# IMPACT OF REPLACING RED AND PROCESSED MEAT WITH NON-MEAT ALTERNATIVES ON METABOLIC HEALTH

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"Our future selves will consider meat eating to be barbaric. I think we'll come to view eating meat in the way we now look back on the Roman games - having crowds of enthusiastic people cheering on the lions as they slaughtered the Christians or gladiators fighting each other to the death."

- Peter Singer

# ABSTRACT

Meat represents an important source of high quality protein and micronutrients. However, excessive consumption, particularly of red and processed meat, may increase risk of chronic diseases, including cardiovascular disease (CVD) and certain cancers. Meat production also represents an inefficient use of finite resources and may be rendered unsustainable by global population growth and the impact of climate change.

This project assessed acute and chronic effects of replacing meat with non-meat alternatives on plasma lipids and lipoproteins, and other markers of cardiovascular health. Two postprandial trials were undertaken in which volunteers consumed two meals (with and without meat), of similar macronutrient content, at least one week apart. Blood was collected every 30 min, for up to 6h post-meal, and chylomicron (CM) and very low density lipoprotein (VLDL) were isolated. Results demonstrated the expected postprandial changes in CM and VLDL triacylglyceride (TAG) but no significant differences between the meat and non-meat meals were observed.

The chronic effects of meat reduction on CVD risk were assessed in a single group, non-randomised, crossover design trial in which healthy volunteers reduced their red and processed meat intake by at least 50%. Volunteers were provided with a range of non-meat alternatives to facilitate the change in diet and aid compliance. Following a four week lead in period, they reduced their meat consumption, by at least 50%, and were assessed at six and 12 weeks. Fasting levels of serum lipids

and lipoproteins were measured together with a range of anthropometric and physiological variables. Reducing meat consumption significantly lowered total serum and low density lipoprotein (LDL) cholesterol in males (P=0.009 and P=0.007 respectively) but not females. No significant differences were seen in any other measurements.

In conclusion, replacing meat in the diet does not impact on postprandial lipid responses, but chronic replacement specifically reduces LDL cholesterol in males.

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# PUBLICATIONS

Some of the data presented in this thesis has been presented at the Nutrition Society Summer Meeting, 2015 as indicated below:

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# ABBREVIATIONS

**Abbreviations for fatty acids**

| | |
|---|---|
| C10:0 | Capric |
| C12:0 | Lauric |
| C14:0 | Myristic |
| C14:1 | Myristoleic |
| C15:0 | Pentadecylic |
| C16:0 | Palmitic |
| C16:1 | Palmitoleic |
| C17:0 | Margaric |
| C17:1 | cis-10-Heptadecanoic |
| C18:0 | Stearic |
| C18:1t9n-9 | Elaidic |
| C18:1c9n-9 | Oleic |
| C18:2 | Linoleic |
| C18:3 | α-Linolenic |
| C20:0 | Arachidic |
| C20:3n-3 | Eicosatrienoic |
| C20:3n-6 | Dihomo-γ-linolenic |
| C22:6 | Docosahexaenoic |

**Other abbreviations**

| | |
|---|---|
| 4-AAP | 4-Aminoantipyrine |
| ANOVA | Analysis of variance |
| BD | Body density |
| BF | Body fat |
| BIA | Bioelectrical impedance analysis |
| BMI | Body mass index |
| BP | Blood pressure |
| CETP | Cholesteryl ester transfer protein |
| CM | Chylomicron |
| CO | Cholesterol oxidase |
| CRC | Colorectal cancer |
| CVD | Cardiovascular disease |
| DSBmT | N,N-bis(4-sulphobutyl)-m-toluidine-disodium |
| EDTA | Ethylenediaminetetraacetic acid |
| eNOS | Endothelial nitric oxide synthase |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| *F. venenatum* | *Fusarium venenatum* |
| FA | Fatty acids |
| FDA | Food and Drug Administration |
| FFQ | Food frequency questionnaire |
| FHS | Framingham Heart study |
| FMD | Flow mediated vasodilation |
| $H_2O_2$ | Hydrogen peroxide |

| | |
|---|---|
| HAA | Heterocyclic aromatic amines |
| HDL | High density lipoprotein |
| IARC | International Agency for Research on Cancer |
| IDF | International Diabetes Federation |
| IDL | Intermediate density lipoprotein |
| IR | Insulin resistance |
| KBr | Potassium Bromide |
| LCAT | Lecithin-cholesterol acyltransferase |
| LDL | Low density lipoprotein |
| MAFF | Ministry of Agriculture, Fisheries and Food |
| MFM | Meat Free Monday |
| mmHg | Millimetres of mercury |
| MTT | Methylthiazolyldiphenyl tetrazolium |
| MUFA | Monounsaturated fatty acids |
| NAD | Nicotinamide dinucleotide |
| NCD | Non-communicable diseases |
| NO | Nitric oxide |
| NOC | N-nitroso compounds |
| PAH | Polycyclicaromatic hydrocarbons |
| PUFA | Polyunsaturated fatty acids |
| QMC | Queens Medical Centre |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| SFA | Saturated fatty acids |
| SLIM | Satiety labelled intensity magnitude |

| | |
|---|---|
| $sum_a$ | Sum of chest, abdomen and thigh skinfolds for males |
| $sum_b$ | Sum of triceps, suprailium and thigh skinfolds for females |
| T1DM | Type 1 diabetes |
| T2DM | Type 2 diabetes mellitus |
| TAG | Triacylglyceride |
| TRL | Triacylglyceride-rich lipoprotein |
| TVP | Textured vegetable protein |
| USDA | United States Department of Agriculture |
| VLDL | Very low density lipoprotein |
| WHO | World Health Organisation |
| YSI | Yellow Springs Instrument |

# Chapter 1 - INTRODUCTION: IMPACT OF MEAT CONSUMPTION ON HEALTH AND THE ENVIRONMENT

## 1.1 BENEFITS OF MEAT CONSUMPTION

Over thousands of years of evolution, humans have become accustomed to consuming significant quantities of meat (McAfee et al., 2010). Meat now represents an important part of a healthy balanced diet for many adults across the globe, supplying high quality protein with low carbohydrate, essential amino acids and various micronutrients such as iron, vitamins A and B12, zinc, selenium and folic acid which are essential for health (Pereira and Vicente, 2013). It is difficult to acquire these micronutrients from plant derived foods as they tend to have poor bioavailability or they are completely non-existent (Biesalski, 2005). Of the hundreds of known amino acids only twenty are required to synthesise proteins (Pereira and Vicente, 2013). Nine of these twenty amino acids are classed as essential because they cannot be produced by the human body (Wu, 2009). It is possible to obtain the essential amino acids from a combination of plant proteins, however animal protein contains all nine essential amino acids (Pereira and Vicente, 2013). The high protein and low carbohydrate properties of meat results in a low glycaemic index which may be beneficial in terms of weight management, insulin sensitivity, and the development of metabolic syndrome and diabetes. This may also lead to improvements in fasting plasma triacylglycerol and cholesterol levels (Paddon-jones et al., 2008), which are associated with risk of developing cardiovascular

disease (CVD). It is thought that the positive effects of a high protein diet on weight management are due the satiating effect of protein, which results in reduced energy intake, and its preservation of lean muscle mass in humans (McAfee et al., 2010). The protection of lean mass is a result of the increased amino acid levels provided by the dietary protein (Mettler et al., 2010). Meat can be separated into three main categories; red meat which includes pork, beef, lamb and veal; white meat which includes chicken, turkey, duck and game; and processed meat which are meats that have been preserved by methods other than freezing including, smoking and curing e.g. bacon, sausages, and deli meats (Rohrmann et al., 2013b).

## 1.2 DIETARY PATTERNS AND HUMAN HEALTH

For many years there have been worldwide concerns regarding the number of adults in the developed world consuming energy-rich diets, leading to the development of obesity and related diseases such as metabolic syndrome, type 2 diabetes mellitus (T2DM), CVD and cancers (Turner and Thompson, 2013). Over the last few years obesity and T2DM are being seen with increasing frequency in children and teenagers, which has led to a greater amount of research into the most appropriate diets required by humans to maintain optimal health. While the majority of modern humans are omnivores, the question still remains as to whether humans are anatomically suited to herbivorous or omnivorous diets (Mills, 2009). Deciding whether humans are essentially omnivorous or herbivorous is not an easy task as there are various contributing factors. Humans have evolved dramatically over the last 2.6 million years through the Palaeolithic period and then the Neolithic age (Turner and Thompson, 2013). Studies that look into the gut microbiota of humans suggest they are similar to that of omnivorous primates (Ley et al., 2008), while other work comparing the anatomy of eating suggest humans are more suited to a herbivorous diet (Mills, 2009). Eating behaviours are also learned through behavioural, social and physiological mechanisms (Turner and Thompson, 2013). Regardless of which diet humans initially evolved to consume, the fact is that the majority of the human population are currently omnivorous and, given free choice are likely to choose to consume a combination of food of animal and plant origin.

## 1.3 IMPACT OF MEAT CONSUMPTION ON HEALTH

As previously mentioned, meat can represent an important part of a healthy diet, supplying high quality protein with low carbohydrate and a range of micronutrients that are difficult to obtain from plant derived foods (Rohrmann et al., 2013b). However, in countries where consumption of meat is high, a number of health concerns have been associated with a diet high in red and processed meat. One of the main disadvantages is that red and processed meat consumption is frequently associated with an increased intake of total fat enriched in saturated fatty acids (SFA) (Rohrmann et al., 2013b). High fat intake can lead to obesity due to excessive energy consumption. SFA are specifically associated with an increase in total plasma and low density lipoprotein (LDL) cholesterol. Obesity leads to an increased risk of developing diseases such as insulin resistance (IR), metabolic syndrome, and T2DM (Feskens et al., 2013), all of which can further increase the risk of CVD. More recently, red and processed meat consumption has been linked to a greater risk of developing colorectal cancer (CRC). As with insulin resistance, metabolic syndrome and T2DM, obesity, lack of physical activity, and the global increase of the Westernised diet are all contributing factors to the development of CRC. Furthermore, red and processed meat has also been associated with carcinogenic properties which are major factors in the development of CRC (Bouvard et al., 2015).

CVD and T2DM have been long established as diseases of the developed world. These diseases have been associated with sedentary lifestyles and excessive consumption of energy dense foods that are often associated with increased wealth and prosperity (Feskens et al., 2013; WHO, 2010; WHO, 2014; Lambrinoudaki et al., 2013). It is tempting to associate an increased vulnerability to these non-communicable diseases (NCD) with high consumption of animal products because, in many populations, animal product consumption increases in conjunction with economic development (Feskens et al., 2013). However, NCD such as CVD and T2DM occur throughout the global population, not only in well developed countries. In fact, approximately 80% of NCD deaths occur in low- and middle-income countries (WHO, 2014). Salter (2012) demonstrated that on an age-standardised bases, premature death from CVD and diabetes is greatest in India and Kenya where consumption of animal products is lowest, compared to the UK and USA where animal product consumption is high. Disease mortality comparisons should be undertaken with caution because data may be significantly influenced by the availability of healthcare facilities (WHO, 2010). Disease prevalence may be a more suitable comparison for assessing the relationship between animal product consumption and NCD. However, it must be understood that these diseases are influenced by a variety of factors including genetic and environmental factors, not solely the consumption of animal products (Salter, 2012; WHO, 2010).

### 1.3.1 Metabolic Syndrome and Type 2 Diabetes Mellitus

Incidences of obesity are increasing to the point of epidemic proportions in developed countries around the world. The primary reason for this is over-consumption of energy-dense foods, such as refined carbohydrates and animal products, accompanied by increasingly sedentary lifestyle choices (Salter, 2012; Van Dam et al., 2002). A substantial number of obese individuals in this population will eventually become insulin resistant and develop the metabolic syndrome. IR is a decreased responsiveness or sensitivity to the metabolic actions of insulin. During IR the pancreas produces insulin in response to glucose, as seen in normal insulin sensitivity, however, the cells of the body do not respond to the release of insulin, resulting in the glucose not being absorbed by the body's cells. This lack of response leads to high levels of glucose in the blood. The body then senses the high glucose levels, which subsequently causes the pancreas to increase production of insulin to attempt to lower the glucose, therefore resulting in high insulin and high glucose levels in the blood (Katz et al., 2000). Although IR is determined by genetic factors, it is also determined by environmental factors such as diet and physical activity, and, if undetected, IR can result in the development of T2DM (Chiu et al., 2007). The metabolic syndrome can be described as a group of physiological risk factors that can lead to an individual developing CVD and T2DM. These risk factors are visceral obesity, hypertension, glucose intolerance, low high-density lipoprotein (HDL) cholesterol and elevated plasma triacylglycerol levels. The metabolic syndrome is deemed present if a person displays three

or more of the risk factors, and these people are significantly more inclined to develop T2DM than those displaying no risk factors (Wilson et al., 2005; Salter, 2012). The International Diabetes Federation (IDF) annual report 2014 estimated that 387 million people aged 20 – 79 years old were living with diabetes. This estimate is expected to increase to 592 million by 2035 (IDF, 2014). Although the IDF annual report does not distinguish between type 1 diabetes (T1DM) and T2DM, approximately ninety percent of people diagnosed with diabetes have T2DM (Diabetes UK, 2015; Aune et al., 2009). T2DM is one of the major public health concerns in the European Union. Along with obesity and the other metabolic syndrome risk factors mentioned above, lifestyle, age, genetics and ethnicity also play a role in the development of T2DM (Feskens et al., 2013; Bendinelli et al., 2013). Until recently, there has not been much focus on the role specific foods play in the development of T2DM, and given the present worldwide discussions on the sustainable production of food, it is important to understand, as much as possible, the impact that meat consumption has on health and nutritional status (Fung et al., 2004). As mentioned earlier, meat is an important source of protein and other nutrients. However, the intake of meat in Western countries is high and that in the developing countries is rapidly increasing. Therefore, it is important to fully understand the causes, processes, development and consequences of T2DM and the role meat plays in this (Aune et al., 2009; Feskens et al., 2013). Dietary proteins, carbohydrates and fats all provide energy which is measured as calories. However macronutrients impact differently on a range of

metabolic parameters, including protein synthesis, fat oxidation, insulinemia and glycaemia (Comerford and Pasin, 2016). For example, although individuals with T2DM tend to lose their ability to secrete insulin in response to carbohydrate consumption, they can maintain, or even increase, their ability to secrete insulin in response to protein consumption (Saeed et al., 2002; Van Loon et al., 2003). However, differences in amino acid composition mean that not all protein sources have the same effect on postprandial insulin secretion and insulin sensitivity and therefore do not have the same protective effects on T2DM risk (Comerford and Pasin, 2016). Each protein source has different properties and functions that produce different effects on the human body. In general, plant proteins have been shown to have protective effects on T2DM risk; however, these may not be due to the type and amount of actual protein in them but more a result of the non-protein components of the product, particularly complex carbohydrate and fibre (Comerford and Pasin, 2016). Snowdon and Phillips (1985) suggested that a vegetarian diet could be protective of T2DM after reporting that meat consumption was an independent risk factor of T2DM in a group of Seventh-Day Adventists. There have been various studies conducted since, which confirm that a diet rich in red and/or processed meat is a significant and independent risk factor for T2DM (Van Dam et al., 2002; Fung et al., 2004; Lajous et al., 2012; Micha et al., 2010; Montonen et al., 2005; Pan et al., 2011; Schulze et al., 2003; Song et al., 2004; Van Woudenbergh et al., 2012). The EPIC-InterAct study, as described by Riboli et al. (2002), is a large prospective case-

cohort which presented data in 2013 showing an overall positive association between meat consumption and T2DM risk (Bendinelli et al., 2013). Although studies looking into T2DM have demonstrated a positive association with red and/or processed meat intake, the underlying mechanisms are still unclear (Lajous et al., 2012). In general, high meat consumption is frequently associated with excessive energy intake leading to obesity. Also, people that consume high amounts of red and processed meat tend to live unhealthier lifestyles including higher levels of smoking, alcohol consumption, and lack of physical exercise (Pan et al., 2012). As such, it remains to be established whether there are specific effects of components of red and/or processed meat which are associated with risk of developing T2DM beyond these general lifestyle factors.

### 1.3.2 Cardiovascular Disease

CVD can be described as an arterial disease that affects the blood supply to the heart, brain or peripheral regions of the body (Frayn and Stanner, 2005). CVD, which includes coronary heart disease, strokes, and a range of diseases affecting other arteries, are a major global cause of disability and early death (McAfee et al., 2010; Williamson et al., 2005; Lambrinoudaki et al., 2013). Diet, hypertension, high blood cholesterol levels, and smoking have been identified as major risk factors for the development of CVD (Thom et al., 2004), and years of scientific research has established that alterations of diet and lifestyle can make considerable contributions to reducing the risk of CVD (Williamson et al., 2005; Lambrinoudaki et al., 2013). However, it has also been established that these are not the only factors involved. Over the last several years a variety of other factors have emerged as potential predictors of CVD risk: decrease in blood vessel function; variations of postprandial lipids in the bloodstream; decreased resistance to oxidative stress and elements involved in clotting and inflammation (Frayn and Stanner, 2005). Intervention studies have shown that reducing body weight, decreasing dietary SFA intake, increasing polyunsaturated fatty acid (PUFA) intake, and increasing physical activity provide overall benefits in reducing CVD risk (Salter, 2012; Lambrinoudaki et al., 2013). A link between red and processed meat consumption and the development of CVD has been established in various studies. Although the exact link is still unknown, the generally high SFA content of red and processed meat, along with the high salt

content of processed meat, is thought to be a major contributing factor (McAfee et al., 2010; Williamson et al., 2005). In developed countries, a significant amount of total SFA intake is associated with animal products including meat and dairy products (Williamson et al., 2005). For example, **Figure 1.1** shows that in the UK, animal products contribute ~50% of SFA intake (Salter, 2012).

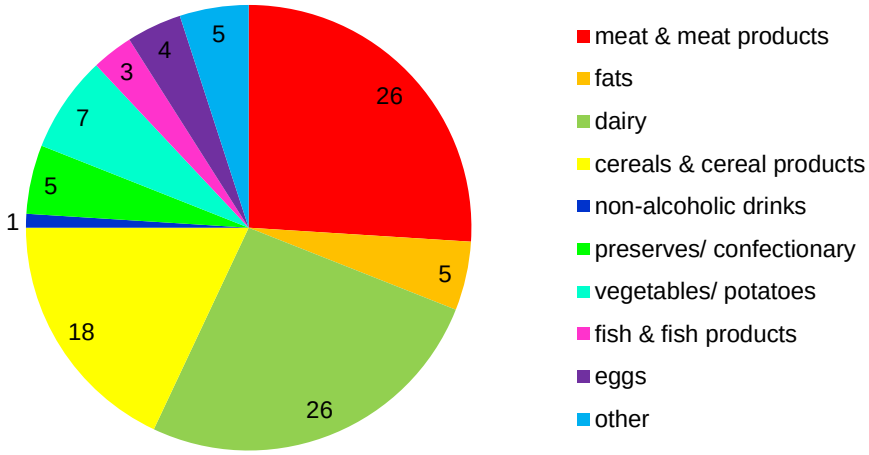


**Figure 1.1 Contribution of different food groups to SFA intake in the UK in 2008-2009.**

(redrawn from Salter, 2012)

It has also been suggested that people who consume large quantities of red and processed meat tend to have a relatively unhealthy diet high in total and SFA-rich fats and refined carbohydrate, so this may be a reason for the higher CVD risk in this population (Williamson et al., 2005; McAfee et al., 2010). CVD risk has been repeatedly shown to significantly decrease when substituting SFA with unsaturated fatty

acids (FA) (Salter, 2012; Williamson et al., 2005; Lambrinoudaki et al., 2013), with the benefits generally being greater for PUFA than monounsaturated fatty acids (MUFA). The last twenty years has provided countless evidence from controlled dietary studies demonstrating the metabolic effects of replacing dietary SFA with unsaturated FA (Williams and Salter, 2016). Professor Julie Lovegrove, from the University of Reading, UK, recently investigated the impact of both MUFA and PUFA on CVD risk. Lovegrove concluded that substitution of SFA with both PUFA and MUFA is associated with a reduction in lipid markers of cardiovascular risk; substitution by PUFA is associated with a reduction in CVD risk and mortality; and substitution by MUFA is associated with a reduction in risk markers of CVD such as systolic blood pressure. Although there is not enough data to determine the effect of substituting SFA with MUFA on CVD mortality, MUFA are available from both plant and animal sources, therefore, the food source may have an impact on the association with CVD mortality (Nettleton et al., 2016). As red meat is naturally very low in PUFA, the meat industry has been researching techniques to manipulate the PUFA profile of red meat by altering feed sources, in turn, tackling a major health issue associated with red meat consumption (Enser et al., 2000; Kouba et al., 2003; Leskanich et al., 1994; Myer et al., 1992; Wood et al., 2003). Although this may address some of the health implications of meat consumption, it does not address the sustainability and environmental issues.

A review by Hooper et al. (2011) showed a 14% decrease in CVD risk when substituting SFA for unsaturated FA in the human diet. A systematic review by Micha et al. (2010) suggested no association between red meat consumption and CVD risk. However, their meta-analysis of four studies suggested that each 50g serving of processed meat per day increased CVD risk by 42%. Although the meta-analysis by Micha et al. (2010) demonstrated no relationship between fresh red meat consumption and CVD, other studies have presented evidence of this relationship. Bernstein et al. (2010) conducted an analysis using data from the Nurse's Health Study. Results showed that both red and processed meat were associated with an increased risk of CVD. Pan et al. (2012) later combined the data from Bernstein's 2010 analyses with data from the Health Professionals Study. Results showed that although the association between processed meat consumption and CVD risk was higher, fresh red meat consumption was also associated with an increased risk. Pan's 2012 study also suggested that increased CVD risk may not only be linked with SFA intake, but it may also be associated with heme-iron intake. However, more studies are required to ascertain this link.

As mentioned above, hypertension is a major risk factor for the development of CVD. It is well known that diet plays an important role in the regulation of blood pressure (BP), and the main dietary factors affecting hypertension are, high intakes of sodium and alcohol, obesity, and low potassium intake (Appleby et al., 2002). A trial by the Dietary

Approaches to Stop Hypertension (DASH) group in 1997 discovered that a diet rich in a combination of fruit, vegetables, and low in SFA, can significantly lower systolic and diastolic BP (Appel et al., 1997).

The sodium and nitrite content of processed red meat is significantly higher than unprocessed red meat due to the common use of these products in preservatives that are used for curing the meat (Paik et al., 2005). Sodium consumption is a well-known contributor to increased BP, therefore the strong association observed between processed meat and CVD risk may be mediated by its effects on BP (Lajous et al., 2014).

More recently, a novel study by Koeth et al. (2013) produced data suggesting that the intestinal microbiota metabolism of L-carnitine, an amino acid derivative abundant in red meat, may have a significant impact on CVD risk. Along with dietary ingestion being a major source of L-carnitine in omnivores, it is also produced endogenously from lysine and methionine. L-carnitine plays an essential role in transporting FA into mitochondria. The supplementation of this product in the bodybuilding community in Westernised societies has increased dramatically over the last decade, due to suggestions of fat loss and increased muscle mass (Wutzke and Lorenz, 2004; Karlic and Lohninger, 2004). However, the evidence behind these reports is lacking. Therefore, more research into the protective effects of L-carnitine on CVD risk is required.

### 1.3.3 Cancer

There has been an increasing amount of research into the association of red and processed meat consumption and the risk of developing various cancers (Salehi et al., 2013; Wallin et al., 2011; Wie et al., 2014; Choi et al., 2013; Di Maso et al., 2013; De Stefani et al., 2012; Zhu et al., 2014; Lippi et al., 2016; Cross et al., 2007; Larsson and Wolk, 2012). The cancer most frequently associated with meat consumption, for there is the most evidence for an association with meat consumption, is CRC. Multiple studies have shown a positive association between meat intake and CRC (Chan et al., 2011 and 2013; Joshi et al., 2015; Demeyer et al., 2015; Ananthakrishnan et al., 2014; Bernstein et al., 2015; Parr et al., 2013). CRC incidence has risen significantly over the last 50 years and was relatively uncommon before the 1900s (Pericleous et al., 2013). It now represents one of the primary causes of death, particularly amongst men, worldwide. Jemal et al. (2011) estimated that there are more than one million newly diagnosed cases and over 600,000 deaths every year relating to CRC.

Although the causes of CRC have been found to be of both genetic and environmental origin, only ~20% of risk is thought to be related to genetics, with the majority of risk associated with environmental factors (Kim et al., 2013). Environmental risk factors include obesity, lack of physical activity, and the global increase of the Westernised diet (Wynder and Shigematsu, 1967; Center et al., 2009; Ahmed, 2004). It has been suggested that diet accounts for approximately 70% to 90% of

all CRC cases (Shannon et al., 1996). Therefore, modifying the populations' diet may help to reduce the occurrence of this form of cancer (Pericleous et al., 2013). It is important for the body to get a sufficient amount of iron to prevent anaemia. The type of iron in meat is known as heme iron, and is more efficiently absorbed than plant-based non-heme iron. However, a high intake of heme iron is associated with the development of carcinogenic N-nitroso compounds (NOC) in the gastro-intestinal tract, and therefore may be associated with the development of CRC (Rohrmann et al., 2013a). The processing of meat products can also result in the formation of NOC, along with another carcinogenic chemical called polycyclicaromatic hydrocarbons (PAH) (Bouvard et al., 2015). A review by Pericleous et al. (2013) concluded that red and processed meats, especially when cooked at high temperatures, should not be consumed in high quantities due to the production of carcinogens such as heterocyclic aromatic amines (HAA) and PAH. It was suggested that these meat products be replaced by fish and white meats. More recently, in late 2015, the International Agency for Research on Cancer (IARC), the cancer agency for the World Health Organisation (WHO), employed 22 experts from ten different countries to assess the carcinogenicity of the consumption of red and processed meat. More than 800 worldwide epidemiological studies that examined the link between cancer and red and processed meat consumption were assessed (WHO, 2015). The IARC concluded that, based on the large amount of data, and the consistent worldwide associations between processed meat consumption and colorectal

cancer, processed meat should be classified as “carcinogenic to humans”. The IARC also stated that, based on limited evidence, red meat is “probably carcinogenic to humans” (Bouvard et al., 2015).

## 1.4 ENVIRONMENTAL IMPACT OF MEAT CONSUMPTION

The exchange of gases in the atmosphere is what determines the Earth's climate (Falkowski et al., 2000). Throughout history, natural processed have caused the Earth's climate to change significantly. However, the last 50 to 100 years have seen a much greater and more rapid change in the warming of the Earth's climate (GAO, 2007). It has been stated that human activity is extremely likely to be the main cause of this global warming (IPCC, 2007). The livestock sector has now been recognised as one of the major contributors to climate change and occupies ~30% of the ice-free terrestrial surface of the planet due to feed crop production and grazing (Steinfeld et al., 2006; FAO, 2006). The environmental issues caused by the livestock sector include deforestation, soil degradation, desertification, eutrophication, land- and water-based pollution, water scarcity and emission of greenhouse gas (GHG) from animals. The agricultural sector, more particularly, the production of livestock, accounts for approximately one fifth of the world's GHG emissions (Reisch et al., 2013). Increases in the production of livestock, due to the global increase in demand for animal products, has potentially serious consequences for the environment. It has been suggested that the worlds growing population, along with improved prosperity of many developing countries, will double global meat consumption from the year 2000 to 2050 (FAO, 2006). The increase in livestock production results in the requirement for large quantities feed, and in turn, results in substantial areas of land being used to grow the required cereals for feed. Rather than using land to

produce grains that can be fed directly to humans, developing countries are using increasing amounts of land for grains that will be used as animal feed. It is estimated that developing countries will, in fact, surpass the rest of the world, in terms of grain production used for animal feed, by 2030 (**Figure 1.2**). Developing countries currently account for 39% of the world's non-food grain production, and this is expected to increase to 56% by 2050 (FAO, 2006). The inefficiency of animals in converting feed to meat results in meat production having the greatest impact on the environment. Approximately 75 – 90% of energy consumed by the animals is either lost in manure and by-products, such as skin and bones, or required for body maintenance (Steinfeld et al., 2006). Therefore, the use of these grains and cereals as a direct feed source for humans may be a far more efficient strategy.

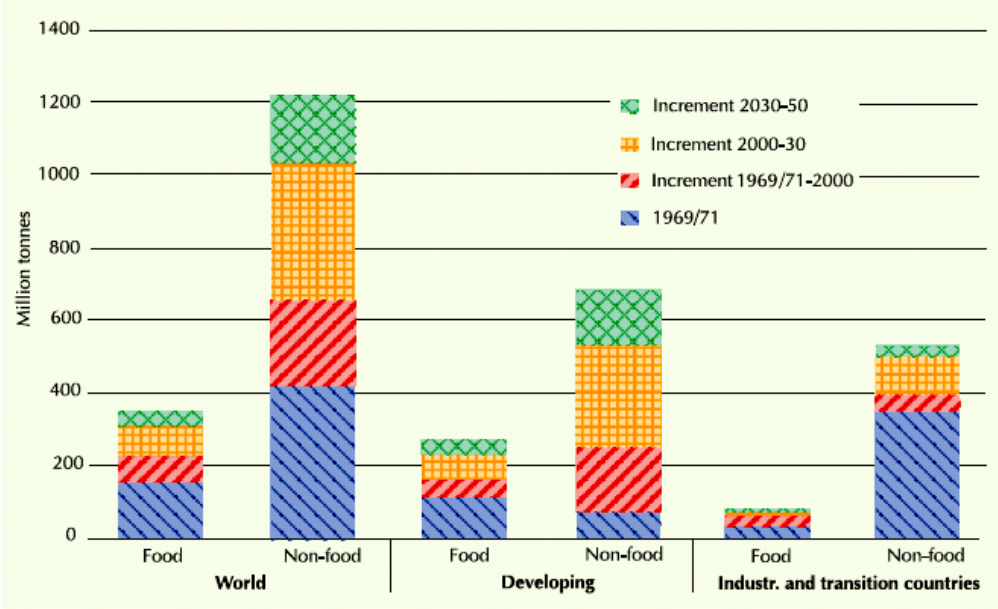


**Figure 1.2 Global food and non-food grain production rates.**

(FAO, 2006)

Methane from the digestive processes of ruminants, nitrous oxide from excreted nitrogen and the fertilisers used in production of crops for the use as feed for animals, and carbon dioxide from deforestation are produced during the raising of livestock. Methane and nitrous oxide account for 80% of all GHG emissions in the agricultural sector (Friel et al., 2009). The use of pesticides on crops causes toxic substances to leach into surface and ground waters. Freshwater reserves are being overused by the livestock sector in many regions, which is causing serious water shortage problems (Steinfeld et al., 2006). Although these environmental issues are well recognised, the livestock sector is estimated to increase substantially over the next 30 years. Therefore, it is important to investigate technologies to reduce GHG emissions from livestock and to create strategies that will reduce the consumption of livestock products (McMichael et al., 2007).

## 1.5 TRENDS ON MEAT CONSUMPTION

Trends in meat consumption have changed dramatically over the recent decades. Data shows that red and processed meat consumption in the developed world peaked around 20 years ago (Salter, 2012). Although meat consumption is still high in developed countries, with the UK consuming 851 kcal/person/day of livestock, and USA consuming 900 kcal/person/day (FAO, 2009), red and processed meat consumption in these areas now appears to be decreasing, and the consumption of poultry is increasing (Scarborough et al., 2011; Salter, 2012). Although poultry is a meat product, it does not have the same adverse effects on health as red and processed meat. In the developing world however, the increase in consumption of red and processed meat has been quite dramatic (Delgado, 2003). For example, China and Brazil, which have seen dramatic economic development, consume the largest amount of calories (in the developing world) from livestock products per person, per day; with China consuming 610 kcal/person/day and Brazil not far behind, consuming 603 kcal/person/day (FAO, 2015; Salter, 2012). As previously mentioned, a major contributor to climate change is the livestock sector. The ever-increasing population and economic growth of developing countries, coinciding with the increasing production of meat, means that climate change presents a major challenge for our future way of life on Earth (IPCC, 2007).

## 1.6 ALTERNATIVES TO EATING MEAT

As discussed in previous sections, consumption of excessive amounts of red and/or processed meat is associated with a number of adverse effects on health. Furthermore, as consumption increases in the developing world, livestock production poses serious risks to the environment. In the developed world, increasing interest has been shown in strategies to reduce meat consumption (Asgar et al., 2010). Although the majority of people, especially in developed countries, are meat-eaters, there are those who choose not to eat animal-based foods. The reasons people choose to avoid meat, and other animal products, include concerns about animal welfare, environmental and sustainability issues, religious beliefs, distaste, and their impact on health (Asgar et al., 2010). There are several categories of such individuals depending on their specific eating habits (Ruby, 2012).

- Vegetarians: consume no meat, but may or may not consume and use animal by-products such as eggs, milk, cheese, leather and wool.
- Vegans: avoid all meat and animal by-products including eggs, milk, cheese, wool and leather.
- Pescetarians: do not consume meat from land animals or birds, but do consume fish, molluscs, and crustaceans. Eggs and dairy may or may not be consumed in the pescetarian diet.
- Pollotarians: avoid red meat, but do consume poultry. Seafood and animal by-products may or may not be excluded from the pollotarian diet (King, 2014).

It is therefore clear individuals can adopt a variety of meat-reduced or meat-free diets. In most industrialised countries, the number of individuals opting to adopt a vegetarian diet is relatively low (**Figure 1.3**), with approximately 2% of the UK population (FSA, 2014). These lifestyles are more common in populations where religion is involved in decisions on meat consumption (Kalverkamp et al., 2014).

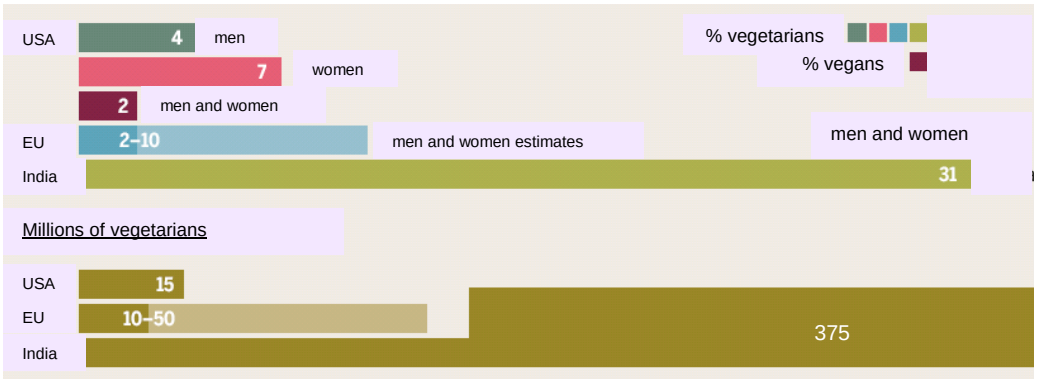


**Figure 1.3 Vegetarians in the West versus India.**

(Kalverkamp et al., 2014)

However, it has recently been shown that as many as one third of individuals in the UK are attempting to reduce their meat consumption (Penny et al, 2015). Such individuals have a variety of alternative food types with which to replace meat. In many parts of the world vegetarianism is enforced, through lack of availability of animal products, and populations are often dependent on a very limited range of crop species often leading to deficiencies in specific nutrients (Sabaté, 2003; Bender, 1992). By contrast in those who elect to become vegetarian, by selecting a wide range of plant materials, including grains, beans and pulses, all nutritional requirements can be

met (Dwyer, 1988). As noted above, many vegetarians will opt to supplement their diets with dairy products, egg and fish. There is considerable evidence to suggest that adoption of a nutritionally adequate vegetarian diet is protective from metabolic disease, including obesity, diabetes and CVD (Appleby and Key, 2016; Dwyer, 1988; Sabaté, 2003). In addition to the broad range of naturally occurring plants, an increasingly wide range of foods are being developed to address the demand for non-meat alternatives. Some of the most popular are reviewed below.

### 1.6.1 Plant-based Materials as Meat Substitutes

Meat substitutes, which are also referred to as analogues, alternatives or replacers, are predominantly plant-based foods that contain protein from sources such as pulses, cereal or fungi (Hoek et al., 2011). Meat substitutes are produced in various forms, such as sausages, burgers, mince, pieces and sandwich slices; and have been developed to directly replace animal meat in the diet (Asgar et al., 2010). One of the most established meat substitutes, which is still widely consumed by the vegetarian market, is tofu. Tofu is a soya-based product, and has been produced for approximately two thousand years in China (Elzerman et al., 2013). Its consumption in Western Europe and the USA started in the 1960s. Also in this decade, a soy protein-based meat substitute was developed and introduced to the market. This product was created by spinning soy protein into fibres to form products that imitated meat, and was termed textured vegetable protein (TVP) (Singh et al., 2008).

Although the first TVP products were soya-based, further products made from legumes, wheat, and milk proteins were subsequently developed (Elzerman et al., 2013). Along with this expansion came improvements in agricultural production and human nutrition, which in turn, increased the interest in TVP. In 1999, the United States Food and Drug Administration (FDA) confirmed that soy protein may lower cholesterol and reduce the risk of coronary heart disease due to the low content of SFA and high content of PUFA, fibre, vitamins, and minerals. This claim resulted in the widespread acceptance of soy products throughout Western cultures (Asgar et al., 2010). TVP can be consumed as whole food products, designed to look, taste and feel like real meat products, or it can be used as a meat extender. A meat extender is TVP that is mixed with meat to reduce the meat content of a product and improve the nutritional properties of the product (Singh et al., 2008; Asgar et al., 2010). TVPs frequently contain additives, such as flavours, colours, emulsifiers and pH modifiers, to improve the texture, colour and flavour of the product, in an attempt to replicate the flavour and sensory properties of meat as closely as possible (Asgar et al., 2010).

### 1.6.2 Entomophagy

The consumption of insects is termed entomophagy, and is traditionally exercised in 113 countries worldwide. More than two thousand species of insects are considered edible and highly nutritious due to their high protein and essential amino acid content, together with a range of minerals, and relatively high unsaturated FA content (Rumpold and Schlüter, 2013). A study by Nakagaki and Defoliart (1991) demonstrated that, when compared to livestock, crickets have a higher food conversion efficiency than chicks, pigs, sheep and cattle. This means that crickets require less feed for the production of 1kg biomass than livestock. Insects also have a higher fertility than livestock, require less space in the breeding process, and can also be raised on organic waste due to their omnivorous diets (Verbeke, 2015). The energy content of 100g of insects is similar to 100g of fresh weight meat. Some insects, such as caterpillars, grasshoppers, termites and weevils, have been shown to be better sources of protein than livestock. Insects also exhibit a high quality amino acid profile, generally meeting all of the essential amino acid requirements, except for methionine (Rumpold and Schlüter, 2013). There is currently considerable interest in developing agricultural systems for the large scale production of insect products for human consumption, which are not only cost-effective and energy efficient, but also adhere to food safety regulations (Rumpold and Schlüter, 2013).The development of such facilities may ultimately provide products, including protein-rich flours and oils, that are acceptable to a mass global market.

### 1.6.3 Cultured Meat

Cultured meat, also referred to as in-vitro meat or lab grown meat, is meat that has been grown in vitro using satellite cells to create skeletal muscle tissue (Edelman et al., 2005). Cultured meat is produced in a similar way to tissue engineering in regenerative medicine and has been studied for the last 20 years (Post, 2012; Goodwin and Shoulders, 2013). Cultured meat could provide several benefits such as the consumption of all of the nutrients contained in meat without the need to slaughter animals, more favourable fatty acid profile, and reduced environmental impact (Goodwin and Shoulders, 2013). Post (2012) claims that if lab grown meat is to serve as a credible alternative to meat from animal sources, it should be designed to mimic all aspects of meat from livestock, such as taste, texture, smell and visual appearance. In 2013 Dr Mark Post was the first to produce a cell cultured beef burger, however, due to the challenges of cost, large scale production and consumer reactions, cultured meat has not yet been manufactured for commercial use (Verbeke et al., 2015).

### 1.6.4 Fungi

Although fungi products have been used as food for man for many thousands of years, it was not until the 1980s that a filamentous fungus was intentionally cultured and widely marketed for use as a significant source of protein for the population. The British company Rank Hovis McDougall first decided to develop a meat substitute based on fungal mycelium in the 1960s (Wiebe, 2004). Over 3000 fungal isolates from around the world were tested for protein, toxins and appropriate growth and morphology. The specific fungus that was selected is known as *Fusarium venenatum* (*F. venenatum*) A3/5 and was initially identified as *F. graminearum* in 1967 (Denny et al., 2008). After *F. venenatum* was selected as the most ideal organism to use it was put through the fermentation, toxicity testing and product development process. It took more than ten years of research to determine that it was safe and acceptable to be consumed by human volunteers and lab animals (Wiebe, 2002). *F. venenatum* is grown in continuous flow culture to create mycoprotein. During the testing period it was discovered that the amino acid profile of mycoprotein was similar to that of eggs, but had sizable fibre content and contained no cholesterol. Mycoprotein also has naturally low sodium content and a similar FA profile and fibre content as other vegetarian protein sources. Mycoprotein was ultimately marketed under the brand name 'Quorn™'. In this commercial product mycoprotein is combined with egg albumin and various spices and flavouring to create a high protein, high fibre, low fat product of desirable texture, taste and aroma, which could be manufactured to

resemble a number of meat products including beef, chicken and pork. Food processing technology is used to shape the mycoprotein into various products such as mince, sausages, fillets, steaks, sandwich-style slices and tasteless chunks (Asgar et al., 2010). The British Ministry of Agriculture, Fisheries and Food (MAFF) approved the sale of the product in 1985. It is now being sold in the UK, USA and at least eight other European countries (Wiebe, 2002). Since 1994, rather than being presented solely as a vegetarian product, the marketing of Quorn™ began to focus more on the potential benefits of replacing meat with Quorn™ on health. Studies by Turnbull et al. (1990, 1992 and 1995) have shown that the consumption of mycoprotein may have beneficial effects on blood cholesterol concentrations and may also induce greater satiety, resulting in reduced energy intake. While these results suggest that substituting meat with Quorn™ may have long term benefits in maintaining a healthy body weight and reducing CVD risk, it should be noted that studies to date have had relatively small sample sizes and been of short study durations. Quorn™ remains the only commercially available source of mycoprotein for human consumption on the market (Wiebe, 2002).

### 1.6.5 Changing Consumer Behaviour

There is increasing recognition that reducing meat consumption represents an important strategy in ensuring sufficient food will be available to the growing global population (faced with the potential impact of climate change) in coming the decades. This is most likely to be achieved if new meat-free food products can be developed that are appealing to omnivorous consumers (Hoek et al., 2011). Studies investigating the acceptance of meat substitute products are few and far between; however, those that have been published suggest that the acceptance of meat substitutes relies on various factors such as nutritional and sensory qualities, price, image, and convenience (Elzerman et al., 2013). Although technological advancement is incredibly important in the development of consumer acceptable products, this alone will not be enough to address the environmental issues related to meat production; behaviour also needs to change. Worldwide economic and cultural transformations are required to facilitate the changes required to redress the issues. There are two main strategies proposed by de Bakker and Dagevos (2012) relating to consumer alliance, that can be used to facilitate the change in meat consumption.

The first strategy, sustainability by stealth, is aimed at consumers that are described as passive when it comes to food. Passive consumers generally do not, and feel no need, to concern themselves with difficult questions about food. It is suggested that the consumers in this group

will accept new food innovations, especially if they are not very noticeable. An example of sustainability by stealth can be seen in the Dutch food market. Meat extenders were used in mince, sausages, and burgers to create hybrid products that looked and tasted like the original meat products. These hybrid products were advertised as lean meat products to focus on the value of health rather than sustainability. This allowed the gradual introduction of sustainable products into the food industry. Although this strategy is beneficial in terms of gaining consumer acceptability and attention, it can lead to the consumers feeling misled about these new products, and result in a loss of trust. Lack of trust is a major concern as it can lead to difficulties in marketing and acceptance of future products aimed at improving sustainability (de Bakker and Dagevos, 2012).

The second strategy, moderate involvement, is aimed at consumers that are active and engaged, who form part of a society with common interests. The idea behind this strategy is that consumers are involved in the incorporation of small steps, that appear feasible, to create a more sustainable environment. An example of this is the Meat Free Monday (MFM) campaign designed by Paul, Mary and Stella McCartney in 2009. The MFM campaign aims to raise awareness about the environmental impact of meat production and consumption. MFM encourages people to have one meat-free day a week to help prevent climate change and improve their health (Meat Free Monday, 2016). The challenge of this strategy is convincing the consumers to

incorporate meat-free and low-meat days into their week using meat alternatives that taste good, are healthy, and sustainable (de Bakker and Dagevos, 2012).

A combination of these two strategies appears to be the most likely way in which wide-spread reduction in meat consumption can be achieved. Most consumers are aware of the health impact of consuming excessive amounts of animal products and this needs to be coupled with an increasing awareness of the impact of animal production on the environment. There are many consumers that would like to participate in the various methods of meat reduction, but they just aren't sure how to incorporate it into their daily lives. Others will accept novel meat-reduced products as long as perceived quality is not affected. Coupled, with an almost inevitable increased in the cost of animal products as demand starts to outstrip supply, such strategies should help to achieve the reduction in consumption required. Overall, consumers need to be accepted as allies that can be trusted with the challenge of understanding how meat reduction can contribute to both better health, and improved global food security (de Bakker and Dagevos, 2012).

## 1.7 FACTORS CONTROLLING FOOD INTAKE

Knowledge of the factors that control food intake is crucial to an understanding of adequate energy regulation the control of body weight and obesity (Hopkins et al., 2016). Satiety has been shown to be controlled by both behavioural and physiological factors that inhibit further consumption of food until the signals of hunger return (Bellisle, 2008). The satiety cascade (**Figure 1.4**) was designed by Blundell et al. (1987) to demonstrate the process of satiation and satiety when a food or drink is consumed; the process of such food entering the gastrointestinal tract, along with digestion and absorption (Blundell et al., 1987). When food or drink is ingested signals travel to specific areas of the brain that are associated with triggering satiation (Benelam, 2009). Functional foods, those which provide additional health promotion or disease prevention, may affect satiety by acting at various points in the satiety cascade (Bellisle, 2008). There are certain characteristics of foods that could potentially enhance the intensity and duration of satiety following consumption; this could potentially impact on the control of body weight (Bellisle, 2008). Satiety is not only regulated by the internal signals mentioned above, but also by portion size, palatability of the food, time of day of consumption, and also the current social situation the person is in (Benelam, 2009). As previously mentioned in section 1.1, protein has been shown to be the most satiating macronutrient (McAfee et al., 2010), so it is important to discover if certain proteins affect satiety different to others.

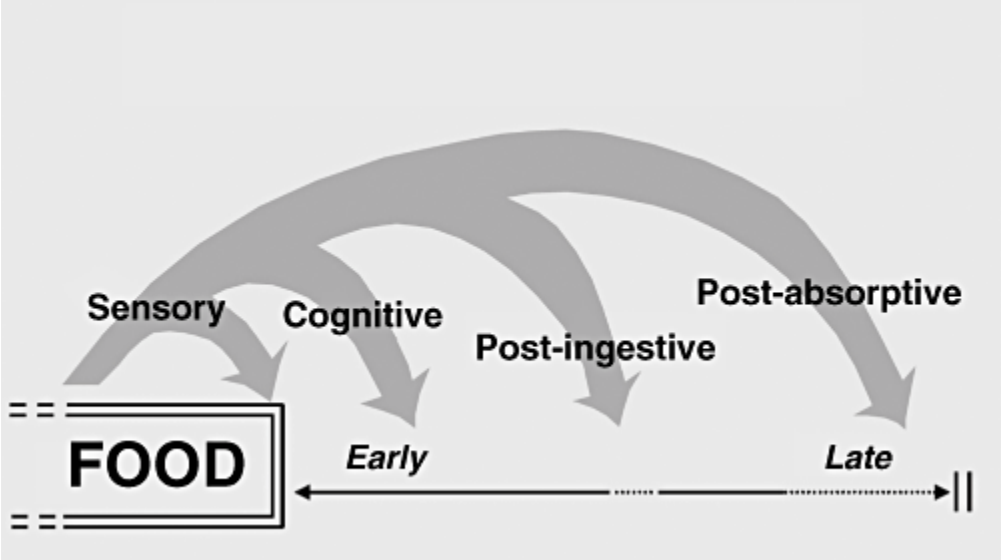


**Figure 1.4 The satiety cascade showing the influences on satiation and satiety over time.**

(adapted from Blundell et al., 1987)

## 1.8 LIPID METABOLISM

### 1.8.1 Lipoproteins

Cholesterol, cholesterol ester and TAG have several important functions within the body but they are insoluble so they must be packaged into biochemical particles called lipoproteins so they can circulate in the plasma. Lipoproteins consist of a lipid rich hydrophobic core surrounded by a hydrophilic membrane made up of phospholipids. The phospholipid membrane also contains apolipoproteins which not only stabilise the particle, but also determine its fate. Lipoproteins have been have been divided into four main classes, determined by their size and density: chylomicrons (CM), very low density lipoproteins (VLDL), LDL and HDL. CM and VLDL are the main transport for TAG, and LDL is the main transport for cholesterol. The various lipoprotein classes differ greatly in both size and lipid content (Eisenberg, 1979). The characteristics of the main lipoproteins can be seen in **Table 1.1**.

**Table 1.1 Characteristics of main lipoprotein classes.**

| Class | Density range (g/mL) | Diameter (nm) | Major apolipoproteins | Composition (% by weight) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Pro. | TAG | Chol. | PL |
| CM | <0.950 | 80-1000 | B48, A1, A4, C, E | 1 | 90 | 5 | 4 |
| VLDL | 0.950-1.006 | 30-80 | B100, C, E | 10 | 65 | 13 | 13 |
| LDL | 1.019-1.063 | 20-25 | B100 | 20 | 10 | 45 | 23 |
| HDL | 1.063-1.210 | 9-13 | A1, A2, C, E | 50 | 2 | 18 | 30 |

**Pro. = protein, TAG = triacylglycerol, Chol. = cholesterol, PL = phospholipid. Notes: 1. There are other fractions and sub-fractions not distinguished here. 2. The proportions shown are approximate only and vary within each major class. 3. Apolipoprotein C refers to the presence of apolipoproteins C1, C2 and C3.**

(adapted from Frayn, 2010)

### 1.8.2 Apolipoproteins

Apolipoproteins are proteins that are found in the phospholipid membrane of the lipoproteins. Apolipoproteins have an essential role in lipoprotein metabolism by supporting the structure of the lipoproteins, acting as cell-surface receptor ligands for lipoproteins, and serving as enzyme cofactors. There are six main classes of apolipoproteins (A, B, C, D, E and H), and various other subclasses within these.

### 1.8.3 Lipoprotein Metabolism

A diagram showing lipoprotein metabolism can be seen in **Figure 1.5**. CM and VLDL are the largest of the lipoproteins and are the primary transport for TAG. When the fat from a meal is digested, the FA are released then packaged into TAG in the intestine. The CM particles, containing apolipoprotein B48, collect these TAG and dietary cholesterol and transport them to muscle cells and adipose tissue. The CM are metabolised by an enzyme called lipoprotein lipase (LPL) and release the FA so they can enter the cells (Rader, 2004). Some of the FA transported by the CM are taken into the liver by the hepatic LDL receptor; these are known as CM remnants. Hepatocytes in the liver re-package the CM remnants with apolipoprotein B100 and sends them back out into circulation as TAG-rich VLDL particles (Packard, 1996). Whilst in the circulation, VLDL becomes hydrolysed by LPL to release the FA. Most of the FA in VLDL are taken up by the tissues and, as with CM, those which are not taken up by tissues become VLDL remnants, also known as intermediate density lipoprotein (IDL). These VLDL remnants, or IDL, are either cleared by the liver or converted into cholesterol-rich LDL (Rader, 2004). LDL particles carry the majority of the total cholesterol through the blood and are the source of cholesterol for the cells that require it (Szablewski, 2011). The LDL particles bind to the hepatic LDL receptor and is taken up by the liver. Excessive energy consumption leads to an increase of these cholesterol-rich LDL particles, which in turn, leads to an increased risk of developing diseases such as metabolic syndrome, CVD, insulin-resistance, and

T2DM (Feskens et al., 2013). HDL are the most dense lipoproteins and are involved in the transport of cholesterol from the peripheral cells back to the liver for excretion and catabolism. This is known as reverse cholesterol transport and occurs in three stages (Eisenberg, 1984). The first extracellular stage is where small apolipoprotein A1-containing particles remove unesterified cholesterol from cell membranes in interstitial fluid. Binding of the particles to cell surface receptors may facilitate this stage. The second intravascular stage occurs when the HDL particles enter into the blood through the peripheral lymph. Lecithin-cholesterol acyltransferase (LCAT) esterifies the cholesterol in the HDL particles, and some of the resulting cholesterol esters are transferred, via a transfer protein, to CM and VLDL, while the other cholesterol esters are incorporated into the core of the HDL particle. The third intrahepatic stage is where the cholesterol esters are removed from the circulation by receptor-mediated uptake of HDL, and by direct transfer of cholesterol esters from HDL into hepatocytes. The hepatocytes then eliminate the cholesterol by secretion into the bile ducts (Miller, 1990). HDL has been recognised as being protective against the development of CVD and strokes, and has been shown to remove cholesterol from developing atherosclerotic plaques (Lund-Katz and Phillips, 2010; Salter, 2011).

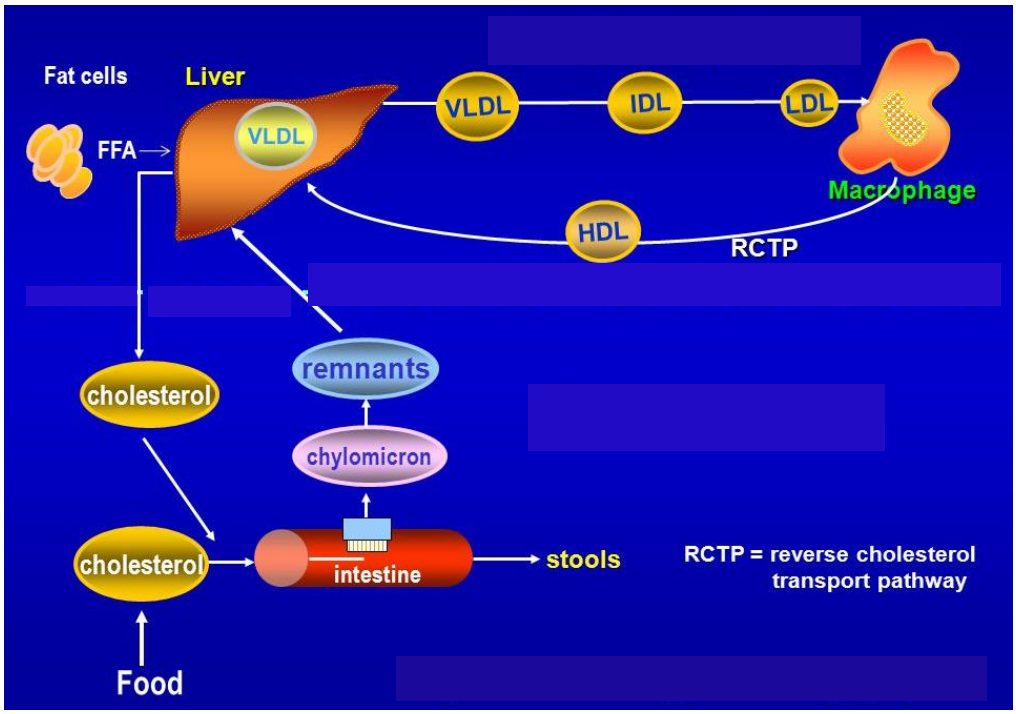


**Figure 1.5 Lipoprotein metabolism pathway.**

(Sheperd, 2001)

### 1.8.4 Neutral Lipid Exchange

Neutral lipid exchange is the process by which cholesteryl esters are transferred, by a hetero-exchange, from cholesterol-rich LDL and HDL in exchange for TAG from CM and VLDL (Roche and Gibney, 2000). This process is initiated by cholesteryl ester transfer protein (CETP) (Karpe, 2016). CETP is the only protein able to transfer neutral lipids, cholesteryl esters and TAG, in human blood. This CETP-mediated lipid exchange is significantly increased during the postprandial state with the concentration of CM and VLDL determining the degree of hetero-exchange that occurs between TRL and cholesterol-rich lipoproteins (LDL and HDL) (Karpe, 2016). In particular, the movement of cholesterol from HDL to chylomicrons and VLDL and its ultimate accumulation in their remnants (CM remnants and IDL/LDL) could be viewed as a pro-atherosclerotic mechanism. Indeed, inhibition of CETP has been shown to aid in the prevention of the development of atherosclerosis by reducing plasma LDL and increasing plasma HDL concentrations (Koivuniemi et al., 2012).

## 1.9 POSTPRANDIAL PERIOD

Circulating carbohydrates usually show temporary elevations as glucose following a meal, whereas TAG levels display a distinct increase within an hour of consuming a meal and may remain raised for up to eight hours. The characteristics of the postprandial period can be described as a “traffic jam” of circulating lipoprotein particles provided by the small intestine and the liver (Lairon et al., 2007). It has been recognised that raised TAG-rich lipoproteins (TRL), such as CM and VLDL, are a potential risk factor for a number of lifestyle related diseases such as obesity, T2DM, metabolic syndrome and hypertriglyceridemia (Sethi et al., 1993). This discovery has revealed the limited number of dietary studies evaluating postprandial lipoprotein metabolism. The fasting state, the measurement typically used in epidemiological studies, does not reflect the normal nutritional state for most individuals. TAG levels reflecting a 12 hr fast can only be seen naturally at around 0300 and 0600 hrs (Williams, 1997). Most individuals remain in a postprandial state for the majority of a 24 hr period as they tend to consume fat-containing meals and snacks at regular intervals throughout the day (Lopez-Miranda et al., 2007). The postprandial response can reflect the ability of an individual’s body to process fat-containing meals. It can also be used to represent acute metabolic responses to a meal and specific nutrients. The ability of individuals to control circulating TAG and clear TRLs is an important reflection on their metabolic efficiency (Lairon et al., 2007). Therefore,

there is a need for greater understanding of the postprandial effects of diet on lipid metabolism.

## 1.10 EXPERIMENTAL OBJECTIVES

Preliminary work at the University of Nottingham has identified some of the barriers faced in transitioning to a meat-reduced diet. Most 'non-meat alternatives' have been developed for the vegetarian market and are frequently not well accepted by the general population. Reducing meat consumption by replacing meat with high quality and convenient non-meat alternative ingredients and reduced-meat/non-meat products could have significant health benefits for consumers, a positive impact on the environment, build a sustainable food model and provide products that address consumer concerns over food safety in relation to animal produce.

Therefore, the aims and objectives of this project were;

1. Directly compare the impact of meat and Quorn™-based meals on postprandial glycaemia and lipaemia.
2. To investigate whether, when provided with a range of non/reduced meat alternatives and motivational support, a group of free-living individuals could reduce their red and process meat intake by 50% over a three month period.
3. To determine whether such a reduction would have specific benefits in terms of reducing CVD risk.

This project was broken down into three separate trials with the first trial comparing the postprandial lipid response of consuming a meat-containing breakfast meal compared with a mycoprotein-containing breakfast meal. The second trial went on to compare the postprandial lipid response and satiety effects of a meat-containing mid-day meal compared with a mycoprotein-containing mid-day meal. The third trial aimed to investigate the chronic effects of replacing at least 50% of red and processed meat intake with non-meat replacement products on cardiovascular health.

## 1.11 INTRODUCTION TO THE PROJECT

Initially this project was funded by the University of Nottingham. Marlow Foods funded the second trial, and were partners with the University of Nottingham, New Food Innovation, JEM Innovation Ltd, Naturis and Poortman (London) Ltd in an Innovate UK project which funded the third trial. Whilst I played a major role in study design, advertising, recruitment, blood sampling, anthropometry measurements, lipoprotein separation, lipoprotein and glucose analysis, data collection and statistical analysis, various other people contributed to the project. The study design of all three trials was organised with the help of Professor Andrew Salter and Dr Liz Simpson. Chris Essex and Gill West helped with the macronutrient analysis of the food products used in the project. Cannulation and flow mediated vasodilation measurements were undertaken by Dr Liz Simpson. Serum insulin samples were analysed by Karen Swift following trial one and two. Sally Cordon analysed lipoprotein cholesterol and triacylglycerol and serum insulin following the third trial. The third intervention trial, which was funded by Innovate UK, included several parts, one of which was the development and sensory testing of the novel reduced meat products used in the trial by Michelle Neville. Jane Penny designed a questionnaire which was used to determine the opinions of the general public on consuming such products, and also their willingness to reduce meat consumption. Dr Azlina Razak took over my role of advertising, recruitment, blood sampling, anthropometry measurements, glucose analysis and data collection half way through the third trial so I could focus on writing this

thesis. All statistical analysis presented in this thesis was performed by myself, and diet diary analysis is currently being undertaken by Dr Liz Simpson and Maab Ali.

## 1.12 KEY TECHNIQUES USED IN THE PROJECT

### 1.12.1 Blood Pressure

BP is the pressure exerted by circulating blood upon the walls of blood vessels. BP is expressed in terms of the systolic (maximum) pressure over diastolic (minimum) pressure and is measured in millimetres of mercury (mmHg) (Health Unlocked, 2016; URMC, 2016). The systolic pressure is the force at which your heart pumps blood around your body, and the diastolic pressure is the resistance to the blood flow in the blood vessels (AHA, 2016). Ideal BP is considered to be between 90/60 mmHg and 120/80 mmHg (**Figure 1.6**), however, a person's BP can vary depending on situation, activity and disease states, such as stress, nutrition, drugs and fitness. BP below optimal range, due to a disease state, is referred to as hypotension, and BP that is constantly high is known as hypertension. Both hypotension and hypertension can be either an acute or chronic issue. Chronic hypertension is a risk factor for CVD, including strokes and heart disease, and it often goes undetected due to the lack of symptoms (URMC, 2016).

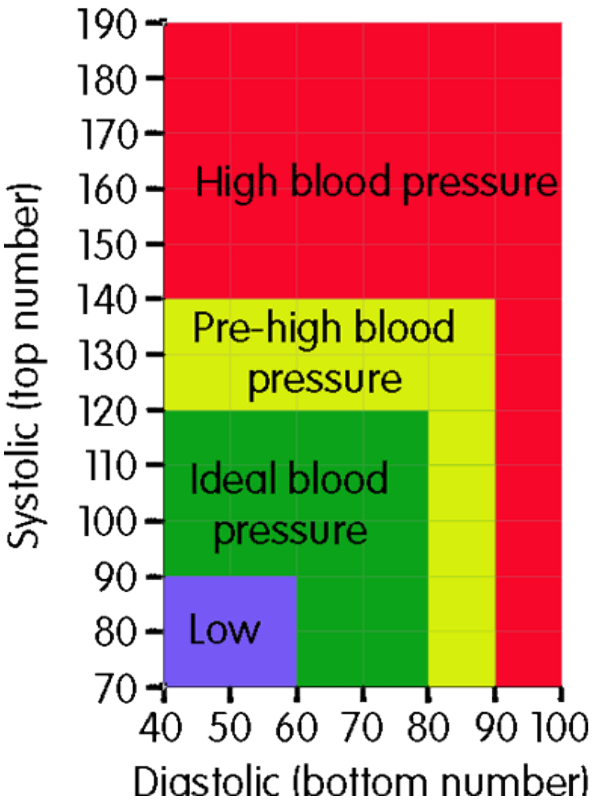


**Figure 1.6 Blood pressure chart for adults.**

(Blood Pressure Association, 2008)

### 1.12.2 Blood Sampling and Preparation of Plasma

The standard method for obtaining arterial blood samples is via a catheter inserted into an artery. However arterial blood sampling has been associated with small but significant morbidity rates and therefore it is an inappropriate method to use in volunteer trials (Jensen and Heiling, 1991; Zello et al., 1990). Although venous blood is easily obtainable, the metabolite concentrations of venous blood can vary significantly from the metabolite concentrations in arterial blood. When blood passes through an organ bed, such as the liver or muscle, metabolites are both extracted and added to the blood (Abumrad et al.,

1981). For example, glucose and oxygen may be removed and the end products of metabolism, lactate, $CO_2$ and alanine may be added as the blood passes through the muscle bed (Matthews, 2015). The method of heating the hand, in air temperatures between 50 – 55°C, to obtain 'arterialised' blood has been deemed a suitable substitute for arterial blood sampling when measuring post-prandial blood glucose concentration as it helps to avoid the variability that is introduced by changing insulin stimulated tissue extraction that arises after eating (Jensen and Heiling, 1991; McGuire et al., 1976). The term 'arterialised' is used instead of 'arterial' because the venous blood that has been obtained from a heated hand is not actually arterial blood, but a good representation of it. Getting the skin temperature of the hand well above 37°C within an even heat flow is the main factor to obtaining arterialised blood (Zello et al., 1990). If the flow of air is too fast or too concentrated, such as using a hair dryer or heated pad, it can result in local areas of heat injury. The use of heating pads causes considerable heat transfer, therefore increasing the temperature of the skin and core, along with increasing the blood flow of the forearm, whereas a box that is warmed using almost static air, does not seem to have the same effect (Frayn and Macdonald, 1992; Gallen and Macdonald, 1990).

### 1.12.3 Blood Glucose Analysis via HemoCue Glucose 201+

The HemoCue is a photometric system based on a glucose dehydrogenase method as described by Deshpande et al. (1996). The system is comprised of a photometer in which disposable plastic microcuvettes, containing dry chemicals, are used to hold a 5 µl sample of whole blood. The microcuvette is placed in the cuvette chamber where saponin lyses the erythrocytes, and Mutarotase converts the α-D-Glucose to β-D-Glucose. Glucose dehydrogenase then catalyses the conversion of β-D-Glucose and nicotinamide dinucleotide (NAD) to gluconolactone, and NADH. Methylthiazolyldiphenyl tetrazolium (MTT) is then reduced by diaphorase, in the presence of NADH, which results in the formation of a coloured formazan dye. The dye is measured, by bi-chromatic monitoring, at wavelengths of 660 nm and 840 nm, resulting in the endpoint reaction being reached, and producing a concentration of glucose.

**Basis of glucose measurement**

*Glucose dehydrogenase*

1. β-D-Glucose + NAD ⟶ Gluconolactone + NADH

*Diaphorase*

2. NADH + MTT ⟶ Formazan + NAD

(Deshpande et al., 1996)

### 1.12.4 Blood Glucose Analysis via Yellow Springs Instrument 2300

The YSI 2300 sensor probe technology is based on the principles designed by Clark and Lyons (1962), with the immobilised enzyme membrane being developed by YSI. The probes are fitted with a membrane consisting of three layers, with the middle layer containing immobilised enzyme. A magnified view of the sensor probe's relationship with the enzyme membrane can be seen in **Figure 1.7**.

The membrane covered probe is situated in a sample chamber which is filled with buffer. A blood sample is injected into the sample chamber where some of the substrate diffuses through the membrane, makes contact with the immobilised enzyme, and is rapidly oxidised causing the production of hydrogen peroxide ($H_2O_2$). The $H_2O_2$ is then oxidised at the platinum anode which, in turn, produces electrons (**Figure 1.8**). When the rate of $H_2O_2$ production and the rate at which the $H_2O_2$ leaves the immobilised enzyme layer are constant, a dynamic equilibrium is achieved which is indicated 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.

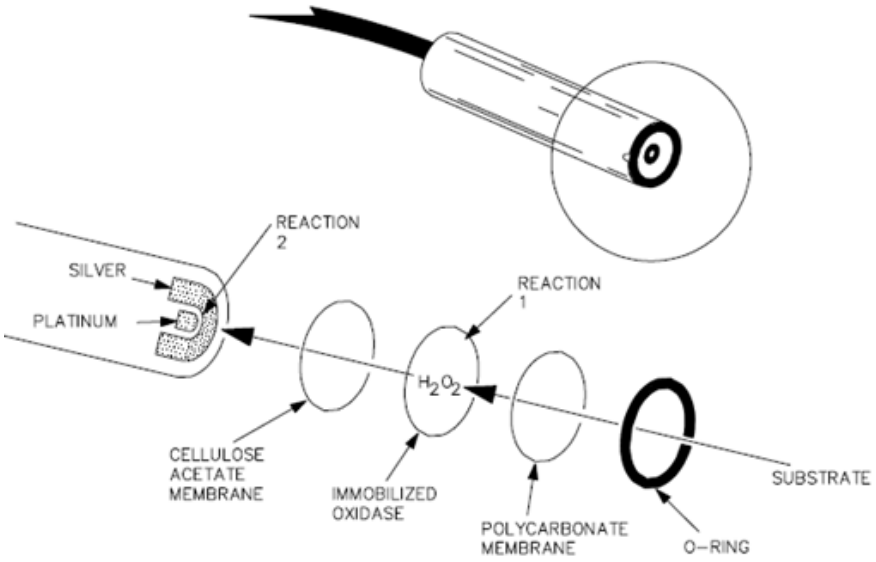


**Figure 1.7 Sensor probe and enzyme membrane relationship.**

(YSI, 2009)

1. β-D-glucose + $O_2$ $\xrightarrow[GOx]{}$ Glucono-δ-lactone + $H_2O_2$

2. $H_2O_2$ $\xrightarrow[Pt\ anode]{}$ $2H^+ + O_2 + 2e^-$

**Figure 1.8 Reaction sequence for determining glucose concentration using YSI 2300.**

**$O_2$ = Oxygen, GOx = Glucose Oxidase, $H_2O_2$ = Hydrogen Peroxide, Pt anode = Platinum anode.**

(YSI, 2009)

### 1.12.5 Serum Insulin Analysis via Radioimmunoassay

The RIA method is a two-step procedure:

Step 1. Insulin forms a soluble complex with a specific immunised guinea pig antibody.

Step 2. An antibody obtained from immunised rabbits is then used to precipitate this soluble complex.

A radioactive insulin tracer is then used to measure the amount of radioactivity in the precipitate. The amount of radioactivity in the precipitate inversely corresponds to the concentration of insulin in the reaction mixture; e.g. as the concentration of unlabelled insulin increases, the amount of radioactive insulin in the precipitate decreases (Morgan, 1977).

### 1.12.6 Satiety Labelled Intensity Magnitude Scale

The SLIM scale, designed by Cardello et al. (2005), has been shown to be an easy to use and reliable method for measuring perceived hunger and fullness. During the development of the SLIM scale, authors recognised the importance of a balanced number of commonly used adjective phrases to represent the varying levels of hunger and fullness. It was also important that there was consistency within the adjectives that were used to describe the varying levels of hunger/fullness. With these factors in mind ten symmetrical phrases, plus one neutral phrase to anchor the scale at the zero point, were chosen to create the scale. A group of panellists assigned a free range number to each phrase to indicate their expressed magnitude of hunger/fullness. In order to create the SLIM scale, a multiplicative constant was used to transform the magnitude estimate to a value between +100 and -100 (**Table 1.2**). The SLIM scale was then constructed as a bi-directional visual analogue scale with zero at the midpoint, +100 at the top and -100 at the bottom (**Figure 1.9**). The varying phrases of hunger/fullness were placed at the appropriate distance along the scale.

**Table 1.2 Point locations on a -100 to +100 scale for phrases used on the SLIM scale.**

| | |
|---|---|
| Greatest imaginable fullness | 100.0 |
| Extremely full | 79.4 |
| Very full | 74.3 |
| Moderately full | 46.7 |
| Slightly full | 31.9 |
| Neither hungry nor full | 0.0 |
| Slightly hungry | -18.6 |
| Moderately hungry | -38.2 |
| Very hungry | -56.2 |
| Extremely hungry | -67.4 |
| Greatest imaginable hunger | -100.0 |

(Cardello et al., 2005)

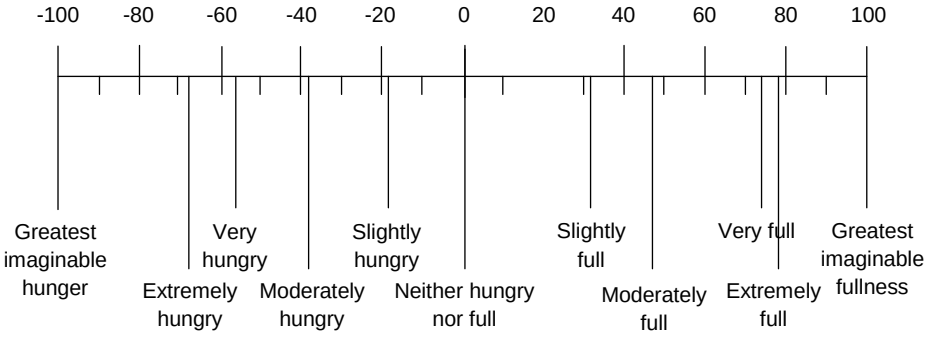


**Figure 1.9 Satiety Labelled Intensity Magnitude scale used to measure volunteers hunger/fullness.**

(Cardello et al., 2005)

### 1.12.7 Lipid Analysis via Pentra 400 Clinical Chemistry Analyser

The clinical chemistry analyser determines total cholesterol and total TAG by performing an enzymatic photometric test employing the same reaction as described in section 2.11. LDL cholesterol is determined using a two reagent format. Reagent one solubilises the non-LDL particles which cause the cholesterol particles to be released and absorbed by cholesterol esterase and cholesterol oxidase, and reagent two solubilises the remaining LDL particles creating a colour reaction. The enzymatic reaction, in the presence of a chromogenic coupler, yields a colour that is relative to the amount of LDL cholesterol in the sample. HDL cholesterol is determined using a two reagent format similar to LDL cholesterol. Reagent one causes the non-HDL particles to be subject to an enzymatic reaction which causes the generated peroxide to be consumed by a peroxidase reaction, and reagent two solubilises the remaining HDL particles. Cholesterol esterase, along with a chromogenic coupler, is used to develop a colour that is proportional to the amount of HDL cholesterol in the sample. This process can be referred to as the Accelerator Selective Detergent method (**Figure 1.10**).

*Accelerator + CO*

1. HDL, LDL, VLDL, CM - - - - - ▶ Non-reactive LDL, VLDL, CM

*DSBmT + Peroxidase*

*HDL Specific Detergent*

2. HDL - - - - - - - - - - - - - - - - - - - - - - ▶ HDL Disrupted

*Cholesterol esterase*

3. HDL Cholesterol - - - - - - - - ▶ $\Delta^4$ Cholestenone + $H_2O_2$

*Cholesterol oxidase*

4. H2O2 + DSBmT + 4-AAP - - - - - - - ▶ Colour Development

*Peroxidase*

**Figure 1.10 Accelerator Selective Detergent method.**

**4-AAP = 4-Aminoantipyrine, CO = Cholesterol Oxidase,**

**DSBmT = N,N-bis(4-sulphobutyl)-m-toluidine-disodium.**

(ABX Pentra, 2009)

### 1.12.8 Insulin Sensitivity

As previously mentioned in section 1.3.1, insulin sensitivity determines how responsive the body is to the effects of insulin. Therefore it is necessary to be able to quantitatively measure changes in insulin sensitivity throughout various intervention studies (Chen et al., 2005). There are several methods available for measuring insulin sensitivity/resistance in individuals. The most well established techniques in clinical practice are the glucose clamp, which is deemed the “gold standard” technique, and minimal model analysis. However, these techniques are not suitable for large-scale trials due to the complexity and expense involved ( Hrebicek et al., 2002). Therefore, it was important to develop an accurate and easy-to-use technique for measuring insulin sensitivity following intervention, which can be implemented on a large scale studies (Bonora et al., 2000). Several alternative techniques for measuring insulin sensitivity/resistance in these populations have been developed. Two of the main techniques are the Homeostasis Model Assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) (Chen et al., 2005).

### 1.12.8.1 Homeostasis model assessment

Matthews et al. (1985) designed the HOMA method for assessing insulin sensitivity, which is based on the interactions of insulin and glucose concentrations on a feedback loop (**Figure 1.11**). This feedback loop demonstrates that blood glucose rises and insulin is released from the beta cells of the pancreas, which results in the uptake of glucose in the cells and a storage of glycogen in the liver. The lowering of blood glucose, due to the release of insulin, causes alpha cells in the pancreas to produce glucagon, resulting in the breakdown of glycogen in the liver, and the re-release of glucose (Hrebicek et al., 2002; Matthews et al.,1985).

The HOMA mathematical model was designed to be simple and inexpensive, and has proven to correlate well with the glucose clamp (Bonora et al., 2000; Radziuk, 2014; Matthews et al., 1985). The HOMA score, which estimates insulin sensitivity, is calculated with the formula:

$$\frac{\text{fasting insulin (mIU/L)} \times \text{fasting glucose (mmol/L)}}{22.5}$$

The resulting score relates to the insulin sensitivity of the individual. The higher the score, the less insulin sensitive the individual is. A score of <1.0 is optimal insulin sensitivity range. A score >1.9 indicates early IR, and a score of >2.9 indicates significant IR (Matthews et al.,1985).

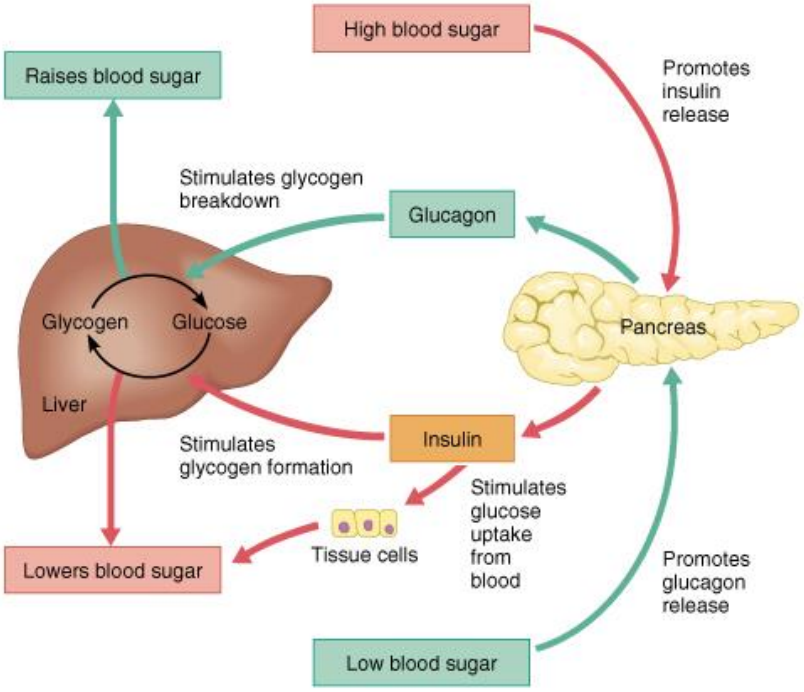


**Figure 1.11 Insulin and glucose feedback loop.**

(Fox, 2008)

#### 1.12.8.2 Quantitative insulin sensitivity check index

QUICKI was designed by Katz et al. (2000) as an index for assessment of insulin sensitivity. QUICKI, which is determined by a mathematical transformation of fasting insulin and glucose levels, demonstrated a better correlation with glucose clamp and minimal model methods than other alternatives, including HOMA (Chen et al., 2005; Katz et al., 2000). QUICKI is obtained from fasting blood samples. The QUICKI score, which estimates insulin sensitivity, is calculated with the formula:

QUICKI = 1 / [log(fasting insulin (μIU/mL))+log(fasting glucose (mg/dL))]

The resulting score relates to the insulin sensitivity of the individual. Scores range from 0.45 in extremely healthy individuals to 0.30 in diabetic individuals. Therefore, the lower the score, the greater the insulin resistance (Katz et al., 2000).

### 1.12.9 Skin-Fold Thickness for Measuring Body Density

Anthropometry has been common practice for measuring body density for many years. Regression equations using anthropometric variables to predict body density was first published by Brozek and Keys (1951). Thereafter, various equations were published by numerous investigators, using various combinations of skinfold thickness and body circumference, to determine body density. Although common practice, there have been shown to be certain limitations when predicting body density from anthropometric equations. These equations have been shown to be population specific, and different equations are required for individuals of varying sex, age, and body fatness (Brozek et al., 1963, Flint et al., 1977; Lesser et al., 1963; Malina, 1969; Novak, 1972; Durnin and Womersley, 1974; Pollock et al., 1976; Pollock et al., 1977). Another limitation with anthropometric equations is that linear regression models have been used to create the equations, while research has shown that the relationship between skinfold fat and body density is, in fact, curvilinear (Allen et al., 1956; Chen et al., 1975; Durnin and Womersley, 1974). Jackson and Pollock (1978) designed a generalised regression equation for men, of various age and body composition, that provided an unbiased estimate of body density (**Figure 1.12**). Jackson et al. (1980) then went on to develop a similar generalised regression equation for women (**Figure 1.13**).

Callipers were used to measure skinfold thickness at three points and body fat percentage was calculated, using the equation described by Jackson and Pollock (1978) for males and Jackson et al. (1980) for females. Each point was measured in triplicate and the average value was used for the equation.

$BD\ (g/ml) = 1.1093800 - 0.0008267*sum_a + 0.0000016*sum_a^2 - 0.0002574*age$

$BF\ (\%) = (495 / BD) - 450$

**Figure 1.12 Body fat percentage equation for males.**

**BD = Body density, $sum_a$ = sum of chest, abdomen and thigh skinfolds, BF = Body fat.**

(Jackson and Pollock, 1978)

$BD\ (g/ml) = 1.0994921 - 0.0009929*sum_b + 0.0000023*sum_b^2 - 0.0001392*age$

$BF\ (\%) = (495 / BD) - 450$

**Figure 1.13 Body fat percentage equation for females.**

**BD = Body density, $sum_b$ = sum of triceps, suprailium and thigh skinfolds, BF = Body fat.**

(Jackson et al., 1980)

### 1.12.10 Bioelectrical Impedance Analysis

BIA is a widely used method for determining body composition, particularly body fat, due to its ease of use, portability, and affordability (Dehghan and Merchant, 2008). BIA can be used to calculate body fat by determining the flow of an electrical current through body tissues. The theory behind BIA is that an electrical current passes through the body at a differential rate depending on the composition of the body. The human body contains intracellular and extracellular water that act as electrical conductors, and cell membranes that behave as electrical condensers. There are also non-conducting materials in the body, such as fat, which provide resistance to the flow of the electric current. Adipose tissue is significantly less conductive than muscle or bone (Dehghan and Merchant, 2008). Early research demonstrated many limitations to BIA such as variability and inaccuracy in measurements. Improvements in technology over the years has made BIA more reliable, however, it is not a “gold standard” method for measuring body composition (Kyle et al., 2004). Some of the things that influence the output of BIA and provide inaccurate readings are dehydration, food consumption prior to analysis, and exercise prior to analysis.

### 1.12.11 Flow Mediated Vasodilation

Blood vessels have the ability to self-regulate tone and adjust blood flow distribution, according to changes in the local environment, due to a response to chemical and physical stimuli in the lumen. In a healthy vascular endothelium, an equilibrium exists between factors that mediate vessel constriction and those that bring about dilation (Marchesi et al., 2000). Vascular endothelial function is thought to be an important indicator of cardiovascular risk with adverse metabolic changes resulting in a vascular environment that favours constriction (Corretti et al., 2002; Raitakari et al., 2004; Lairon et al., 2007; Raitakari and Celermajer, 2009). The development of endothelial dysfunction has been associated with various cardiovascular risk factors such as obesity, T2DM, hypertension, smoking, and hypercholesterolemia (Raitakari et al., 2004; Marchesi et al., 2000). Although a number of methods have been developed for studying endothelial function in vivo, a technique called ‘flow mediated vasodilation’ (FMD), which uses high frequency ultra-sonographic imaging of the brachial artery to assess vasodilatory response to a shear stress stimulus, has been developed over the last 15 – 20 years (Anderson and Mark, 1989; Celermajer et al., 1992; Laurent et al., 1990; Sorensen et al., 1995). The dilated brachial artery is imaged and quantitated as an index of vascular function (Lairon et al., 2007; Raitakari and Celermajer, 2009; Marchesi et al., 2000; Corretti et al., 2002). Endothelium-derived NO is a primary mediator of FMD, with application of shear stress to an artery provoking the release of nitric oxide (NO) and dilation of the vessel. Although the

exact process of detecting shear forces, and subsequent signal transduction, to regulate vascular tone is not fully understood, the endothelial cell membrane contains specialised ion channels, such as potassium channels, that open in response to shear stress, which causes hyperpolarisation of the endothelial cell, and in turn, increases the driving force for calcium entry. Calcium activates the enzyme endothelial nitric oxide synthase (eNOS), and the following production of NO appears to account for FMD (Corretti et al., 2002).

This technique, termed FMD, is positively associated with cardiovascular health (Corretti et al., 2002; Lairon et al., 2007). The benefits of using FMD to determine endothelial function, opposed to other methods, are that FMD is non-invasive and allows repeated measurements, which can be useful in trials that are investigating the effect of an intervention on vascular health (Marchesi et al., 2000). However, despite its wide-spread use, FMD of the brachial artery is technically challenging. It requires an operator that has been trained extensively in 2D and Doppler ultrasonography, ideally with at least 100 supervised measurements completed before independent scanning and reading is attempted (Corretti et al., 2002).

FMD has been used to detect changes in endothelial function in postprandial lipid studies cholesterol reduction studies and weight reduction studies (Anderson et al., 2001; Dupuis et al., 1999; Lairon et al., 2007; Marchesi et al., 2000; Plotnick et al., 1997; Raitakari et al.,

2004; Vogel et al., 1997 and 2000); but as of yet there has been no work, that could be found, on the effects of meat reduction on endothelial function using FMD.

### 1.12.12 World Health Organisation Quality of Life Questionnaires

The World Health Organisation (WHO) Quality of Life (QOL) group define QOL as “individuals’ perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns” (The WHOQOL Group, 1998). This definition suggests that QOL refers to a personal assessment that is rooted in a cultural, social and environmental context. The WHOQOL Group incorporated fifteen international field centres in an attempted to design a QOL questionnaire that could be employed cross-culturally (WHO, 1997). The WHOQOL-100 questionnaire was developed when the fifteen field centres decided on facets of life that were important in the assessment of QOL. Originally including 236 questions relating to QOL, the WHOQOL-100 questionnaire was eventually revised to contain 100 questions separated into four domains, physical health, psychological, social relationships, and environment, and 24 facets (Power et al., 1999). While the WHOQOL-100 questionnaire allows a detailed assessment of an individuals’ QOL, the WHOQOL Group decided that it may be too lengthy for certain uses, such as intervention trials that are incorporating QOL assessment as a secondary measurement. Questionnaires are more likely to be incorporated into trials if they are

brief, convenient and accurate. This observation led to the development of the WHOQOL-BREF questionnaire, an abbreviated version of the WHOQOL-100 questionnaire (WHO, 1997)(O'Carroll et al., 2000). The WHOQOL-BREF questionnaire was still separated into four domains and 24 facets (**Table 1.3**), but it only contained 26 questions. The WHOQOL Group agreed that the questions selected for the WHOQOL-BREF questionnaire would be based on the following criteria.

1. Questions selected to represent a certain domain should express a large amount of variance within that domain.
2. Questions included should express a considerable amount of variance within the general facet relating to overall QOL and general health perceptions.
3. Structural integrity, in terms of confirmatory factor analysis, should be demonstrated in the final assessment.
4. The final assessment should be able to differentiate between the different groups of subjects, for example, ill versus well subjects.

(The WHOQOL Group, 1998)

**Table 1.3 WHOQOL-BREF domains and facets of quality of life.**

| Domain | Facets incorporated within domains |
|---|---|
| Physical health | Pain and discomfort |
| | Sleep and rest |
| | Energy and fatigue |
| | Mobility |
| | Activities of daily living |
| | Dependence on medicinal substances and medical aids |
| | Work capacity |
| Psychological | Positive feelings |
| | Thinking, learning, memory and concentration |
| | Self-esteem |
| | Bodily image and appearance |
| | Negative feelings |
| | Spirituality/religion/personal beliefs |
| Social relationships | Personal relationships |
| | Social support |
| | Sexual activity |
| Environment | Freedom, physical safety and security |
| | Home environment |
| | Financial resources |
| | Health and social care: accessibility and quality |
| | Opportunities for acquiring new information and skills |
| | Participation in and opportunities for recreation/leisure activity |
| | Physical environment (pollution/noise/traffic/climate) |
| | Transport |

(The WHOQOL Group, 1998)

Completion of the questionnaire produces four domain scores and two independently scored questions. The independently scored questions ask about the individual's overall perception of QOL, and overall perception of their health. The four domain scores represent an individual's perception of QOL in each specific domain. Domain scores are scaled in a positive direction, for example, higher scores indicate a higher QOL. The mean score of the questions within each domain is used to calculate the domain score. Mean scores are then multiplied by four in order to make domain scores comparable with the scores used in the WHOQOL-100 questionnaire (**Table 1.4**). Conversion tables (**Appendix A**) are then used to convert raw scores to transformed scores. The first transformation converts scores to range between four and twenty, comparable with the WHOQOL-100, while the second transformation converts the domain scores to a 0 – 100 scale (WHO, 1996).

**Table 1.4 Equations for computing domain scores from the WHOQOL-BREF.**

| | Equations for computing domain scores | Raw score | Transformed score | |
|---|---|---|---|---|
| | | | 4-20 | 0-100 |
| Domain 1 | (6-Q3) + (6-Q4) + Q10 + Q15 + Q16 + Q17 + Q18<br>□ + □ + □ + □ + □ + □ + □ | = | | |
| Domain 2 | Q5 + Q6 + Q7 + Q11 + Q19 + (6-Q26)<br>□ + □ + □ + □ + □ + □ | = | | |
| Domain 3 | Q20 + Q21 + Q22<br>□ + □ + □ | = | | |
| Domain 4 | Q8 + Q9 + Q12 + Q13 + Q14 + Q23 + Q24 + Q25<br>□ + □ + □ + □ + □ + □ + □ + □ | = | | |

(WHO, 1996)

# Chapter 2 - IMPACT OF REPLACING MEAT WITH MYCOPROTEIN AS A BREAKFAST MEAL

## 2.1 INTRODUCTION

As mentioned in section 1.8, it has been recognised that raised TRL, including CM and VLDL, are potential risk factors for a number of lifestyle related diseases (Sethi et al., 1993). The postprandial response reflects the ability of a person's body to process fat-containing meals. Most individuals consume various meals and snacks throughout the day, which result in the majority of a 24 hr period being spent in a postprandial state (Lopez-Miranda et al., 2007). It is, therefore, important to understand the postprandial effects of diet on lipid metabolism.

Previous work from the University of Nottingham investigated the effects of meat reduction on lipid profiles. Volunteers were instructed to reduce their meat intake by 50% over a four week period, by using meat replacement products that were provided for them. Results showed a reduction in total TAG and cholesterol concentrations (Holloway, 2014). This reduction in TAG and cholesterol concentrations may be a result of reduced SFA intake. However, since the majority of the meat replacement products used throughout the trial were Quorn™, these results could possibly be due to this unique protein source.

Previous studies by Turnbull et al. (1990, 1992 and 1995) have demonstrated beneficial effects of replacing meat with Quorn™ products on plasma lipids, insulinemia, and glycaemia. It has been suggested that one of the reasons for these effects may be due to the fibre content of the product. The fibre in mycoprotein consists of polymeric N-acetyl glucosamine (chitin) and beta-1,3 and 1,6 glucan. Chitin accounts for 35% of the fibre and insoluble B-glucan accounts for the other 65% (Turnbull and Ward, 1995). As the majority of fibre in mycoprotein is insoluble and not viscous, the reduced glucose and insulin response demonstrated in Turnbull's study would not normally be expected. Fibre solubility determines the viscosity of a fibre containing meal. There have been many studies showing that postprandial glycaemia and insulinemia is reduced by viscous polysaccharides (Jenkins et al, 1978; Bourdon et al, 1999; Würsch and Pi-Sunyer, 1997; Braaten et al., 1991; Tappy et al., 1996). Although the fibre in mycoprotein is not viscous, it is possible that chitin may produce the viscous polysaccharide chitosan by undergoing alkaline deacetylation somewhere in the gastrointestinal tract (Turnbull and Ward, 1995). However, this has not been shown in vivo. If the fibre in mycoprotein is being converted into a viscous fibre, this may be the reason for the glycaemic and insulinemic response. Therefore, it has been suggested that Quorn™ may be significant in long-term control of diabetes as it can result in a decrease in fasting glycaemia and also lower insulin requirements (Turnbull and Ward, 1995). Studies investigating the lipid response of replacing meat with Quorn™ have

been chronic interventions. To date, there has been no research to investigate the acute effects of mycoprotein on postprandial lipids when compared to meat.

## 2.2 HYPOTHESIS AND OBJECTIVES

Hypothesis: A mycoprotein-enriched (Quorn™) breakfast meal will reduce postprandial lipaemia when compared to an equivalent meat-containing meal.

Main objective:

To determine the relative effects of the meat versus Quorn™ meals on postprandial changes in CM and VLDL lipid concentrations.

Secondary objective:

To determine the relative effects of the meat and Quorn™ meals on postprandial changes in blood glucose and serum insulin concentrations.

## 2.3 STUDY DESIGN

The study design can be seen in **Figure 2.1**. In a randomised cross-over design, ten volunteers were asked to consume two cooked breakfast meals of similar macronutrient content, within a three week period, with at least seven days between meals. One meal contained Quorn™ 'bacon' and 'sausage' products and the other meal contained regular bacon and pork sausage. The meals were freshly prepared on site, and were closely balanced for protein, carbohydrate, SFA, MUFA, PUFA and fibre content. Volunteers were instructed to refrain from exercise and alcohol consumption for 24 hrs before each assessment, and were required to fast from 2200 hrs the night before their assessment. On the day of the trial, arterialised venous blood samples were taken at baseline and then every 30 min for six hours following the meal. Blood samples were used to analyse total plasma, CM and VLDL TAG and cholesterol, serum insulin and blood glucose. Randomisation of the meals was achieved by drawing pieces of paper out of a cup. One had meat and the other Quorn™ written on it, the one drawn first represented the volunteers' first meal.

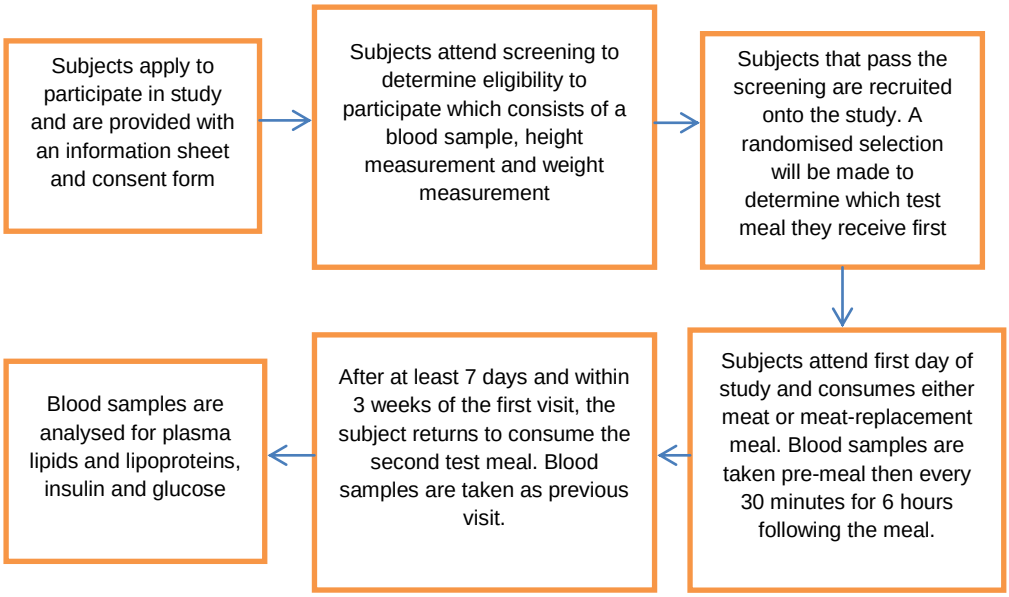


**Figure 2.1 Study design flow diagram.**

## 2.4 MACRONUTRIENT CONTENT OF MEALS

The fat, protein and carbohydrate content of the two meals were matched as closely as possible (**Table 2.1 and Table 2.2**), using information that was provided on the food packets. The percentage macronutrients for the consumed meat meal were 21% protein, 32% carbohydrate and 47% fat. The percentage macronutrients for the consumed Quorn™ meal were 21% protein, 31% carbohydrate and 48% fat.

## 2.5 ETHICAL APPROVAL

Ethical approval was obtained from the University of Nottingham Faculty of Medicine and Health Sciences, Medical School Research Ethics Committee. Ethics Reference No: E14022013 SBS Nutrition (**Appendix B**)

## 2.6 RECRUITMENT

Information dissemination using posters (**Appendix C**) was used to attract volunteers. Recruitment criteria included omnivorous male and female students of the University of Nottingham, aged 18 – 35 years old, not taking lipid lowering drugs, not diagnosed with any chronic diseases, such as diabetes mellitus, and with a BMI less than 26 $kg/m^2$. Interested volunteers were provided an information sheet (**Appendix D**) which described the study in detail. After reading the information sheet, the volunteers who still wished to participate were invited to attend a screening visit.

**Table 2.1 Nutrition content of breakfast meal containing meat products.**

| | Energy (kcal) | Protein (g) | Carbohydrate (g) | Sugars (g) | Fat (g) | Saturates (g) | MUFA (g) | PUFA (g) | Fibre (g) | Sodium (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Tesco Butchers Choice ½ Fat Pork Sausage x1.5 (85.13g uncooked) | 144.71 | 13.36 | 6.90 | 3.75 | 6.47 | 2.30 | 2.72 | 1.11 | 2.04 | 0.43 |
| Tesco Smoked Back Bacon x1 (37.5g uncooked) | 91.88 | 5.96 | 0.00 | 0.00 | 7.43 | 2.48 | 2.96 | 1.13 | 0.00 | 0.45 |
| Tesco Delicious Hash Browns x3 (120g oven baked) | 240.00 | 2.90 | 29.00 | 0.40 | 11.20 | 1.30 | 3.00 | 6.80 | 3.80 | 0.40 |
| Tesco Baked Beans (140g) | 113.40 | 6.72 | 21.14 | 8.12 | 0.84 | 0.14 | 0.14 | 0.42 | 6.58 | 0.77 |
| Mushrooms (100g uncooked) | 16.00 | 1.80 | 0.40 | 0.20 | 0.50 | 0.10 | 0.00 | 0.30 | 1.10 | 0.00 |
| Eggs x2 (100g in shell) | 151.00 | 12.50 | 0.00 | 0.00 | 11.20 | 3.20 | 4.40 | 1.70 | 0.00 | 0.10 |
| Tesco Ds Gluten Free Herb and Onion Crackers x1 (6g) | 25.00 | 0.18 | 3.60 | 0.10 | 0.70 | 0.30 | n/a | n/a | 1.02 | n/a |
| Tesco Pure Vegetable Oil 2tsp (10g) | 90.00 | 0.00 | 0.00 | 0.00 | 10.00 | 0.70 | 6.10 | 3.10 | 0.00 | 0.00 |
| **Total** | **781.99** | **43.43** | **61.04** | **12.57** | **38.33** | **9.81** | **13.23** | **11.45** | **14.54** | **2.15** |

n/a = values were not available

**Table 2.2 Nutrition content of breakfast meal containing Quorn products.**

| | Energy (kcal) | Protein (g) | Carbohydrate (g) | Sugars (g) | Fat (g) | Saturates (g) | MUFA (g) | PUFA (g) | Fibre (g) | Sodium (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Quorn Sausages x2 (85.2g uncooked) | 140.58 | 10.74 | 9.88 | 0.26 | 5.79 | 0.51 | 3.66 | 1.62 | 2.98 | 0.34 |
| Quorn Bacon x1 (20.5g uncooked) | 44.90 | 2.48 | 0.62 | 0.43 | 3.30 | 0.37 | 2.03 | 0.90 | 1.44 | 0.18 |
| Tesco Delicious Hash Browns x3 (120g oven baked) | 240.00 | 2.90 | 29.00 | 0.40 | 11.20 | 1.30 | 3.00 | 6.80 | 3.80 | 0.40 |
| Baked Beans Reduced Sugar (120g) | 88.80 | 6.48 | 15.36 | 3.72 | 0.72 | 0.12 | 0.12 | 0.36 | 6.12 | 0.66 |
| Mushrooms (60g uncooked) | 9.60 | 1.08 | 0.24 | 0.12 | 0.30 | 0.06 | 0.00 | 0.18 | 0.66 | 0.00 |
| Eggs x2 (100g in shell) | 151.00 | 12.50 | 0.00 | 0.00 | 11.20 | 3.20 | 4.40 | 1.70 | 0.00 | 0.10 |
| Tesco Whole Milk (120ml) | 76.80 | 3.96 | 5.64 | 5.64 | 4.32 | 2.88 | n/a | n/a | 0.00 | 0.00 |
| Tesco Pure Vegetable Oil 2tsp (10g) | 90.00 | 0.00 | 0.00 | 0.00 | 10.00 | 0.70 | 6.10 | 3.10 | 0.00 | 0.00 |
| Tesco Table Salt (0.71g) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.28 |
| **Total** | **751.68** | **40.14** | **60.74** | **10.57** | **36.83** | **8.44** | **13.21** | **11.56** | **15.00** | **1.96** |

n/a = values were not available

## 2.7 SCREENING VISIT

After being provided with an information sheet describing the study in detail, and a screening questionnaire (**Appendix E**), to determine the persons current health status, volunteers were required to fast overnight prior to a screening session in which verbal and written consent was obtained (**Appendix F**). Following consent, the volunteers weight, height, body mass index and blood pressure (in triplicate) was measured. An 8 ml blood sample was taken via venepuncture of the antecubital vein to assess full blood count, urea and electrolytes. If the volunteer fulfilled all inclusion criteria, they were invited to take part in the study.

## 2.8 BLOOD PRESSURE MEASUREMENTS

BP was measured, in triplicate, at the screening using a Dinamap Pro 1000 (GE Medical, Milwaukee, USA) automated oscillometry machine to ensure the volunteer was healthy and could participate in the trial.

## 2.9 BLOOD SAMPLING AND PREPARATION OF PLASMA

Fasted volunteers arrived at QMC, Nottingham at approximately 0800 hrs and were instructed to lay semi supine on a hospital bed. Their hand was placed in a 55°C hot box at least ten minutes prior to cannulation and blood sampling so the hand could reach optimum temperature for producing arterialised blood. After cannulation the volunteer rested for 15 min to allow any changes in blood variables, which may have occurred as a result of the cannulation, to return to baseline levels. Two consecutive 10 ml baseline blood samples were taken before the volunteers were fed breakfast at approximately 0830 hrs. Following the meal, 10 ml blood samples were taken every 30 min for six hours. Following blood sampling, a drop of blood was immediately placed in a HemoCue Glucose 201+ analyser (Radiometer, West Sussex, UK) for bedside analysis of glucose using the glucose hydrogenase method (section 1.12.3). Approximately 1 ml blood was transferred into SST™II serum separator vacutainer tubes (BD, Oxford, UK), and left to clot at room temperature for 15 min. After this time they were spun in a chilled centrifuge (Eppendorf UK, Stevenage) at 4400 rpm for 10 min at 4°C. The top layer of serum was transferred into 1 ml cryotubes and stored at -20°C until ready for insulin analysis. The remaining blood was transferred to ETDA vacutainer tubes, spun in a centrifuge at 4400 rpm for 10 min at 4°C and the top plasma layer was refrigerated overnight in LP4 tubes until lipoprotein separation via ultracentrifugation (section 2.10). The plasma, CM and VLDL fractions were frozen at -20°C until analysis of TAG and cholesterol

concentrations at the end of the trial. Insulin analysis of serum samples was performed using a human insulin specific radio immunoassay (Millipore UK Ltd, Watford, UK).

## 2.10 LIPOPROTEIN SEPARATION VIA ULTRACENTRIFUGATION

Lipoproteins were separated by preparative ultracentrifugation by established techniques based on those originally described by Havel et al (1955). CM and VLDL were obtained from plasma and separated within 24 hrs of collection by sequential non-equilibrium density-gradient ultracentrifugation. Up to 3 ml plasma was pipetted into labelled Beckman quick-seal ultracentrifuge tubes (Beckman Coulter, Inc., High Wycombe, UK) using a 5 ml syringe and 21GA needle. A separate syringe was used for each sample. The ultracentrifuge tubes were topped up with 1.006 g/ml potassium bromide (KBr) solution and sealed using a Beckman handheld quick-seal cordless tube topper (Beckman Coulter, Inc., High Wycombe, UK). The tubes were spun in a Beckman Optima ultracentrifuge XL-70 under vacuum at 12°C using rotor 50.4 for 20 min at 12,000 rpm with full acceleration and no break. Following ultracentrifugation each tube was cut approximately 1.5 cm from the top using a Kontron tube slicer (Kontron, Watford, UK). The top CM layer was transferred to 2 ml volumetric flasks which were then topped up to 2 ml with 1.006 g/ml KBr solution. The CM samples were then transferred to labelled 2 ml screw top tubes and stored at -20°C until required for cholesterol and TAG analysis. The top of the tube slicer blade was dried with tissue paper then drawn back to expose the remaining lipoprotein fractions. The lower layer was then transferred to fresh ultracentrifuge tubes using a syringe and needle then topped up with 1.006 g/ml KBr solution. The tubes were sealed as before then spun in the ultracentrifuge under vacuum at 12°C using rotor 50.4 for 16

hrs at 39,000 rpm with full acceleration and no break. Following ultracentrifugation the tubes were cut and the top VLDL layer was separated as described above, transferred to screw top tubes then stored at -20°C until further analysis. The lower layer was transferred to labelled LP4 tubes then stored at -20°C until required for further analysis.

## 2.11 TRIACYLGLYCEROL AND CHOLESTEROL ASSAYS

Following ultracentrifugation, quantitative determination of TAG and cholesterol was carried out on the plasma, CM and VLDL fractions that were obtained from each volunteer by the techniques described by McGowan et al. (1983) and Allain et al. (1974) using Infinity™ TAG and Cholesterol Liquid Stable Reagent kits (Thermo Fisher Scientific, Loughborough, UK).

Suitable volumes of blanks (distilled water), standards and samples (plasma, CM and VLDL) were aliquoted, in duplicate, to the appropriate wells of a 96-well microassay plate. 200 µl of Infinity™ triacylclyceride or cholesterol reagent was then added to all wells containing blanks, standards and samples using a multichannel pipette. The plates were incubated at 37°C for 15 min then read on a Bio-Rad 680XR microplate reader at 550 nm with a background wavelength of 650 nm.

**Basis of a TAG assay**

1. Triacylglycerides $+H_2O$ —*Lipase*→ Glycerol + FFA

2. Glycerol +ATP —*GK*→ Glycerol-3-phosphate + ADP

3. Glycerol-3-phosphate + $O_2$ —*GPO*→ DHAP + $2H_2O_2$

4. $H_2O_2$ + 4-AAP + 3,5 DCHBS —*Peroxidase*→ Quinoneimine Dye + $2H_2O$

(Mackness and Durrington, 1992)

**Basis of a cholesterol assay**

5. Cholesterol Esters —*CE*→ Cholesterol + FFA

6. Cholesterol + $O_2$ —*CO*→ Cholest-4-en-3-one + $H_2O_2$

7. $2H_2O_2$ + HBA + 4-AAP —*Peroxidase*→ Quinoneimine Dye + $4H_2O$

(Laker and Game, 1990)

## 2.12 GLUCOSE ANALYSIS

Glucose concentration was measured each time a blood sample was taken. HemoCue Glucose 201+ (Radiometer Ltd, West Sussex, UK) was used for the determination of glucose in whole blood.

## 2.13 INSULIN ANALYSIS

Serum insulin was analysed using insulin radioimmunoassay (RIA), as described by Morgan and Lazarow (1963), using a HI-14k Millipore kit.

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## 2.14 STATISTICAL ANALYSIS

Data were analysed using Statistical Package for Social Sciences (SPSS) version 22. All data were assessed for normal distribution (using Skewness and Kurtosis z score between -1.96 and 1.96), and boxplots were used to assess data for outliers. Data are presented as means ± standard deviation (SD) in text and tables, and means ± standard error of the mean (SEM) in figures. Baseline characteristics between gender were analysed using independent-samples t-test. Repeated measures analysis of variance (ANOVA) was used to measure differences in lipid, glucose and insulin profiles. Following outliers assessment, one volunteer was removed from statistical analysis due to one of their visits producing above normal range in all lipid measurements. Differences were considered significant at $P=<0.05$.

## 2.15 RESULTS

## 2.16 BASELINE CHARACTERISTICS

**Table 2.3** summarises the baseline characteristics of the volunteers measured at the screening visit. There were significant differences seen between the genders that were expected. Males were generally taller and heavier than females (P=0.005 and P=0.004 respectively). However, no differences were seen in BMI between the two sexes. Systolic BP was significantly lower in females, which is also expected in this age group (P=0.009).

**Table 2.3 Baseline characteristics of volunteers.**

| | Males n=4 | Females n=6 | Total n=10 | P value males vs females |
|---|---|---|---|---|
| Age (years) | 27.8 ± 4.6 | 23.2 ± 4.1 | 25.0 ± 4.7 | 0.139 |
| Height (cm) | 179.9 ± 7.7 | 163.3 ± 6.2 | 170.0 ± 10.7 | 0.005 |
| Weight (kg) | 73.3 ± 9.6 | 56.1 ± 3.9 | 63.0 ± 10.9 | 0.004 |
| BMI | 22.6 ± 2.3 | 21.2 ± 1.6 | 21.8 ± 1.9 | 0.299 |
| Systolic BP (mmHg) | 126.7 ± 8.2 | 109.3 ± 7.6 | 116.3 ± 11.6 | 0.009 |
| Diastolic BP (mmHg) | 75.2 ± 5.4 | 71.4 ± 3.1 | 72.9 ± 4.3 | 0.178 |

Data are presented as means ± SD. Data were analysed using independent-samples t-test.

## 2.17 PLASMA LIPID ANALYSIS

TAG and cholesterol concentrations of total plasma, CM and VLDL samples were analysed, via enzymatic assays (section 2.11), to determine the lipaemic response to the meals.

### 2.17.1 Total Plasma Lipid Response

Observation of the data showed there was a clear difference in baseline values for total plasma cholesterol with the meat meal baseline points being higher than the Quorn™ meal (3.87 ± 0.51 and 3.57 ± 0.48 respectively). Therefore, the response was analysed as change from baseline. **Figure 2.2** shows that when change from baseline values were used for analysis of total plasma cholesterol, there is no significant difference between the two meals and no significant change over time. **Figure 2.3** shows the total plasma lipid TAG and cholesterol response to the meals without change from baseline. The plasma TAG changed significantly over time (P=<0.001), reaching a maximal value at approximately 180 min. No significant difference was seen between the two meals. There is no significant change over time with regards to the plasma cholesterol; there is however, as expected following data observation, a significant difference between the meals. The meat meal induced significantly higher (P=0.014) cholesterol levels than the Quorn™ meal.

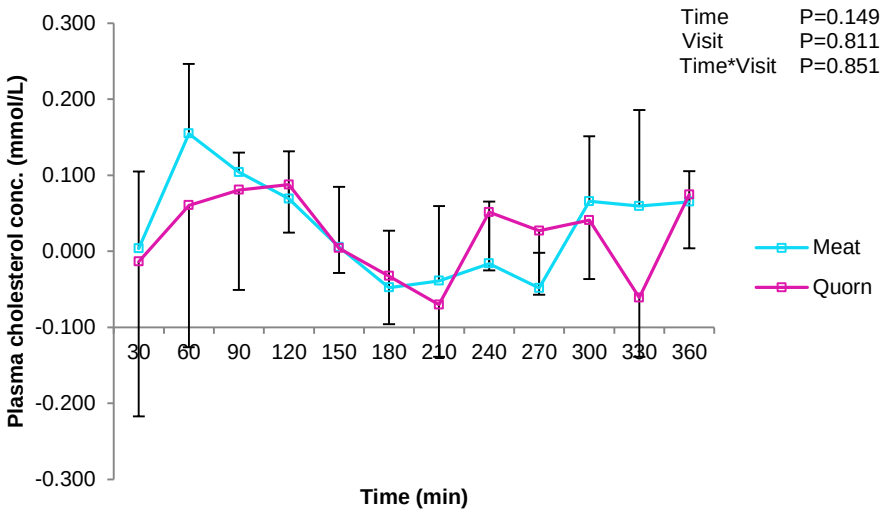


**Figure 2.2 Total plasma cholesterol change from baseline response to the meals over 6 hours. Data are presented as means ± SEM for n = 9 samples per group. Data were analysed using two-way ANOVA with repeated measures.**

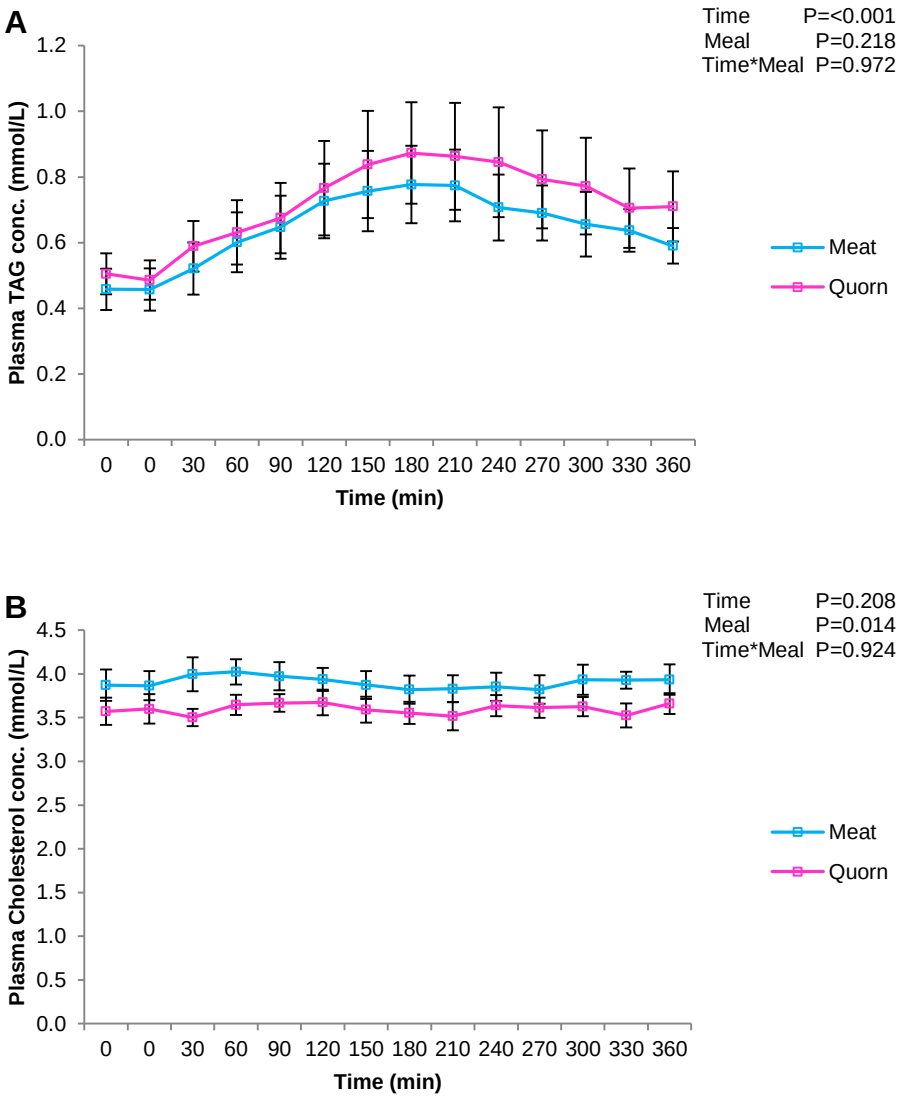


**Figure 2.3 Total plasma TAG and cholesterol response to the meals over 6 hours. Data are presented as means ± SEM for n = 9 samples per group. A = Total plasma TAG response, B = Total plasma cholesterol response. Data were analysed using two-way ANOVA with repeated measures.**

### 2.17.2 Chylomicron and Very Low Density Lipoprotein Triacylglycerol Response

**Figure 2.4** shows the CM and VLDL TAG response to the meals. Both the CM and VLDL TAG changed significantly over time (P=<0.001 for both). As expected, CM TAG increased following the meals peaking at approximately 120 – 150 min. VLDL TAG increased steadily following the meals before starting to decrease back to baseline levels at approximately 210 min. No significant differences were seen between the meals in either CM TAG or VLDL TAG.

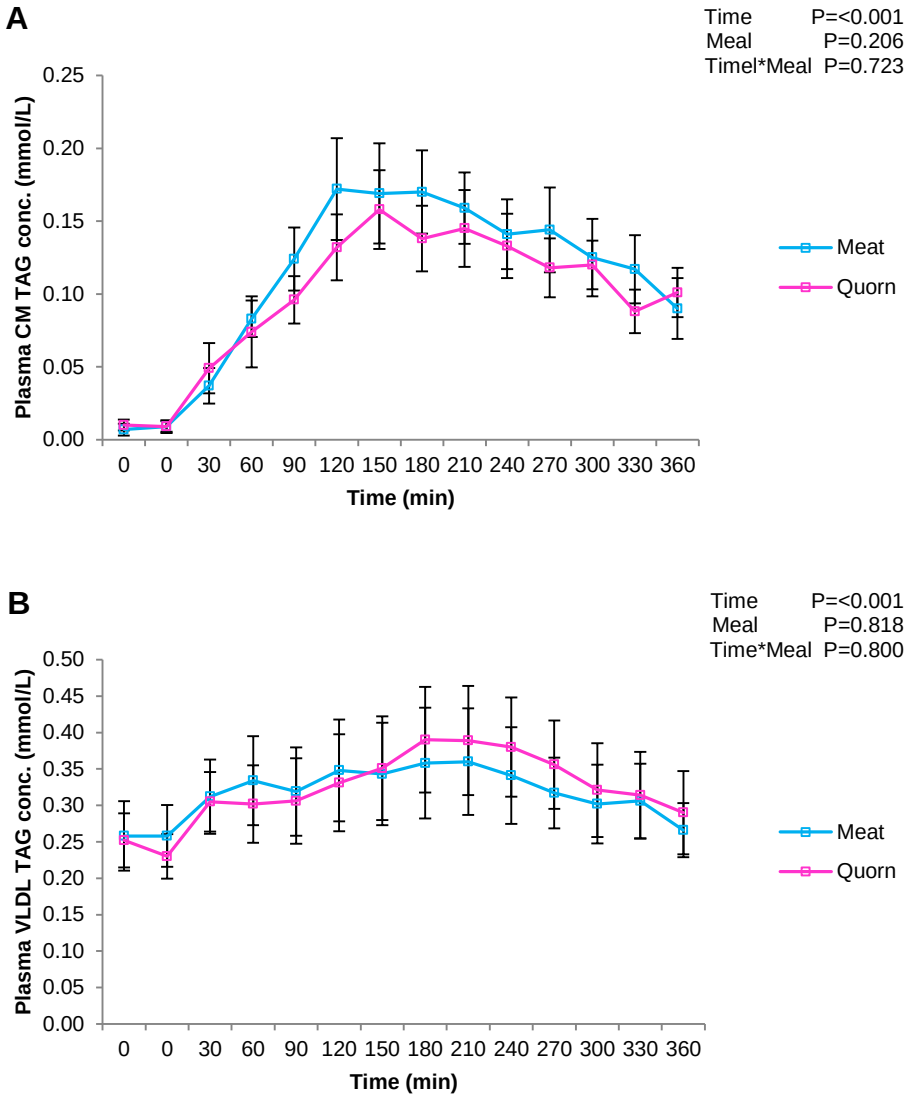


**Figure 2.4 CM and VLDL TAG response to the meals over 6 hours. Data are presented as means ± SEM for n = 9 samples per group. A = CM TAG response, B = VLDL TAG response. Data were analysed using two-way ANOVA with repeated measures.**

### 2.17.3 Chylomicron and Very Low Density Lipoprotein Cholesterol Response

**Figure 2.5** shows the CM and VLDL cholesterol response to the meals. The CM response displays a significant change over time (P=<0.001), with both meals peaking at approximately 120 min. The Quorn™ meal shows a significantly higher concentration of CM cholesterol than the meat meal (P=0.029). VLDL cholesterol also shows a significant change over time (P=<0.001). VLDL cholesterol slowly increases until it reaches a peak at approximately 180 – 240 min when it slowly starts to decrease back down to baseline. There is no significant difference between the meals.

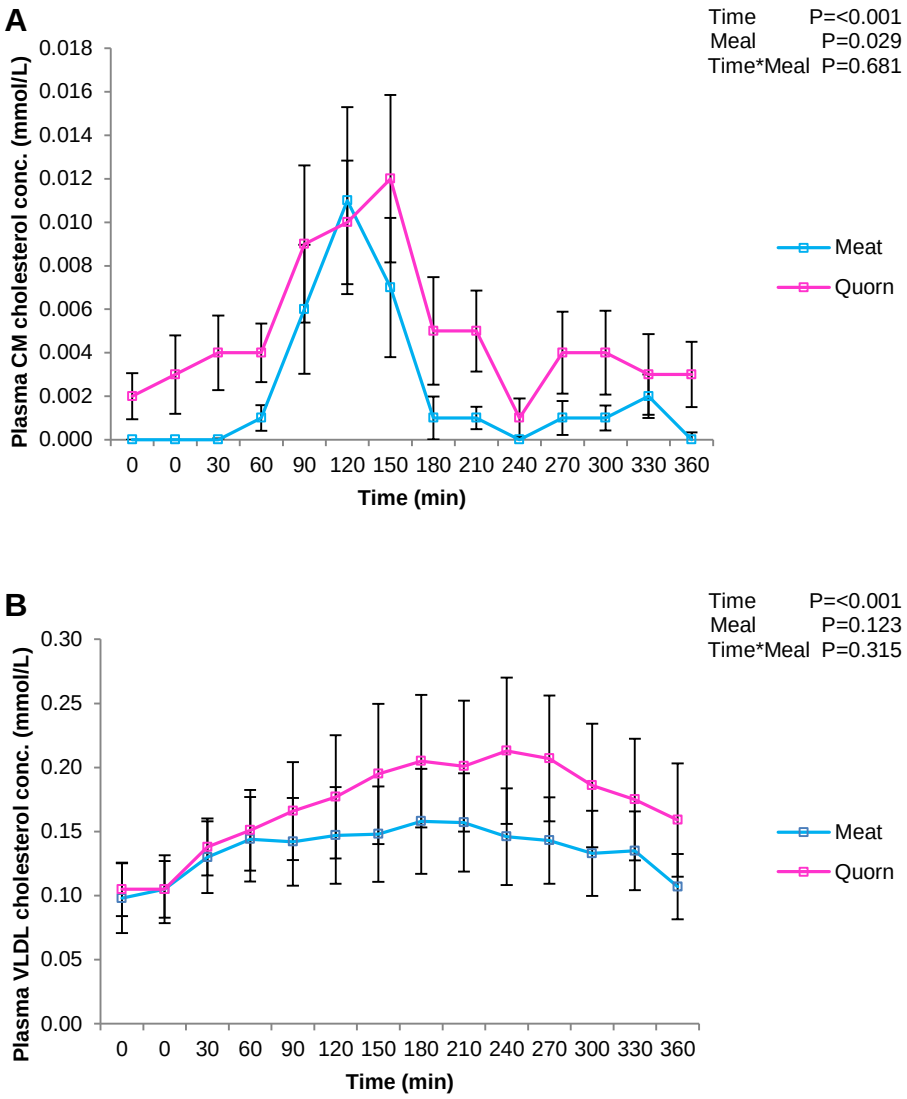


**Figure 2.5 CM and VLDL cholesterol response to the meals over 6 hours. Data are presented as means ± SEM for n = 9 samples per group. A = CM cholesterol response, B = VLDL cholesterol response. Data were analysed using two-way ANOVA with repeated measures.**

## 2.18 BLOOD GLUCOSE

Glucose was analysed immediately following blood collection to compare the blood glucose response of the two meals. The effect the two meals had on blood glucose concentrations can be seen in **Figure 2.6**. Both meals displayed a significant increase (P=<0.001) in glucose concentration immediately after consuming the meal. There was however, no significant difference between the two meals.

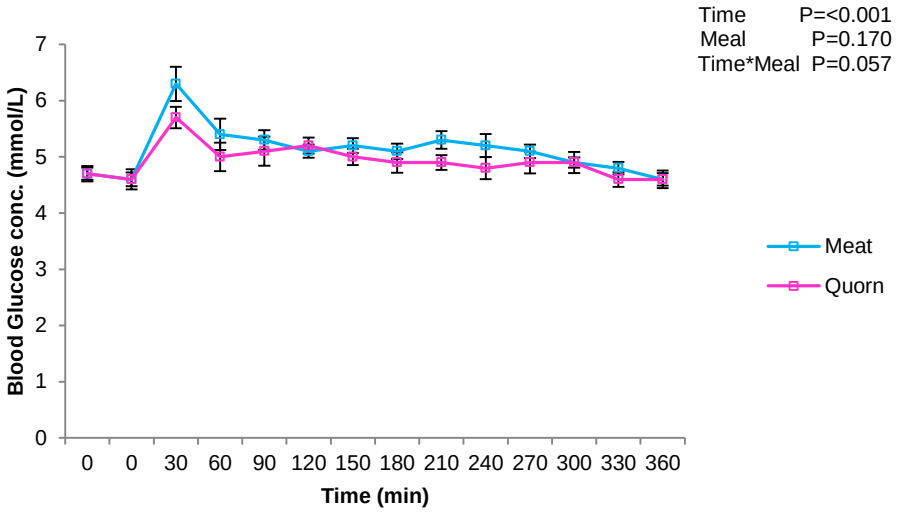


**Figure 2.6 Glucose response to the meals over 6 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures.**

## 2.19 SERUM INSULIN

Serum insulin was analysed to determine any differences in the insulin response of the two meals. **Figure 2.7** shows the effect of the two meals on serum insulin levels. There is a significant increase (P<0.001) in insulin levels at 30 min after both meals. There is no significant difference between the two meals.

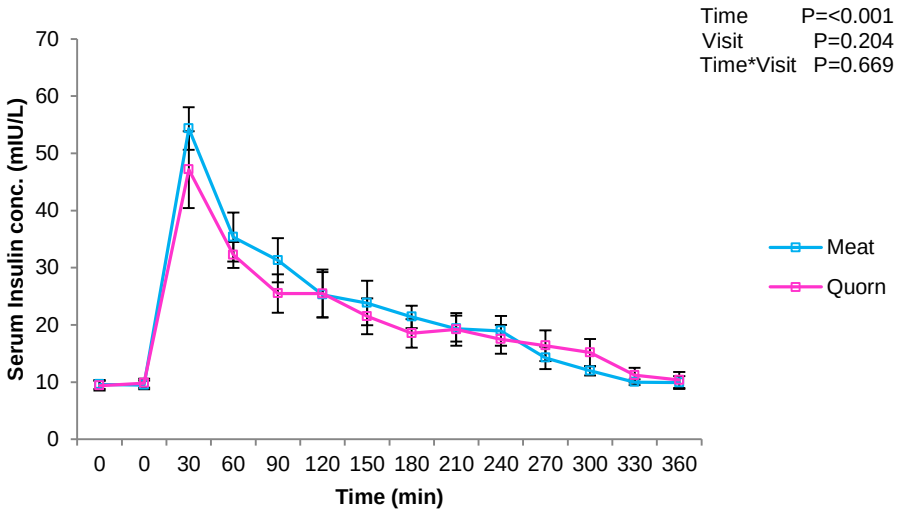


**Figure 2.7 Insulin response to the meals over 6 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures.**

## 2.20 CONCLUSIONS

The plasma lipid results demonstrate the expected postprandial lipaemic response. As mentioned in the introduction (section 1.8.3), after consuming a fatty meal, CM are synthesized within the intestinal epithelial cells, secreted into the lymphatic system and then enter the bloodstream, which produces a peak in CM TAG and cholesterol at approximately 120 min. The CM then associate with lipoprotein lipase in the capillaries of adipose tissue and muscle, TAG is hydrolysed and the resulting FA enter the tissue cells. The CM remnants are then transported into the liver, delivering residual TAG and cholesterol which can then be repackaged into VLDL particles and secreted back into the blood (Frayn, 2010). The results demonstrate this happening by producing a peak in VLDL TAG and cholesterol at approximately 210 – 240 min post-meal. When examining the CM cholesterol response to the meals it is important to recognise that, even at the peak of absorption, concentration represented less than 1% of total plasma cholesterol. As such, even though there was a significant difference between the meals, the physiological significance of this may be negligible. It was also intriguing that starting total plasma cholesterol was apparently higher prior to the meat meal. No obvious explanation for this result is apparent and it may simply represent a statistical anomaly. When data was normalized for fasting plasma cholesterol no differences were seen in postprandial total cholesterol following the two meals.

Both meals produced the expected glycaemic response and associated changes in serum insulin. However, no significant difference was observed between the meal types. This is in contrast to previous work which has shown Quorn™ to reduce postprandial glycaemia and insulinemia (Turnbull and Ward, 1995). These authors suggested that the fibre in Quorn™ was the cause of the reduced glucose and insulin response. In the present trial, diets were designed to contain similar amounts of total fibre, with the meat meal supplemented with plant fibre. The results therefore suggest that the total fibre content may be more important than the specific nature of the fibre in attenuating the glycaemic response to a meal. Another difference between these two experiments is the total mycoprotein content of the meals. In the present experiment this represented approximately 8% of the total weight of the meal compared to 6% in the experiment by Turnbull and Ward (1995). Although the Quorn™ meal in the current trial contained a greater amount of mycoprotein, the Turnbull experiment used freeze-dried blended mycoprotein rather than the cooked Quorn™ products. Perhaps the cooking process interferes with the quality of the mycoprotein. Furthermore, the total fat content of the two meals was relatively low and, as a result, the lipaemic response was relatively modest. However, the aim of the present study was to investigate the effects of replacing the meat component of a typical ‘English’ breakfast with available mycoprotein-containing alternatives, while maintaining the overall nutritional composition of the meal.

The results indicate that in this situation the meat-free, mycoprotein enriched, meal produced similar lipaemic and glycaemic response to the meat -containing meal. It remains to be seen whether further enrichment of a meal with mycoprotein would elicit a different response. However, using commercially available products this would be difficult to achieve in the context of a breakfast meal. Thus, it was decided to perform a further intervention comparing lunch-time meals in which meat is replaced with a significantly higher content of mycoprotein.

# Chapter 3 - IMPACT OF REPLACING MEAT WITH MYCOPROTEIN AS A MID-DAY MEAL

## 3.1 INTRODUCTION

The previous trial investigated the differences in the postprandial response of a breakfast meal containing meat products with a breakfast meal containing Quorn™ products. Although no significant differences were found between the two meals, there were several limitations to the trial. As previously mentioned in chapter 2, the mixed gender cohort was small in size. Another limitation with the breakfast trial was the amount of fat in each of the meals. The 37g in the Quorn™ meal and 38g in the meat meal may not have been enough to observe a distinct postprandial effect. Research into the recommended fat content of meals being used to asses postprandial lipaemia revealed that at least 50g of fat is required to elicit a lipaemic response (Patsch et al 1983 and 1992; Cohen et al 1988; Weintraub et al 1988; Lovegrove et al 1997; Guerci et al 2001; Dallongeville et al 2002). The 8% mycoprotein content of the Quorn™ meal may have also been too low to cause any significant effects. Therefore, to confirm the results seen in the previous trial, a second, more robust postprandial trial was designed to compare the postprandial response of a meat meal versus a Quorn™ meal.

A closer look at the data from the previous trial in chapter 2 revealed that, although not significant, males produced a higher postprandial lipid response to the meals, than females. Evidence has shown that this is

expected in the age group measured in this trial due to premenopausal females having a higher clearance capacity of postprandial lipoproteins due to an increase in lipoprotein lipase activity (Lairon et al., 2007; Lopez-Miranda et al., 2007). Therefore the cohort for this second postprandial trial included only male volunteers in order to minimise inter-individual variation. Quorn™ mince has the highest mycoprotein content (98%) of the entire Quorn™ range, therefore, the mince was incorporated into the second intervention trial as a lunch meal to ensure an adequate amount of mycoprotein was consumed. The fat content of the meals was also increased significantly to ensure a postprandial lipaemic response was observed. In order to elicit normal eating behaviour, the meals were served at lunch time because it would be unrealistic for individuals to consume such a meal at breakfast.

## 3.2 HYPOTHESIS AND OBJECTIVES

Hypothesis: Mycoprotein-enriched (Quorn™) mid-day meal will reduce postprandial lipaemia when compared to equivalent meat-containing meal.

Main objectives:

To determine the relative effects of the meat and Quorn™ meals (consumed as a second meal of the day) on postprandial changes in CM and VLDL lipid concentrations.

Secondary objectives:

To determine the relative effects of the meat and Quorn™ meals on postprandial changes in circulating glucose and insulin concentrations.

To determine the satiating effects of the meat and Quorn™ meals, and compare differences.

### 3.3 STUDY DESIGN

The study design can be seen in **Figure 3.1**. In a randomised cross-over design, 11 male volunteers were asked to consume two spaghetti bolognese meals of similar macronutrient content. One meal contained Quorn™ mince and the other meal contained beef mince. Meals were closely balanced in terms of protein, carbohydrate, SFA, MUFA and PUFA. Both meals included a vanilla milkshake to increase, and enable balance of the fat content of the meals, and thereby induce an appropriate lipaemic response. Volunteers were instructed to refrain from exercise and alcohol consumption for 24 hrs before and fast from 2200 hrs the evening before their assessment. Volunteers were provided a standard breakfast to consume at 0800 hrs the morning of the trial (**Table 3.1 and 3.2**). On the day of the trial, blood samples were taken at baseline and then every 30 min for five hours following the meal. Immediately before the meal, immediately after the meal, and every 60 min following the meal the volunteers were asked to rate their satiety (hunger/fullness) on a Satiety Labelled Intensity Magnitude (SLIM) scale (Cardello et al., 2005). Blood samples were used to analyse total plasma TAG and cholesterol, CM, VLDL, serum insulin and blood glucose as described in the previous trial.


**Figure 3.1 Study design flow diagram.**

### 3.4 MACRONUTRIENT CONTENT OF MEALS

The fat, protein and carbohydrate content of the two meals were matched as closely as possible to determine any differences in postprandial response attributable to the protein source. Initially the macronutrient content was matched via the information provided on the food packets (**Table 3.1 and 3.2**), however, since many of the food companies did not provide information on the MUFA and PUFA content, the spaghetti bolognese meals were analysed for FA composition. This was achieved by both spaghetti bolognese meals being cooked, weighed, blended, frozen then freeze dried (RC 3 SuperModulyo freeze-dryer; Biopharma Process Systems Ltd, Winchester) to remove moisture. The samples were then crushed to a powder using a coffee bean grinder (Braun) and stored in a desiccator until required for analysis. The percentage macronutrients for the consumed meat meal were 18% protein, 34% carbohydrate and 48% fat. The percentage macronutrients for the consumed Quorn™ meal were 17% protein, 37% carbohydrate and 46% fat.

**Table 3.1 Nutrition content of mid-day meal containing meat products.**

| **Spaghetti Bolognese** | **Energy (kcal)** | **Protein (g)** | **Carbohydrate (g)** | **Fat (g)** | **Saturates (g)** | **Fibre (g)** | **Sodium (g)** |
|---|---|---|---|---|---|---|---|
| Tesco Beef Lean Steak Mince (118g uncooked) | 129.80 | 24.66 | 0.00 | 3.19 | 1.53 | 0.00 | n/a |
| Tesco Organic Whole Wheat Spaghetti (85g dried) | 289.00 | 10.80 | 52.87 | 2.13 | 0.17 | 7.65 | n/a |
| Dolmio Original Bolognese Sauce (135g) | 54.00 | 1.73 | 8.75 | 0.97 | 0.11 | 2.03 | 0.68 |
| Tesco Pure Vegetable Oil (14g) | 126.00 | 0.00 | 0.00 | 14.00 | 0.98 | 0.00 | 0.00 |
| Tesco Grated Parmesan Cheese (7g) | 27.30 | 2.31 | 0.00 | 1.99 | 1.46 | 0.00 | 0.05 |
| **Milkshake** | | | | | | | |
| Tesco British Whole Milk (200ml) | 130.00 | 6.40 | 9.40 | 7.20 | 4.60 | 0.00 | 0.30 |
| Tesco Fresh British Double Cream (20ml) | 93.33 | 0.33 | 0.33 | 10.13 | 6.27 | 0.00 | n/a |
| Haagen-Dazs Vanilla Ice Cream (150g) | 336.00 | 5.70 | 27.15 | 22.80 | 13.95 | 0.00 | 0.08 |
| Nielsen-Massey Pure Vanilla Extract (1/2 tsp) | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **Total** | **1185.43** | **51.93** | **98.50** | **62.40** | **29.06** | **9.68** | **1.82** |
| **Breakfast** | | | | | | | |
| Nestle Nido Milk Powder (35g) | 175.54 | 9.05 | 12.82 | 9.91 | n/a | n/a | n/a |
| Tesco British Whole Milk (300ml) | 195.00 | 9.60 | 14.10 | 10.80 | 6.90 | n/a | n/a |
| Kellogg's Cornflakes (70g) | 401.33 | 14.00 | 72.33 | 5.83 | 3.50 | 2.10 | 0.47 |
| **Total** | **771.87** | **32.65** | **99.25** | **26.54** | **10.40** | **2.10** | **0.47** |

n/a = values were not available

**Table 3.2 Nutrition content of mid-day meal containing Quorn™ products.**

| **Spaghetti Bolognese** | **Energy (kcal)** | **Protein (g)** | **Carbohydrate (g)** | **Fat (g)** | **Saturates (g)** | **Fibre (g)** | **Sodium (g)** |
|---|---|---|---|---|---|---|---|
| Quorn Mince (125g uncooked) | 131.25 | 18.13 | 5.63 | 2.50 | 0.63 | 6.88 | 0.13 |
| Tesco Spaghetti (70g dried) | 252.00 | 8.75 | 51.10 | 0.98 | 0.21 | 1.82 | Trace |
| Dolmio Original Bolognese Sauce (125g) | 50.00 | 1.60 | 8.10 | 0.90 | 0.10 | 1.88 | 0.40 |
| Tesco Wholefoods Red Split Lentils (50g cooked) | 52.50 | 3.80 | 8.00 | 0.20 | 0.00 | 0.95 | Trace |
| Tesco Pure Vegetable Oil (15g) | 135.00 | 0.00 | 0.00 | 15.00 | 1.05 | 0.00 | 0.00 |
| Tesco Grated Parmesan Cheese (10g) | 39.00 | 3.30 | 0.00 | 2.84 | 2.08 | 0.00 | 0.07 |
| **Milkshake** | | | | | | | |
| Tesco British Whole Milk (200ml) | 130.00 | 6.40 | 9.40 | 7.20 | 4.60 | 0.00 | 0.30 |
| Tesco Fresh British Double Cream (20ml) | 93.33 | 0.33 | 0.33 | 10.13 | 6.27 | 0.00 | n/a |
| Haagen-Dazs Vanilla Ice Cream (150g) | 336.00 | 5.70 | 27.15 | 22.80 | 13.95 | 0.00 | 0.08 |
| Nielsen-Massey Pure Vanilla Extract (1/2 tsp) | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **Total** | **1219.08** | **48.01** | **109.71** | **62.55** | **28.88** | **11.52** | **1.57** |
| **Breakfast** | | | | | | | |
| Nestle Nido Milk Powder (35g) | 175.54 | 9.05 | 12.82 | 9.91 | n/a | n/a | n/a |
| Tesco British Whole Milk (300ml) | 195.00 | 9.60 | 14.10 | 10.80 | 6.90 | n/a | n/a |
| Kellogg's Cornflakes (70g) | 401.33 | 14.00 | 72.33 | 5.83 | 3.50 | 2.10 | n/a |
| **Total** | **771.87** | **32.65** | **99.25** | **26.54** | **10.40** | **2.10** | **0.47** |

n/a = values were not available

### 3.4.1 Fat Extraction

Gerhardt Soxtherm 2000 (Gerhardt UK Ltd, Brackley) was used to extract the fat from the freeze-dried samples. In duplicates, 1 – 2g of each sample was weighed onto 11 cm Qualitative Q125 filter paper and gently folded into an envelope, placed into an extraction thimble then topped with absorbent cotton wool. Thimbles were placed in a wire holder in a pre-weighed extraction beaker containing three boiling stones and 162 ml petroleum ether. The beakers were then sealed in place on the hotplates in the Soxtherm machine then set to run using the operating conditions in **Table 3.3**. Once removed from the oven the beakers were placed in a desiccator to cool. Once cool, the beakers were re-weighed and total fat of sample was calculated.

**Calculation for measuring total fat (g) per meal**

$$\text{Fat content (\%DM)} = \frac{\text{Weight of extract (g)}}{\text{Dried sample weight (g)}} \times 100$$

$$\text{Fat content (\% fresh)} = \text{Fat (\%DM)} \times \frac{\text{FD-DM (\%)}}{100}$$

$$\text{Total fat of meal (g)} = \text{Fat (\% fresh)} \times \frac{\text{Wet sample weight (g)}}{100}$$

Fat content was measured using freeze-dried material

DM = Dry matter

FD = Freeze-dried

$$\text{FD-DM} = \frac{\text{weight of dried sample (g)}}{\text{weight of wet sample (g)}} \times 100$$

1 ml hexane was added to the beakers with the boiling stones and was gently agitated in order to collect the fat from the stones and beaker wall. The hexane/fat mixture was then transferred, via glass pipettes, into solvent resistant screw top 4 ml tubes ready for fatty acid methyl ester (FAME) synthesis and analysis.

**Table 3.3 Operating conditions for the Gerhardt Soxtherm 2000**

| | |
|---|---|
| Running temperature | 150°C |
| Temperature limit | 200°C |
| Boiling time | 35 minutes |
| Solvent recovery A | 6 x 15 ml |
| Extraction time | 50 minutes |
| Solvent recovery B | 15 minutes |
| Solvent recovery C | Nil |
| Interval | 4 minutes |
| Pulse | 4 seconds |

### 3.4.2 Direct Synthesis of Fatty Acid Methyl Esters

FA were extracted and methylated by a method based on that of O'Fallon et al. (2007). 0.2 ml hexane/fat mixture that was collected via fat extraction (section 3.4.1) was pipetted into methylation tubes. 0.7 ml 10M potassium hydroxide and 5.2 ml methanol was then added to the methylation tubes. The samples were incubated in a 55°C water bath for 90 min with vigorous shaking for five seconds every 20 min. The samples were then cooled in a cold tap water bath for 10 min then vortexed. The hot and cold water baths were then repeated as described. 3 ml hexane was then added to the samples and they were

vortexed for 30 sec each before being centrifuged at 1500 rpm for five minutes at room temperature to separate the FA hexane layer. The top FA hexane layer was transferred via glass pipette to solvent resistant 4 ml tubes. 1 ml of sample was transferred into gas chromatography (GC) vials (PerkinElmer Inc., Buckinghamshire, UK), capped, and stored at -20°C until ready to analyse on the GC machine.

### 3.4.3 Fatty Acid Methyl Ester Analysis by Gas Chromatography

FAME analysis was carried out using a Perkin Elmer Clarus 500 GC (PerkinElmer Inc., Buckinghamshire, UK) fitted with an autosampler and flame ionization detector running on TotalChrom software. Analysis was carried out using a 100m CP-Sil 88 capillary column. See **Table 3.4** for operating conditions. The different FA components were identified by reference to a C4-C24 standard of known composition (Supelco 18919-1AMP). The output report displayed the proportion of each individual FAME as a percentage of the total FAME reported.

**Table 3.4 Operating conditions for the Perkin Elmer Clarus 500 GC for measurement of FAME**

| | |
|---|---|
| Column specification | |
| Type | CP-Sil 88 (FAME) |
| Manufacturer | Varian (Walton on Thames, Surrey) |
| Length | 100 m |
| Internal diameter | 0.25 mm |
| Film thickness | 0.20 µm |
| | |
| Injector temperature | 250°C |
| Detector temperature | 250°C |
| | |
| Air flow rate | 450 ml/min |
| Hydrogen flow rate | 45 ml/min |
| | |
| Carrier gas | Hydrogen |
| Inlet pressure | 28PSIG |
| Split ratio | 10:1 |
| | |
| Injection speed | Normal |
| Injection volume | 1.0 µl |
| Syringe capacity | 5.0 µl |
| Pre-injection solvent washes | 0 |
| Pre-injection sample washes | 2 |
| Sample pumps | 6 |
| Post-injection solvent washes | 2 |
| | |
| Oven program | |
| Initial temperature | 45°C – hold for 4 min |
| Ramp 1 | 13°C/min to 175°C – hold for 27 min |
| Ramp 2 | 4°C/min to 215°C – hold for 35 min |
| Total run time | 86 min |
| Maximum temperature | 255°C |
| Equilibration time | 2 min |

## 3.5 ETHICAL APPROVAL

Ethical approval was obtained from the University of Nottingham Faculty of Medicine and Health Sciences, Medical School Research Ethics Committee. Ethics Reference No: C14082014 SoBS NS (**Appendix G**)

## 3.6 RECRUITMENT

Information dissemination using posters (**Appendix H**) was used to attract volunteers. Recruitment criteria were omnivorous males aged 18 – 50 years old, not taking lipid lowering drugs, not diagnosed with any chronic diseases, such as diabetes mellitus, and with a BMI less than 28 $kg/m^2$. Interested volunteers were provided an information sheet (**Appendix I**) which described the study in detail. If, after reading the information sheet, the volunteers still wished to participate they were invited to attend a screening visit at the Medical School at QMC in Nottingham.

### 3.7 SCREENING VISIT

Screening was performed as in the previous trial (section 2.7). The consent form for the current trial can be found in **Appendix J**. If the volunteer passed the screening they were invited to take part in the study. Each volunteer would consume two meals, one with Quorn™ products and the other with meat products, within a three week period, with at least seven days between meals.

### 3.8 BLOOD SAMPLING AND PREPARATION OF PLASMA

Volunteers arrived at QMC, Nottingham at approximately 1100 hrs. Cannulation was performed as described in section 2.9. Two consecutive 10 ml baseline blood samples were taken before the volunteers were fed their meal at approximately 1140 hrs. Following the meal, 10 ml blood samples were taken every 30 min for five hours. Plasma lipids and lipoproteins, serum insulin, and blood glucose were determined as previously described in chapter 2.

### 3.9 SATIETY MEASUREMENTS USING THE SATIETY LABELLED INTENSITY MAGNITUDE SCALE

The SLIM scale was used to measure hunger and fullness at baseline, immediately after the meal, and at 60 min intervals following the meal. Volunteers were instructed to mark the scale at a point that corresponded to their level of hunger/fullness.

## 3.10 STATISTICAL ANALYSIS

Data were analysed using SPSS version 22. All data were assessed for normal distribution (using Skewness and Kurtosis z score between -1.96 and 1.96), and boxplots were used to assess data for outliers. Unless otherwise stated, data are presented as means ± SD in text and tables, and means ± SEM in figures. Data that were not normally distributed are presented, in text and tables, as medians with 25th and 75th percentiles in parentheses. FA composition of the meals and baseline characteristics between genders were analysed using independent-samples t-test for parametric data, and Mann-Whitney U test for non-parametric data. Repeated measures ANOVA was used to measure differences in satiety, lipid, glucose and insulin profiles. Differences were considered significant at $P=<0.05$.

## 3.11 RESULTS

## 3.12 FATTY ACID COMPOSITION OF MEALS

FA composition of the meals was analysed via GC (section 3.4.3), to ensure the MUFA and PUFA were matched as closely as possible between the two meals, and the results are displayed in **Figure 3.2**. A full list of the FA (% FAME) of each meal can be seen in **Table 3.5**. When comparing total SFA, MUFA and PUFA, there was no significant difference between the meals.

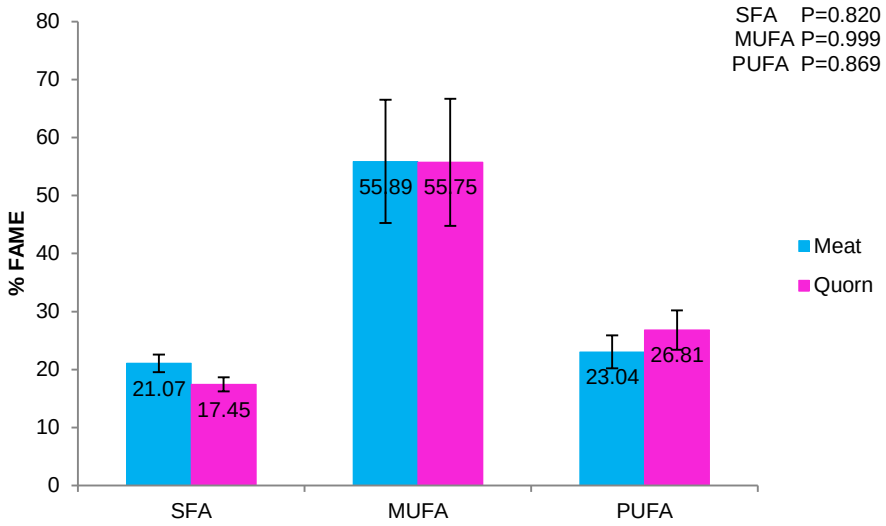


**Figure 3.2 Sum of FA groups (% FAME) from meals.**

**Data are presented as means ± SEM for n = 2 samples per group. Data were analysed using independent-samples t-test. SFA meal comparison P=0.820. MUFA meal comparison P=0.999. PUFA meal comparison P=0.869.**

**SFA = saturated fatty acids: C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0**

**MUFA = mono- unsaturated fatty acids: C14:1+C16:1+C17:1+C18:1t9n-9+C18:1c9n-9**

**PUFA = poly- unsaturated fatty acids: C18:2+C18:3+C20:3n-6+C20:3n-3+C22:6**

**Table 3.5 Complete listing of fatty acids (% FAME) from spaghetti bolognese meals**

| | Meat | Quorn™ | *P* value |
|---|---|---|---|
| C10:0 Capric | 0.28 ± 0.00 | 0.39 ± 0.00 | **0.030** |
| C12:0 Lauric | 0.67 ± 0.01 | 0.75 ± 0.01 | **0.042** |
| C14:0 Myristic | 1.89 ± 0.01 | 1.86 ± 0.01 | 0.140 |
| C14:1 Myristoleic | 0.23 ± 0.00 | 0.12 ± 0.00 | n.c |
| C15:0 Pentadecylic | 0.28 ± 0.00 | 0.24 ± 0.00 | 0.070 |
| C16:0 Palmitic | 12.58 ± 0.02 | 10.26 ± 0.06 | **0.011** |
| C16:1 Palmitoleic | 1.18 ± 0.00 | 0.45 ± 0.01 | **<0.001** |
| C17:0 Margaric | 0.27 ± 0.00 | 0.14 ± 0.00 | **0.025** |
| C17:1 cis-10-Heptadecanoic | 0.18 ± 0.00 | n.d | - |
| C18:0 Stearic | 4.78 ± 0.02 | 3.43 ± 0.03 | **0.001** |
| C18:1*t9 n-9* Elaidic | 0.56 ± 0.00 | 0.20 ± 0.05 | 0.088 |
| C18:1*c9 n-9* Oleic | 53.74 ± 0.06 | 54.98 ± 0.19 | 0.078 |
| C18:2 Linoleic | 15.08 ± 0.02 | 17.80 ± 0.12 | **0.024** |
| C20:0 Arachidic | 0.33 ± 0.01 | 0.39 ± 0.04 | 0.410 |
| C18:3 α-Linolenic | 6.05 ± 0.03 | 7.50 ± 0.05 | **0.006** |
| C20:3*n-6* Dihomo-γ-linolenic | 0.18 ± 0.00 | 0.26 ± 0.00 | **0.042** |
| C20:3*n-3* Eicosatrienoic | 0.26 ± 0.05 | 0.37 ± 0.00 | 0.280 |
| C22:6 Docosahexaenoic | 1.47 ± 0.01 | 0.89 ± 0.38 | 0.372 |
| Sum of SFA[a] | 21.07 ± 1.52 | 17.45 ± 1.22 | 0.820 |
| Sum of MUFA[b] | 55.89 ± 10.64 | 55.75 ± 10.96 | 0.999 |
| Sum of PUFA[c] | 23.04 ± 2.83 | 26.81 ± 3.39 | 0.869 |
| Sum of total fats | 100 | 100 | - |

Data are presented as means ± S.E.M; n = 2 samples per group; n.d = non detectable; n.c = not computable because SEM for both groups is 0. Data were analysed using independent-samples t-test.

[a]Sum of saturated fatty acids:
C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0

[b]Sum of mono- unsaturated fatty acids:
C14:1+C16:1+C17:1+C18:1*t9n-9*+C18:1*c9n-9*

[c]Sum of poly- unsaturated fatty acids:
C18:2+C18:3+C20:3*n-6*+C20:3*n-3*+C22:6

### 3.13 BASELINE CHARACTERISTICS

**Table 3.6** summarises the baseline characteristics, which were measured at the screening visit, of the volunteers that participated in the trial. All volunteers were healthy with BMI and BP measures in the normal range.

**Table 3.6 Baseline characteristics of volunteers.**

| | |
|---|---|
| Age (years) | 26(20-33) |
| Height (cm) | 177.3 ± 8.5 |
| Weight (kg) | 74.1 ± 6.8 |
| BMI | 23.7 ± 2.4 |
| Systolic BP (mmHg) | 127.7 ± 11.6 |
| Diastolic BP (mmHg) | 73.4 ± 5.0 |

Normally distributed data are presented as means ± SD. Data not normally distributed are presented as medians with $25^{th}$ and $75^{th}$ percentiles in parentheses for n = 10.

## 3.14 PLASMA LIPID ANALYSIS

TAG and cholesterol concentrations of total plasma, CM and VLDL samples were analysed, via enzymatic assays (section 2.11), to determine the lipaemic response to the meals.

### 3.14.1 Total Plasma Lipid Response

**Figure 3.3** shows the total plasma lipid response to the meals. The total TAG response to the meals show a significant change over time (P=<0.001) with a peak shown at around 150 – 180 min. There was no significant difference between the two meals. When comparing the total TAG response of the current trial with the previous trial (section 2.17.1, **Figure 2.2**), the current trial produced a more pronounced lipemic response to the meals. The mean TAG response of the breakfast meals at the highest peak was 0.82 mmol/L, whereas the mean TAG response of the mid-day meals at the highest peak was 2.6 mmol/L. With regards to total plasma cholesterol response, there was a significant change over time (P=0.005), but as with the TAG, there was no significant difference between the meals.

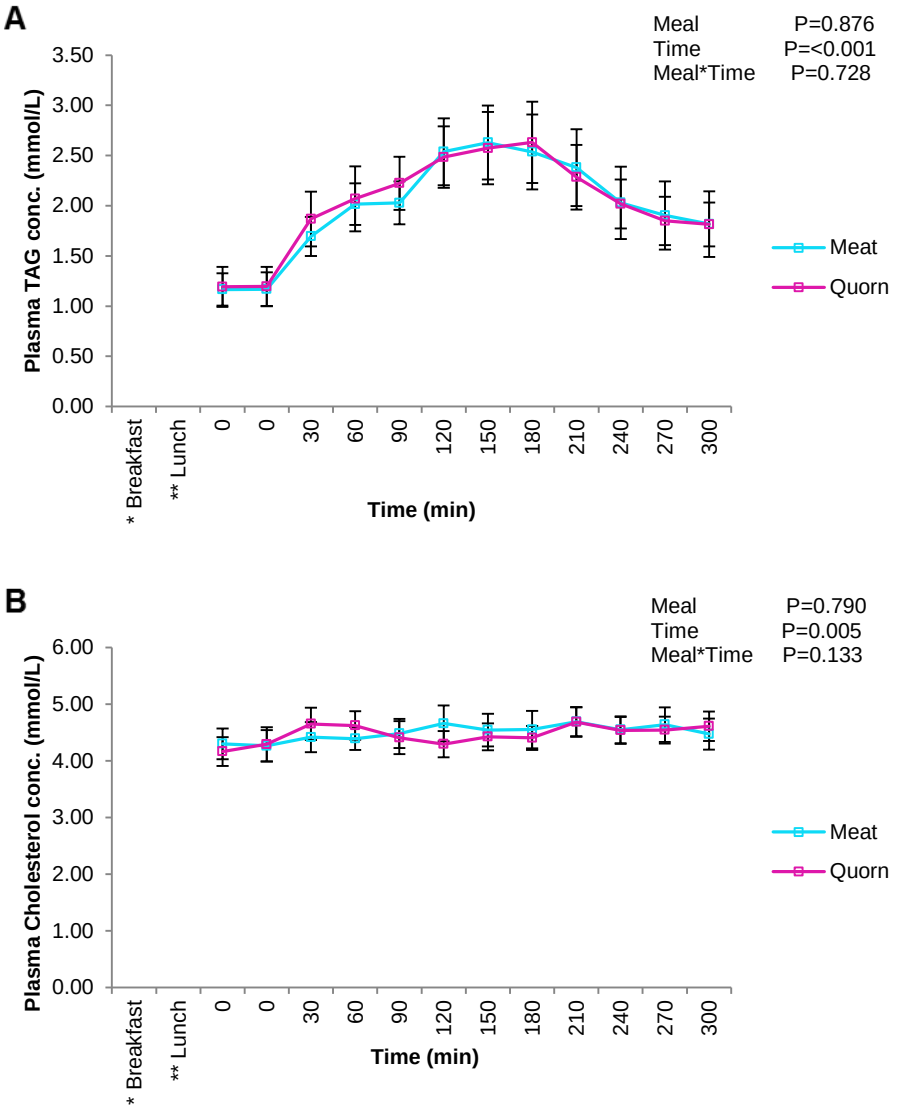


**Figure 3.3 Plasma TAG and Cholesterol response to the meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately followed by first baseline measurement, A = Plasma TAG response, B = Plasma cholesterol response.**

### 3.14.2 Chylomicron and Very Low Density Lipoprotein Triacylglycerol Response

**Figure 3.4** shows the CM and VLDL TAG response to the meals. Both the CM and VLDL TAG significantly change over time (P=<0.001 and P=0.001 respectively). As expected, CM TAG increased following the meal peaking at approximately 150 min. VLDL TAG increased steadily following the meal before starting to decrease back to baseline levels at approximately 180 – 210 min. No significant differences were seen between the meals in either CM TAG or VLDL TAG. As with total TAG, CM and VLDL TAG response was more pronounced in the current trial when compared to the previous trial (section 2.17.2). The mean CM TAG response of the breakfast meals at the highest peak was 0.17 mmol/L, whereas the mean CM TAG response of the mid-day meals at the highest peak was 0.7 mmol/L. The mean VLDL TAG response of the breakfast meals at the highest peak was 0.38 mmol/L, whereas the mean CM TAG response of the mid-day meals at the highest peak was 1.15 mmol/L.

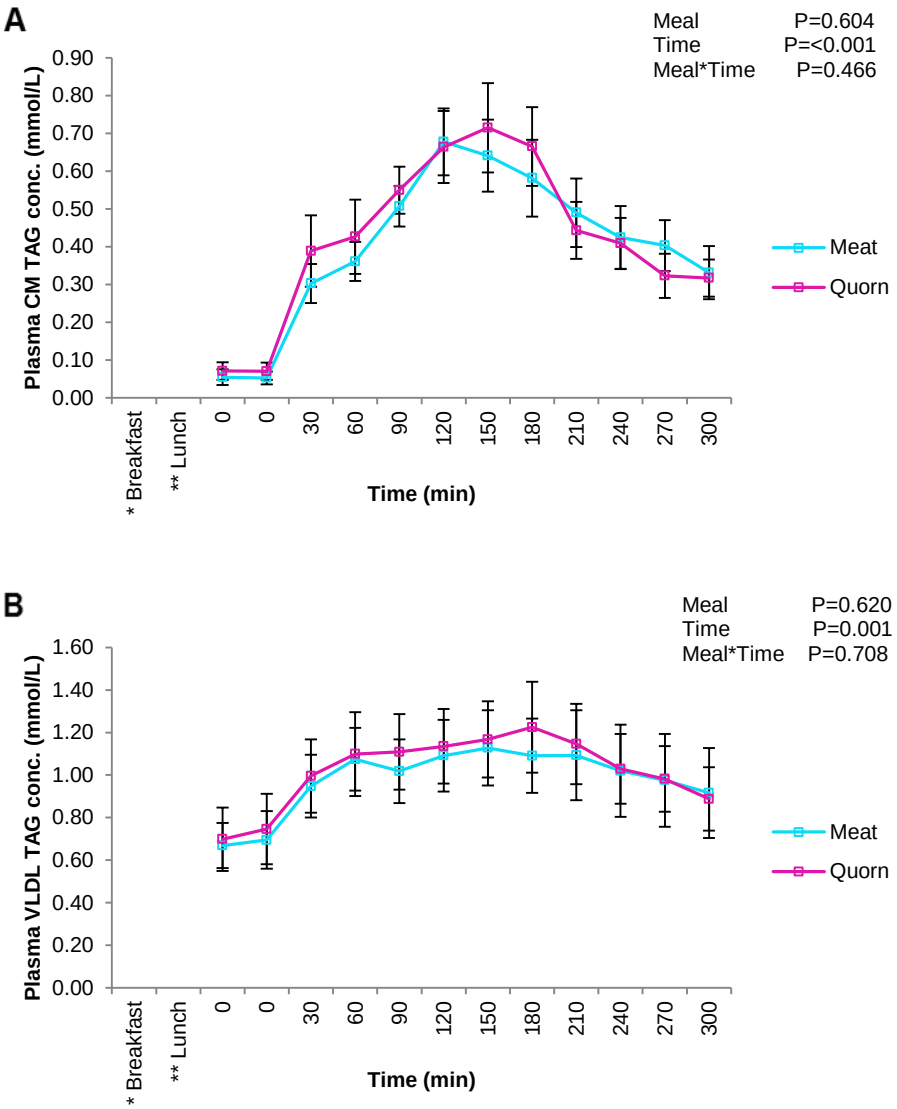


**Figure 3.4 CM and VLDL TAG response to the meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately followed by first baseline measurement, A = CM TAG response, B = VLDL TAG response.**

### 3.14.3 Chylomicron and Very Low Density Lipoprotein Cholesterol Response

**Figure 3.5** shows the CM and VLDL cholesterol response to the meals changing significantly over time (P=0.001 and P=0.017 respectively) however, there was no significant differences between the meals.

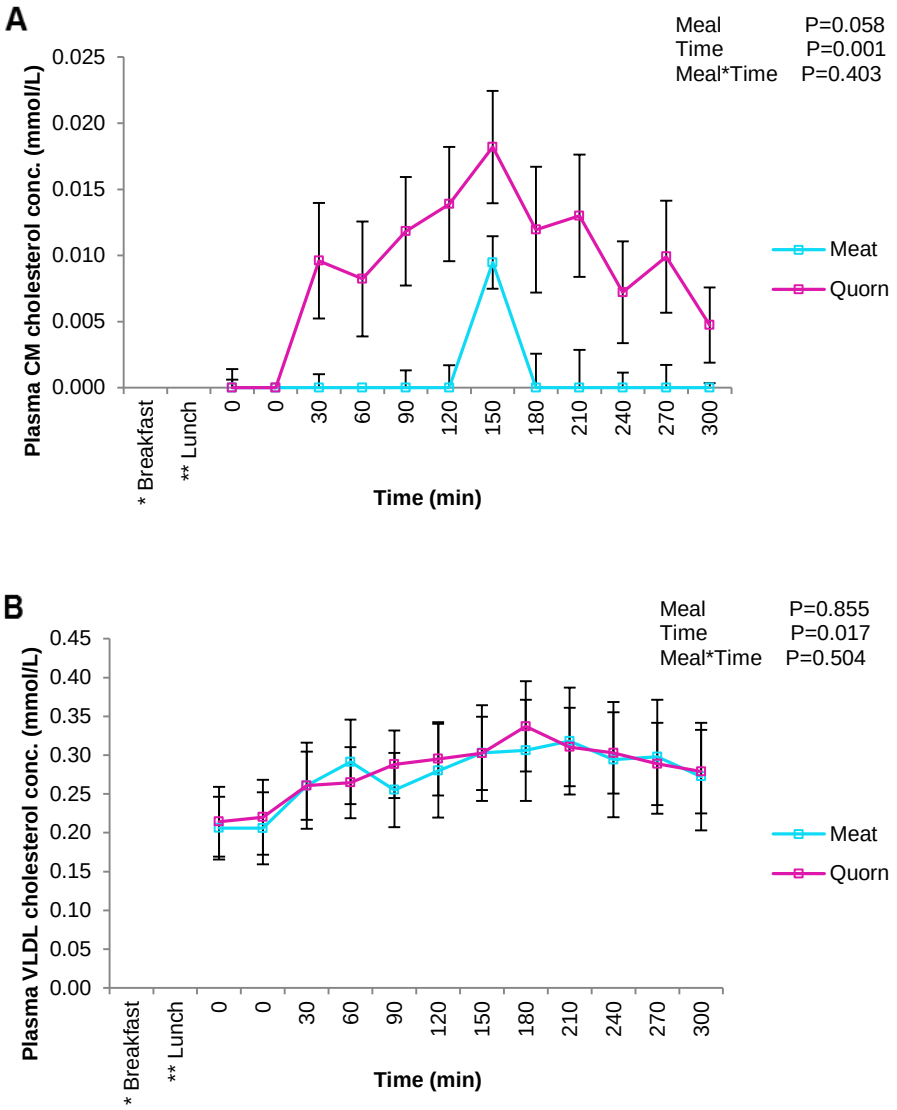


**Figure 3.5 CM and VLDL cholesterol response to the meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately followed by first baseline measurement, A = CM cholesterol response, B = VLDL cholesterol response.**

### 3.15 BLOOD GLUCOSE

Glucose was analysed immediately following blood collection to compare the blood glucose response of the two meals. The effect the two meals had on blood glucose concentrations can be seen in **Figure 3.6**. Both meals, as expected, produced a significant increase in blood glucose levels immediately following the meal (P=<0.001). There was no significant difference found between the two meals.

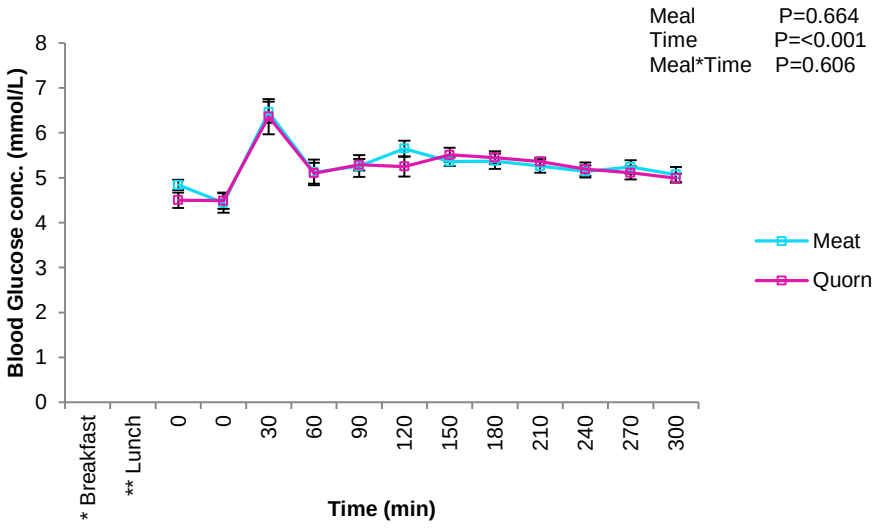


**Figure 3.6 Glucose response to meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately followed by first baseline measurement.**

### 3.16 SERUM INSULIN

Serum insulin was analysed to determine any differences in the insulin response of the two meals. **Figure 3.7** shows the effect of the two meals on serum insulin levels. There is a significant increase (P<0.001) in insulin levels at 30 min after both meals, but there is no significant difference between the two meals.

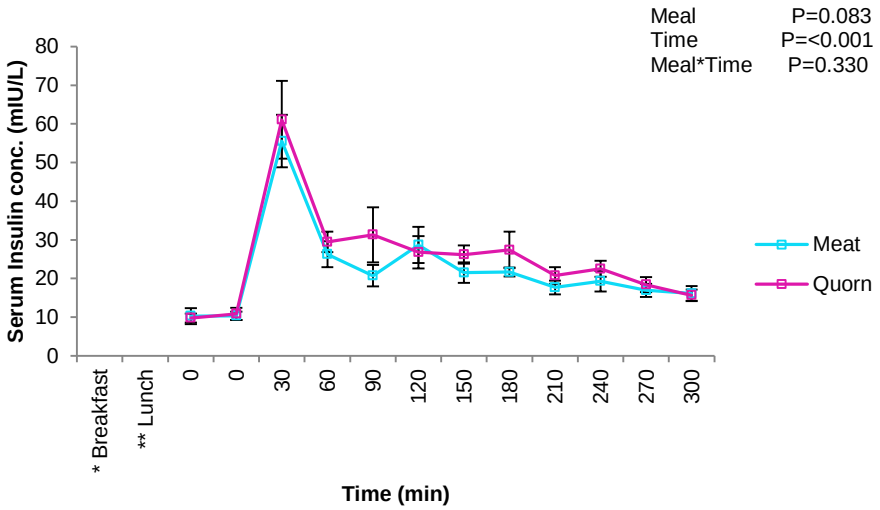


**Figure 3.7 Insulin response to the meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately followed by first baseline measurement.**

### 3.17 SATIETY

The volunteers' satiety response to the meals can be seen in **Figure 3.8**. Both meals significantly increased satiety (P=<0.001), of a similar magnitude, immediately after consumption. There was no significant difference between the two meals.

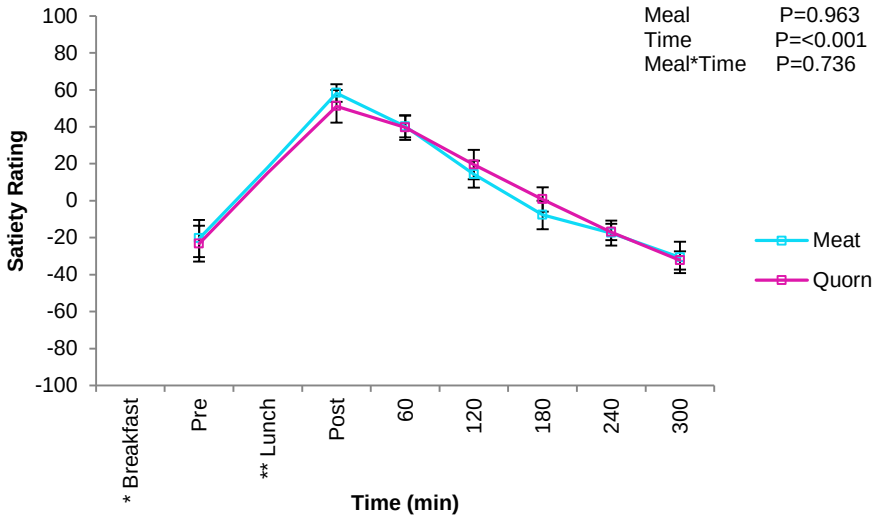


**Figure 3.8 Satiety response to the meals over 5 hours. Data are presented as means ± SEM for n = 10 samples per group. Data were analysed using two-way ANOVA with repeated measures. *Standardised breakfast consumed at 0800, **Test meal lunch was consumed at 1130 immediately following pre-meal satiety rating. Post-meal satiety rating was taken immediately following test meal lunch.**

## 3.18 CONCLUSIONS

As with the previous breakfast trial, the plasma lipid results from the mid-day trial demonstrate the expected postprandial lipaemic response. The previous breakfast trial showed little significant difference in postprandial lipid, glucose, and insulin profiles when comparing meat and Quorn™ meals. However, there were several limitations to the trial such as the small sample size, the low mycoprotein content of the meals, and the low fat content of the meals. Therefore, having taken into account all of these limitations, the current mid-day trial was designed.

After examining data from the previous trial (chapter 2) it was clear that, although not significant, males produced a higher postprandial lipid response to the meals than females. Therefore, to minimise inter-individual variation, only males were recruited for the current trial. The age group was also changed from 18 – 35 to 18 – 50 years in order to avoid recruiting undergraduate students so that we could study a more free-living population. The mycoprotein content was increased from 8% in the breakfast meal to 15.2% in the mid-day meal. The fat content was increased from 37g in the breakfast meal to 62g in the mid-day meal. Prior to consumption of the mid-day meal subjects were provided with a standard breakfast which contained 27g fat. The increase in the fat content of the mid-day meal helped produce a more pronounced lipaemic response, when compared with the breakfast trial. If there is not enough fat in the meal, then the postprandial lipaemic response can

show similar results to if the volunteers were in a fasted state (this is what happened in the breakfast trial). Although the addition of a standardised breakfast was used in this trial to increase the fat load, the general eating habits of the volunteers should have been taken into consideration. If the volunteers do not usually consume breakfast each day then forcing them to change their eating habits and consume a breakfast on the day of the trial may impact their postprandial response. The macronutrient content of the test meal in this trial was analysed in the laboratory rather than solely using the information provided on the packets as in the breakfast trial. However, the batch of the test meal that was analysed was not the same as the batch used in the trial itself. This may implicate the results due to the batches possibly containing a different macronutrient composition. A previous study by Turnbull and Ward (1995) claims mycoprotein reduces glycaemia and insulinemia. However, neither the breakfast nor mid-day meal trial demonstrated this effect.

Although longer term mycoprotein intervention trials have been shown to reduce total plasma and LDL cholesterol, and increase HDL concentrations (Ruxton and McMillan, 2010; Turnbull et al., 1990 and 1992; Holloway, 2014), to date there are no published reports of any changes to postprandial lipaemia when replacing meat with Quorn™.

Total plasma, CM, VLDL, glucose and satiety all showed significant changes over time for both meals. However, there were no significant differences between the meals shown in any of the measured parameters. This trial shows that when meals are closely match to contain similar macronutrient content including total fat and fibre content and similar FA compositions, Quorn™ has no effect on postprandial lipaemia. However, when consumed as a direct replacement for meat, Quorn containing meals are likely to be lower in energy and total fat, higher in fibre and contain less SFA. All of these factors are likely to impact on the postprandial response. The present trials indicate that there are no intrinsic properties of the Quorn™ protein, or associated fibre which specifically impacts on the glycaemic or lipaemic response to a meal. The mid-day meal trial does demonstrate that Quorn™ can be used to replace meat without any adverse effect or impact on satiety.

One of the reasons for conducting these two pilot-scale experiments was to investigate whether a postprandial challenge should be included in the fully-powered, long-term intervention study described in the following chapter. The lack of any suggestion of significant differences in postprandial responses led to the conclusion that this was not appropriate.

# Chapter 4 - CHRONIC EFFECTS OF REPLACING RED AND PROCESSED MEAT WITH NON/REDUCED MEAT ALTERNATIVES

## 4.1 INTRODUCTION

Meat can represent an important part of a healthy diet, supplying high quality protein and a range of micronutrients. Red meat also plays a significant role in the intake of Omega-3 FA, particularly in people that do not have a diet containing oily fish (Salter, 2012). However, excessive consumption, particularly of processed meat products, has been associated with increased risk of a range of chronic diseases including CVD, T2DM and certain cancers (Wyness et al., 2011; Micha et al., 2010).

The livestock sector has now been recognised as one of the major contributors to climate change and occupies ~30% of the ice-free terrestrial surface of the planet due to feed crop production and grazing (Steinfeld et al., 2006). The environmental issues caused by the livestock sector include deforestation, soil degradation, desertification, eutrophication, land- and water-based pollution, water scarcity and emission of GHG from animals (Reisch et al., 2013). Although these environmental issues are well recognised, the livestock sector is estimated to continue to increase substantially over the next 30 years (FAO, 2006). Therefore, it is important to investigate technologies to reduce GHG emissions from livestock and to create strategies that will reduce the consumption of livestock products (McMichael et al., 2007).

The link between developing CVD and the intake of animal products such as meat and dairy is largely associated with the intake of SFA in the diet (Larsson and Orsini, 2014; McAfee et al., 2010; Rohrmann et al., 2013b; Pereira and Vicente, 2013), with approximately half of the intake of SFA in the UK being contributed by meat and animal products (Salter, 2012). Moreover, individuals consuming an energy-dense diet, abundant in meat and animal products, along with leading a sedentary lifestyle, can lead to obesity which can, in turn, increase the risk of manifesting metabolic syndrome (McAfee et al., 2010; Salter, 2012; Williamson et al., 2005). There has also been an increasing amount of research into the association of red and processed meat consumption and the risk of developing various cancers (Salehi et al., 2013; Wallin et al., 2011; Wie et al., 2014; Choi et al., 2013; Di Maso et al., 2013; De Stefani et al., 2012; Zhu et al., 2014; Lippi et al., 2016; Cross et al., 2007; Larsson and Wolk, 2012), with multiple studies showing a positive association between meat intake and CRC (Chan et al., 2011; Joshi et al., 2015; Demeyer et al., 2015; Chan et al., 2013; Ananthakrishnan et al., 2014; Bernstein et al., 2015; Parr et al., 2013). Thus in terms of health, reducing meat intake is seen as a positive goal.

A pilot study was recently completed which aimed to reduce the meat consumption of a group of 24 university students by 50% over four weeks. This was achieved by conducting pre-interventional motivational events providing information relating to the impact of meat production on the environment and introducing participants to a range of meat

replacement products. Participants were then provided with sufficient meat replacement products, such as Quorn™, to replace 50% of their habitual energy intake from meat. All subjects completed the study without any significant change in body weight or body composition, thus suggesting that energy intakes were maintained. Data on plasma lipids indicated a number of beneficial changes in response to meat reduction, such as lowered total plasma cholesterol and TAG (Holloway, 2014). This study indicates that it is possible for free-living individuals to significantly reduce meat intake over a short period of time with potentially beneficial effects on their health. However, it remains to be established whether such a change can be maintained over a longer period, whether this negatively impacts on quality of life and whether the changes in CVD risk factors persist. The intervention group (largely undergraduate students) was also very homogenous and perhaps not representative of the general population and their more varied lifestyle patterns. Thus, a further study was planned to investigate the impact of specifically reducing red and processed meat over a 12 week period in a group of older (non-undergraduate) subjects.

## 4.2 HYPOTHESIS AND OBJECTIVES

Hypothesis: With the provision of meat replacement products and appropriate support, it is possible for adult men and women to reduce their meat intake by 50% and this will have a positive impact on risk factors for CVD.

Main objectives:

Reduce red/processed meat intake of adult men and women by 50% and assess the impact of such dietary changes on blood lipids, insulin sensitivity, and cardiovascular measures.

## 4.3 SAMPLE SIZE AND JUSTIFICATION

Using paired sample t-test model, power calculated at the level of 0.8 (Lenth, 2006)

Primary variable = improvement in total plasma cholesterol concentration.

Using data (generated from a previous study carried out in this research group) to inform the population variability (Holloway, 2014), a minimum of 26 subjects would give the statistical power to detect a difference in mean plasma cholesterol of 0.3 mmol/L. It seems feasible to suggest that a three month intervention period could cause a change of this magnitude, as Holloway (2014) demonstrated a 0.4 mmol/L reduction in total plasma cholesterol after one month of 50% meat reduction. Considering that the study group may be more heterogeneous than the student group previously studied and that the substantially increased length of the study may lead to significant drop-out it was decided to aim to recruit 40 individuals (20 of each gender).

## 4.4 STUDY DESIGN

The study design can be seen in **Figure 4.1**. In a single group, non-randomised, crossover design, 37 healthy, normal weight volunteers (16 males and 21 females) were asked to reduce their red and processed meat consumption by at least 50% for three months. The volunteer consort statement is shown in **Figure 4.2**. Volunteers were provided with a range of meat replacement products (**Table 4.1**) and recipes (**Appendix K**) to facilitate the change in diet and aid compliance.

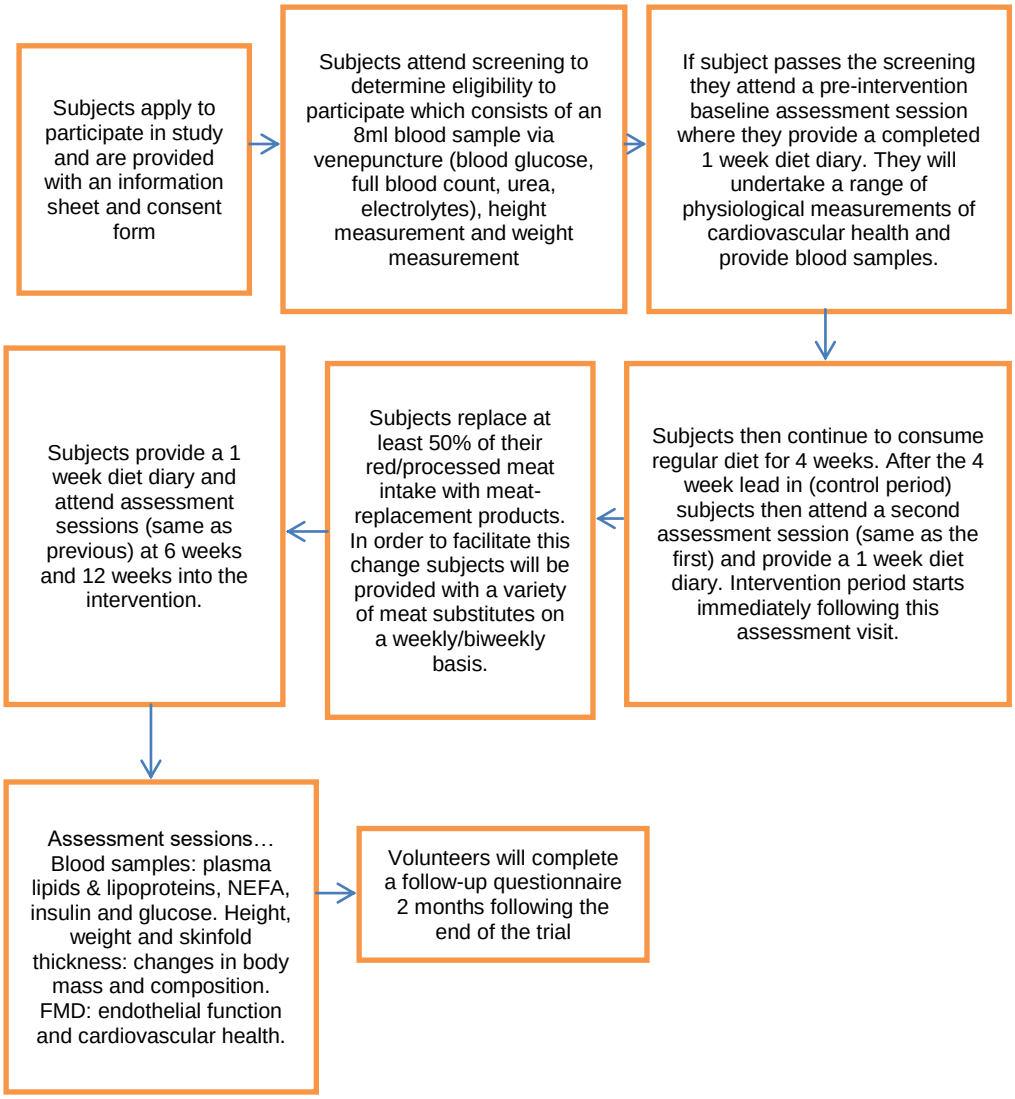


**Figure 4.1 Study design flow diagram.**

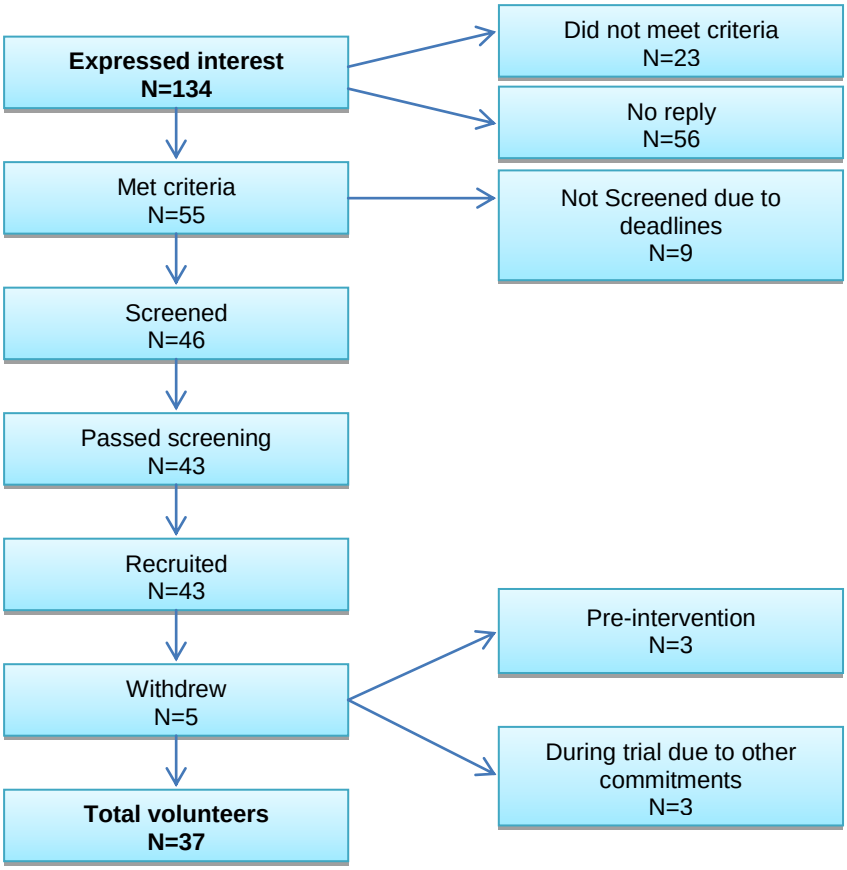


**Figure 4.2 Consort statement for intervention trial.**

**Table 4.1 Meat replacement products incorporated into the trial.**

| Sausages | Burgers& Meatballs | Mince | Beans | Lentils | Other |
|---|---|---|---|---|---|
| Pork & Quorn™ mix | Beef & Quorn™ mix | Beef & Quorn™ mix | Black turtle | Red split | Quorn™ ham slices |
| Pork & Soya mix | Beef & Soya mix | Soya | Flageolet | Speckled green | Quorn™ turkey slices |
| Quorn™ | Quorn™ | Quorn™ | Red kidney | | Quorn™ steak strip |
| | | | Chickpeas | | |

## 4.5 RECRUITMENT

Information dissemination using posters (**Appendix L**) was used in the first instance to attract volunteers. Posters with tear off slips displaying contact details were placed on several locations on the University of Nottingham's campus and QMC. Recruitment also took place via newspaper advertising, the University of Nottingham intranet and social media pages, and Nottingham University Hospitals health & wellbeing e-newsletter. Recruitment criteria can be seen in **Table 4.2**. Interested volunteers were provided an information sheet (**Appendix M**) which described the study in detail. If, after reading the information sheet, the volunteers still wished to participate they were invited to attend a screening visit at the QMC Medical School in Nottingham.

**Table 4.2 Recruitment criteria for intervention trial**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Aged 21 – 50 years | Smoking |
| BMI 18 – 28 kg/m$^2$ | Chronic disease |
| Healthy | Diabetes mellitus |
| Men and Premenopausal females | Hypertension |
| Good spoken and written English | Pregnancy / Breast feeding |
| Consume 4-5 portions of red and/or processed meat per week -consumption of >70g per day | Chronic medication use (excluding over-the-counter medication & oral contraceptives) |
| | Participation in any other research study three months prior to the screening |
| | Clinically significant findings at screening |

## 4.6 SCREENING VISIT

Screening was performed as in previous trials (section 2.7). The consent form for the current trial can be found in **Appendix N**. If the volunteer fulfilled all inclusion criteria, they were invited to take part in the trial.

## 4.7 PROTOCOL

Following successful recruitment and screening, volunteers were required to attend two pre-intervention assessment visits one month apart. This one month lead-in period provided the control data. A pre-trial food frequency questionnaire (FFQ) was completed at visit 1 to assess meat consumption prior to the intervention. The intervention and 50% meat reduction began immediately following visit 2. Volunteers were instructed to reduce their red and processed meat intake by at least 50% using any alternatives they wished, such as the products provided by the study (**Table 4.**1), chicken, fish, beans, pulses and other vegetarian products. The sausages provided by the study were designed to be larger than commercially available sausages due to the difference in energy content. Volunteers were sent frequent emails and monthly newsletters to motivate and aid compliance (**Appendix O**). Volunteers then attended assessment visit 3 at six weeks after the start of intervention, and the final assessment, visit 4, at 12 weeks following the start of intervention. Volunteers completed a four-day food diary the week prior to each assessment visit and all four assessment visits required the volunteers to fast from midnight the morning of their visit

and refrain from exercise, alcohol and caffeine consumption for 24 hrs before assessments. Volunteers were also instructed to consume the same evening meal the night before each visit, and to avoid foods containing polyphenols such as colourful fruit and vegetables, chocolate, tea and coffee. At each visit, blood samples were taken to determine total serum cholesterol, TAG, LDL-cholesterol, HDL-cholesterol, insulin and blood glucose concentrations. Blood pressure was measured to assess changes in cardiovascular health; height, weight, waist circumference, skinfold thickness and bioelectrical impedance analysis (BIA) was used to calculate any changes in body mass and body composition during the intervention. In a subset of volunteers, flow mediated vasodilation (FMD) was measured at visits 1, 2 and 4 and used as an index of endothelial function and cardiovascular health. Compliance was assessed by conversing with the volunteers during each assessment visit. Quality of life questionnaires were completed at each visit and a follow-up FFQ was completed by volunteers two months after completing the trial to determine lifestyle changes in meat consumption.

### 4.8 ETHICAL APPROVAL

Ethical approval was obtained from the University of Nottingham Faculty of Medicine and Health Sciences, Medical School Research Ethics Committee. Ethics Reference No: A18062015 SoBS ELM (**Appendix P**)

## 4.9 DIETARY ASSESSMENT

One of the most fundamental problems with nutritional research is assessing dietary intake accurately due to a tendency for participants to under-report (Macdiarmid and Blundell, 1998). Therefore, this trial incorporated two methods, FFQ and food diaries, to assess meat consumption.

FFQs are the most commonly used tool for assessing dietary intake of specific foods in large epidemiologic studies of diet and health (Sichieri and Everhart, 1998; Subar et al., 2001). The FFQs used in the meat reduction trial (**Appendix Q**) required volunteers to report the frequency of consumption and portion size of various food items over four weeks prior to taking part in the intervention trial, and two months after completing the trial in order to investigate changes in dietary behaviour pre- and post- intervention. The responses from the FFQ were compiled in a spreadsheet and descriptive statistics were used to present the outcomes.

Although FFQs have been largely the instrument of choice in large intervention studies, food diaries are becoming increasingly popular for analysing food consumption (Day et al., 2001). Food diaries were used in the current trial to measure compliance, and also to determine if any significant differences identified throughout the trial could be due to macronutrient changes in the diet. In order to get a representation of a standard week of food intake, volunteers were instructed to record, in

as much detail as possible, all food and beverages consumed over a four-day period (including one weekend day) the week prior to an assessment visit. The food diaries provided to the volunteers (**Appendix R**) contained extensive instruction on how to complete the diary, along with illustrations and examples to aid the completion of the diaries. Volunteers were encouraged to contact organisers of the trial if they had any queries.

Food diaries are currently under analysis and therefore, no data will be present in the results section.

## 4.10 BLOOD SAMPLING

A 16 ml blood sample was taken via venepuncture of the antecubital vein at each of the four assessment visits. Approximately 9 ml blood was transferred to SST™II serum separator vacutainer tubes (Becton, Dickinson and Co, USA) and allowed to clot for 15 min at room temperature. Approximately 6 ml blood was transferred to Ethylenediaminetetraacetic acid (ETDA) vacutainer tubes (Becton, Dickinson and Co, USA); and immediately centrifuged; and approximately 1 ml blood was transferred to into a fluoride heparin microtube (Sarstedt, Germany). The SST™II serum separator vacutainer tubes and one of the EDTA vacutainer tubes (approx. 3 ml) were spun in a centrifuge at 4400 rpm for ten minutes at 4°C. The top layer of serum was transferred from the SST™II tubes into 1 ml eppendorf tubes and stored at -20°C until ready for total cholesterol, total TAG, LDL, HDL and insulin analysis. The top layer of plasma was transferred from the spun EDTA vacutainer tube into a 1 ml eppendorf tube and stored at -20°C. This plasma sample was collected and stored in case any further analysis was required. The second EDTA vacutainer tube, containing whole blood, was sent to the pathology department at QMC and analysed for full blood count, as described for the screening visit, to monitor for anaemia during the trial. The 1 ml whole blood that was collected in the fluoride heparin microtube was analysed within ten minutes for blood glucose concentration.

## 4.11 LIPID ANALYSIS

Total cholesterol, total TAG, LDL-cholesterol and HDL-cholesterol was determined using a Pentra 400 clinical chemistry analyser (Horiba Medical). ABX Pentra reagents for determination of total cholesterol (Ref.A11A01634), total TAG (Ref. A11A01640), LDL cholesterol (Ref. A11A01638) and HDL cholesterol (Ref. A11A01636) were used following manufacturer's instructions.

## 4.12 INSULIN SENSITIVITY

HOMA and QUICKI were used to determine volunteers' changes in insulin sensitivity throughout the intervention trial. Outputs from both methods were compared to determine any differences.

### 4.12.3 Insulin Analysis

Serum insulin was analysed using the radio-immunoassay method as described in section 2.13.

### 4.12.4 Glucose Analysis

Blood glucose concentration was measured at each visit using a Yellow Springs Instrument (YSI) 2300 (Yellowsprings Inc. CO, USA).

## 4.13 PHYSIOLOGICAL MEASUREMENTS FOR BODY COMPOSITION

A series of physiological measurements were undertaken at each visit to determine if any changes in the lipids could be due to changes in body mass and composition.

### 4.13.1 Height Measurements

Height was measured at the initial screening using a SECA Stadiometer (SECA Ltd, Birmingham, UK). Measurements were used to calculate BMI.

### 4.13.2 Weight Measurements

Weight was measured at every visit using a calibrated Seca 882 Digital Scale (SECA Ltd, Birmingham, UK). Weight measurements were also used to calculate BMI.

### 4.13.3 Waist and Hip Circumference Measurements

Waist and hip circumference were assessed, with the participant standing, mid-way between the lowest rib and the iliac crest, and at the greater trochanters, respectively. Volunteers were instructed to relax and breathe normally during the measurement.

### 4.13.4 Skin-Fold Thickness for Measuring Body Density

Callipers were used to measure skinfold thickness at three points and body fat percentage was calculated, using the equation described by Jackson and Pollock (1978) for males and Jackson et al. (1980) for females. Each point was measured in triplicate and the average value was used for the equation.

### 4.13.5 Bioelectrical Impedance Analysis

Bioelectrical Impedance (BodyStat Ltd, Douglas, Isle of Man) was used as a secondary method for determining body fat percentage in the volunteers. Volunteers were instructed to lie down and rest for 15 min prior to analysis while surface electrodes were placed on hands and feet. A 50 kHz current is passed between the electrodes and a weighted sum of extracellular water and intracellular water resistivity is measured. The BIA machine produces results that are based on algorithms that have been designed for analysis.

## 4.14 BLOOD PRESSURE MEASUREMENTS

BP was measured at approximately the same time (within 30 min) each visit, using the method described in section 2.8, to determine any changes in cardiovascular health throughout the intervention.

## 4.15 FLOW MEDIATED VASODILATION

FMD was used as an index of endothelial function and cardiovascular health, and was measured at visits 1, 2 and 4 to determine any changes in cardiovascular health throughout the intervention trial. As there are many things that can influence FMD, volunteers were advised to follow the preparation details listed in **Table 4.3** prior to attending an assessment visit. The volunteers were also advised not to walk, run, or cycle to QMC for their visit, and should travel to their visit using a mode of transport that would not elevate their heart rate. On reaching QMC they were advised to take the elevator, not the stairs, to the assessment room. Measurements took place in a quiet, temperature controlled room. The temperature was set at 27°C for all FMD measurements taken throughout the trial. On arrival to the assessment room, volunteers were weighed then laid on a bed with the headrest positioned 12 cm above the top of the mattress (**Figure 4.3**).

**Table 4.3 FMD preparation for volunteers prior to assessment visit.**

| |
|---|
| - No food or drink (except water) for 8-12 hrs prior to assessment |
| - No strenuous exercise for 24 hrs prior to assessment |
| - No caffeine or vitamin C for 18 hrs prior to assessment |
| - No alcohol for 24 hrs prior to assessment |
| - No foods containing polyphenols for 18 hrs prior to assessment |
| - Consume the same pre-assessment evening meal for each visit |
| - Attend each assessment visit at the same time of day |

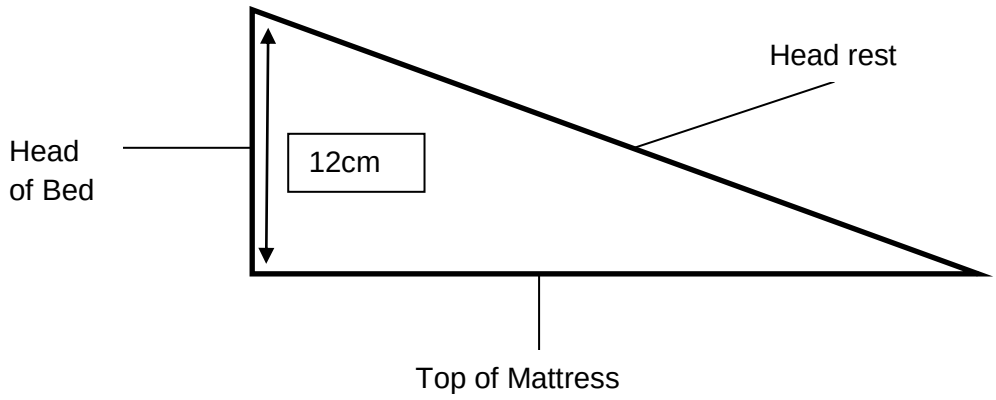


**Figure 4.3 Diagram showing the angle of the headrest on the bed.**

The volunteer was positioned supine on the bed with one pillow under their head and back, and a pillow under each arm so that their arms are resting at equal height relative to the heart. The volunteers remained resting in the position for 45 min prior to commencing the baseline scans. Volunteers were not allowed to fall asleep during the FMD measurement. A sphygmomanometric cuff was placed on the lower part of the volunteers right arm, between the antecubital fossa and the ulna. The cuff is placed around the area with the most tissue. The distance between the bony prominence of the wrist and the lower margin of the cuff was measured and recorded so that the cuff was placed in the same position at each visit. A Dinamap Pro 1000 BP cuff was placed around the left upper arm of the volunteer in the correct position for taking a BP measurement. The ultrasound operator then took three baseline diameter measurements of the brachial artery by applying ultrasound transmission gel to the scanning probe and locating the brachial artery, above the antecubital fossa in the longitudinal plane (**Figure 4.4**), on the volunteer's right arm with the probe. A segment that has clear anterior and posterior intimal interfaces between the lumen and the vessel wall was selected for imaging. Anatomical landmarks, such as veins, were noted to help maintain the same image of the artery throughout the trial.

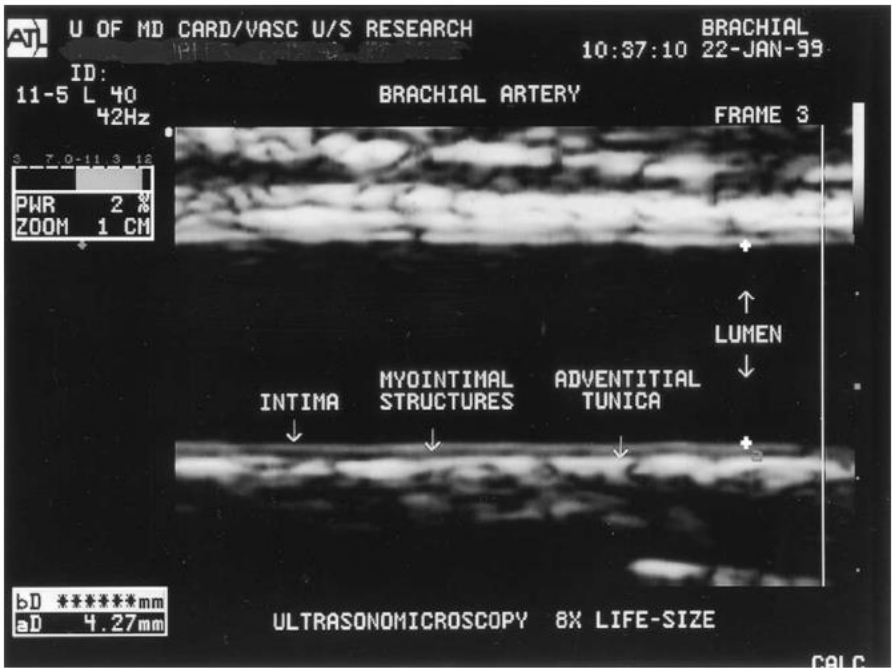


**Figure 4.4 Longitudinal ultrasound image of the brachial artery displaying clear anterior and posterior intimal interfaces between the lumen and the vessel wall.**

(Corretti et al., 2002)

A Toshiba Diagnostic Ultrasound System SSA-77OA (Toshiba Medical UK Ltd, West Sussex) was used to record the diameter and velocity measurements of the brachial artery. The volunteer's arm was marked where the probe should be placed to locate the brachial artery when taking measurements. Following baseline diameter and velocity measurements, three BP readings were taken from the volunteers left arm, as described in section 2.8, and recorded. In order to create a flow stimulus in the brachial artery, the sphygmomanometric cuff on the right arm was then pumped up to 200 mmHg to cause arterial occlusion, and a stopwatch was started. When the stopwatch reached four minutes, the ultrasound operator prepared the probe with transmission gel and located the brachial artery. When the stopwatch reached five minutes the sphygmomanometric cuff was released and deflated, causing reactive hyperaemia, resulting in shear stress which causes the brachial artery to dilate (**Figure 4.5**). 15 sec after the sphygmomanometric cuff was released, the operator measured and recorded the velocity of the artery. When the stopwatch reached 5 min 50 sec the first diameter measurement was recorded (**Figure 4.6**). A further seven diameter measurements were taken, each separated by ten seconds, and recorded in the same way until the stopwatch read seven minutes.

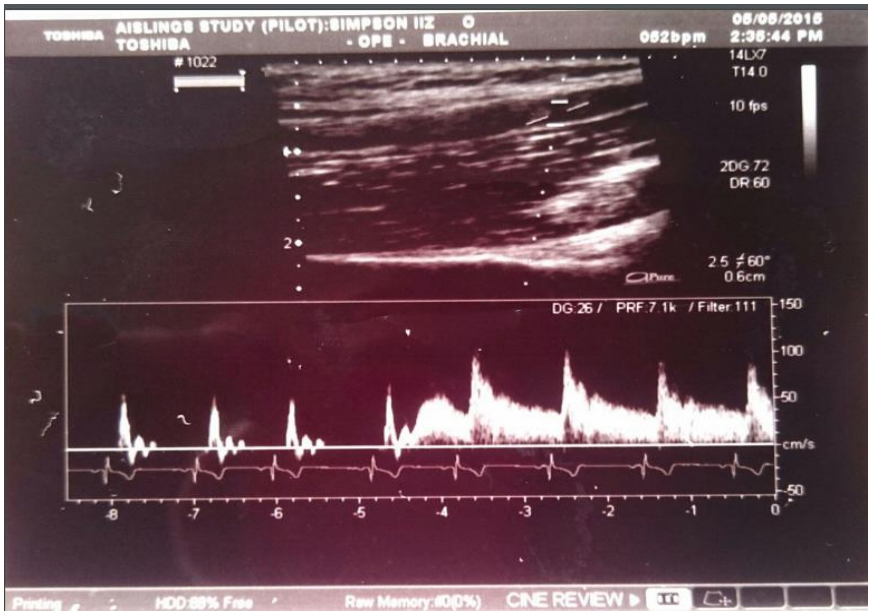


**Figure 4.5 Blood vessel reaction when occlusion is released.**

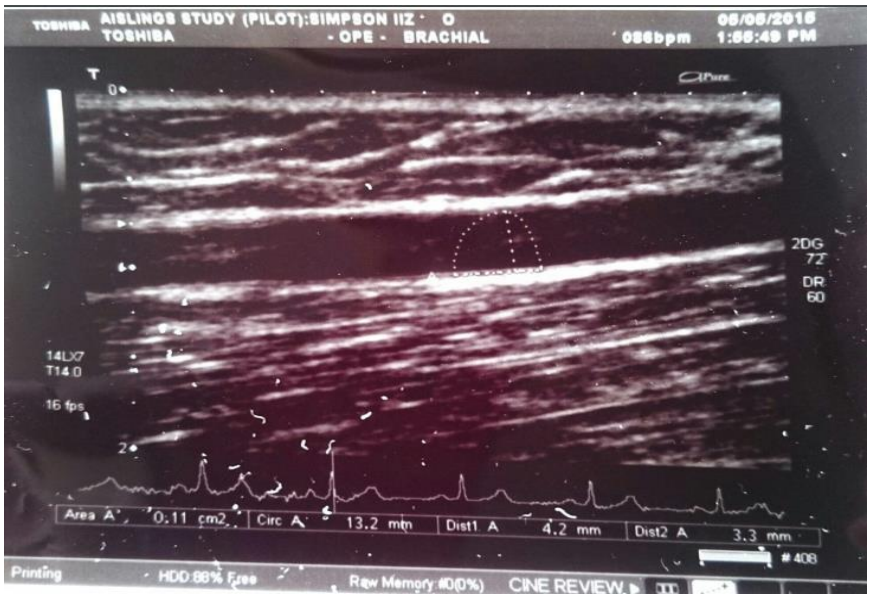


**Figure 4.6 Measurement of vessel diameter.**

The volunteer then remained resting on the bed for one hour until a second measurement process was completed. The diameter measurements were obtained from the ultrasound machine and the FMD was calculated and expressed as a percentage change from baseline diameter (**Figure 4.7**)

B – A = C

C / A*100 = FMD %

**Figure 4.7 Equation to calculate FMD expressed as a percentage change from baseline diameter.**

**A = mean of the three baseline diameter measurements, B = mean of the diameter measurements taken at 6 min, 6 min 10 sec, and 6 min 20 sec.**

## 4.16 WORLD HEALTH ORGANISATION QUALITY OF LIFE QUESTIONNAIRES

WHOQOL-BREF questionnaires (**Appendix S**) were completed at each visit to assess changes in QOL over the course of the intervention trial. The questionnaires could also help determine if any changes in the results are possibly due to changes in QOL throughout the trial.

## 4.17 STATISTICAL ANALYSIS

Data were analysed using SPSS version 22 (Statistical Package for the Social Sciences 2013). All data were assessed for normal distribution (using Skewness and Kurtosis z score between -1.96 and 1.96), and boxplots were used to assess data for outliers. Unless otherwise stated, data are presented as means ± SD in text and tables, and means ± SEM in figures. Data that were not normally distributed are presented, in text and tables, as medians with $25^{th}$ and $75^{th}$ percentiles in parentheses. All data were analysed as follows, unless otherwise stated. Baseline characteristics between gender were analysed using independent-samples t-test for parametric data, and Mann-Whitney U test for non-parametric data. Data from the whole cohort were analysed using paired-samples t-test (or non-parametric equivalent) to determine if any change had occurred in a variable during the control period (V1 – V2) and therefore whether the control period was to be factored in to subsequent analysis to investigate an intervention effect. If no change in a variable was observed over the control period, the data from the 'meat reduction' period were analysed as an intervention effect over time using one-way repeated measures ANOVA. If there was a significant change in a variable during the control period, data were analysed by comparing the control period (V1 – V2) with the intervention period (V2 – V4) using two-way repeated measures ANOVA. Where the control period was not required within the analysis of the intervention period, gender differences were investigated using one-way mixed model ANOVA with gender as Between Subject Factor.

If data from the control period was required as a factor within the intervention analysis, gender differences were analysed using two-way mixed model ANOVA with gender as Between Subject Factor. Within each ANOVA analysis, violation of the assumption of sphericity was assessed using Mauchly's test, and where appropriate the Greenhouse-Geisser statistic was used to determine the P value. All analysis used a two-tailed assessment and statistical significance was assumed where $P=<0.05$.

## 4.18 RESULTS

## 4.19 BASELINE CHARACTERISTICS

Comparisons between males and females, at baseline, were analysed, and the data is presented in **Table 4.4**. There were various significant differences observed between the genders, but these were not unexpected. Males are generally taller, heavier, and leaner than females, and this was reflected in the baseline data with height, weight, BF percentage for both BIA and callipers, displaying a significant difference (P=<0.001). Systolic BP was significantly lower (P=0.024) in these pre-menopausal females, when compared to men, an observation which has previously been reported (Reckelhoff, 2001; Benetos et al., 1999; Convertino, 1998). HDL cholesterol was significantly higher in females (P=<0.001), which has also been previously reported in premenopausal females (Castelli et al., 1977; Heiss et al., 1980; Schaefer et al., 1983).

**Table 4.4 Baseline characteristics of volunteers.**

| | Males n=16 | Females n=21 | Total n=37 | P value males vs females |
|---|---|---|---|---|
| Age (years) | 27 (24-30) | 27 (24-32) | 27 (24-32) | 0.964 |
| Height (cm) | 180.5 ± 8.0 | 164.6 ± 5.5 | 171.5 ± 10.3 | <0.001 |
| BMI | 23.4 ± 1.7 | 22.9 ± 2.7 | 23.1 ± 2.3 | 0.503 |
| Weight (kg) | 76.9 ± 9.6 | 62.0 ± 8.4 | 68.4 ± 11.6 | <0.001 |
| HOMA | 1.67 ± 0.59 | 1.90 ± 0.59 | 1.80 ± 0.59 | 0.241 |
| QUICKI | 0.35 (0.35-0.37) | 0.35 (0.34-0.36) | 0.35 (0.34-0.36) | 0.138 |
| Systolic BP (mmHg) | 116.3 ±8.7 | 108.5 ± 10.8 | 111.9 ± 10.7 | 0.024 |
| Diastolic BP (mmHg) | 69.4±5.5 | 67.6 ± 7.4 | 68.4 ± 6.7 | 0.610 |
| Body fat via BIA (%) | 13.9 ± 3.4 | 26.7 ± 6.3 | 21.4 ± 8.3 | <0.001 |
| Body fat via callipers (%) | 16.2 ± 5.2 | 25.2 ± 5.6 | 21.3 ± 7.0 | <0.001 |
| Total cholesterol (mmol/L) | 3.7 ± 0.9 | 4.0 ± 0.7 | 3.9 ± 0.7 | 0.408 |
| LDL cholesterol (mmol/L) | 2.2 ± 0.7 | 2.0 ± 0.5 | 2.1 ± 0.6 | 0.303 |
| HDL cholesterol (mmol/L) | 1.1 ± 0.3 | 1.5 ± 0.3 | 1.4 ± 0.3 | <0.001 |
| TAG (mmol/L) | 0.7 ± 0.2 | 0.7 ± 0.3 | 0.7 ± 0.2 | 0.945 |
| FMD (%) | 5.8 ± 2.3 | 8.3 ± 4.1 | 7.3 ± 3.5 | 0.069 |

Normally distributed data are presented as means ± SD. Data not normally distributed are presented as medians with $25^{th}$ and $75^{th}$ percentiles in parentheses. FMD n=25 (12 males, 13 females). Normally distributed data were analysed using independent-samples t-test. Data not normally distributed were analysed using Mann-Whitney U test.

## 4.20 SERUM LIPID ANALYSIS

Statistical analysis of the control periods (V1 – V2), of all lipid variables, showed no change had occurred during the control period; therefore, data were analysed to compare the intervention effect over time.

### 4.20.1 Total Serum Cholesterol

Total cholesterol was the primary variable in the trial and although **Figure 4.8** shows total cholesterol concentration did not significantly change over time, there is an indication of a trend in reduction (P=0.082). Further investigation showed that while total cholesterol of the whole cohort reduced by 6.2% over the intervention period, the reduction in the male cohort was a significant 12.8% (P=0.009), while females demonstrated a non-significant 1.1% reduction (P=0.950) over the intervention period (**Figure 4.9**).

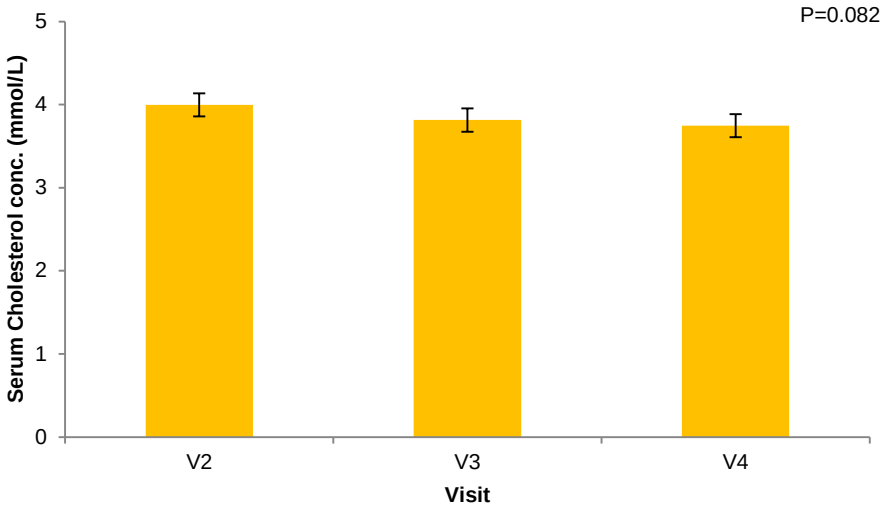


**Figure 4.8 Total cholesterol response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.082.**

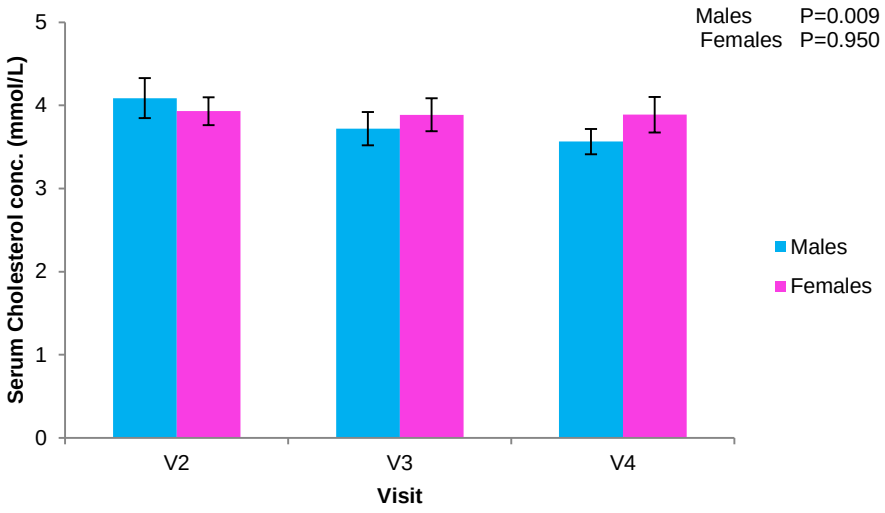


**Figure 4.9 Total cholesterol gender response throughout intervention. Data are presented as means ± SEM for males n = 16 and females n = 21. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time for males P=0.009. Intervention effect over time for females P=0.950.**

### 4.20.2 Serum Triacylglyceride

Total TAG does not change throughout the intervention period (**Figure 4.10**).

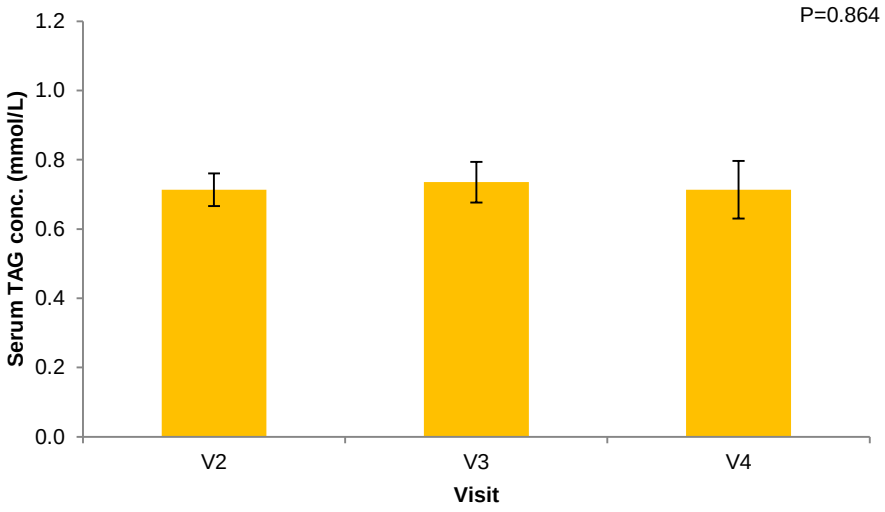


**Figure 4.10 TAG response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.864.**

### 4.20.3 Lipoprotein Response

Serum LDL cholesterol results are presented in **Figure 4.11** and show a significant reduction over the intervention period (P=0.012), which equates to a 9.7% reduction for the whole cohort. As with total cholesterol concentration, LDL cholesterol also showed a more pronounced reduction in the males (**Figure 4.12**). The males reduced their LDL cholesterol by a significant 16.4% (P=0.007), while the females only reduced theirs by 3.4% (P=0.694).

The HDL cholesterol response to the intervention period is shown in **Figure 4.13**. HDL cholesterol levels did not change over the intervention period (P=0.807). The significant gender difference seen in the baseline characteristics in section 4.19 continued throughout the intervention period.

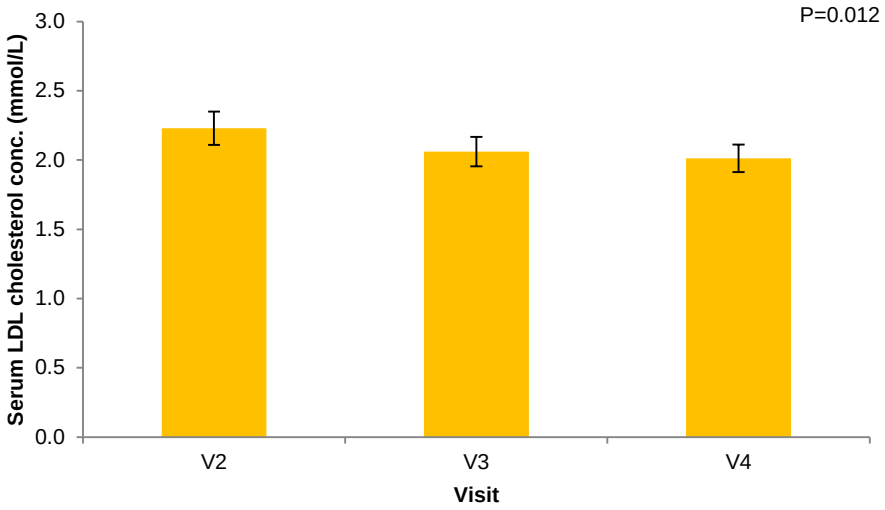


**Figure 4.11 LDL cholesterol response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.012.**

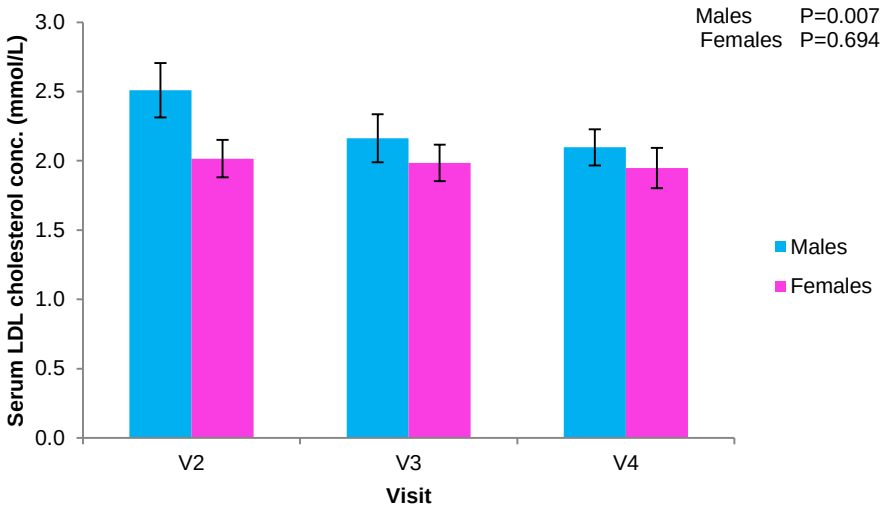


**Figure 4.12 LDL cholesterol gender response throughout intervention. Data are presented as means ± SEM for males n = 16 and females n = 21. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time for males P=0.007. Intervention effect over time for females P=0.694.**

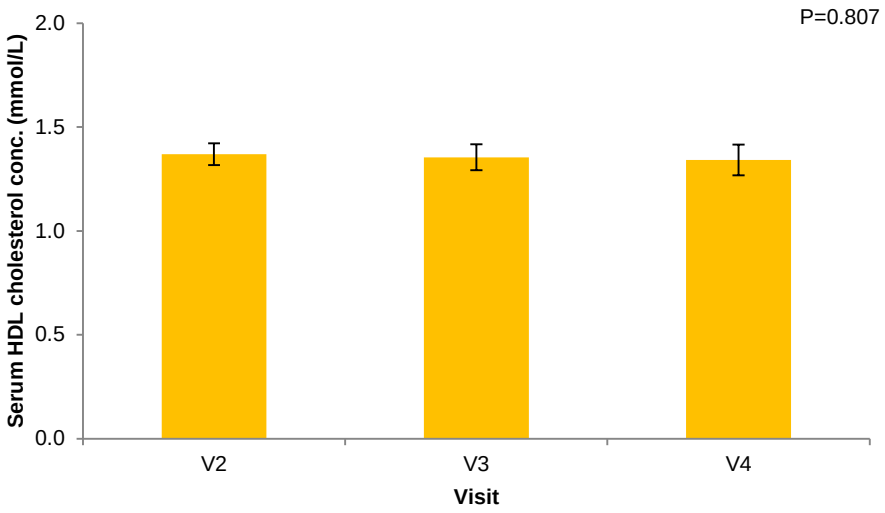


**Figure 4.13 HDL cholesterol response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.807.**

## 4.21 INSULIN SENSITIVITY

Insulin sensitivity was assessed using HOMA and QUICKI methods described in section 1.12.8

Statistical analysis of the control period (V1 – V2), of HOMA and QUICKI response, showed no change had occurred during the control period; therefore, data were analysed to compare the intervention effect over time and the results can be seen in **Figure 4.14**.

There was a trend for HOMA response to change over the intervention period (P=0.070), with the numerical elevation of HOMA at visit 3 being higher than visit 2 and visit 4. QUICKI response to the intervention showed a significant change over the intervention period (P=0.009) with the QUICKI value being lower at visit 3 than visit 2 and visit 4. FMD was not measured at visit 3; therefore, to determine if the differences at visit 3 were influenced by the timing of the fasting blood sample, data from the volunteers that did not participate in FMD throughout the entire intervention were analysed, and the results are shown in **Figure 4.15**. The trend and significant effect over the intervention that was originally found, was removed when analysing HOMA and QUICKI scores from no-FMD volunteers (HOMA: P=0.846, QUICKI: P=0.959).

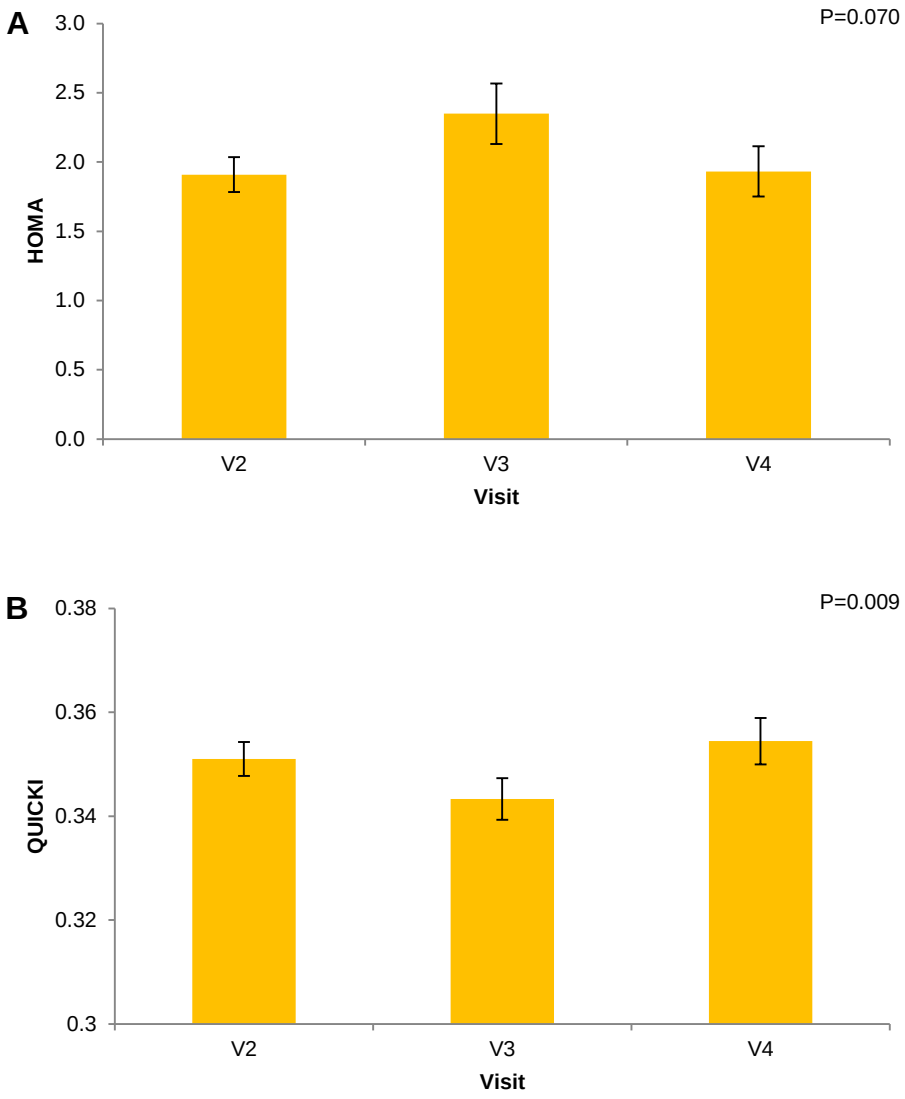


**Figure 4.14 HOMA and QUICKI response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. A = HOMA response P=0.070, B = QUICKI response P=0.009.**

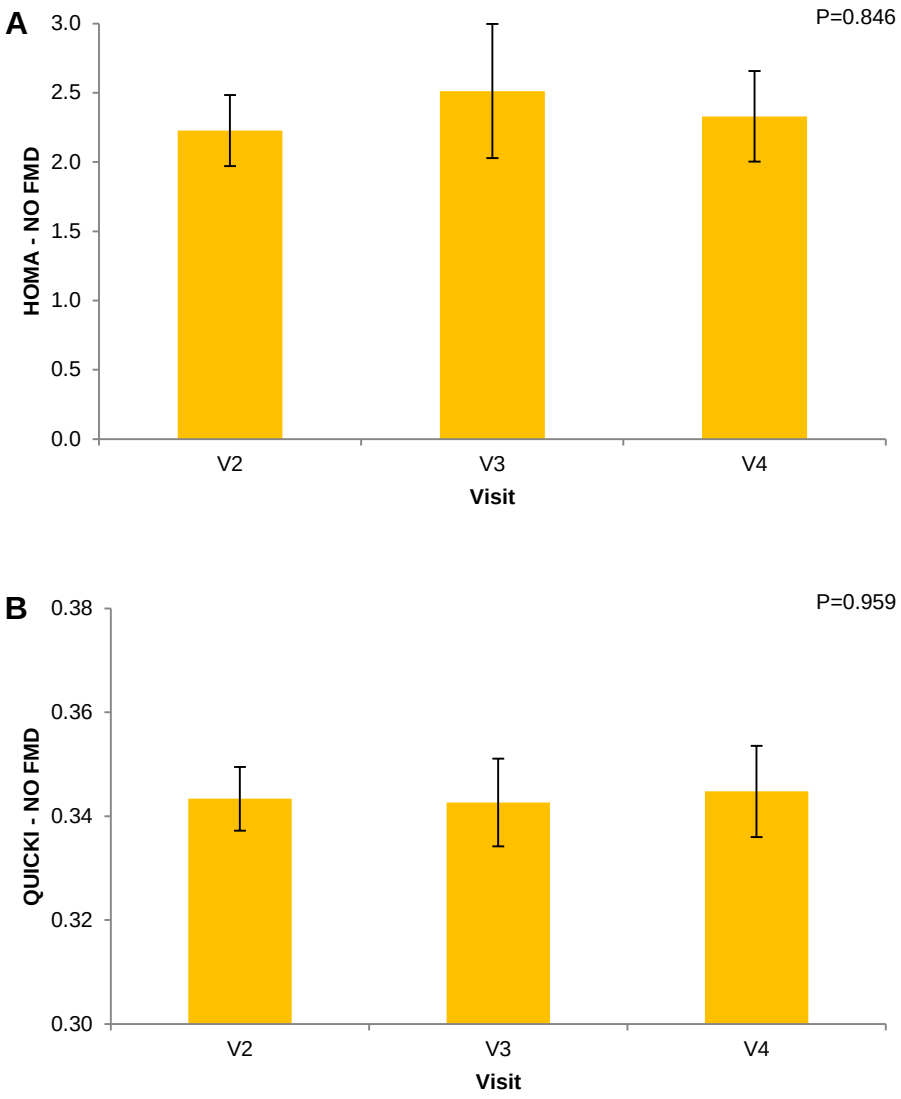


**Figure 4.15 No-FMD HOMA and QUICKI response throughout intervention. Data are presented as means ± SEM for n = 12. Data were analysed using one-way ANOVA with repeated measures. A = HOMA response P=0.846, B = QUICKI response P=0.959.**

## 4.22 BODY COMPOSITION MEASUREMENTS

Measurements were undertaken at each visit to determine if any changes in the primary variable over intervention could be due to changes in body mass or body composition.

Statistical analysis of the control period (V1 – V2), of weight and BF% with callipers, showed a significant change over the control period (P=0.041 and P=0.006 respectively). Therefore, data were analysed by comparing the control period (V1 – V2) with the intervention period (V2 – V4). All other body composition data showed no change during the control period and were analysed to compare the intervention effect over time.

### 4.22.1 Weight

**Figure 4.16** shows the intervention did not have a significant effect on weight when compared to the control period (P=0.227). The weight differences between gender that were seen at baseline (section 4.19) were maintained throughout the intervention.

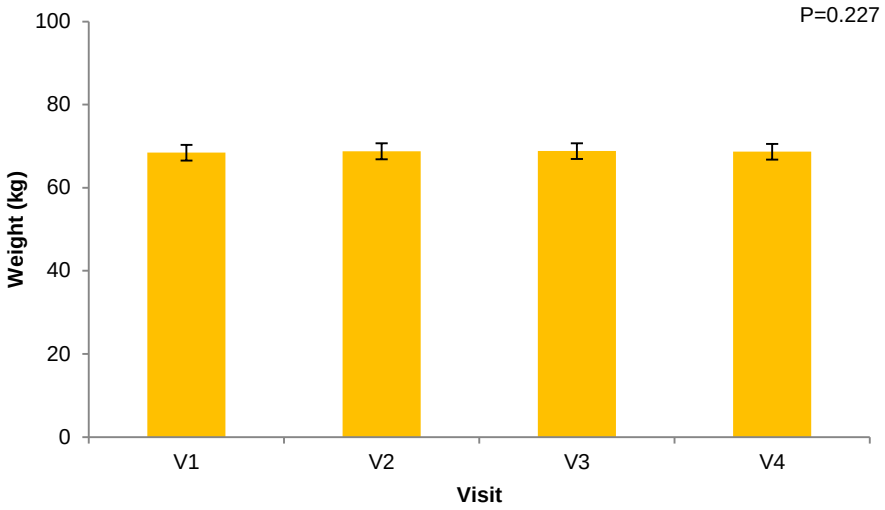


**Figure 4.16 Weight during control period compared with intervention period. Data are presented as means ± SEM for n = 37. Data were analysed using two-way ANOVA with repeated measures. Control compared with intervention P=0.227.**

### 4.22.2 Body Fat Percentage

**Figure 4.17** illustrates that BF percentage, when measured by callipers, is significantly higher in the control period compared to the intervention period (P=0.033). However, when investigated further, the significant difference lies with visit 1, which showed higher BF percentage when compared with visit 2. There is no significance when comparing visit 2, 3 and 4 (P=0.182).

BF percentage was also measured using BIA, and the response to the intervention can be seen in **Figure 4.18**. There was no significant change in BF percentage throughout the intervention period (P=0.190); and as with calliper BF percentage and weight, the gender differences seen at baseline were maintained throughout intervention.

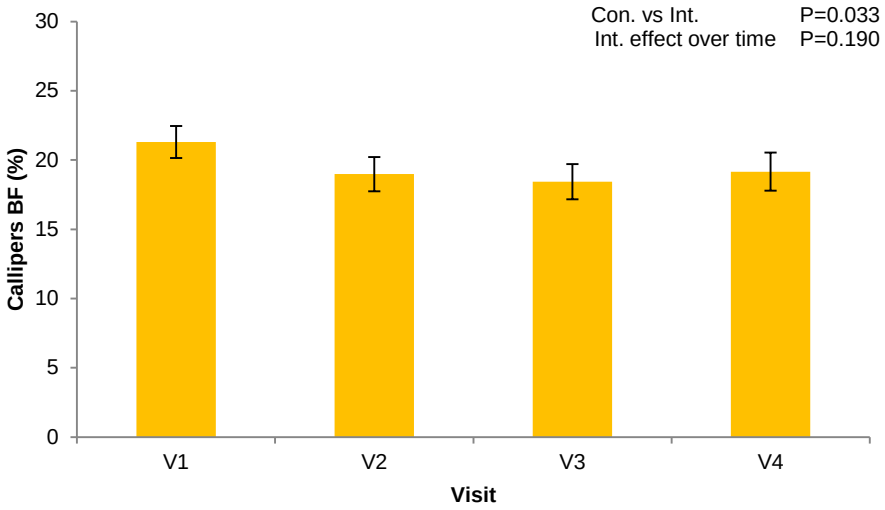


**Figure 4.17 Callipers measurement of BF% during control compared with intervention period. Data are presented as means ± SEM for n = 37. Data were analysed using two-way ANOVA with repeated measures. Control compared with intervention P=0.033. Intervention effect over time was analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.190).**

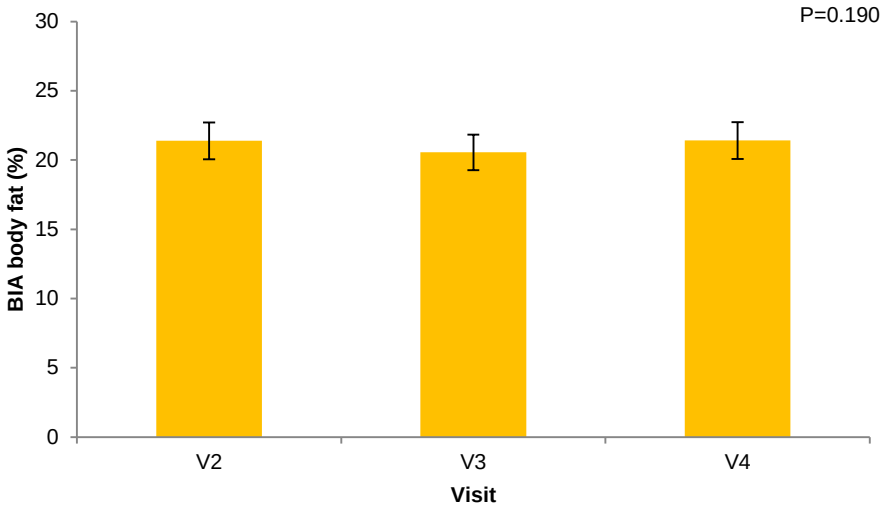


**Figure 4.18 BIA measurement of BF% throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. Intervention effect over time P=0.190.**

## 4.23 BLOOD PRESSURE

BP was measured at all visits to determine any changes on indices of cardiovascular health as a result of the intervention.

Statistical analysis of the control period (V1 – V2), of systolic and diastolic BP, showed no change had occurred during the control period; therefore, data were analysed to compare the intervention effect over time.

Neither systolic nor diastolic BP (**Figure 4.19**) changed significantly throughout the intervention (P=0.200 and P=0.317 respectively). When comparing gender differences, males had consistently higher BP than females throughout the intervention.

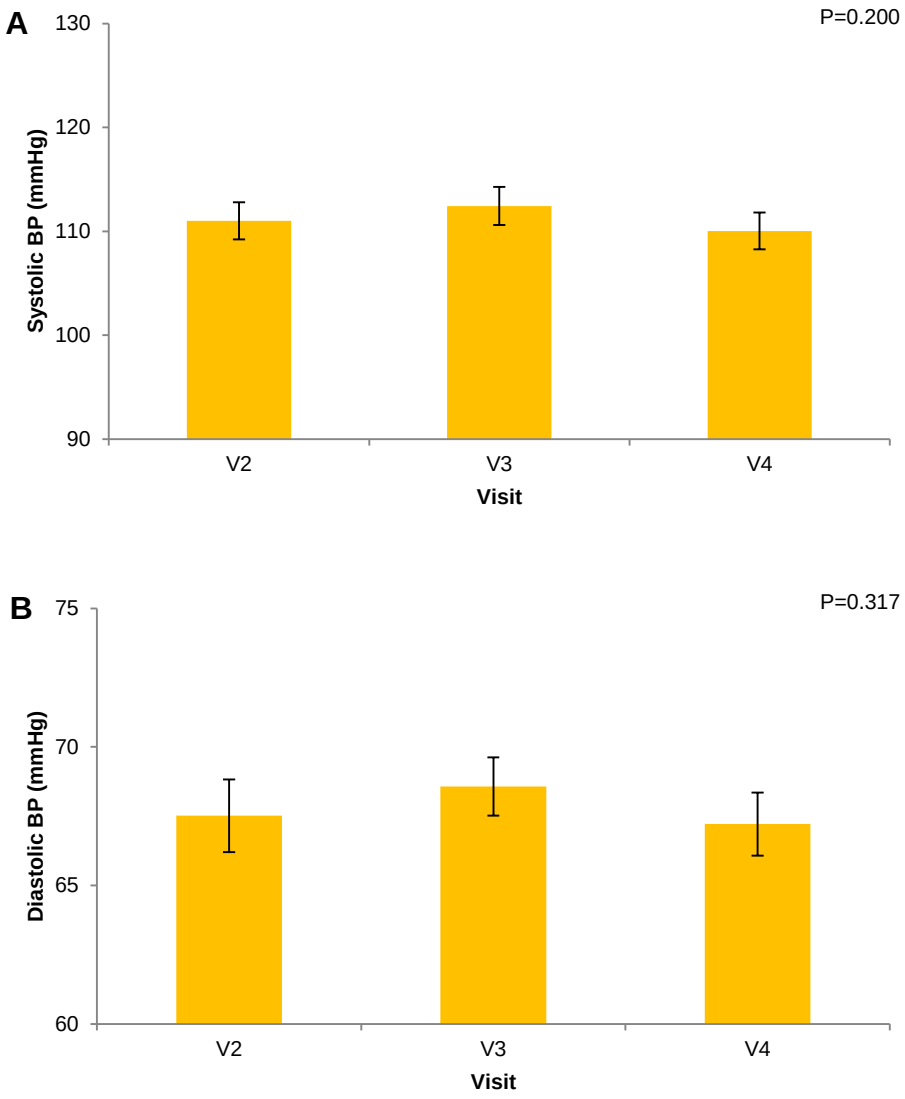


**Figure 4.19 BP response throughout intervention. Data are presented as means ± SEM for n = 37. Data were analysed using one-way ANOVA with repeated measures. A = Systolic BP response P=0.200, B = Diastolic BP response P=0.317.**

## 4.24 FLOW MEDIATED VASODILATION

FMD was measured at visits 1, 2 and 4, on a subset of volunteers, to determine any changes in cardiovascular health throughout the intervention trial. Statistical analysis of the control period (V1 – V2), of FMD response, showed no change had occurred during the control period; therefore, data were analysed to compare the intervention effect over time. As FMD was only measured at visit 1, 2 and 4, the intervention effect over time was analysed using paired-samples t-test to compare V2 – V4. FMD response can be seen in **Figure 4.20**. The intervention had no significant effect on the percentage change in diameter of the brachial artery (P=0.661). **Figure 4.21** shows, when comparing genders, females showed a significantly higher FMD response than males at the start of the intervention (P=0.026) and a trend for higher FMD at the end (P=0.059).

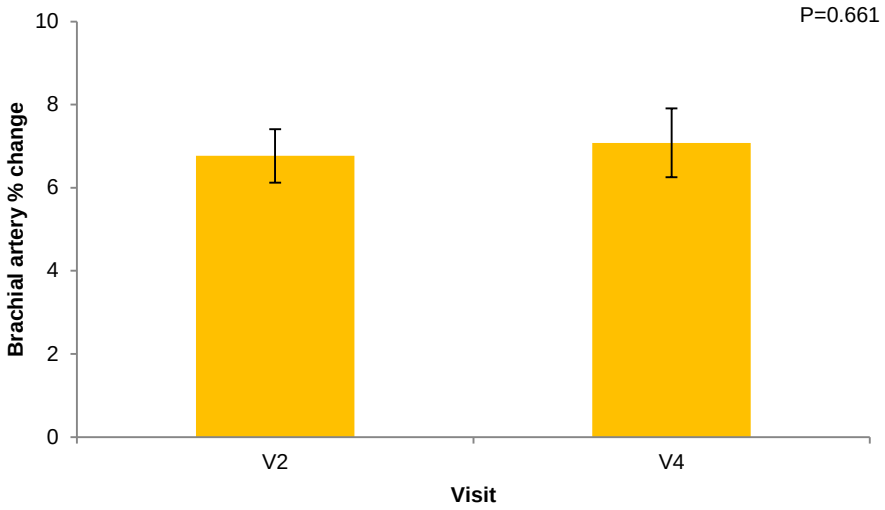


**Figure 4.20 FMD response of the brachial artery throughout intervention, displaying % change in diameter from baseline. Data are presented as means ± SEM for n = 25. Data were analysed using paired-samples t-test. Intervention effect over time P=0.661.**

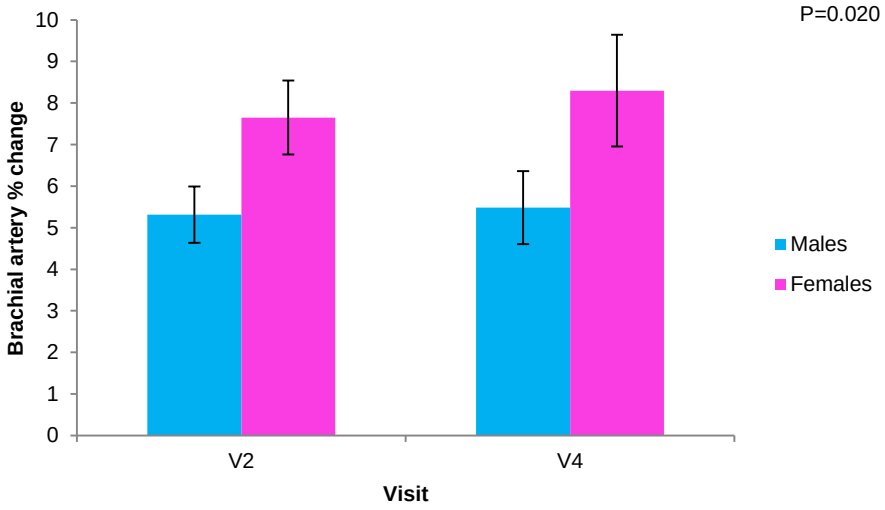


**Figure 4.21 FMD gender response of the brachial artery throughout intervention, displaying % change in diameter from baseline. Data are presented as means ± SEM for males n = 12 and females n = 13. Data were analysed using independent samples t-test. Gender difference at visit 2 P=0.026. Gender difference at visit 4 P=0.059.**

## 4.25 FOOD FREQUENCY QUESTIONNAIRES

FFQ were completed by a subset of volunteers (n = 29), pre- and post-intervention, to determine changes in meat consumption habits two months after completing the intervention (**Figure 4.22**).

Data, pre- and post- intervention, showed that the majority of volunteers' food choices were influenced by their partner (Pre = 51.7%, Post = 44.8%). Post- intervention data showed, over the last month, five volunteers (17.2%) reported that they purposefully restricted meat in their diet, while seven volunteers (24.1%) reported no meat consumption over this period. The main reason for the restriction is reported as "health reasons". When assessing meat consumption pre-intervention, 22 volunteers (75.9%) reported their frequency of meat consumption as at least five to six times per week, with 12 volunteers (41.4%) consuming meat more than once per day. Post-intervention, the main frequency of meat consumption, of those that had eaten meat in the last month, with 8 volunteers (36.4%), was two to four times per week. Five volunteers (22.7%) reported meat consumption of five to six times per week, and two volunteers (9.1%) reported consumption as more than once per day. When asked if participating in the trial made them change their behaviour towards meat consumption 13 volunteers (44.8%) said yes.

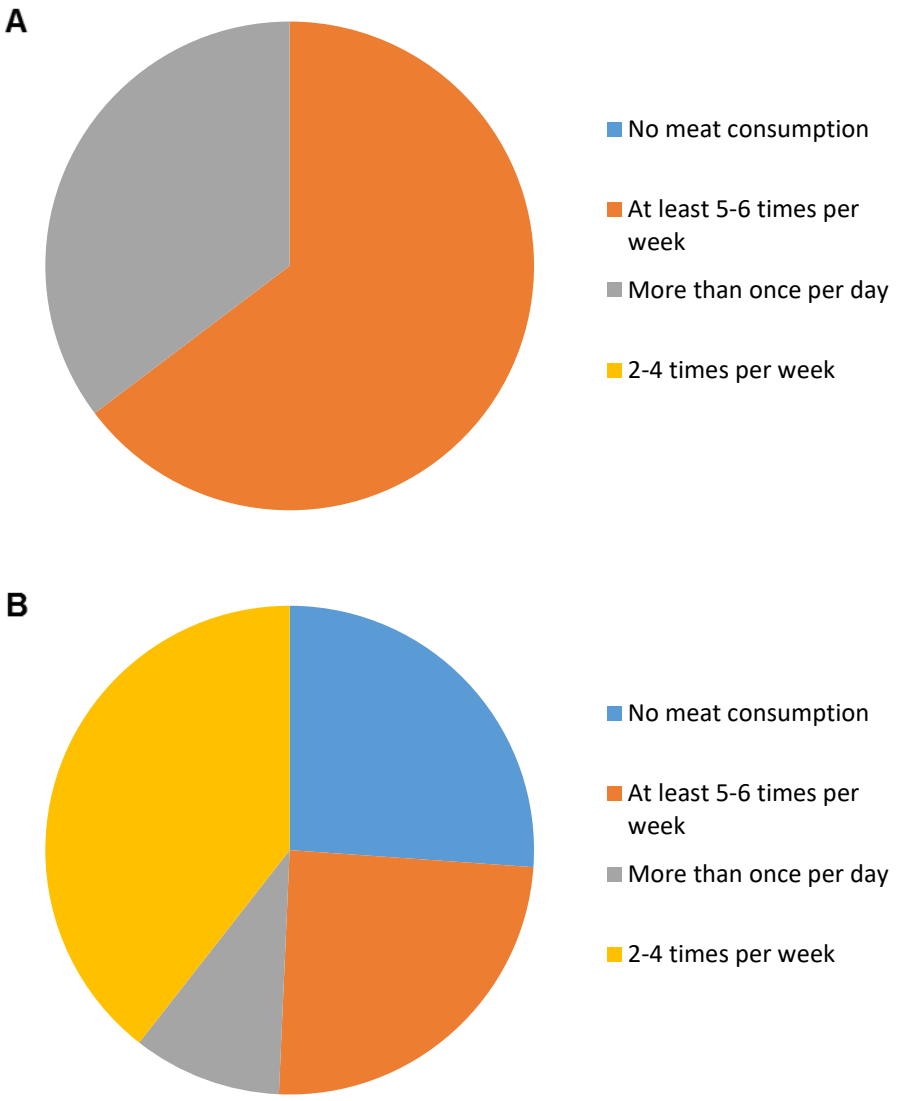


**Figure 4.22 Meat consumption habits of volunteers pre- and post-intervention. N = 29. A = Pre-intervention meat consumption, B = Post-intervention meat consumption.**

If behaviour towards meat consumption had changed, the volunteers were asked to comment on how they felt their behaviour had changed since taking part in the trial. A number of volunteers found reducing meat consumption a lot easier than they thought it would be, with

comments like “I thought it would be harder to not eat meat but it wasn’t” and “I didn’t miss it as much as I thought I would”

Comments such as “I can see that I can eat less meat and it doesn’t affect my mood or lifestyle” and “not noticed any differences in my gym performance” demonstrate meat reduction had no negative impact on energy levels. Several volunteers stated that they are more likely to consume a lot more meat replacement products after participating in the trial, because they realised they are not just designed for vegetarians. One volunteer said, “Before I wouldn’t have tried Quorn™ products or certain dried beans because I had a perception of Quorn™ and soya products as being for vegetarians only, and not very tasty. Now I would readily buy these products in a supermarket, and have two to three days a week (if not more) where I would go completely meat free”. Another volunteer stated that the trial made them “more aware of the substitutes available”. Participation in the trial also appeared to make the volunteers more aware of the amount of meat they were consuming, with comments like, “I am much more aware of what I eat and my meat consumption in general” and “will most likely eat less (meat) from now on”. Comments such as, “when I go out for dinner I am now considering the vegetarian options” and “Participating has definitely changed my behaviour and attitude towards my meat consumption” demonstrates that volunteers appear to no longer think negatively towards meat-free substitutes, or feel like meat is an essential part of their diet.

## 4.26 QUALITY OF LIFE

WHOQOL-BREF questionnaires were completed at each visit to assess changes in QOL over the course of the intervention. Statistical analysis of the control periods (V1 – V2), of each domain, showed no change had occurred during the control period; therefore, data were analysed to compare the intervention effect over time and the results can be seen in **Figure 4.23**. Physical, psychological and environmental QOL all showed no significant change throughout the intervention (P=0.201, P=0.695 and P=0.388 respectively). However social relationships showed a significant change over intervention (P=0.025). Further investigation showed visit 3 produced a significantly lower QOL score than visit 2 (P=0.025), but not visit 4 (P=0.160).

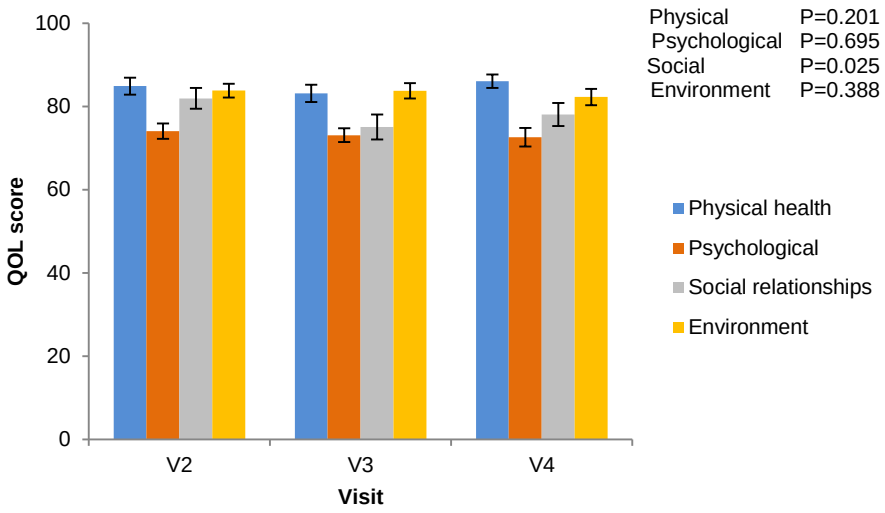


**Figure 4.23 QOL response throughout intervention. Data are presented as means ± SEM for n = 31. Data were analysed using one-way ANOVA with repeated measures. Physical health response P=0.201, Psychological response P=0.695, Social relationship response P=0.025, Environmental response P=0.388. Paired samples t-test was used to compare social relationships V3 with V2 and V4. Social relationships V3 compared with V2 P=0.025, Social relationships V3 compared with V4 P=0.160.**

## 4.27 CONCLUSIONS

### 4.27.1 Baseline Characteristics

The differences found in the baseline data (height, weight, BF percentage, and BP) were all expected in the population that was recruited for the trial. Males are generally taller, heavier and leaner than females; and although women showed significantly lower systolic BP than males, this is normal in premenopausal women (Reckelhoff, 2001; Benetos et al., 1999; Convertino, 1998). Females did show significantly higher HDL cholesterol at baseline than males. This is not unexpected, as from puberty onward, males have been shown to have lower HDL cholesterol levels than females (Hazzard and Applebaum-Bowden, 1990). This is due to the female sex hormone oestrogen raising the HDL cholesterol levels. Oestrogen levels are highest at premenopausal age which explains this difference (Bittner, 2001; Herrington and Parks, 1998; Kushwaha et al., 1991). All baseline lipid and BP data, for males and females, fall into the optimum range; Total cholesterol <5.0 mmol/L, Total TAG <1.7 mmol/L, LDL cholesterol <3.0 mmol/L, HDL cholesterol >1.0 mmol/L for males and >1.5 mmol/L for females, BP <120/80 mmHg (Perk et al., 2012).

### 4.27.2 Lipids

Although not significant, total cholesterol did show a 6.2% reduction in the whole cohort. This reduction was primarily due to changes in the males rather than females. The males presented a significant 12.8% reduction, while the females showed a reduction of just 1.1%. This gender difference may be due to the males consuming healthier diets throughout the intervention, eating a less healthy diet pre-intervention, or perhaps males are more responsive to lifestyle changes. Future analysis of diet diaries should confirm dietary changes during intervention and may explain the differing responses in males and females. Although a 6.2% or 12.8% reduction in total cholesterol may not be clinically significant in this cohort of healthy volunteers, it may be beneficial in populations with cholesterol levels outside of the normal range (Law et al., 1994). Law et al. (1994) demonstrated that a 10% (0.6 mmol/L) reduction of total cholesterol concentration reduced the risk of ischaemic heart disease by 54%. The reduction in total cholesterol in the current trial reflects previous work by Turnbull et al. (1990 and 1992). Although Turnbull's studies demonstrated significant reductions, whereas the current trial did not, the volunteers recruited for these trials had baseline total cholesterol concentrations >5.2 mmol/L. Perhaps the significant reduction was due to the starting concentrations being substantially higher than the current trial.

TAG concentration changes in conjunction with macronutrient changes in the diet, e.g. high carbohydrate diets can promote elevated TAG (Connor and Connor, 1997; Hudgins, 2000; Katan, 2006; Parks et al., 1999). However, the aim of the intervention was to replace the protein source of the diets, not the carbohydrate source. The TAG response seen in the intervention trial may indicate that carbohydrate consumption did not change throughout the intervention. As explained in section 1.8, dietary fat also influences TAG concentrations. If the volunteers replaced their fat consumption from meat sources with other sources, such as omega-3 PUFA from oily fish, this may also explain the unchanged TAG concentrations. However, neither of these possible dietary changes can be confirmed or denied until analysis of the food diaries is complete. Previous work by Turnbull et al., (1990 and 1992) has produced mixed TAG responses when replacing meat with mycoprotein-containing products. Although Turnbull et al. (1990) demonstrated a significant reduction in TAG levels when replacing meat with mycoprotein-containing products, the unchanged TAG concentrations seen in the current trial are similar to Turnbull et al. (1992) where no changes were observed. The significant reduction in the Turnbull et al. (1990) trial was suggested to be “due to the nutritionally balanced diet” given to the volunteers.

The significant LDL reduction of 9.7% for the whole cohort confirms the findings from previous trials (Holloway, 2014, Turnbull et al., 1990 and 1992). Holloway (2014) designed a meat reduction trial which took place in a smaller cohort and resulted in a 10.4% reduction in LDL cholesterol. The studies by Turnbull et al. (1990 and 1992) also demonstrated significant reductions in LDL cholesterol (9% and 22% respectively) when replacing meat with mycoprotein-containing alternatives. After further investigation into gender differences in the current trial, the significant reduction appears to be due to the male population of the group, which showed a significant reduction of 16.4% over the intervention period. Meat consumption is associated with SFA intake, therefore by removing the meat from the diet, SFA intake will likely be reduced, and PUFA intake increased, which could explain the reduction in LDL cholesterol. However, this cannot be determined until analysis of the diet diaries is complete. As for the gender differences, males had higher baseline LDL cholesterol levels than the females, and the greatest reduction in LDL cholesterol levels were seen in those with higher levels at baseline. The reason for the lack of reduction in the female population may be due to their baseline LDL cholesterol levels being too low to produce a response to the intervention. Another possibility is perhaps the females maintained their SFA intake through other food choices, whereas males may have consumed a healthier diet throughout the intervention. Analysis of the food diaries will investigate this further. Also, perhaps women's lipid control may be more influenced by internal mechanisms, such as sex hormones, than males.

The HDL cholesterol response in the current trial confirms findings from previous work (Holloway, 2014; Turnbull et al., 1992). Although HDL cholesterol did not change over the intervention period, women consistently demonstrated significantly higher HDL cholesterol levels throughout the trial than males. As previously mentioned above, this may be due to the oestrogen in premenopausal women protecting the HDL from oxidation (Bittner, 2001). There is recent evidence suggesting that the dietary carnitine content of meat may have an effect on reducing HDL (Koeth et al., 2013); however, the HDL cholesterol response to meat reduction in the current trial suggests that this is not the case.

### 4.27.3 Insulin Sensitivity

Although HOMA showed no significant change over the intervention period, insulin resistance did appear to be increased at visit 3. When analysed using the QUICKI method, the reduction in insulin sensitivity at visit 3 was shown to be significant. This was surprising; why was insulin sensitivity decreasing at visit 3 then improving back to baseline at visit 4? Fasted blood samples were taken at around 1100 – 1200 hrs, following FMD, at each visit except for visit 3. FMD was measured on a subset of volunteers at visits 1, 2 and 4. Although every effort was made to take the fasting blood sample at V3 at the same time as other visits, fasted blood samples at visit 3 were measured as early as 0900 hrs in some cases. Perhaps the different response at visit 3 is down to the volunteers not being as fasted as they were at the other visits. To test this theory, the HOMA and QUICKI scores from the volunteers that did not participate in the FMD (n = 12) were analysed. Although the pattern of change in this smaller cohort looked similar to that of the whole, results from these volunteers showed no significant change in indices of insulin sensitivity throughout the intervention; however, the sample size of the no-FMD cohort was very small, this may be the reason for the removed effect. There was no reason to expect a reduction in insulin sensitivity (or increased insulin resistance) throughout the intervention, as literature suggests that meat reduction produces an increase in insulin sensitivity (Bottin et al., 2016; Turnbull and Ward, 1995); however this was not observed in the current trial.

### 4.27.4 Anthropometry

The slight, but significant, increase in weight at visit 2 theoretically may be due to a change in eating behaviour during the control period, e.g. volunteers may be consuming all of the meat products in their freezers before starting the intervention to make space for the replacement products, or just to make the most of eating meat while they could. Food diary analysis will help assess changes in eating habits over the control period. Generally, ready-to-eat meat-replacement products, such as Quorn™, tend to contain less fat and lower energy than the meat versions. The fact that volunteers' weight was maintained throughout the intervention is a positive finding, as this indicates that the reduction in LDL cholesterol which occurred over the intervention was not due to caloric intake, but rather as a result of the type of food they were consuming. Although BF%, when measured with callipers, was significantly higher at visit 1 compared with other visits, this finding was not replicated in the BIA BF% data. The technicians taking the BF measurements with callipers were new to the technique, so this significant difference may be due to the skill of the technician improving over the study period. Measurement of BF% with BIA supports this theory as there was no significant change over the control period. Both BIA BF% and BF% measured with callipers was consistently significantly higher in females throughout control and intervention periods. This is expected as females in general have a higher BF% than males (Taylor et al., 1997).

### 4.27.5 Blood Pressure

Data from the intervention trial reflects the current understanding that BP is generally higher in males than premenopausal females (Reckelhoff, 2001; Benetos et al., 1999; Convertino, 1998). Although the exact mechanisms responsible for the gender differences in the regulation of BP remain unclear, there is evidence that sex hormones play an important role (Lopez-Ruiz et al., 2008; Reckelhoff, 2001). Studies monitoring BP regulation in children have shown that BP increases with age in both genders. However, after the onset of puberty, males have higher BP than age-matched females (Bachmann et al., 1987; Harshfield et al., 1994). This could be due to the male sex hormones, such as testosterone, increasing the development of hypertension in some way; or the female sex hormones, such as oestrogen, having a protective effect on BP regulation (Reckelhoff, 2001). What is interesting is that once females reach menopause, this protective effect disappears; which may be due a reduction of circulating oestrogen. Oestrogen has been shown to stimulate the production of the powerful vasodilator, NO. It is possible that, when females reach menopause and their oestrogen levels decrease, along with the production of NO, an increase in BP occurs (Reckelhoff, 2001). Although BP was higher in males than females, overall there was no significant change throughout the intervention. However, all volunteers in the current trial were very healthy with all baseline characteristics within the normal range; therefore the lack of change in BP was not unexpected.

### 4.27.6 Flow Mediated Vasodilation

When analysing the entire cohort, FMD showed no change over the intervention period. In agreement with previous studies (Gavin et al., 2009; Sader and Celermajer, 2002), females showed a higher response than males at all time points throughout the intervention. It has been suggested that oestrogen promotes the production of NO, resulting in lower BP in females than males, thereby displaying that females have a 'healthier' cardiovascular profile than males (Reckelhoff, 2001). Since FMD has not been used in chronic intervention studies to investigate the effects of meat reduction on this variable, it was an exploratory method to use in this instance. This trial indicated, in this relatively small cohort, meat reduction had no effect on endothelial function, as measured by FMD.

### 4.27.7 Quality of Life

Results show that meat reduction has no effect on physical, psychological or environmental QOL. However, this trial demonstrates that meat reduction may affect social relationships. This may be due to the structure of the trial; volunteers wanted to ensure they complied with rules of the trial and therefore struggled to make decisions, when out with friends and family, on food choices. The effect on social relationships may also be a result of the friends and family of the volunteers being large meat consumers; therefore, meals with friends and relatives may be primarily meat-based.

### 4.27.8 Discussion

The present trial demonstrated that it is possible to successfully reduce meat consumption, which resulted in reduced LDL cholesterol in males. Data from the post-intervention FFQ and QOL surveys showed that the majority of the volunteers found this transition into a meat-reduced diet relatively easy, though meat reduction may have an effect on social relationships. The fact that volunteers maintained their weight, BF and BP throughout the intervention suggests the effects seen on LDL cholesterol were not confounded by physiological changes. It is of note that, in general, the volunteers in this trial were metabolically healthy and all CVD risk factors tended to be in optimum range. Despite this, there was a decrease in LDL cholesterol and this was primarily seen in males who presented with higher baseline levels. There was no evidence of any impact on insulin sensitivity or improvements in specific factors associated with metabolic syndrome (TAG, HDL or BP). Again, this may be a reflection of the metabolic health of the volunteers at the start of the trial. A follow-up study in a group of metabolically-challenged' individuals may present more specific changes in risk factors associated with insulin sensitivity. Many of the suggestions in this results section rely on analysis of the food diaries. The food diaries will provide important insights into how volunteers reduced their meat intake (reduced portion sizes, meat-free days, blended products) throughout the intervention, which may have impacted the results. The food diaries will also allow analysis of the impact of dietary changes on

carbon footprint, which could potentially aid in the development of strategies for global meat reduction.

# Chapter 5 - General Discussion

## 5.1 INTRODUCTION

Red and process meat has been a significant dietary component of populations around the world for thousands of years, supplying high quality protein with low carbohydrate, essential amino acids, and important micronutrients. Over the last fifty years, as a result of population growth, urbanisation, and rising income in developing countries, the demand for meat and other animal products has increased worldwide (Givens, Kliem et al 2006).

It is well established that diets rich in SFA have the capacity to raise LDL cholesterol concentrations and thereby CVD risk. While it is less certain what SFA should be replaced with, a number of systematic reviews have reported that increasing dietary intake of PUFA may be more beneficial than replacement with carbohydrate (Hooper et al., 2011; Kuipers et al., 2011; Micha and Mozaffarian, 2010; Siri-Tarino et al., 2015). Meat, particularly red and processed meat, represent a major source of SFA with products derived from ruminant animals (cattle, sheep, goats) being relatively poor in PUFA (Salter, 2012). This research, along with the new evidence of the impact of red, and particularly processed, meat consumption on cancer risk (section 1.3.3), has led to most national public health nutrition agencies suggesting a reduction in the consumption of red and processed meat. Thus, there is a growing consensus that those individuals consuming excessive

amounts of red and processed meat would see significant benefits of moderating intake.

In parallel to the health concerns related to meat consumption, there is a growing recognition that the predicted global increase in animal product consumption associated with population growth, increased life expectancy and improved economic status of many developing countries will place unsustainable demands on the environment which is also being challenged by the impact of climate change. Animal agriculture is relatively inefficient in terms of land and water use and makes a significant contribution to GHG emissions, and therefore, climate change (FAO, 2006).

Despite all of the above, red and processed meat still represents a major dietary component of the worldwide population and in many emerging economies consumption is growing significantly (Reisch et al., 2013). By contrast, there is evidence to suggest that in developed countries consumers are aware of the health, and to a lesser extent environmental impacts of excessive meat consumption. In a project linked to the meat reduction trial described in chapter 4 an on-line survey indicated that up to a third of respondents were actively trying to reduce meat consumption, largely on the basis of reducing weight and other health effects (Penny et al, 2015). The study also showed that meat replacement products, such as Quorn™ and soya, are an acceptable alternative to many individuals trying to reduce meat

consumption. Another linked project showed that in blind tests, blended products containing Quorn™ or soya together with meat were well accepted in terms of flavour and sensory properties (Neville et al., n.d.).

The aims of the current study were to:

1. Directly compare the impact of meat and Quorn™-based meals on postprandial glycaemia and lipaemia.
2. To investigate whether, when provided with a range of non/reduced meat alternatives and motivational support, a group of free-living individuals could reduce their red and process meat intake by 50% over a 3 month period.
3. To determine whether such a reduction would have specific benefits in terms of reducing CVD risk.

## 5.2 POSTPRANDIAL LIPIDS

A previous study, on a group of students at the University of Nottingham, demonstrated that a fifty percent reduction in meat consumption over a four week period reduces total plasma cholesterol and TAG concentrations. As the majority of meat-replacement products consumed throughout the intervention were Quorn™ products, the following questions were raised. What was it about the meat reduced diet that produced these beneficial effects on the lipid profiles? Was it something in the Quorn products themselves, or was it just a result of removing meat from the diet? Although longer term intervention trials, using Quorn™ products, have shown to produce beneficial effects on cardiovascular risk (Ruxton and McMillan, 2010; Turnbull et al., 1990 and 1992; Holloway, 2014), to date there are no published reports of changes to postprandial lipaemia when replacing meat with Quorn™. There is a growing recognition that in addition to fasting lipid concentrations, postprandial TAG response may represent important risk factors for CVD (Jackson et al., 2012).

The data in this thesis clearly show that replacing a meat meal with a macronutrient-matched Quorn™ meal has no effect on postprandial lipids, glucose, insulin, or satiety. Contrary to these results, previous studies have demonstrated that Quorn™ is more satiating than meat (Turnbull et al., 1992 and 1993). However, further investigation showed that neither of the previous studies matched the fibre content of the meals.

The two postprandial studies presented in this thesis carefully matched meals in terms of macronutrient content. This was done so as to investigate whether there were specific properties associated with the protein, or fibre, content of Quorn™ which would impact on postprandial responses. It should be noted that, in general, many meat substitute products contain less calories, total and saturated fat, and more PUFA than equivalent meat products, in which case one would expect the postprandial glycaemic and lipaemic response to differ. It should also be noted that the makeup of the Quorn™ products used in the breakfast trial was not taken into consideration. Investigation showed that although the Quorn™ sausages are made up of very similar ingredients to meat sausages (containing seasoning and rusk), the Quorn™ sausages do also contain rehydrated egg white. Perhaps this added ingredient has an effect on the metabolization of the sausages and therefore a potential impact of results. While the volunteers were asked to fast overnight (from 2200 hrs), for both postprandial trials, it is possible that the timing, size and composition of the previous evening meal did have some impact on the postprandial response to the test breakfast meal. It seems less likely that this could be the case with the lunchtime meal which was consumed considerably longer after the previous evening meal and following a standard breakfast.

Power calculations are undertaken prior to recruitment in order to decide the minimum number of volunteers required to detect a significant difference (McCrum-Gardner, 2010). Without the use of a

power calculation the trial may be under powered which may result in true effects not being detected, or on the other hand, it may result in the production of statistically significant results that do not represent the true effect (Button et al., 2013). Power calculations were not incorporated into either postprandial trials. This was primarily because no preliminary data was available on which to base such analysis. Based on the data collected, if a trend toward a significant effect had been seen, a retrospective power analysis could have been performed to investigate the number of subjects required to reach statistical significance. A decision could have then been made whether to extend the studies to achieve a significant outcome. However, as in both cases, based on the postprandial increases in chylomicron TAG, there was no clear trend toward a significant difference between the two meal types it was felt unnecessary to conduct such an analysis.

Although the data from the postprandial trials were analysed via repeated measures ANOVA, incremental area under the curve (iAUC) may have also been used. iAUC is a beneficial analysis technique for such postprandial studies because it reflects the total changes in the parameter of interest (Venn and Green, 2007; Carstensen et al., 2003). The iAUC of the two test meals can then be statistically analysed. If the data had indicated a trend towards significant differences in response to the meals this analysis would have been performed. However, as the postprandial responses to the meals were essentially identical it was decided unnecessary to complete this further analysis.

## 5.3 MEAT REDUCTION INTERVENTION TRIAL

The third study described in this thesis investigated whether prolonged reduction in red and processed meat consumption would have beneficial effects of CVD risk factors. It was also interesting to see if the volunteers continued on a reduced meat diet following the completion of the trial, or if they reverted back to diets high in red and processed meat.

Weight loss is often associated with a reduction in red and processed meat consumption due to the reduced fat, and thus caloric intake. The meat reduction trial required volunteers to maintain the same caloric intake throughout the intervention as in the control period to ensure the results were associated specifically with meat reduction and not due to changes in caloric intake and resulting weight loss. To facilitate the maintenance of caloric intake, the meat replacement products provided to the volunteers during the intervention were produced larger in size than the products normally purchased from the supermarket.

The reduction in LDL and total cholesterol concentration was shown to be associated with the males, who tended to have higher levels of LDL cholesterol at baseline. This was an interesting finding and raises questions as to why this happened. As mentioned in section 4.27.2, perhaps this finding is a result of the baseline LDL cholesterol levels. Those volunteers with higher LDL cholesterol levels to start, which were the males, appeared to have the largest decrease throughout the

intervention. It is possible that in this healthy cohort, the females' baseline LDL cholesterol levels were already too low to produce a significant change over the intervention period. However, the results may also be due to the types of food the volunteers were consuming throughout the intervention. Analysis of the food diaries will be able to tell us more about food consumption throughout the trial.

As seen from the comments above, some of the data was presented by gender. This was done by using a one-way mixed model ANOVA with gender as Between Subject Factor. Although difficult in this particular trial, as there are repeated measures involved, the first instance analysis ideally would have been a two-way ANOVA with gender and visit as factors to investigate if there was an interaction between gender before comparing males and females. It is important that the correct analyses are undertaken because the more statistical tests that are performed, the more likelihood there is of finding a significant difference by chance.

The results from this intervention demonstrate that with the appropriate support, it is possible to reduce red and processed meat consumption by at least half, and in some individuals this results in reduced LDL cholesterol concentrations. As previously mentioned, compliance was assessed by conversing with the volunteers during each assessment visit. However, perhaps a more systematic approach to measuring compliance would have been more suitable. The use of tick sheets,

where volunteers note what food products they have consumed each day, or a post-trial verbally administered questionnaire would have been more appropriate. As mentioned in section 4.27.1, all volunteers were healthy and had fasting lipid levels in the optimum range. So the changes that have been observed in this cohort are a positive sign. It would be interesting to see what the outcome of the same trial would be in a population displaying plasma cholesterol values which would place them at a more significant risk of developing CVD. The study also showed that a meat-reduced diet was generally accepted to this group of regular meat consumers. Anecdotally, volunteers used a range of techniques to reduce their meat intake including reduced portion sizes, meat-free days and use of meat replacers and/or beans and pulses. Although the volunteers were given instructions on how to reduce their meat intake at the screening visit, a future study should include a script to be cited to the volunteers explaining exactly what is required of them with detail about how to reduce their meat intake. This will ensure that if other investigators become involved in the trial consistency will be maintained with all volunteers. Future analysis of diet diaries from these volunteers may provide some evidence of the relative efficacy of these strategies in reducing LDL cholesterol. It will also allow an analysis of the potential environmental impacts of such dietary changes.

## 5.4 FUTURE WORK

There is still further work to be done on the meat reduction trial presented in this thesis. Analysis of the food diaries will provide information on the replacement products that the volunteers used throughout the intervention. The food diaries will also be useful for examining the macronutrient content of the volunteers' diets. Data can be used to determine which food groups were the most prominent before and during the intervention. Were volunteers replacing red and processed meat with other meat or animal products such as poultry, fish and cheese, or were they using plant-based alternatives? This will also give insight into the acceptance of the plant-based meat alternatives in this cohort, which can, in turn, give guidance to future product development or future strategies on changing consumer behaviour towards meat reduction. If it can be shown that the volunteers accepted and enjoyed the novel meat-reduced and meat-free products that were designed for the trial, perhaps there should be further steps taken to develop these products on a larger scale and promote them as a way to reduce red meat consumption without having to completely cut meat out of the diet. The intervention trial presented in this thesis showed that the biggest reduction in serum cholesterol occurred in those volunteers with higher baseline cholesterol levels. Therefore, future work outside of this thesis should assess the effects of meat reduction on CVD risk in a much larger cohort of 'metabolically challenged' volunteers, as this is the population at the greatest risk of developing diseases such as T2DM and CVD. Although the postprandial trials presented in this thesis

showed no significant differences when comparing meals of the same macronutrient content, it may be interesting to investigate the effects of a mycoprotein meal on a second non-mycoprotein meal using a bottomless bowl technique. Participants would be presented with a bowl of food, and it would be refilled when approximately one third remained, in order to avoid the empty bowl acting as a cue to finish the meal. As mentioned in section 1.6.4 mycoprotein has been shown to have a more favourable fatty acid profile than meat, therefore it would also be interesting to compare the effects of a mycoprotein-containing lunch with a meat-containing lunch of realistic portions and not matching macronutrient content.

## 5.5 OVERALL CONCLUSION

1. Replacement of meat with mycoprotein, in a meal balanced for macronutrient content, produces similar postprandial responses.
2. With appropriate support, regular red and processed meat consumers can significantly reduce their intake for a prolonged period of time.
3. Such changes may have beneficial effects on LDL cholesterol, particularly in those with higher baseline levels.

These results suggest that the development of novel meat-free and meat-blended products represents a viable commercial opportunity that may be popular with a growing number of consumers who are actively trying to reduce their meat consumption. While at the present time most of this consumer concern is being driven by health concerns, with increased public awareness, concerns relating to the environmental impacts of meat production may lead to further increases in this potential market.

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# APPENDICES

**Appendix A:** Conversion tables to calculate WHOQOL scores, as described in **Chapter 1**.

| Domain 1 | | | Domain 2 | | | Domain 3 | | | Domain 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Raw Score | Transformed scores | | Raw Score | Transformed scores | | Raw Score | Transformed scores | | Raw Score | Transformed scores | |
| | 4-20 | 0-100 | | 4-20 | 0-100 | | 4-20 | 0-100 | | 4-20 | 0-100 |
| 7 | 4 | 0 | 6 | 4 | 0 | 3 | 4 | 0 | 8 | 4 | 0 |
| 8 | 5 | 6 | 7 | 5 | 6 | 4 | 5 | 6 | 9 | 5 | 6 |
| 9 | 5 | 6 | 8 | 5 | 6 | 5 | 7 | 19 | 10 | 5 | 6 |
| 10 | 6 | 13 | 9 | 6 | 13 | 6 | 8 | 25 | 11 | 6 | 13 |
| 11 | 6 | 13 | 10 | 7 | 19 | 7 | 9 | 31 | 12 | 6 | 13 |
| 12 | 7 | 19 | 11 | 7 | 19 | 8 | 11 | 44 | 13 | 7 | 19 |
| 13 | 7 | 19 | 12 | 8 | 25 | 9 | 12 | 50 | 14 | 7 | 19 |
| 14 | 8 | 25 | 13 | 9 | 31 | 10 | 13 | 56 | 15 | 8 | 25 |
| 15 | 9 | 31 | 14 | 9 | 31 | 11 | 15 | 69 | 16 | 8 | 25 |
| 16 | 9 | 31 | 15 | 10 | 38 | 12 | 16 | 75 | 17 | 9 | 31 |
| 17 | 10 | 38 | 16 | 11 | 44 | 13 | 17 | 81 | 18 | 9 | 31 |
| 18 | 10 | 38 | 17 | 11 | 44 | 14 | 19 | 94 | 19 | 10 | 38 |
| 19 | 11 | 44 | 18 | 12 | 50 | 15 | 20 | 100 | 20 | 10 | 38 |
| 20 | 11 | 44 | 19 | 13 | 56 | | | | 21 | 11 | 44 |
| 21 | 12 | 50 | 20 | 13 | 56 | | | | 22 | 11 | 44 |
| 22 | 13 | 56 | 21 | 14 | 63 | | | | 23 | 12 | 50 |
| 23 | 13 | 56 | 22 | 15 | 69 | | | | 24 | 12 | 50 |
| 24 | 14 | 63 | 23 | 15 | 69 | | | | 25 | 13 | 56 |
| 25 | 14 | 63 | 24 | 16 | 75 | | | | 26 | 13 | 56 |
| 26 | 15 | 69 | 25 | 17 | 81 | | | | 27 | 14 | 63 |
| 27 | 15 | 69 | 26 | 17 | 81 | | | | 28 | 14 | 63 |
| 28 | 16 | 75 | 27 | 18 | 88 | | | | 29 | 15 | 69 |
| 29 | 17 | 81 | 28 | 19 | 94 | | | | 30 | 15 | 69 |
| 30 | 17 | 81 | 29 | 19 | 94 | | | | 31 | 16 | 75 |
| 31 | 18 | 88 | 30 | 20 | 100 | | | | 32 | 16 | 75 |
| 32 | 18 | 88 | | | | | | | 33 | 17 | 81 |
| 33 | 19 | 94 | | | | | | | 34 | 17 | 81 |
| 34 | 19 | 94 | | | | | | | 35 | 18 | 88 |
| 35 | 20 | 100 | | | | | | | 36 | 18 | 88 |
| | | | | | | | | | 37 | 19 | 94 |
| | | | | | | | | | 38 | 19 | 94 |
| | | | | | | | | | 39 | 20 | 100 |
| | | | | | | | | | 40 | 20 | 100 |

**Appendix B:** Breakfast trial approval letter from the Medical School Research Ethics Committee as mentioned in **Chapter 2**.

Direct line/e-mail
+44 (0) 115 8231063
Louise.Sabir@nottingham.ac.uk

The University of Nottingham
**Faculty of Medicine and Health Sciences**

Medical School Research Ethics Committee
Division of Therapeutics & Molecular Medicine
D Floor, South Block
Queen's Medical Centre
Nottingham
NG7 2UH

Tel: +44 (0) 115 8231063
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6th March 2013

Dr Fiona McCullough
Associate Professor
Division of Nutritional Sciences
School of Biosciences
University of Nottingham
Sutton Bonington

Dear Dr McCullough

**Ethics Reference No: E14022013 SBS Nutrition**
**Study Title:** Impact of mycoprotein on Postprandial lipidaemia.
**Lead Investigator:** Marie Clark, MRes student, Nutritional Sciences, School of Biosciences.
**Chief Investigator/Supervisor:** Professor Andrew Salter and Fiona McCullough Lecturer, Nutritional Sciences, School of Biosciences, Sutton Bonington Campus
**Duration of Study:** 14/3/2013-14/6/2012 4mths **No of Subjects:** 12

Thank you for your letter dated 27th February 2013 responding to the issues raised by the committee and enclosing the following revised documents:

- Impact of Mycoprotein study: Application form version 2.0:27.02.2013
- Impact of Mycoprotein study: Experimental Protocol version 2.0: 27.02.2013
- Impact of Mycoprotein study: Information Sheet version 2.0: 27.02.2013
- Impact of Mycoprotein study: Consent form version 2.0:27.02.2013
- Impact of Mycoprotein study: Poster version 2.0:27.02.2013

The following documents were previously reviewed:

- Impact of Mycoprotein study: Application form version 1.0:30.01.2013
- Impact of Mycoprotein study: Experimental Protocol version 1.0: 30.01.2013
- Impact of Mycoprotein study: Information Sheet version 1.0: 30.01.2013
- Impact of Mycoprotein study: Consent form version 1.0:30.01.2013
- Impact of Mycoprotein study: Poster version 1.0:30.01.2013

These have been reviewed and are satisfactory and the study is approved.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

**Conditions of Approval**

You must follow the protocol agreed and any changes to the protocol will require prior Ethics' Committee approval. Please use the attached notification of amendment form.

**Appendix C:** Poster used to advertise the Breakfast trial in **Chapter 2**.

THE UNIVERSITY OF NOTTINGHAM
SUTTON BONINGTON CAMPUS

## MALE AND FEMALE VOLUNTEERS WANTED

## IMPACT OF MYCOPROTEIN ON POSTPRANDIAL LIPIDAEMIA

Want to find out if replacing meat products with mycoprotein–containing meat substitutes can improve your health?

Take part in a study that provides you with two full cooked breakfasts!

Breakfast one: Pork Sausages, Bacon, Hash Browns, Baked Beans, Mushrooms, Fried eggs, High fibre Cracker
Breakfast two: Meat alternative Sausages and Bacon, Hash Browns, Baked Beans, Mushrooms, Scrambled Eggs

Blood samples will be taken every 30 minutes for up to 6 hours after each meal and used to determine plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose levels.

Volunteers must be aged between 18 - 35 and have a BMI between 18 - 26

INCONVENIENCE ALLOWANCE OFFERED

Want to find out more???

Contact: Marie Clark
Email: stxmc8@nottingham.ac.uk

## **Appendix D:** Information sheet from the breakfast trial, supplied to volunteers as described in **Chapter 2**.

**UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL ETHICS COMMITTEE**
**Information Sheet for Normal Health Volunteers**

**University of Nottingham School of Biosciences**
**Division of Nutritional Sciences**
**Sutton Bonington Campus**
**Loughborough**
**Leicestershire**
**LE12 5RD**
**Impact of Mycoprotein on Postprandial Lipidaemia**
**Name of Investigators: Marie Clark, Dr. Fiona McCullough and Prof. Andrew Salter**

**Healthy Volunteers Information Sheet**
You have been invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part or not. If you wish to take part, you may keep this leaflet. Thank you for reading this.

**Background**
It has become increasingly clear that diet has a major impact on life-long health and wellbeing. Thus, it is vital to consume a balanced diet consisting of an appropriate balance of carbohydrates, protein, fat, vitamins, minerals, salts and fibre in the correct proportions to maintain an appropriate bodyweight and minimize risk of developing chronic diseases such as cardiovascular disease (CVD) and diabetes..
The present study will investigate the changes in CVD risk factors associated with consuming a different type of protein (mycoprotein) than meat. Mycoprotein is a commonly used ingredient present in a wide range of meat alternatives sold widely through the UK and many other countries. The volunteers will be asked to consume two meals (cooked breakfast). One meal will contain meat (bacon and sausages) and the other mycoprotein- containing meat alternatives. Meals will be balanced in terms of total fat, fatty acid composition, carbohydrate and total protein content. Blood samples will be taken every 30 minutes for up to 4 hours after the meal. Samples will be used for determining plasma lipids and lipoproteins, insulin and glucose.

**What does the study involve?**
The study involves completing a short initial form to indicate willingness to participate.

**Why have you been chosen?**
The study will include healthly, normal weight, University staff/students of both sexes ages 18-35 years and all ethnic backgrounds. You have been randomly chosen as a member of one of these groups.

**Do you have to take part?**
It is up to you whether or not you choose to take part. If you do choose to take part, you will be given this information sheet to keep and asked to complete a consent form. If you decide to take part, you are free to withdraw at any time and without giving a reason.

**What do I have to do?**

1) Attend a screening process prior to the study where we will assess your height, weight, body mass index, blood pressure and heart rate. A blood sample (8ml) will be taken via the anticubital vein (inside of elbow) to assess for blood glucose, full blood count, urea and electrolytes.
2) Consume two cooked breakfasts, one containing meat and the other mycoprotein-containing meat alternatives.
3) Provide blood samples for analysis

Blood samples will be obtained by venepuncture in the screening process and via a cannula during the experimental visits by a trained and experienced person. There can be some slight discomfort when the needle enters the skin, and on rare occasions you may be left with some bruising. Approximately 15 ml of blood will be obtained every 30 minutes for up to 6 hours each study day, totalling less than 500ml for the entire study (including 8ml on the screening visit). A small amount of local anaesthetic will be injected under the skin before each cannula is inserted. This may sting a little, but almost immediately will make

the area numb. You may feel a movement under the skin when the cannula is inserted, but you should not experience any discomfort. All procedures are carried out by experienced personnel routinely within our research group and are audited in all of our studies.

If you do suffer any other symptoms or side-effects you should report them next time you meet. If you become in any way concerned prior to your next visit or after the study has finished you should contact the lead investigator (see end of sheet). If for any reason you are excluded from the study following your medical screening, you shall be informed of the reason straight away. The blood sample is not screened for any diseases or viruses.

**What are the possible disadvantages and risks of taking part?**
You may be trying unfamiliar foods. While attending the exercise will inevitably take some of your time, it has been kept as short as possible to minimize any inconvenience incurred.
Taking a blood sample my cause minor discomfort in the form of a sharp scratch when taking blood and may result in some bruising afterwards. If you do have any concerns please contact: Marie Clark on 07790735870 or email stxmc8@nottingham.ac.uk

**What if something goes wrong?**
In case you have a complaint about your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator, Marie Clark. If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary, Ms. Louise Sabir, Division of Therapeutics and Molecular Medicine, D Floor, South Block, Queen's Medical Centre, Nottingham, NG7 2UH. Telephone 0115 8231063. Email louise.sabir@nottibgham.ac.uk.

**Will my taking part in this study be kept confidential?**
The information will be available only to research investigators.

**What will happen to the results of the research study?**
The findings will be published in aMRes thesis and may also be published in a scientific journal article

**Who has reviewed the study?**
The study has been reviewed by the University of Nottingham Ethics Committee.

**Contact for further information**

Dr. Fiona McCullough RD, RPHNutr, PhD
Division of Nutritional Sciences, University of Nottingham
Sutton Bonington, LE 12 5RD
Fiona.Mccullough@nottingham.ac.uk
Telephone 0115 9516118
Fiona.Mccullough@nottingham.ac.uk

Professor Andrew Salter
Professor of Nutritional Biochemistry
School of Biosciences,
University of Nottingham,
Sutton Bonington Campus
Loughborough
Leicestershire, LE12 5RD

Tel 44(0)115 951 6120
Fax 44 (0) 0115 951 6122
andrew.salter@nottingham.ac.uk

Thank you for volunteering to take part in this study. You will be given a copy of the information sheet and signed consent form to keep.

Marie Clark
stxmc8@nottingham.ac.uk

**Appendix E:** Screening questionnaire, used in all three trials, as mentioned in **Chapters 2, 3 and 4**.

PERSONAL INFORMATION

Volunteer Number _____ Date ___/____/___

1. **Name** ……..

2. **Contact Address** …….....

3. **Contact Telephone number** .....……...

4. **Email address** ……..

5. **Date of Birth** ……./……./ 19…….

GENERAL HEALTH

1. In general would you say your health is;
**Please tick one response**

a. ☐ *Excellent*
b. ☐ *Very good*
c. ☐ *Good*
d ☐ *Fair*
e. ☐ *Poor*

2. Compared to one year ago, how would you rate your health in general now?
**Please tick one response**

a. ☐ *Much better than a year ago*
b. ☐ *Somewhat better than a year ago*
c. ☐ *About the same*
d. ☐ *Somewhat worse than a year ago*
e. ☐ *Much worse than a year ago*

3. The following questions are concerned with any medical conditions you might have. Do you suffer from any of the following?

**Please tick one box on each line**

| | ***No*** | ***Yes*** | ***Don't Know*** |
|---|---|---|---|
| **Diabetes** | | | |
| **Epilepsy** | | | |
| **Depression** | | | |
| **Hayfever** | | | |
| **Asthma** | | | |
| **Eczema** | | | |
| **High blood pressure** | | | |
| **Heart problems** | | | |
| **Breathing problems *** | | | |
| **Indigestion / heartburn** | | | |
| **Irritable bowel Syndrome** | | | |
| **Any other medical condition not listed above (please detail below)** | | | |

*** other than asthma**

Please detail any other medical condition/s
……
……

If you answered '*yes*' to any of the above, when did you first start having the problem and what medication (if any) do you use?
……
……
……

4. Have you had any operations in the last year?
If '*Yes*, please detail;
……
……

5. Have you ever had any operations on your stomach/ abdomen?
If '*Yes*, please detail;
……
……

6. **Do you have a tendency to faint in certain situations?** ☐ ***Yes*** ☐ ***No*** ☐ ***Don't Know***

eg. on standing, in a warm room, after fasting

DIET AND HEALTH

1. For whatever reason, do you **avoid eating** any of these foods?

**Please tick all boxes that apply**

| | *No* | *Yes, Don't like* | *Yes, for health reasons* | *Yes, allergic* | *Yes, for ethical reasons* | *Yes, for religious reasons* |
|---|---|---|---|---|---|---|
| **Red meat eg beef, lamb, pork** | 0 | 1 | 2 | 3 | 4 | 5 |
| **White meat eg chicken** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Fish** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Eggs** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Dairy products eg milk, cheese, yoghurt** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Wheat products eg flour, bread, pasta, cereals** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Fried food, crisps, pastry, chips etc** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Cakes, biscuits, sweets** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Chocolate** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Tea / coffee/cola** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Nuts eg Peanuts** | 0 | 1 | 2 | 3 | 4 | 5 |
| **Other, Please detail; …… ……** | 0 | 1 | 2 | 3 | 4 | 5 |

If you would like to include any other information, please use the space below;
……
……
……
……
……

The following questions relate to your diet and your health
**Please tick one box on each line**

| | | | | |
|---|---|---|---|---|
| a. | Would you consider yourself a **vegetarian**? ie eat no meat or fish, but eat dairy products | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |
| b. | Would you consider yourself a **vegan**? ie eat no meat or fish or dairy products | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |
| c. | Are you on **a medically prescribed diet**? eg diabetic, gluten-free etc | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |

If '*yes*', what diet are you following?
……..……
……..……

| | | | | |
|---|---|---|---|---|
| d. | Do you suffer from any **food allergies**? Eg dairy products, wheat, nuts | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |

If '*yes*', which foods are you allergic to?
……..……
……..……

| | | | | |
|---|---|---|---|---|
| e. | Do you regularly (at least once a week) take **vitamin** and / or **mineral** supplements | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |

If '*yes*', which supplements do you take?
……..……
……..……

| | | | | |
|---|---|---|---|---|
| f. | Do you regularly (at least once a week) take any other supplements for your health? Eg. Creatine, Carnitine, Echinacea, St John's Wort, etc | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |

If '*yes*', which supplements do you take?
……..……
……..……

| | | | | |
|---|---|---|---|---|
| g. | Are you currently 'dieting' to control your weight? | ☐ ***Yes*** | ☐ ***No*** | ☐ ***Don't Know*** |

**Have you taken part, or do you intend to take part in any other trials within the three months prior to or following this particular study?** ☐ ***Yes*** ☐ ***No***

PHYSIOLOGICAL MEASUREMENTS

1. **Height** ……cm
2. **Weight** ……kg
3. **BP** **……./…….** **Lying**
   **……./…….** **Standing (1min)**
   **……./…….** **Standing (3 min)**
4. **HR:** **……bpm**
5. **BMI:** **……..kg/m$^2$**

**Recruited?** ☐ **Yes** ☐ **No**

**Appendix F:** Consent form given to the volunteers from the breakfast trial, as seen in **Chapter 2**.

**CONSENT FORM**
**(Final version 1.0: 30/01/13)**

**Title of Study:** Impact of Mycoprotein on Postprandial Lipidaemia

**REC ref:** ***(to be added after approval given)***

**Name of Researcher**: Marie Clark

**Name of Participant:**

**Please initial box**

| | | |
|---|---|---|
| 1. | I confirm that I have read and understand the information sheet version number ……dated..... for the above study and have had the opportunity to ask questions. | ☐ |
| 2. | I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. | ☐ |
| 3. | I understand that relevant sections of data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. | ☐ |
| 4. | I understand and agree that a 15ml blood sample will be taken for analysis of plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose every 30 minutes for 6 hours. | ☐ |
| 5. | I understand that my GP may be informed if any of my blood tests show any significant abnormality. | ☐ |
| 6. | I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation. | ☐ |
| 7. | I voluntarily agree to take part in the above study. | ☐ |

| | | |
|---|---|---|
| Name of Participant | Date | Signature |
| Name of Person taking consent (if different from Principal Investigator) | Date | Signature |
| Name of Principal Investigator | Date | Signature |

2 copies: 1 for participant, 1 for the project notes.

**Appendix G:** Mid-day trial approval letter from the Medical School Research Ethics Committee as mentioned in **Chapter 3**.

The University of Nottingham

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

**Faculty of Medicine and Health Sciences**

Research Ethics Committee
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

13th August 2014

Marie Clark
PhD Student
c/o Professor Andrew Salter
Professor of Nutritional Biochemistry
Head of Division of Nutritional Sciences
School of Biosciences
Sutton Bonington Campus
University of Nottingham
Loughborough
LE12 5RD

Dear Marie

**Ethics Reference No:** C14082014 SoBS NS – please always quote
**Study Title:** Impact of Mycoprotein on Postprandial Lipemia.
**Short Title:** Mycoprotein Study
**Chief Researcher/Supervisors:** Professor Professor Andrew Salter Professor of Nutritional Biochemistry, Head of Division, Dr Fiona McCullough, Associate Professor, Nutritional Sciences, School of Biosciences, Sutton Bonington Campus
**Co Researchers/Student:** Marie Clark, PhD Student, Nutritional Sciences, School of Biosciences
**Duration of Study:** 1/09/14-01/01/15 3mths **No of Subjects:** 20-30

Thank you for the above application which has been reviewed by the Committee and the following documents were received:

Mycoprotein Study:

- Med Sch REC Application form, version 1.0, Date: 08/07/2014.
- Mycoprotein Study Protocol, Final Version 1.0 date 08/07/14
- Recruitment Poster, Final Version 1.0 Date 08/07/2014
- Participant Information Sheet Final Version 1.0, Date 08/07/2014
- Consent Form Final Version 1.0: 08/07/2014

These have been reviewed and are satisfactory and the study is approved.

**Appendix H:** Poster used to advertise the mid-day trial in **Chapter 3**.

THE UNIVERSITY OF NOTTINGHAM
SUTTON BONINGTON CAMPUS

MALE AND FEMALE VOLUNTEERS WANTED

IMPACT OF MYCOPROTEIN ON POSTPRANDIAL LIPIDAEMIA

Want to find out if replacing meat products with mycoprotein–containing meat substitutes can improve your health?

Take part in a study that provides you with two Spaghetti Bolognese meals!

Blood samples will be taken every 30 minutes for up to 6 hours after each meal and used to determine plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose levels.

Volunteers must be aged between 18 - 50 and have a healthy body fat % / BMI

INCONVENIENCE ALLOWANCE OFFERED

Want to find out more???

Contact: Marie Clark
Email: stxmc8@nottingham.ac.uk

## **Appendix I:** Information sheet from the mid-day trial, supplied to volunteers as described in **Chapter 3**.

Participant Information Sheet
Final version 1.0: 08/07/2014)

Title of Study: Impact of Mycoprotein on Postprandial Lipemia
Study ID – Mycoprotein Study

Name of Researcher(s): Professor Andrew Salter, Chief Researcher Dr Fiona McCullough, Research Supervisor and Marie Clark, PhD Research Student.

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

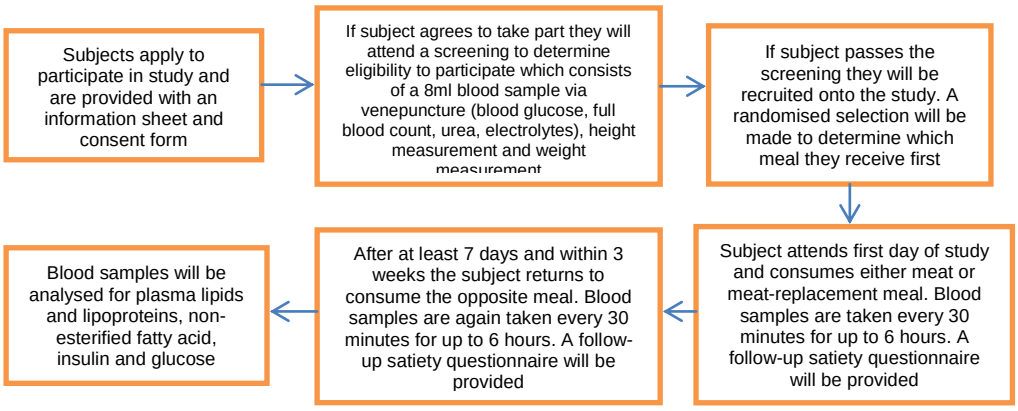
**What is the purpose of the study?**
It has become increasingly clear that diet has a major impact on life-long health and wellbeing. Thus, it is vital to consume a balanced diet consisting of an appropriate balance of carbohydrates, protein, fat, vitamins, minerals, salts and fibre in the correct proportions to maintain an appropriate bodyweight and minimize risk of developing chronic diseases such as cardiovascular disease (CVD) and diabetes.
The present study will investigate the changes in CVD risk factors associated with consuming a different type of protein (mycoprotein) than meat. Mycoprotein is a commonly used ingredient present in a wide range of meat alternatives sold widely through the UK and many other countries. The volunteers will be asked to consume two meals (Spaghetti Bolognese). One meal will contain meat (beef mince) and the other mycoprotein- containing meat alternatives. Meals will be balanced in terms of total fat, fatty acid composition, carbohydrate and total protein content. Blood samples will be taken every 30 minutes for up to 6 hours after the meal. Samples will be used for determining plasma lipids and lipoproteins, insulin and glucose.

**Why have I been invited?**
The study will include healthy, normal weight, University staff/students of both sexes ages 18-50 years and all ethnic backgrounds. You have been randomly chosen as a member of one of these groups. We are inviting 20-30 participants like you to take part

**Do I have to take part?**
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

**What will happen to me if I take part?**

1) Attend a screening process prior to the study where we will assess your height, weight, body fat %, body mass index, blood pressure and heart rate. A blood sample (8ml) will be taken via the anticubital vein (inside of elbow) to assess for blood glucose, full blood count, urea and electrolytes.
2) On the morning of trial day one, consume a standard breakfast provided by the investigators
3) Return at approx. 11.30am and consume a spaghetti bolognese meal containing either meat or the mycoprotein- containing meat alternative.
4) Provide 15 ml blood samples every 30 minutes for up to 6 hours for analysis.
5) Fill out a follow-up questionnaire regarding the satiety of the meal.
6) On the morning of trial day two (at least 7 days after trial day one), consume a standard breakfast provided by the investigators
7) Return at approx. 11.30am and consume a spaghetti bolognese meal containing either meat or the mycoprotein- containing meat alternative
8) Provide 15 ml blood samples every 30 minutes for up to 6 hours for analysis.
9) Fill out a follow-up questionnaire regarding the satiety of the meal.



Blood samples will be obtained by venepuncture in the screening process and via a cannula during the experimental visits by a trained and experienced person. There can be some slight discomfort when the needle enters the skin, and on rare occasions you may be left with some bruising. Approximately 15 ml of blood will be obtained every 30 minutes for up to 6 hours each study day, totalling less than 500ml for the entire study (including 8ml on the screening visit). A small amount of local anaesthetic will be injected under the skin before each cannula is inserted. This may sting a little, but almost immediately will make the area numb. You may feel a movement under the skin when the cannula is inserted, but you should not experience any discomfort. All procedures are carried out by experienced personnel routinely within our research group and are audited in all of our studies.

If you do suffer any other symptoms or side-effects you should report them next time you meet. If you become in any way concerned prior to your next visit or after the study has finished you should contact the lead investigator (see end of sheet). If for any reason you are excluded from the study following your medical screening, you shall be informed of the reason straight away. The blood sample is not screened for any diseases or viruses.

**Expenses and inconvenience allowance**
Participants will be paid an inconvenience allowance to participate in the study (£100). Travel expenses will not be offered for any visits incurred as a result of participation.

**What are the possible disadvantages and risks of taking part?**
You may be trying unfamiliar foods. Participating in the study will inevitably take up some of your time, it has been designed to be kept as short as possible to minimize any inconvenience.

Taking a blood sample my cause minor discomfort in the form of a sharp scratch when taking blood and may result in some bruising afterwards. If you do have any concerns please contact: Marie Clark via email stxmc8@nottingham.ac.uk

It is recommended that you should avoid donating more than 500 ml blood over a 6 month period and allow at least 3 months between participating in further studies which involves giving blood. If you are a regular blood donor you should also bear this in mind.

Exclusion criteria including the following:

If you have been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation.

**What are the possible benefits of taking part?**
The information we get from your participation in this study may help expand the knowledge on the benefits of reducing meat intake. The information collected may also explain specific benefits of mycoprotein containing meals and contribute to the existing pool of knowledge around this area.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you should then contact the FMHS Research Ethics Committee Administrator, School of Medicine Education Centre, B Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH. E-mail louise.sabir@nottingham.ac.uk."

**Will my taking part in the study be kept confidential?**
We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the institution will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept secure after the end of the study so that we are able to contact you about the findings of the study *and possible follow-up studies* (unless you advise us that you do not wish to be contacted). All research data will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

**What will happen if I don't want to carry on with the study?**
You participation is voluntary and you are free to withdraw at any time, without giving any reason.

You may be withdrawn from the study for:
- Safety reasons (including pregnancy or illness)
- Failure to adhere to protocol requirements
- Withdrawal of consent

You will be withdrawn from study if consent is withdrawn – it should be noted that data collected up to the point of withdrawal will not be destroyed because once the anonymised data has been entered into the databases on the University secure servers and back up servers it becomes impractical to extract and erase.

**Involvement of the General Practitioner/Family doctor (GP)**
You GP (or other health care practitioner) does not need to be notified of your participation. However if any of your blood samples show any significant abnormality your GP may be informed with your permission.

**What will happen to any samples I give?**
Samples will be used for determining plasma lipids and lipoproteins, insulin and glucose.

We would also like to seek your consent so that any remaining samples may be stored and used in possible future research – this is optional (please indicate you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the University of Nottingham in accordance with the Human Tissue Act (HTA) regulations (Licence no 12265).

Some of these future studies may be carried out by researchers other than current team of Prof. Andrew Salter, Dr. Fiona McCullough and Marie Clark, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and you will not be identified in anyway. If you do not agree to this any remaining samples will be disposed of in accordance with the Human Tissue Authorities codes of practice.

**What will happen to the results of the research study**
The findings will be published in a PhD thesis and may also be published in a scientific journal article.

**Who is organising and funding the research?**
This research is being organised by the University of Nottingham and is being funded by the University of Nottingham

**Who has reviewed the study?**
All research in the University of Nottingham is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by UoN Faculty of Medicine & Health Sciences Research Ethics Committee.

**Further information and contact details**
Professor Andrew Salter
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Fiona.Mccullough@nottingham.ac.uk
Telephone 0115 9516118

Marie Clark
stxmc8@nottingham.ac.uk

## **Appendix J:** Consent form given to the volunteers from the mid-day trial, as seen in **Chapter 3**.

**CONSENT FORM**
**(Final version 1.0: 08/07/2014)**

**Title of Study: Impact of Mycoprotein on Postprandial Lipemia**
**REC ref:** C14082014 SoBS NS
**Name of Researcher(s)**: Professor Andrew Salter, Chief Researcher Dr Fiona McCullough, Research Supervisor and Marie Clark, PhD Research Student.

**Name of Participant:** **Please initial box**

1. I confirm that I have read and understand the information sheet Final version number 1.0 dated 08/07/2014 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study but not my name. I understand that my personal details will be kept confidential. ☐
4. I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published and then destroyed securely. ☐
5. I understand and agree that a 15ml blood sample will be taken for analysis of plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose every 30 minutes for 6 hours. ☐
6. Consent for storage and use in possible future research (Optional)

I agree that the samples I have given and the information gathered about me can be stored by Professor Andrew Salter at the School of Biosciences, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Professor Andrew Salter, Fiona McCullough and Marie Clark, who ran the first study, including researchers working for commercial companies. Any blood samples or data used will be anonymised, and I will not be identified in anyway. ☐

7. I understand that I will be informed if any of my blood tests show any significant abnormality and my GP may be informed with my permission. ☐
8. I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation. ☐
9. I shall receive an inconvenience allowance of £100 for completing the study. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising investigator. ☐
10. I voluntarily agree to take part in the above study. ☐

| | | |
|---|---|---|
| Name of Participant | Date | Signature |
| Name of Person taking consent | Date | Signature |

**Appendix K:** List of recipes from the recipe book, provided to the volunteers in **Chapter 4** to aid compliance of meat reduction.

**Mince Recipes**



**Sausage Recipes**



**Meatball Recipes**



**Meat-free Recipes**

**Appendix L:** Poster advertising the intervention trial in **Chapter 4**.

THE UNIVERSITY OF NOTTINGHAM

MALE & FEMALE VOLUNTEERS WANTED

(COUPLES WELCOME)

# EAT LESS MEAT


Want to find out if replacing 50% of your red and/or processed meat intake can improve your health?

Take part in a study that provides you with free main ingredients and recipes ideas for meat free meals for 3 months!

Inclusion criteria:

- Aged 21-50 years
- BMI 18-28kg/m²
- Non-smoker
- Females must be premenopausal
- Good spoken and written English
- Habitual consumption of 4-5 portions of red and/or processed meat per week (3-4 of these being main meals)

Want to find out more???

Contact: Marie Clark
Email: stxmc8@nottingham.ac.uk

## **Appendix M:** Information sheet from the intervention trial, supplied to volunteers as described in **Chapter 4**.

Participant Information Sheet
Final version 2.1: 01/12/2015

Title of Study: Eat Less Meat
Study ID – A18062015 SoBS ELM

Name of Researcher(s): Professor Andrew Salter, Chief Researcher Dr Fiona McCullough, Research Supervisor Dr Liz Simpson, Research Supervisor, Marie Clark, PhD Research Student and Azlina A Razak, Research Student.

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

**What is the purpose of the study?**
Meat can represent an important part of a healthy diet, supplying high quality protein and a range of micronutrients. Red meat also plays a significant role in the intake of Omega-3 fatty acids, particularly in people that do not have a diet containing oily fish. However, excessive consumption, particularly of processed meat products, has been associated with increased risk of a range of chronic diseases including cardiovascular disease (CVD), type 2 diabetes and certain cancers. Reducing meat consumption by developing high quality and convenient meat alternative ingredients, products and hybrid meat/non meat products could have significant health benefits for consumers, a positive impact on the environment, build a sustainable food model and provide products that address consumer concerns over food safety in relation to animal produce. Preliminary work, at the University of Nottingham has identified some of the barriers faced in transitioning to a meat-reduced diet. Most 'meat alternatives' have been developed for the vegetarian market and are frequently not well accepted by the general population.
The present study will investigate whether with appropriate non/reduced-meat alternatives and motivational support, free living subjects can significantly reduce their meat intake and whether such dietary changes positively impact on a range of cardiovascular risk factors. The volunteers will be asked to replace at least 50% of their current meat (red and/or processed) intake with these meat-replacement products over a 3 month period. In order to facilitate this change subjects will be provided with a variety of meat substitutes on a weekly/biweekly basis. Volunteers will attend a fasted screening session. If they pass the screening they are eligible to take part in the intervention. Volunteers will then attend an assessment session 1 month prior to the start of the intervention, immediately before the start of the intervention, at 6 weeks and 12 weeks during intervention (4 in total). Volunteers will undertake a range of physiological measurements of cardiovascular health and provide blood samples. Diet diaries will be completed one week prior to each assessment session. Blood samples will be used for determining plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose. Height, weight and skinfold thickness will be used to calculate any changes in body mass and body composition during the intervention. Flow mediated vasodilation (via ultrasound) will be used as an index of endothelial function and cardiovascular health.
**Why have I been invited?**
The study will include male and (premenopausal) female volunteers (couples welcome) aged 25–50years with a BMI 18–28kg/$m^2$ with a habitual consumption of 4-5 portions of red and/or processed meat per week (3-4 of these being main meals). Volunteers will not be taking lipid lowering drugs and not diagnosed diabetic. You have been randomly chosen as a member of one of these groups. We are inviting 40 participants like you to take part.

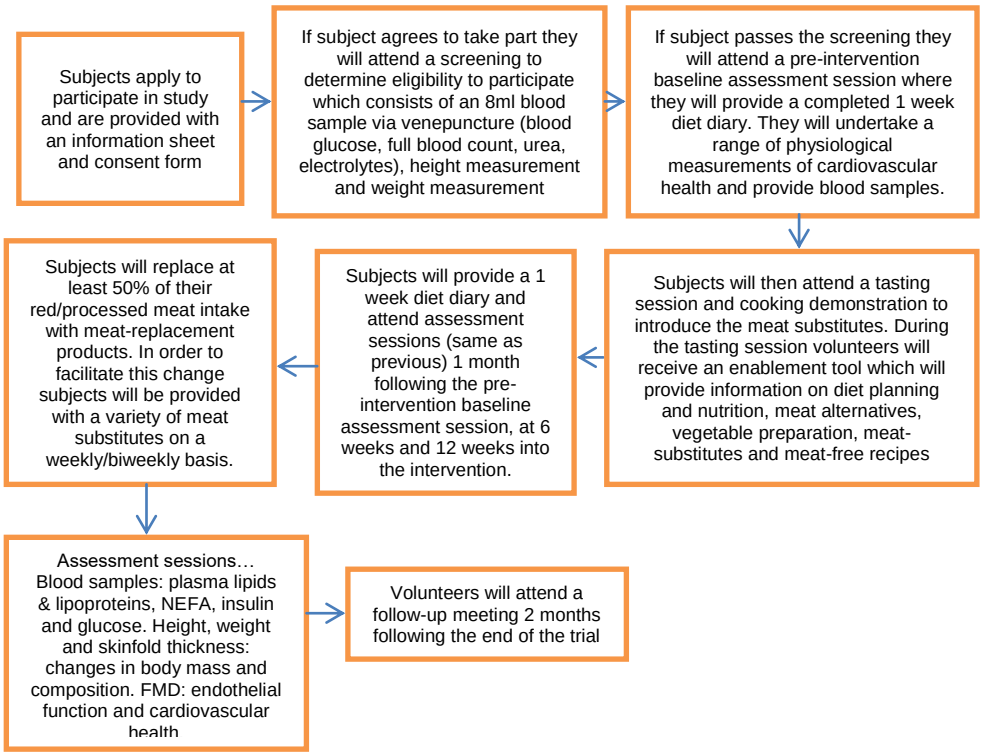
**Do I have to take part?**
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

**What will happen to me if I take part?**

10) Attend a screening process prior to the study where we will assess your height, weight, body mass index, blood pressure and heart rate. A blood sample (8ml) will be taken via the antecubital vein (inside of elbow) to assess for blood glucose, full blood count, urea and electrolytes.
11) Attend a pre-intervention baseline assessment session 1 month prior to the start of the intervention trial where you will provide a completed 1 week diet diary. You will undertake a range of physiological measurements of cardiovascular health and provide blood samples (discussed in no. 6 below).
12) Attend an information-based motivational event where you will be informed on the health and environmental benefits of meat reduction. You will also attend a tasting session which includes an introduction to the meat substitutes and a cooking demonstration. During the tasting session you will receive an enablement tool which will provide information on diet planning and nutrition, meat alternatives, vegetable preparation, meat-substitutes and meat-free recipes
13) You will be provided with a variety of meat substitutes on a weekly/biweekly basis along with a recipe book with ideas to try. Over a 3 month period you will reduce your red and/or processed meat intake by at least 50% (we provide you with meat alternative products). The meat substitutes will include products such as Quorn, soya, pulses, beans and '50-50' meat products (part meat, part quorn/soya).

14) You will attend assessment sessions (lasting approximately 2 hours) at 4 points throughout the trial. 1 month prior to the start of the intervention (mentioned above), at the start of the intervention, and at the 6 week and 12 week time points. During these assessment sessions you will undertake a range of physiological measurements of cardiovascular health and provide a 16ml blood sample.
   - Blood samples will be used for determining plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose.
   - Height, weight and skinfold thickness will be used to calculate any changes in body mass and body composition during the intervention.
   - Your blood vessel function will be assessed. This involves having a blood pressure cuff placed around your forearm which is inflated to a pressure above that of your blood pressure, for 5mins and the artery that can be felt at your elbow will be imaged at the same time, using an ultrasound machine. The ultrasound technique is similar to that used to observe babies during pregnancy. The scan will take no more than 10 minutes to complete.

15) You will complete a diet diary one week prior to each of the assessment sessions (above).
16) You will attend a follow-up meeting 2 months after you have completed the trial.




Blood samples will be obtained by venepuncture by a trained and experienced person. There can be some slight discomfort when the needle enters the skin, and on rare occasions you may be left with some bruising. Approximately 16 ml of blood will be obtained during assessment sessions (8ml on the screening visit). All procedures are carried out by experienced personnel routinely within our research group and are audited in all of our studies.

If you do suffer any other symptoms or side-effects you should report them next time you meet. If you become in any way concerned prior to your next visit or after the study has finished you should contact the lead investigator (see end of sheet). If for any reason you are excluded from the study following your medical screening, you shall be informed of the reason straight away. The blood sample is not screened for any diseases or viruses.

**Expenses and inconvenience allowance**
Participants will not be paid an inconvenience allowance to participate in the study.
Travel expenses will not be offered for any visits incurred as a result of participation.

**What are the possible disadvantages and risks of taking part?**
You may be trying unfamiliar foods. Participating in the study will inevitably take up some of your time; it has been designed to be kept as short as possible to minimize any inconvenience.

Taking a blood sample my cause minor discomfort in the form of a sharp scratch when taking blood and may result in some bruising afterwards. If you do have any concerns please contact: Marie Clark via email stxmc8@nottingham.ac.uk

It is recommended that you should avoid donating more than 500 ml blood over a 6 month period and allow at least 3 months between participating in further studies which involves giving blood. If you are a regular blood donor you should also bear this in mind.

**Exclusion criteria including the following:**

- Perimenopausal females (around menopause)
- Chronic disease
- Cardiovascular risk
- Statins
- High blood pressure
- Diabetes
- Taken part in any other research study three months prior which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml, muscle biopsies) or exposure to ionising radiation.

**What are the possible benefits of taking part?**
The information we get from your participation in this study may help expand the knowledge on the benefits of reducing meat intake.

**What if there is a problem?**
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you should then contact the FMHS Research Ethics Committee Administrator, School of Medicine Education Centre, B Floor, Medical School, Queen's Medical Centre Campus, Nottingham University Hospitals, Nottingham, NG7 2UH. E-mail louise.sabir@nottingham.ac.uk."

**Will my taking part in the study be kept confidential?**
We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the institution will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept secure after the end of the study so that we are able to contact you about the findings of the study *and possible follow-up studies* (unless you advise us that you do not wish to be contacted). All research data will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

**What will happen if I don't want to carry on with the study?**
You participation is voluntary and you are free to withdraw at any time, without giving any reason.

You may be withdrawn from the study for:

- Safety reasons (including pregnancy or illness)
- Failure to adhere to protocol requirements
- Withdrawal of consent

You will be withdrawn from study if consent is withdrawn – it should be noted that data collected up to the point of withdrawal will not be destroyed because once the anonymised data has been entered into the databases on the University secure servers and back up servers it becomes impractical to extract and erase.

**Involvement of the General Practitioner/Family doctor (GP)**
Your GP (or other health care practitioner) does not need to be notified of your participation. However if any of your blood samples show any significant abnormality your GP may be informed with your permission.

**What will happen to any samples I give?**
Samples will be used for determining plasma lipids and lipoproteins, insulin and glucose.

We would also like to seek your consent so that any remaining samples may be stored and used in possible future research – this is optional (please indicate you agree to this on the consent form). The samples will be stored with a code unique to you and securely at the University of Nottingham in accordance with the Human Tissue Act (HTA) regulations (Licence no 12265).

Some of these future studies may be carried out by researchers other than current team of Prof. Andrew Salter, Dr. Fiona McCullough, Dr Liz Simpson and Marie Clark, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and you will not be identified in anyway. If you do not agree to this any remaining samples will be disposed of in accordance with the Human Tissue Authorities codes of practice.

**What will happen to the results of the research study**
The findings will be published in a PhD thesis and may also be published in a scientific journal article.

**Who is organising and funding the research?**
This research is being organised by the University of Nottingham and is being funded by BBSRC

**Who has reviewed the study?**

All research in the University of Nottingham is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by UoN Faculty of Medicine & Health Sciences Research Ethics Committee.

**Further information and contact details**

Professor Andrew Salter
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Azlina A Razak
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**Appendix N:** Consent form given to the volunteers from the intervention trial, as seen in **Chapter 4**.

**CONSENT FORM**
**(Final version 1.1: 01/12/2015)**

**Title of Study: Eat Less Meat**
**REC ref: A18062015 SoBS ELM**
**Name of Researcher(s)**: Professor Andrew Salter, Chief Researcher Dr Fiona McCullough, Research Supervisor Dr Liz Simpson, Research Supervisor, Marie Clark, PhD Research Student and Azlina A Razak, Research Student.

**Name of Participant:**

**Please initial box**

11. I confirm that I have read and understand the information sheet Final version number 1.1 dated 01/12/15 for the above study and have had the opportunity to ask questions. ☐
12. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
13. I understand that relevant sections of data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study but not my name. I understand that my personal details will be kept confidential. ☐
14. I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published and then destroyed securely. ☐
15. I understand and agree that a 16ml blood sample will be taken for analysis of plasma lipids and lipoproteins, non-esterified fatty acid, insulin and glucose 1 month prior, pre-, mid- and post-trial. ☐
16. Consent for storage and use in possible future research (Optional)

I agree that the samples I have given and the information gathered about me can be stored by Professor Andrew Salter at the School of Biosciences, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Professor Andrew Salter, Dr Fiona McCullough, Dr Liz Simpson and Marie Clark, who ran the study, including researchers working for commercial companies. Any blood samples or data used will be anonymised, and I will not be identified in anyway. ☐

17. I understand that I will be informed if any of my blood tests show any significant abnormality and my GP may be informed with my permission. ☐
18. I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation. ☐
19. I voluntarily agree to take part in the above study. ☐

| Name of Participant | Date | Signature |
|---|---|---|
| Name of Person taking consent | Date | Signature |

**Appendix O:** Intervention trial motivational newsletter as mentioned in **Chapter 4**.



## Welcome to the 'Eat Less Meat' Project

This project is being run at the University of Nottingham, and its aim is to look at the potential health benefits of reducing your red and processed meat intake.

However, what you may not know is that we are pleased to be working with partners from the food industry who have developed and provided some reduced meat, and vegetarian alternatives to meat, to help you to lower your meat intake.

Quorn

Tivertown

The University of Nottingham

## Health benefits of reducing meat intake

In UK adults who eat meat, meat provides approximately 40% of the average daily protein intake and makes a significant contribution to the intake of thiamin, niacin, vitamin B12, iron, zinc, potassium, and phosphorus found in the diet.

However, excessive consumption, particularly of red and processed meat, is associated with an increased risk of developing a range of chronic diseases, including cardiovascular disease, obesity, type 2 diabetes and some cancers. Fortunately, nutrients found in meat are also found in plants and fungi, and with proper planning, meat can safely be replaced in the diet without compromising dietary intake of these nutrients.

Increasing fruit and vegetables in the diet also provides other components, not found in meat, that are thought to confer additional health benefits. These include dietary fibre, which is essential for maintaining the health of the bowel and may help to lower cholesterol levels in the blood. Fruit and vegetables generally also provide lower amounts of fat and calories than meat.

The Oxford part of the European Prospective Investigation into Cancer and Nutrition showed that the risk of heart disease in vegetarians is reduced by over 30%, but it is suggested that more modest reductions in meat intake would still improve an individual's health. By taking part in this project you are helping us to find out whether this is true.

Thank you for volunteering for this study.

**Professor Andrew Salter,**
**(Principal investigator for the 'Eat Less Meat' Study)**

## You are currently one of 0039 people taking part in this study

**Flageolet Beans** are immature haricot beans (the bean you find in a tin of Baked Beans). They come from the 'French Bean' plant (Passeolus vulgaris), and are much prized in France for their delicate flavour.

Before use, soak for 8 hours (or overnight) in a large bowl with twice the volume of water. Or, if you don't have as much time, hot soak them. Put the beans into a large saucepan with twice the volume of water and bring to the boil. Boil for 2 mins, remove from the heat and leave to soak in the pan for 1 hour. Drain and rinse well in cold water.

To cook, boil rapidly in plenty of water for 10 mins, then simmer for 30-60 mins, on their own or in your choice of recipe.

Great in salads, stews or soups

Storage: 9 months in dark dry conditions.



Flageolet Beans



DID YOU KNOW?

In Britain we consume more than double the world's meat average: in excess of 80kg per person per year. To put this into context, Morocco consumes 21kg per person and India just 5kg.

The UN Food and Agriculture Organization estimates that the meat industry generates nearly one-fifth of the man-made 'greenhouse gas' emissions that are thought to be accelerating climate change worldwide.

This is far more than transportation emissions. If everyone in the U.S. went meatless one day a week, it would be equivalent to taking 7.6 million cars off the road.

**Appendix P:** Intervention trial approval letter from the Medical School Research Ethics Committee as mentioned in **Chapter 4**.

Direct line/e-mail
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The University of Nottingham

**Faculty of Medicine and Health Sciences**

Research Ethics Committee
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

22nd June 2015

Marie Clark
PhD Student Nutritional Sciences
c/o Andrew Salter, Professor of Nutritional Biochemistry
Head of Division of Nutritional Sciences
School of Biosciences
Sutton Bonington Campus
University of Nottingham
Loughborough
LE12 5RD

Dear Marie

**Ethics Reference No:** A18062015 SoBS ELM **- please always quote**
**Study Title:** Impact of red and processed meat reduction on cardiovascular risk factors involving healthy participants.
**Chief Researcher/Academic Supervisors:** Professor Andrew Salter, Professor of Nutritional Biochemistry, Head of Nutritional Sciences, School of Biosciences.
**Lead Researcher/Student:** Marie Clark, PhD Student, Nutritional Sciences, School of Biosciences.
Other key researchers: Dr Fiona McCullough, , Dr Judy Swift, Study Statistician, Nutritional Sciences, School of Biosciences, Dr Liz Simpson , School of Life Sciences,
**Duration of Study:** 01/07/2015-30/09/2016 12 mths **No of Subjects:** 40 (25-50 yrs)

Thank you for your recent application which was considered by the Committee at its meeting on 18th June 2015 and the following documents were received:

ELM Study:

- Med Sch REC Application Form version 1.0, Date: 14/05/2015
- Protocol, Final Version 2.0, Date 14/05/15
- Recruitment Poster, Version 1.0: 14/05/2015
- Participant Information Sheet Final Version 1.0: 14/05/3015
- Consent Form Final Version 1.0: 14/05/2015

These have been reviewed and are satisfactory and the study is approved subject to the following comments being addressed:

1. **Exclusion Criteria:** Please expand what you mean by "Cardio vascular risk" i.e taking the oral contraceptive pill, smokers etc.

2. **Information Sheet:**
   - p2 no 6 under What will happen to if I take part: Please revise the term "Flow mediated vasodilation" to a lay term i.e a cuff will be put on your arm to measure your blood pressure.
   - Please state how long each assessment session will take to give volunteers an idea of the time commitment required.

## **Appendix Q:** Food frequency questionnaire given to volunteers from the intervention trial as mentioned in **Chapter 4**

**Current eating habits**

A portion of fruit (80g) is roughly equivalent to: a slice or half a large fruit (e.g. a slice of melon or half a grapefruit), 1 medium size fruit (e.g. an apple), or 2 small size fruits (e.g. 2 plums or satsumas).

1. How many servings of fruit and vegetables (excluding potatoes) do you think you consume, on average, per day?
   - ☐ 0
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ 4
   - ☐ 5
   - ☐ 6+
2. When deciding what to eat, who, if anybody, influences your decisions on food choice? (Tick all that apply)
   - ☐ my partner
   - ☐ my parent/s, carer or guardian
   - ☐ children or other family members
   - ☐ friend/s or housemate/s
   - ☐ N/A

For the purposes of this survey, meat is defined as any meat (animal flesh) or meat product, but does not include fish.

3. Do you purposefully exclude or restrict meat in your diet?
   - ☐ Yes
   - ☐ No

   If yes - go to question **4**
   If no - go to question **5**
4. What is/are the reason/s you exclude or restrict meat in your diet? (Tick all that apply)
   - ☐ Health reasons
   - ☐ To improve mood
   - ☐ For convenience
   - ☐ Taste/texture/attractiveness of meat or substitutes
   - ☐ Concerns over artificial ingredients or additives
   - ☐ Cost
   - ☐ Other (please specify)
   - ☐ Weight control
   - ☐ Familiarity or habit
   - ☐ Animal welfare reasons
   - ☐ Environmental concerns
   - ☐ Political values
   - ☐ Religious reasons
5. Have you eaten meat in the last 4 weeks?
   - ☐ Yes
   - ☐ No

   If yes - go to question **6**
   If no - go to question **8**
6. Over the last 4 weeks, how frequently did you eat meat or meat products?
   - ☐ More than once a day
   - ☐ Once a day
   - ☐ 5-6 times per week
   - ☐ 2-4 times per week
   - ☐ Once a week
   - ☐ Less than once a week

**Food Frequency Questionnaire (FFQ adapted from EPIC Norfolk trial)**

7. Thinking back to what you ate over the last 4 weeks, please estimate how many times you ate each meat product below. If you have eaten it at all over the last year, please estimate the average portion size. As a guide, a **medium serving** is around 4oz (around 100g) of meat/1 burger/1 sausage/2 slices lunch meat/3 strips of bacon/1 pie/1 large piece of chicken or 6 nuggets.

**Beef burgers**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Other beef (e.g. roast, steak, mince, stew or casserole, or in dishes, such as lasagne or pizza)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Pork (e.g. roast, chops, stew or slices)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Lamb (e.g. roast, chops or stew)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Chicken or other poultry (e.g. turkey)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Bacon**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Ham**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Corned beef, spam or other luncheon meats**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Sausages (including pepperoni and hotdogs)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Savoury meat pies (e.g. pork pie, pasties, steak & kidney pie, sausage rolls)**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Liver, liver pate, liver sausage**

| Average consumption over the last 4 weeks | Average portion size |
|---|---|
| ☐ Never | ☐ N/A |
| ☐ Once per week | ☐ Small |
| ☐ 2-4 times per week | ☐ Medium |
| ☐ 5-6 times per week | ☐ Large |
| ☐ Once a day | |
| ☐ More than once a day | |

**Meat Substitutes – current consumption**

8. On the days on which you do not eat meat, do you purposefully substitute the meat with something else?
   - ☐ Yes
   - ☐ No – I do not purposefully replace the meat with anything else
   - ☐ N/A – I eat meat everyday

   If yes - go to question **9**
   If no or N/A – go to question **17**
9. When would you usually substitute meat with something else? (please tick all that apply)
   - ☐ Breakfast
   - ☐ Lunch
   - ☐ Dinner
   - ☐ Snack
   - ☐ other meal
10. On average, how much of the portion of meat in a meal do you typically substitute?
   - ☐ all of it
   - ☐ three quarters
   - ☐ a half
   - ☐ a quarter
   - ☐ less than a quarter
11. Over the last 4 weeks, have you ever replaced meat with meat analogues, e.g. Quorn, tofu, or textured vegetable protein, for example, "veggie burgers" or meat-free sausages/mince?
   - ☐ Yes
   - ☐ No

12. Over the last 4 weeks, have you ever replaced meat with cheese (e.g. cheddar, brie, edam, cream cheese or cottage cheese)?
   - ☐ Yes
   - ☐ No
13. Over the last 4 weeks, have you ever replaced meat with egg (e.g. boiled, fried or scrambled, or as part of a meal, e.g. quiche)?
   - ☐ Yes
   - ☐ No
14. Over the last 4 weeks, have you ever replaced meat with nuts or nut butters?
   - ☐ Yes
   - ☐ No
15. Over the last 4 weeks, have you ever replaced meat with fish (e.g. fresh, frozen, tinned or shellfish)?
   - ☐ Yes
   - ☐ No
16. Over the last 4 weeks, have you ever replaced meat with lentils or other legumes?
   - ☐ Yes
   - ☐ No

**Intervention Study – Your participation**

17. Did you find it easy to stick to the meat reduction rules of the study? Please mark on the scale of 1 to 5, where 1 means not easy at all and 5 means completely easy.
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ 4
   - ☐ 5
18. Meat replacement products were provided for you during the study. Did you find the provision of meat replacement products helped you follow the study? Please mark on the scale of 1 to 5, where 1 means the provision of food products did not help you at all and 5 means the provision of food products helped completely.
   - ☐ 1
   - ☐ 2
   - ☐ 3
   - ☐ 4
   - ☐ 5
19. Has your behavior towards meat consumption changed at all since taking part in the study?
   - ☐ Yes
   - ☐ No
20. If you ticked yes to question 19 then please explain briefly how you feel your behavior has changed since taking part in the meat reduction study. If you ticked no then please end the questionnaire.

**Appendix R:** Food diary the volunteers completed before the intervention trial assessment visits, as described in **Chapter 4**.

## *EAT LESS MEAT*

Food Diary

(Household Measures)

Centre:

Subject ID .....

Week -4 0 6 12 (please circle)

Please complete your diary on the following days:

Day 1…….. Date…….

Day 2……. Date…….

Day 3…….. Date…….

Day 4…….. Date…….

**Once the diary is completed, please bring it with you to your next assessment session or email it to stxmc8@nottingham.ac.uk**

## INSTRUCTIONS FOR FILLING IN YOUR FOOD DIARY

- Please keep a food diary for the 4-day period shown on the front cover. The record should include as much detail as possible. Please start a new page each day (ensure the day and date is recorded at the top of the page).

- Fill in the food diary each time you consume a drink, a meal or a snack. Please note the time, and describe everything you eat or drink. Use a further page if you need more space. (Please remember to record the day and date at the top of the new page).

- When you record a food or drink, please give a thorough description and include any Brand names. You cannot give us too much detail! For example:-
    - Was the bread white/wholemeal/granary
    - Did you have a baguette/cob/pitta/slice?
    - Did you use butter or margarine, was the margarine made with olive/sunflower oil?
    - Was the product a diet product- e.g. sugar-free lemonade?
    - Was anything added- e.g. sugar to tea, butter to toast, tomato ketchup or mayonnaise etc?
    - Do you use full fat/semi-skimmed/skimmed milk?
    - How did you cook your food – eg. boiled/fried/baked/raw?
    - How was the food processed – eg. fresh/tinned/frozen?
    - Was the product Heinz/Tesco's own etc.?

- The amounts you have should be described as fully as possible using household measures
    - Use kitchen equipment – if you have a scale and can weigh everything then that is perfect. If not, then use a measuring jug, a mug (of know volume), a teaspoon (see below) and a tablespoon

Level teaspoon

Rounded teaspoon

Heaped teaspoon

    - Look on cans and packets to see whether the weight/volume is given and describe how much of the packet you had (eg. Half a 400g can of Heinz tomato soup)
    - Record the number of sausages/fish fingers/bacon rashers etc. that you ate
    - Describe the size of a piece of food – eg. egg sized potato, small matchbox sized piece of cheese

- Please record each item of food separately. If you drink white tea with sugar, please record the type and amount of tea, milk and sugar separately.

- If you had a packed lunch, each part must be recorded in the diary.

- It is important that you complete the 'leftovers' section with anything you did not actually eat. Please give us as much detail as possible.

- For recipes taken from the Eat Less Meat recipe booklet, record the name of the recipe used. Please tell us how many servings you had (each recipe comes with a serving size).

- For homemade recipes, the raw ingredients should be recorded in the back of the diary in the 'Recipes' section. Please tell us what the meal is composed of and how many it served. You can then put an entry in the diary such as "one quarter of the spaghetti Bolognese made with recipe 1".

- For meals eaten out at restaurants etc. please give as much detail about what you ate, where it was from and an approximate size of the meal.

**AN EXAMPLE OF HOW TO COMPLETE YOUR FOOD DIARY AND RECIPE PAGE IS SHOWN ON THE NEXT PAGE**

| **Subject ID** | Example | **Day-No** | 1 | Monday | **Date** | 01/01/15 | |
|---|---|---|---|---|---|---|---|

| **Time** | **Type of food or drink with brand names and cooking method** | **Amount** | **Food or drink left over and amount** | Office use only: code and weight |
|---|---|---|---|---|
| 7.30am | Beans on toast: | | | |
| | Heinez baked beans - reduced salt and sugar | 1/2 a 415g tin | 1 teaspoon | |
| | White toast - Warburtons thick cut | 2 slices | 1/8th slice | |
| | Flora spread | 2 heaped teaspoons | | |
| | Cup of tea: | | | |
| | PG tips tea | 300ml | | |
| | Semi-skimmed milk | 50ml | | |
| | Sugar | 2 rounded teaspoons | | |
| 10.30am | Dairy milk bar | 40g | | |
| | Diet coke | 330ml can | | |
| 12.45pm | Ham sandwich: | | | |
| | Ham - Tesco honey roast | 2 slices | | |
| | White bread - Warburtons medium cut | 2 slices | | |
| | Flora spread | 2 heaped teaspoons | | |
| | Beef tomato | 1/2 | | |
| | Cup of tea - same as above | 300ml | | |
| | Walkers salt and vinegar crisps | 30g bag | | |
| 4.15pm | Butternut chilli - recipe book | 1 1/2 servings | | |
| 7.15pm | Spaghetti bolognese using recipe 1 | 1/4 of recipe | | |
| | John Smiths Beer | 3 pints | | |
| 10pm | Options belgium hot chocolate | 2 heaped teaspoons | | |
| | Semi-skimmed milk | 300ml | | |

| | | | | | |
|---|---|---|---|---|---|
| **Subject ID** | Example | **Recipe No:** | 1 | **Dates used:** | 01/01/15, 05/01/15 |

| | | | |
|---|---|---|---|
| **Name of recipe:** | Spaghetti bolognese | **Serves:** | 4 |

Please indicate in food diary the portion size you had on each occasion

| **Ingredients with brand names** | **Amount** | **Cooking method** |
|---|---|---|
| Garlic | 2 cloves | Fried in the sunflower oil |
| Onion | 1 medium | Fried in the sunflower oil |
| Sunflower oil | 2 teaspoons | |
| Sainsbury's organic lean minced beef - uncooked | 500g | Added to sunflower oil |
| Ragu original smooth bolognese sauce | 454g jar | Added to mince |
| Dried spaghetti | 250g | Boiled |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

**Appendix S:** WHOQOL-BREF questionnaire completed by volunteers, as described in **Chapter 4**.

# WHOQOL–BREF

UK VERSION

**Department of Mental Health**

**World Health Organisation**

**Geneva**

For Office Use Only

| | Equations for computing domain scores | Raw score | Transformed score | |
|---|---|---|---|---|
| | | | 4-20 | 0-100 |
| Domain 1 | (6-Q3) + (6-Q4) + Q10 + Q15 + Q16 + Q17+ Q18<br>□ + □ + □ + □ + □ + □ + □ | - | | |
| Domain 2 | Q5 + Q6 + Q7 + Q11 + Q19 + (6-Q26)<br>□ + □ + □ + □ + □ + □ | - | | |
| Domain 3 | Q20 + Q21 + Q22<br>□ + □ + □ | - | | |
| Domain 4 | Q8 + Q9 + Q12 + Q13 + Q14 + Q23 + Q24 + Q25<br>□ + □ + □ + □ + □ + □ + □ + □ | - | | |

## ABOUT YOU

Before you begin we would like you to answer a few general questions about yourself: by **circling** the correct answer or by **filling in the space provided**.

What is your gender? **MALE / FEMALE**

What is your date of birth? ____/___/_____. (day/month/year.)

What is the highest education you've received? **None at all**
**Primary school**
**Secondary school**
**Tertiary**

What is your marital status? **Single** **Separated**
**Married** **Divorced**
**Living as married** **Widowed**

Are you currently ill? **YES / NO**

If something is wrong with your health what do you think it is?
Please write your illness(s) or problem here:_____
_____

## Instructions

This questionnaire asks how you feel about your quality of life, health and other areas of your life. **Please answer all the questions**. If you are unsure about which response to give to a question, **please choose the ONE** that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last two weeks**. For example, thinking about the last two weeks, a question might ask:

| | | Not at all | Not much | Moderately | A great deal | Completely |
|---|---|---|---|---|---|---|
| | Do you get the kind of support from others that you need? | 1 | 2 | 3 | 4 | 5 |

You should **circle** the number that best fits how much support you got from others **over the last two weeks**. So you would circle the number 4 if you got a great deal of support from others as follows:

| | | Not at all | Not much | Moderately | A great deal | Completely |
|---|---|---|---|---|---|---|
| | Do you get the kind of support from others that you need? | 1 | 2 | 3 | 4 | 5 |

You would circle the number 1 if you did not get any of the support that you needed from others in the last two weeks. Please read each question, assess you feelings, and **circle** the number on the scale for each question that gives the best answer for you.

| | | Very poor | Poor | Neither poor nor good | Good | Very good |
|---|---|---|---|---|---|---|
| 1 | How would you rate your quality of life? | 1 | 2 | 3 | 4 | 5 |

| | | Very Dissatisfied | Dissatisfied | Neither Satisfied nor Dissatisfied | Satisfied | Very Satisfied |
|---|---|---|---|---|---|---|
| 2 | How satisfied are you with your health? | 1 | 2 | 3 | 4 | 5 |

The following questions ask about **how much** you have experienced certain things **in the last two weeks**.

| | | Not at all | A little | A moderate amount | Very much | An extreme amount |
|---|---|---|---|---|---|---|
| 3 | How much do you feel that pain prevents you from doing what you need to do? | 1 | 2 | 3 | 4 | 5 |
| 4 | How much do you need medical treatment to function in your daily life? | 1 | 2 | 3 | 4 | 5 |
| 5 | How much do you enjoy life? | 1 | 2 | 3 | 4 | 5 |

| | | Not at all | A little | A moderate amount | Very much | Extremely |
|---|---|---|---|---|---|---|
| 6 | To what extent do you feel life to be meaningful? | 1 | 2 | 3 | 4 | 5 |
| 7 | How well are you able to concentrate? | 1 | 2 | 3 | 4 | 5 |
| 8 | How safe do you feel in your daily life? | 1 | 2 | 3 | 4 | 5 |
| 9 | How healthy is your physical environment? | 1 | 2 | 3 | 4 | 5 |

The following questions ask about **how completely** you experience or were able to do certain things **in the last two weeks**.

| | | Not at all | A little | Moderately | Mostly | Completely |
|---|---|---|---|---|---|---|
| 10 | Do you have enough energy for everyday life? | 1 | 2 | 3 | 4 | 5 |
| 11 | Are you able to accept your bodily appearance? | 1 | 2 | 3 | 4 | 5 |
| 12 | To what extent do you have enough money to meet your needs? | 1 | 2 | 3 | 4 | 5 |
| 13 | How available to you is the information that you need in your day-to-day life? | 1 | 2 | 3 | 4 | 5 |
| 14 | To what extent do you have the opportunity for leisure activities? | 1 | 2 | 3 | 4 | 5 |

The following questions ask you to say **how good or satisfied** you have felt about various aspects of your life **over the last two weeks**.

| | | Very poor | Poor | Neither poor nor good | Good | Very good |
|---|---|---|---|---|---|---|
| 15 | How well are you able to get around? | 1 | 2 | 3 | 4 | 5 |

| | | Very dissatisfied | Dissatisfied | Neither satisfied nor dissatisfied | Satisfied | Very satisfied |
|---|---|---|---|---|---|---|
| 16 | How satisfied are you with your sleep? | 1 | 2 | 3 | 4 | 5 |
| 17 | How satisfied are you with your ability to perform daily living activities? | 1 | 2 | 3 | 4 | 5 |
| 18 | How satisfied are you with your capacity for work? | 1 | 2 | 3 | 4 | 5 |
| 19 | How satisfied are you with yourself? | 1 | 2 | 3 | 4 | 5 |
| 20 | How satisfied are you with your personal relationships? | 1 | 2 | 3 | 4 | 5 |
| 21 | How satisfied are you with your sex life? | 1 | 2 | 3 | 4 | 5 |
| 22 | How satisfied are you with the support you get from your friends? | 1 | 2 | 3 | 4 | 5 |
| 23 | How satisfied are you with the conditions of your living place? | 1 | 2 | 3 | 4 | 5 |
| 24 | How satisfied are you with your access to health services? | 1 | 2 | 3 | 4 | 5 |
| 25 | How satisfied are you with your transport? | 1 | 2 | 3 | 4 | 5 |

The following question refers to **how often** you have felt or experienced certain things **in the last two weeks**.

| | | Never | Seldom | Quite often | Very often | Always |
|---|---|---|---|---|---|---|
| 26 | How often do you have negative feelings, such as blue mood, despair, anxiety, depression? | 1 | 2 | 3 | 4 | 5 |

Did someone help you to fill out this form? **YES** / **NO**

**THANK-YOU FOR YOUR HELP**