from any device used in this study were reported. However, standardisation periods need to be more controlled in respect of physical activity, which is a masking factor for circadian measurements. The reason for following this period was to avoid any impact from masking factors on diurnal rhythms including sleep, exposure to light and eating, for which an attempt was made to achieve control in the study protocol through the form of constant routine. Using SWA in all study periods – including the standardisation periods – would be an effective addition to monitor PA. Meanwhile, weight measurement could be undertaken before and after each standardisation period to ensure that the menu, which was provided based on estimated energy requirements, met participants' energy requirements as closely as possible and was not an under- or overestimation.

On the lab day, the calibration time for the GEM indirect calorimetry should be considered when building in the duration of each measurement of energy expenditure and the time between measurements, as the indirect calorimetry had to be recalibrated more than once. In addition, to avoid variations in time between participants for serving the test meal, a small gap should be built into the protocol in the future to allow standardisation between the time after finishing the TEF measurement and offering the pasta. We also noticed that one participant was in a

hurry due to a family task which resulted in them consuming the test meal quickly. It is important to ensure that participants are aware how long they will be required to be available in the lab for each visit, especially the lab day visit, to ensure that they are available for a long enough period.

The collections of study data were applicable, however, the iButton wrist band which was used in the measurement of wrist temperature needs to be secured more tightly to avoid any movement which might affect the accuracy of wrist temperature data. To cover ourselves if this is not successful, a strategy for handling missing data should be outlined in the protocol. For example, if some data are available over the one-hour period used to calculate the hourly mean, these data could be used. The rechargeable battery in all e-Viewer monitors that were used to read the core temperature data from e-Celcius pill failed at the end, and this issue should be raised with the manufacturing company to avoid losing any data during the full study. Losing core temperature data can occur as result of empty batteries in the e-Viewer monitor or the earlier exit of the e-Celsius pill from the body. To overcome this issue, we recommend that an e-Performance Connect bracelet is used alone or as a backup, as it has a longer battery life of 3-5 days, so that there would be no need to recharge it before the next use. In this case, if there are missing data, it would be because of the pill being ejected from the body. Furthermore, the food orders for the study diet were sometimes delivered incomplete as a result of the Covid 19 crisis, so it is important to have enough time between the delivery of the food and the participant attending the study to access food from other suppliers if food items are out of stock.

In terms of the study outcomes, it has been demonstrated that the hypo-energetic diet was effective at achieving weight loss with both the regular and the irregular meal pattern. Each participant in each intervention arm successfully lost weight as a result of providing less energy than the estimated daily energy requirement. The mean of weight loss was 1.25 kg and 1 kg with the regular and irregular meal pattern respectively. This level of weight loss was roughly as would have been expected, although the individual rate of weight loss was different based on BMI classification for each participant. No previous studies have been made

investigating the effect of hypo-energetic diet accompanied by regular and irregular meal patterns on weight. However, it was observed in the nitrogen study (Chapter 5) and in previous studies (Alhussain *et al.*, 2022; 2016; Farshchi *et al.*, 2005) that weight numerically decreased after regular intervention and increased after irregular intervention despite the provision of food, in contrast to the findings of Farshchi *et al.* (2005). The full study would be more conclusive in establishing whether this is the case.

Energy expenditure findings showed that REE (fasting energy expenditure) was numerically higher after the regular meal pattern than the irregular alternative. TEF increased after both interventions, but this increase was numerically greater following the regular intervention than the irregular one. These initial findings are unproven (given the small sample size) but they are in agreement with previous intervention study results (Farshchi *et al.*, 2004a; Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005; Alhussain *et al.*, 2016; Alhussain *et al.*, 2022). Meanwhile, the results of energy intake after the ad libitum test meal showed that after the regular intervention energy intake apparently decreased, in contrast to after the irregular intervention, when the energy intake increased. These results may explain why the weight decreased more after the regular meal pattern compared with irregular meal pattern, but only if participants were complying differently to the instructions to consume all the food provided and no more.

Concentrations of fasting cholesterol, LDL and triglyceride were numerically decreased over the study in both meal patterns, and no change was found in HDL. This is inconsistent with the findings of previous studies, one of which showed no significant differences in fasting serum lipids (Alhussain *et al.*, 2016) while the other revealed that fasting HDL concentrations were lower (P=0.027) and fasting triglyceride concentrations were higher (P=0.028) following the intervention periods compared to before the intervention (Alhussain *et al.*, 2022).

The results for fasting glucose, insulin and gut hormones did not show any consistent trends, but a clearer picture will potentially be demonstrated in the full study. However, in previous studies, the continuous glucose monitors showed that

following the regular meal pattern had a beneficial effect on the IF glucose levels, resulting in greater insulin sensitivity (Alhussain *et al.*, 2016), whereas following irregular meal pattern was associated with a degree of insulin resistance (Farshchi *et al.*, 2004b; Farshchi *et al.*, 2005). Moreover, significant main effects of the visit were detected (ANOVA, $P< 0.05$) in GLP-1 and PYY. Mean fasting plasma GLP-1 and PYY concentrations decreased after both regular and irregular interventions, respectively, compared with before the interventions (Alhussain *et al.*, 2016).

The conflicting results of the effect of meal timing on the circadian system in previous studies inspired us to develop the previous study protocols in order to investigate whether irregular eating habits had an impact on health via the disruption of circadian rhythms. Sarabia *et al.* (2008) observed that a significant increase in wrist temperature was detected when food was consumed at the usual lunch times (between 14:00 and 15:00 h) and a similar increase in wrist temperature was still noticed at this time when food consumption was delayed for three hours (until 17:00 h). Only a negligible elevation was observed after the new lunch time. Another study found that meal timing in constant routine did not influence rhythms of subjective hunger, master clock markers (plasma melatonin and cortisol), plasma triglycerides or clock gene expression in whole blood. However, plasma glucose rhythms were delayed, and average glucose concentration decreased following late meals. This suggests that mealtimes in humans plays a role in synchronising peripheral circadian rhythms (Wehrens *et al.*, 2017). The results for core temperature that are presented in this study show that assessing the influence of irregular eating on the diurnal rhythm through core and wrist temperature was feasible and practical.

6.5. Conclusion

Overall, this study has demonstrated that the protocol to test the hypo-energetic diet based on regular or irregular meal pattern on energy expenditure, metabolism and circadian rhythm is feasible. The process should now be used in a fully powered study to explore the effect of meal pattern irregularity on weight management and to assess the interaction between meal pattern irregularity and circadian rhythm.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

7.1. General Discussion

The overarching purpose of this thesis was to explore the association between meal pattern irregularity and health outcomes. A series of specific aims were set which addressed identified gaps in the knowledge in this area. The extent to which each aim has been achieved will be addressed, broader conclusions drawn and potential application to public health strategy and dietetic practice considered. Finally, an indication of potential future work will be outlined.

The first aim was to undertake a systematic review of the literature relating to the relationship between irregularity of eating and metabolic health and was presented in Chapter 2. Included in this aim was identifying and critiquing the methods of characterising meal pattern that had been used previously. A diverse range of papers were found in both adults and children from a variety of countries. It was concluded that there is some evidence that there is an association between irregularity of meal pattern and many health outcomes including BMI, energy expenditure, metabolic syndrome, blood glucose, insulin sensitivity and lipids profile. A limited number of intervention studies were found which suggested causality between irregular meal pattern and negative health outcomes. However, many different methods, for characterizing irregularity of meal pattern, were identified in the literature limiting comparison. The methods tended not to exploit all the potential dimensions of meal pattern that could have been extracted from available data.

Therefore, the next step in the thesis (Chapter 4) was to propose two novel methods of characterising the irregularity of meal pattern and these methods were applied to the NDNS database in order to explore associations between irregularity and health outcomes. The methods were designed to improve previous methods by incorporating more dimensions of meal pattern irregularity. For example, specific meal pattern (eg with breakfast, lunch and dinner) was not assumed and an 'eating incident' was clearly defined as any energy that was consumed within a 30-minute time period. Although the effect size of the results, using multiple linear regression, was small, there was a significant relationship between day-to-day variation and some health outcomes including BMI, lipids and glucose. Using Method 2 and the

Irregular time index however, the results were unexpected when compared with irregular energy index and the previous literature as irregular time index was significantly negatively associated with BMI, fasting glucose, HbA1C and HDL-cholesterol but positively linked to LDL-cholesterol. This may be because this method is confounded by eating frequency. A participant who tends regularly to eat on less occasions has potentially fewer time slots that can be considered irregular. In contrast to the Irregular Energy Index, the amount of 'irregular' energy is also not taken into account. Two participants might have a similar 'Irregular Time Indices', but in fact one might have a considerably more irregular pattern or energy intake than the other. Whilst two novel methods were successfully proposed, further work, in collaboration with experts in pattern recognition, such as Dr. Daniel Abasolo, would be warranted to further develop the level of sophistication in characterising meal pattern.

The next step in the thesis was to undertake a well-controlled feeding study to explore the impact of meal pattern irregularity on protein balance which had not been considered previously. Garrow *et al.* (1981) had demonstrated that different meal patterns, namely different daily meal frequencies, in conjunction with different dietary protein content, may modify protein metabolism. A crossover randomised controlled study was undertaken successfully, following a similar protocol to that previously used by Alhussain *et al.*, (2016). The impact of meal pattern irregularity on nitrogen balance, a proxy for protein balance, was studied. Regular and irregular meal patterns were provided to investigate the effect of both interventions on nitrogen balance. As 98% of body nitrogen is found in body protein, nitrogen balance was used as a proxy for measuring protein balance. Even though the findings have shown no impact of meal pattern irregularity on nitrogen balance over 7-day period, there was a tendency (P=0.091) for an intervention by time interaction with a higher positive mean nitrogen balance across the 7 days with the regular meal pattern compared with the irregular pattern. Possibly the adaptation of the urea cycle response to the different meal patterns had not occurred fully over a one-week period (Drummen *et al.*, 2018). It may have been that the

nitrogen balance needs over 10 days to establish a new equilibrium when intake of protein was increased or decreased (Millward *et al.*, 1991).

Whilst it is important not to over interpret the results, the current findings, suggesting a tendency for a treatment by time interaction, do warrant investigation, following a similar protocol, but with some improvements. Both nitrogen intake and nitrogen loss are challenging to measure and balance can be affected by wide range of internal and external factors. In the current study, the impact of the Covid 19 crisis further complicated the study process, as previously mentioned. Repeating the study with greater control of confounding factors and having made some adjustments to the protocol would be appropriate. The participants' adherence to the study diet, complete 24-h urine collection and honesty if a deviation from the protocol occurred (for example spilling urine) are important for precise estimation of nitrogen balance. The study might be undertaken in a metabolic unit, or a PABA tablet might be used to assess urinary collection completeness.

It was found that controlling the energy intake in free-living participants was a challenge and needs to be done with more accuracy especially in nitrogen measurement studies (Garrow, 1978). Compliance was poorer in this study, than in previous studies (Alhussain *et al.*, 2016; Alhussain *et al.*, 2022) and in the study described in Chapter 6 in this thesis involving a hypoenergetic diet, however, was not different between interventions. Providing food to the participants aimed to optimise adherence to the diet. However, there was some noncompliance which needs to be addressed in future studies. Introducing more variety in the menu might have improved compliance. There was no significant difference in physical activity between the two intervention periods which was important as in nitrogen balance, it is essential to control this factor and make sure that participants' activity level is the same in both intervention periods. Body weight however was stable, as required, suggesting that estimation of energy requirement was appropriate.

The study would ideally be undertaken over a longer duration and it would be of interest to repeat the study in a broader population, for example, in males, older people and those with specific clinical condition and potentially using a

hypoenergetic diet. It would also be appropriate to consider how stable isotope tracers might be used to measure rates of protein synthesis and breakdown in muscle, for example, as described by Hinde *et al.* (2021), a more direct measure of protein metabolism,

Study 3, described in chapter 6, the hypo energetic study was designed to develop our knowledge of meal pattern irregularity and health outcomes in two important ways. Firstly, a hypoenergetic diet was used, and secondly the feasibility of using a tablet-based method of measuring core body temperature was tested. Establishing the impact of irregularity when imposing an energy restriction is important for potential translation into clinical practice, where weight loss is required in those who are living with obesity. Measuring core body temperature, in free living participants is important in order to subsequently take forward previous work (Farshchi *et al.*, 2005; Alhussain *et al.*, 2016) to consider meal pattern irregularity in the context of the developing field of chrono- disruption. Moreover, participants selected were male and female, in contrast with previous studies which included only females. Hence, this makes the outcomes and conclusions more generalizable to the population at large.

It was demonstrated that the protocol for Study 3 was feasible and effective. The effect of a fixed meal pattern, with no between day variation, on circadian and diurnal rhythm has been considered in a number of previous studies (Sarabia *et al.*, 2008; Wehrens *et al.*, 2017) but the results were inconsistent. As no previous study has investigated the effect of meal pattern irregularity on either circadian or diurnal rhythm, the feasibility study that has been undertaken due to Covid 19, instead of the fully powered study originally planned (Chapter 6) is of considerable value in supporting further work. The feasibility study produced many suggestions and improvements that can be made to the protocol for measuring core and wrist temperatures. The crucial factor in getting a valid results in the measurement of diurnal rhythm is following a constant routine to prevent the masking effect of any other factors, such as irregularity of sleep times, physical activity and light exposure. Whilst weight loss was compatible with the prescribed energy deficit, it would have been beneficial to have measured body weight before and after the pre-

standardisation period to ensure that participants were in energy balance over this period.

In the feasibility study, developments of the protocol were also made in order to avoid limitations seen in previous work. For example, when measuring interstitial glucose by CGM, Alhussain *et al.* (2016) used an iPro™2 for which the sensor can stay in place for three to six days and there is a requirement for a four times per day finger prick glucose readings for calibration. In this study, the freestyle libre 2 CGM was used as effectively as the one previously used without requiring any finger prick glucose reading for calibration. Moreover, the sensor can stay in place and was shown to be patent for 14 days (Kumagai *et al.*, 2019). This gave the opportunity to compare interstitial glucose for longer with less inconvenience. The protocol described in study 3 might also usefully be applied in different participant groups, for example those with prediabetes, or with diagnosed type 2 diabetes. Such individuals might experience greater clinical advantage.

7.1.1. Translation to public health strategy and dietetic practice

The systematic review has identified consistent evidence of an associations between meal pattern irregularity and negative health outcomes in observational studies. However, such studies do not demonstrate causation. However, the results from the small number of intervention studies are compatible with this potential benefit and do not suggest any potential risk from reducing meal pattern irregularity. It would thus seem that encouraging meal pattern regularity in order to maintain health and as a further strategy to aid weight loss in those living with obesity and associated co-morbidities is not contraindicated, and may result in benefit. Furthermore, the thesis has established the feasibility of study protocols that can be used to strengthen the evidence to support more confident translation into public health messages and clinical practice.

7.1.2. Future work

1. Working with those living with obesity and associated co-morbidities, to undertake a clinical study exploring the potential added value of providing advice to increase the regularity of meal pattern. Outcome measures should include weight loss, body fat loss with reference to distribution of body fat, glucose and lipid metabolism, and blood pressure. Other outcome measures might include perception of appetite whilst restricting energy intake.

2. Undertake an expanded study exploring the impact of meal pattern irregularity on nitrogen balance over a longer time period, and with more participants. Stable isotope tracers might be incorporated into the design to explore muscle turnover.

3. Having established the feasibility of the hypo-energetic protocol the planned main study should be undertaken, possibly incorporating more detailed exploration of the impact on insulin resistance. The gold standard for measurement of insulin resistance the hyper-insulinemic euglycemic glucose clamp technique might be utilised.

4. The Gold Standard for testing impact on circadian patterns is to undertake work in a laboratory in which all conditions can be kept constant apart from the intervention of interest, namely meal pattern irregularity. In collaboration with the University of Surrey, in depth studies should be undertaken to explore the impact of regular and irregular meal pattern on main and peripheral circadian rhythms using constant routine protocols.

5. Establish the accumulative effect of the difference in thermogenic response to meals following the irregular meal pattern using indirect chamber calorimetry.

7.2. Conclusion

An investigation of the influence of meal pattern irregularity on obesity and its consequences was found in the literature and the results showed that there is an association between irregularity and metabolic health. The novel methods proposed for characterising meal pattern irregularity suggested that day-to-day variation

appears to contribute to obesity, dyslipidemia and hyperglycemia. These findings suggest that irregularity of meal pattern seems to be a lifestyle factor that negatively impacts on an individual's metabolic health. The impact of eating irregularity on protein balance and circadian system is still inconclusive. However, the altering of usual meal timing may play a role in the chrono-disruption of the peripheral clocks. Therefore, following a regular meal pattern might be a potential additional goal for body weight management as it seems to improve energy expenditure and insulin sensitivity which may indicate a favourable effect on insulin resistance and the risk of cardiovascular diseases. Overall, further intervention studies with controlled protocols and longer duration, including both sexes should be undertaken. These would investigate the impact of irregularity on health including protein balance and circadian rhythm and are necessary to build on the strengthen of evidence that currently exists and to understand the effect of meal pattern irregularity on the circadian rhythm. Moreover, further observational studies using consistent methodology/definition of irregularity of meal pattern would also be beneficial. Especially considering that intervention studies can often be more limited by sample size, and observational studies may provide better real-world data/ less influenced by intervention adherence limitations.

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