IMPACT OF ISOENERGETIC INTAKE OF IRREGULAR MEAL PATTERNS ON ENERGY EXPENDITURE, METABOLISM AND APPETITE REGULATION

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ABSTRACT

Meal pattern has been identified as a factor influencing the thermic effect of food (TEF) and metabolic status, and therefore health.This thesis investigated the effects of an irregular meal pattern, with a controlled energy intake, in normal-weight (Study 1, n=11) and obese with insulin resistance (Study 2, n=9) females over a 14-day period. Measurements were made of the TEF, circulating glucose, insulin, lipids concentration and appetite regulation, using a crossover design.

In both normal-weight and obese studies, there were no significant differences in fasting resting energy expenditure (REE) between a regular and an irregular meal pattern intervention period. However, the irregular intervention period led to a significant reduction in the TEF following a test drink consumption in both normal-weight ($p<0.05$) and obese ($p<0.05$) females.

There were no significant differences in fasting blood glucose and serum insulin concentrations between the two intervention periods in the two studies. Glucose iAUC responses to a test drink measured over 3 h were higher after the irregular compared with the regular meal pattern ($p<0.05$) with no difference in the insulin response in the normal-weight study. However, in the obese study, glucose responses were unaffected by the regular and irregular intervention periods, whilst there was a main effect of meal pattern in insulin responses for 3 h following the test drink.

The measurements of glucose in a free-living environment with the normal-weight participants showed a higher postprandial (after breakfast) iAUC on day 7 during the irregular period than the regular one ($P<0.05$). Postprandial (after lunch and after dinner) iAUC on day 9 in the irregular intervention were also higher than day 9 in the regular intervention ($P<0.05$). On the other hand, no significant differences were observed in these measurements with the obese participants.

A significant increase in serum triglyceride ($P<0.05$) and a significant reduction in fasting serum HDL-cholesterol ($P<0.05$) were observed after both intervention periods in obese, but not normal-weight participants. Fasting serum total and LDL-cholesterol were not significantly different between the two interventions in both normal-weight and obese studies.

Appetite ratings showed no significant differences before or after the test drink and a subsequent *ad-libitum* meal over the two studies, apart from hunger, in the normal-weight study, where there was a significant reduction in hunger ratings for 3 h following the test drink after both intervention periods ($P<0.05$).

In the normal-weight study, fasting plasma GLP-1 decreased ($P<0.05$) after both intervention periods. In contrast, fasting plasma GLP-1 increased ($P<0.05$) after both intervention periods in the obese study. Furthermore, in the obese study, the regular intervention produced a higher plasma GLP-1 iAUC compared with the irregular meal pattern, but there were no such effects in the normal-weight study.

The normal-weight study showed that fasting plasma PYY was lower after the intervention periods compared with before ($P<0.05$). Moreover, iAUC for the 3 h postprandial period increased after the intervention compared with before ($P<0.05$). However, there were no significant differences in both fasting plasma and iAUC PYY responses between the two interventions in the obese study.

A regular meal pattern appears to be associated with greater TEF, which might result in more favourable energy balance for weight maintenance. Also, it is likely that a regular meal pattern improved insulin sensitivity in healthy normal-weight females. Therefore, a regular meal pattern could be a lifestyle factor that may promote an individual's health**.**

PUBLICATIONS

The findings of some of the studies described in this thesis have been published as follows:

Conference Communications

M. Alhussain, M. A. Taylor and I. A. Macdonald. Comparison of ventilated hood indirect calorimetry and the SenseWear Armband for assessing energy expenditure. Presented by the author as a poster at *the International Sports and Exercise Nutrition Conference*, 17-19 December 2013, Newcastle upon Tyne, UK.

M. Alhussain, M. A. Taylor and I. A. Macdonald. Decreased postprandial energy expenditure after an irregular compared with a regular meal pattern in healthy women. Presented orally by the author at *the Nutrition Society Postgraduate Conference*, 1-2 September 2014, Nottingham, UK.

M. Alhussain, M. A. Taylor and I. A. Macdonald. Irregular meal pattern: what is the impact on weight? Presented by the author as a poster at *the 8th Saudi Student Conference*, 31 January-1 February 2015, London, UK.

M. Alhussain, M. A. Taylor and I. A. Macdonald. Association between irregular meal pattern and thermic effect of food in healthy lean women. Presented by the author as a poster at *the 2015 Obesity Summit*, 14-16 April 2015, London, UK.

M. Alhussain, M. A. Taylor and I. A. Macdonald. Effects of an erratic versus a regular eating pattern on appetite regulation in healthy lean women. Presented by the author as a poster at *the 2nd UK Congress on Obesity*, 9-11 September 2015, Glasgow, UK.

M. Alhussain, M. A. Taylor and I. A. Macdonald. Erratic meal pattern and carbohydrate metabolism. Presented by the author as a poster at *the 2nd UK Congress on Obesity*, 9-11 September 2015, Glasgow, UK.

Abstracts

M. Alhussain, M. A. Taylor and I. A. Macdonald (2014). Comparison of ventilated hood indirect calorimetry and the SenseWear Armband for assessing energy expenditure. *International Journal of Sport Nutrition and Exercise Metabolism* 24, S1 -S10.

M. Alhussain, M. A. Taylor and I. A. Macdonald (2015). Influence of the constancy of daily meal pattern on postprandial energy expenditure in healthy weight women. *Proceeding of the Nutrition Society* 74 (OCE1) E141.

Manuscripts

M. Alhussain, M. A. Taylor and I. A. Macdonald. Impact of irregular meal patterns on energy expenditure, metabolism and appetite regulation; a randomised controlled trial in healthy normal-weight women. Under review in *The American Journal of Clinical Nutrition*.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BMI	Body mass index
BMR	Basel metabolic rate
ºC	Degree celsius
CGM	Continuous glucose monitoring
cm	Centimetre
CO_2	Carbon dioxide
CMGs	Continuous glucose monitor system
CONGA	Continuous overlapping net glycemic action
EDTA	Ethylene diamine tetra-acetic acid
g	Gram
GLP-1	Glucagon like peptide 1
h	Hour
HDL	High density lipoprotein
HOMA-IR	Homeostatic model assessment for insulin resistance
iAUC	Incremental area under the curve
IPAQ	International Physical Activity Questionnaire
kcal/day	Kilocalories per day
kg	Kilogram
L	Litre
LDL	Low density lipoprotein
Max	Maximum
METs	Metabolic Equivalent of Task
Min	Minimum
min	Minute
ml	Millilitre
O_2	Oxygen
PAL	Physical activity level
PYY	Peptide YY
REE	Resting energy expenditure
SD	Standard deviation
SEM	Standard error of the mean
SWA	SenseWear armband
TEE	Total energy expenditure
TEF	Thermic effect of food
VAS	Visual analogue scale
VCO_2	Rate of carbon dioxide production
VO_2	Rate of oxygen consumption
WHO	World health organization
YSI	YellowSprings™ glucose analyser

CHAPTER 1 : INTRODUCTION

Obesity is often described as a complex condition with multi-factorial causation (Grundy, 1998, Ogden et al., 2007) and has multiple detrimental effects on health and wellbeing (Wang et al., 2011). Despite being the focus of much research there is no proven unifying hypothesis, nor detailed description of mechanisms that can fully explain the increasing trend in incidence. Modern obesity, sometimes described as an epidemic (Caballero, 2007), seems to be mainly driven by profound environmental changes accompanied by changes in individual behavioural patterns (Crawford and Ball, 2002, Swinburn et al., 2011). Greater emphasis on identifying the key behaviours that contribute to obesity is needed prior to developing efficacious strategies and thereby reversing the epidemic and its negative consequences.

Partly due to a combination of technological advances in production, processing packaging and food preservation, food affordability and availability has increased (Cohen, 2008). Food requiring minimal preparation now can be obtained easily at any time of the day, both inside and outside of the home (Harnack et al., 2000). Moreover, it seems that many people in Western societies no longer comply with traditional family eating times due to more hectic schedules and irregular work patterns (de Assis et al., 2003). These factors have increased the likelihood of people consuming food erratically and may promote greater inter-daily variation in the frequency and distribution of eating than was seen in the past.

In previous studies, such an increase in variation, between days, in for example meal number, resulting in an irregular meal pattern has been associated with

disadvantages to health (Farshchi et al., 2004a, Farshchi et al., 2004b, Farshchi et al., 2005).

In this thesis the effects of regularity of meal patterns on energy expenditure, appetite regulation and cardiometabolic risk factors is considered. First, in the following literature review, the author introduces obesity, energy expenditure, energy intake, and appetite. Previous research concerning the impact of meal patterns on obesity and metabolic health outcomes is then critically reviewed. Finally, the aims of this thesis are described.

1.1 Obesity

1.1.1 Definition and classification of obesity

Obesity refers to an abnormal accumulation of adipose tissue that is large enough to impair health (WHO, 1998). Adipose tissue is difficult to measure via routine examination, so the body mass index (BMI) is used as a widely accepted indicator of obesity in adult populations. BMI is calculated as the weight in kilograms divided by the square of the height in metres (kg/m^2). BMI was firstly introduced in the 19^{th} century by Adolphe Quetelet, and was originally called the Quetelet Index (Eknoyan, 2008). However, this index did not receive much attention until Ancel Keys published a paper in 1972 (Keys et al., 1972) demonstrating that the Quetelet Index largely correlated with adiposity and minimally correlated with height. At that point, it was renamed the BMI.

The World Health Organization (WHO) has adopted the BMI as the international standard for classifying adults as overweight and obese (WHO, 1995). The WHO classification of BMI (Table 1.1) is based on the relationship

between mortality and BMI in the North American and European populations (WHO, 1998). Specifically, individuals with a BMI of 25–29.9 kg/m^2 are classified as overweight and those with a BMI of 30 kg/m^2 or higher are classified as obese. Furthermore, there are three levels of obesity, and individuals with a BMI of 40 kg/m^2 or higher are classified as morbidly obese.

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

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

Although BMI is a simple, non-invasive, and useful index of adiposity at the population level, there are limitations to its use at the individual level. Firstly, BMI does not discriminate between lean and fat mass (Prentice and Jebb, 2001). Therefore, it can provide a misleading estimate of relative adiposity in some individuals. Athletes, for example, who have a relatively higher proportion of lean mass to fat mass (compared with the general population), may be misclassified as overweight or obese (Ode et al., 2007). Alternatively, in older people, BMI values might be within the normal range but there may be

a relatively higher proportion of adipose tissue than in the general population due to muscle atrophy (Villareal et al., 2005, Cook et al., 2005). As such, BMI is not a perfect measurement overall, but it does provide a standard, practical method of classifying adiposity.

The recent trend in obesity assessment has been to incorporate a combination of BMI and other measurements including waist circumference and waist–hip ratio. These are considered additional and valuable measurements for predicting individuals at high risk for obesity-related disease such as cardiovascular disease, as abdominal adiposity has a stronger association with such disease than does adiposity accumulation in other areas of the body (WHO, 2008).The identification of obesity-associated disease risks using waist circumference and waist–hip ratio measurements appears to be population-specific, as such, international cut-offs cannot be identified (WHO, 2008).

1.1.2 Prevalence of obesity

The prevalence of obesity has emerged as a rapidly increasing global problem that has reached epidemic proportions. It is now widely recognised as one of the greatest burdens of ill health facing the populations of both developed and developing countries. Figure 1.1 shows the global prevalence of obesity in adults in 2013. WHO statistics have indicated that, worldwide, around 52% of adults aged 18 and over were overweight and obese (WHO, 2014).

In Europe, the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project (1989) found that the prevalence of obesity has risen by 10-40% in the majority of European countries over the past thirty years. The most significant rise in obesity prevalence occurred in England, where it increased more than twofold over the same period (Prescott-

Clarke and Primatesta, 1997). More recent data from the Health Survey for England (Eastwood, 2014) indicate that the rate of obesity increased from 13.2% and 16.4% for men and women, respectively in 1993 to 24.4% for men and 25.1% for women in 2012. These data also show that the percentage of overweight including obese adults rose from 57.6% and 48.6% for men and women, respectively to 66.6% for men and 57.2% for women between 1993 and 2012. The UK's Foresight analysis projects that 47% and 36% of men and women respectively will be obese by 2025. These numbers are predicted to increase to more than 50% of both gender by 2050 (McPherson et al., 2007).

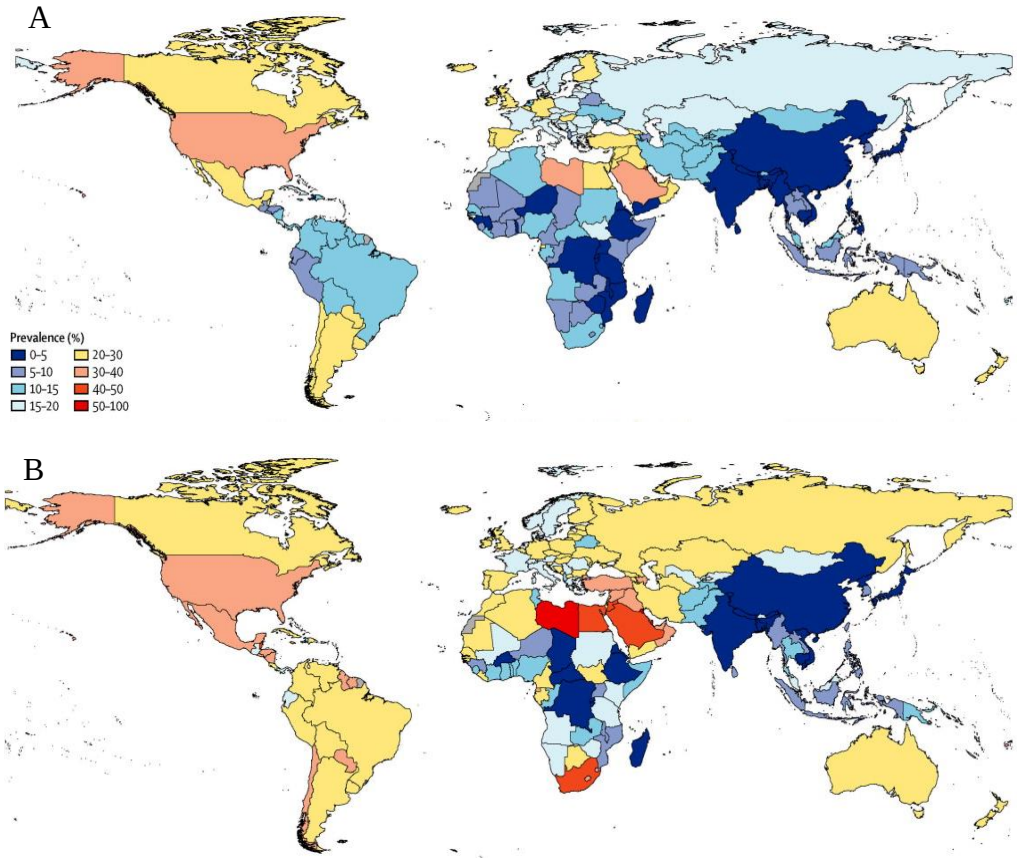

Figure 1.1 Prevalence of obesity (BMI ≥ 30 kg/m^2) worldwide in 2013 in males (A) and females (B) aged ≥ 20 years (Ng et al., 2014).

1.1.3 Health and economic effects of obesity

Overweight and obesity are associated with factors that both increase the risk of subsequent chronic diseases (e.g. insulin resistance, dyslipidaemia and hypertension) and of chronic diseases directly, including cardiovascular disease, diabetes mellitus (type 2), osteoarthritis and cancers (Wyatt et al., 2006, Kopelman, 2007).

Not only does obesity adversely affect individuals' health and quality of life, but it also contributes to a rising financial cost to health services. In 2002, the total cost of obesity and its consequences to the NHS in England was estimated to be between £3340 to £3724 million. If the cost of overweight is also taken into account, the total cost is estimated to be between £6.6 to 7.4 billion (McCormick et al., 2007). Thus, an increase in obesity prevalence is projected to be associated with further increases in health service costs.

1.1.4 Causes of obesity

According to the first law of thermodynamics, energy cannot be created or destroyed, but can be converted into different forms. Thus, the fundamental cause of obesity is a positive energy balance wherein energy intake exceeds energy expenditure for an extended period of time, resulting in fat deposition and weight gain.

1.2 Body weight regulation

The principle of energy balance can be described by the simple equation: energy expenditure =energy intake, by which stable body weight is maintained. The scenario of positive energy balance could arise from either excessive energy intake or low energy expenditure resulting from a sedentary lifestyle, or

both concurrently (Macdonald, 2000). Conversely, fat stores are converted to energy during a period of "deficit" which is defined as a negative energy balance, and results in weight loss over time. Therefore, fat stores are a reflection of the historical difference between energy expenditure and energy intake. Body weight is considered to be a reliable indicator of the state of a person's energy balance (Hall et al., 2012).

1.2.1 Energy expenditure

An adult's total energy expenditure (TEE) can be defined as the 24-h rate of energy expended by the body. TEE has three main components: the basal metabolic rate (BMR), the thermic effect of food (TEF) and the energy cost of physical activity (PA). There are other minor components such as adaptive thermogenesis and energy costs of medications and emotion (Prentice, 2007, Levine, 2005)

1.2.1.1 BMR

The BMR is the first main component of TEE and refers to the amount of daily energy required to maintain basic life processes (e.g., respiration, heart function, neural function, and membrane potentials) (Goran, 2000). The BMR represents the largest component of daily TEE and can account for approximately two-thirds of it (Prentice, 2007).

BMR should be measured under standardized conditions in the morning after a 12-h overnight fast (i.e., while in the post-absorptive state) with a healthy individual who is fully awake but physiologically and mentally at rest in the thermoneutral zone. The thermoneutral zone is defined as the range of ambient temperatures in which a healthy individual can maintain normal body

temperature without needing to use energy above BMR. In humans, the recommendations for a thermoneutral zone, for a lightly clothed individual, is between 23 and 27 °C (Speakman and Keijer, 2013).

The average BMR for an adult is estimated to be approximately 1 kcal per min (Goran, 2000). However, BMR varies with age, gender, body size, and body composition. Indeed, individual variability in BMR is negligible (less than 5%) compared with the variability between individuals (Murgatroyd et al., 1987).

Fat-free mass is the strongest determinant of BMR, with up to 80 % of the variance in BMR being explained by fat-free mass (Nelson et al., 1992). Fat-free mass comprises of two components: low metabolic rate tissue (i.e. bones and connective tissue) and highly metabolic active organs and muscles (Müller et al., 2002). The factors that result in a reduction in the metabolically active component of fat-free mass will accordingly have a negative effect on BMR and vice versa. For example, the decline in BMR with age is primarily due to an age-related decline in fat-free mass (Keys et al., 1973, Visser et al., 1995). BMR is also affected by gender, with females having a lower BMR than do males, due to gender differences in the proportion of fat-free mass to fat mass (Buchholz et al., 2001).

Exercise training leads to an increase in BMR. Training has more implications in terms of sustaining fat-free mass and reducing body fat (Stiegler and Cunliffe, 2006). Furthermore, habitual physical activity purportedly elevates BMR. Among individuals who share the same age, BMI, and fat-free mass, active individuals (who exercise $\geq$ 6 h/week and maintained this level at least 5 years) tend to have a higher BMR than do sedentary individuals (who exercise $\leq$ 2 h/week for a minimum 5 years) (Gilliat-Wimberly et al., 2001). In this

study, although the two groups had the same BMI and fat-free mass, the sedentary group had a higher percentage of body fat that could explain their results.

Should one of the strict measurement conditions for BMR not be met, then the measurement is defined as the resting metabolic rate (RMR). The RMR tends to be slightly higher than BMR (approximately 3% higher) due to subject arousal (Goran, 2000). However this will depends on which of the measurement conditions has not been met. BMR and RMR are often used interchangeably due to their high level of similarity; however this should be done with caution.

Numerous factors may explain RMR variability. For instance, a 30-min acute exercise session results in a significant elevation of 48-h RMR (Dolezal et al., 2000, Speakman and Selman, 2003). Activities of daily living also lead to an increase in RMR. However, a short period of rest (approximately 10-20 min) before the measurements commence is enough for the impact to dissipate (Compher et al., 2006).

Food consumption just before an RMR test can increase energy expenditure if the test is undertaken during the postprandial period. Similarly, caffeine, and alcohol increase RMR for several hours after consumption (Compher et al., 2006). Cigarette smoking has been also shown to increase RMR. This has been suggested as the mechanism behind a lowered body weight in smokers (Audrain et al., 1991).

RMR is also influenced by hormonal state. Thyroid hormones (THs) have long been recognised as having a profound direct impact on metabolism and the regulation of energy balance. Specifically, the dysregulation of THs can lead to

significant alterations in energy balance, which in turn results in hyperthyroidism or hypothyroidism (López et al., 2013). Hypothyroidism is an endocrine disorder characterized by decreased RMR that causes weight gain. In contrast, hyperthyroidism results in an overproduction of THs, which causes an increase in RMR and weight loss (Warner and Mittag, 2012). The mechanism by which THs affect RMR remains unclear.

Shivering due to cold exposure can increase energy expenditure in terms of RMR fivefold (van Marken Lichtenbelt et al., 2014). Furthermore, an increase in RMR accompanied by a gradual decrease in shivering through daily cold exposure (8 h exposure for 31 days) has been noted (Davis, 1961). These findings suggest the existence of non-shivering energy expenditure in humans. However, research findings on the impact of cold exposure on non-shivering energy expenditure have been inconsistent. Although Lean and colleagues (1988) reported an increase in sleeping energy expenditure and TH function in normal-weight females exposed to mild cold (22°C), most other research points to the fact that non-shivering energy expenditure, after cold exposure, varies considerably between individuals and perhaps can be explained by age, gender, physical fitness, diet, acclimatization, and genetic factors (van Marken Lichtenbelt et al., 2014). As RMR is influenced by the environmental temperature, it has been suggested that the typical ambient temperature for measuring RMR should be 22°C - 25°C (Compher et al., 2006).

Technical aspects of the equipment used to measure energy expenditure can also affect RMR measurements. For example, the indirect calorimetry system must be switched on and warmed up 30 min prior to use. In addition, the gas analysers of the indirect calorimetry system must be calibrated using two

cylinders of pressurised gas of known composition and periodically validated with alcohol burning (Pinheiro Volp et al., 2011).

1.2.1.2 TEF

TEF is the additional energy expenditure above BMR that occurs in the short term after ingestion of a meal (Tataranni et al., 1995). The TEF can be divided into two main components: obligatory and facultative thermogenesis. Obligatory thermogenesis, which represents the energy consumed for digestion, absorption, metabolism, and transfer of ingested nutrients in the body during the postprandial phase, accounts for two-thirds of total TEF. Facultative thermogenesis represents the energy expended in excess of obligatory thermogenesis, involving the activation of sympathetic nervous system and accounting for one-third of total TEF (Acheson, 1992).

The TEF makes up only a small percentage of the TEE (approximately 10%) (Van Zant, 1992); nevertheless, the TEF may play a role in body weight regulation and the development of obesity (Schutz et al., 1984). Small but prolonged differences in TEF can account for notable differences in body weight among populations (Granata and Brandon, 2002). Due to the difficulties in measuring TEF of total food intake, the TEF is commonly measured following a single meal, over periods as short as 3 h (Reed and Hill, 1996). It has been reported that TEF has returned to baseline after 3 h post consumption for a meal providing 15% of the total daily energy requirements, and containing around 50%, 16%, and 34% of the energy from carbohydrates, proteins, and fats, respectively (Belko et al., 1986). Weststrate (1993) suggested that the TEF after mixed meals of 1.3–2.6 MJ can be accurately assessed within 3 h.

The energy cost of the TEF is predominantly affected by the quantity and macronutrient composition of the food consumed. Overeating leads to an increase in the TEF which is relative to the degree of overeating; conversely, food restriction decreases TEF (Joosen and Westerterp, 2006). The metabolism of proteins is the most demanding energetically (20% to 30 % of the total energy from protein), followed by carbohydrates (5 % to 15 % of the total energy from carbohydrate) and then fats (0 to 3 % of the total energy from fats) (Raben et al., 2003). The position of alcohol in this metabolism ranking is still unclear (Joosen and Westerterp, 2006). Generally, however, the measured TEF will be higher for meals containing mixed substrates requiring increased enzyme synthesis compared with meals containing one substrate (Barr and Wright, 2010). The TEF response to a mixed substrate or a high-carbohydrate, high-protein meal has been demonstrated to be significantly lower in those who are obese compared with that seen in normal-weight individuals (Pittet et al., 1976, Kaplan and Leveille, 1976, Bessard et al., 1983). The lower TEF that is seen in obese individuals supports the theory that a thermogenic defect may favor obesity. However, this theory was not supported by others (Felig et al., 1983, Nair et al., 1983). The differences in the methodology among laboratories as well as the heterogeneity of the obese participants, mainly with respect to insulin resistance can potentially be a reason for these conflicting findings. There is evidence that insulin resistance is associated with blunted TEF (Ravussin et al., 1985). The mechanism for this effect may partially be that insulin resistance results in a reduction in the rates of glucose uptake and storage, consequently, a decrease in the TEF (Ravussin et al., 1985). It has been also suggested that following ingestion of a meal; insulin resistance

induced a dysfunction in sympathetic nervous activity, hence, lower TEF (Schwartz et al., 1990, Watanabe et al., 2006).

1.2.1.3 Physical activity

The other main component of TEE is the energy cost of physical activity. Here, physical activity is defined as the use of skeletal muscles for body movement, which results in additional energy expenditure (Westerterp, 2008). The energy cost of the physical activity is based on the type and the duration of each activity.

A MET is a unit of physical activity intensity, expressed as a multiple of resting energy expenditure. More specifically, a MET is the RMR in which the amount of O_2 uptake at rest is approximately 3.5 ml O_2/kg/min. For example, working at two METs needs 7 ml O_2/kg/min, twofold the RMR, and so on (Jette et al., 1990).

Physical activity has been categorised as being performed at three levels of intensity, light, moderate, and vigorous, depending on the metabolic cost and difficulty of an activity (Department.of.Health, 1991). Generally, The TEE can be expressed as BMR × physical activity level (PAL). The PAL is the ratio of overall daily energy expenditure to BMR and is determined by an individual's lifestyle (Department.of.Health, 1991). 'Occupational' and 'non- occupational' activity should thus be taken into account (Department.of.Health, 1991).

Physical activity is the most variable portion of TEE among individuals: the energy cost of physical activity can range from negligible to significant based on an individual's activity level (Westerterp, 2008). Thus, the physical activity has an important role in body weight regulation. As mentioned previously, 80 % of the variance in BMR is explained by fat-free mass, and TEF accounts for

10 % of TEE. Hence, the variance in the physical activity level can explain the majority of the remaining difference in TEE, which occurs independently of body weight. It has been shown that the level of physical activity was inversely associated with body weight (Schmitz et al., 2000). An inverse association has been also found between physical activity level and BMI (Remmers et al., 2014). Relatively sedentary individuals have a higher risk of weight gain (Must and Tybor, 2005).

1.2.2 Energy intake

Energy intake is the sum of the energy content of ingested food and beverages as provided predominately by carbohydrates (3.75 kcal/g), proteins (4 kcal/g), fats (9 kcal/g), and alcohol (7 kcal/g). The metabolism energy is estimated using Atwater factors (Maynard, 1944, Southgate, 1981). The metabolism of these dietary components provides an individual with energy (Woo et al., 1985). Unlike energy expenditure, which is a continuous process, energy intake occurs in distinct bouts (i.e. meals and snacks). An individual's daily energy intake is spread over a certain number of meals/snacks, separated by intervals of variable duration across the day. The between day individual variability in energy intake depends upon the variation in the number, volume, and macronutrient composition of meals/snacks on each day.

Energy intake has been suggested as a greater contributor to obesity than energy expenditure (Blundell and Finlayson, 2004), and was indeed cited as the main contributor to weight gain in the US population between the 1970s and 2000s (Swinburn et al., 2009), and in women in the UK between the 1980s and 2000s (Scarborough et al., 2011).

1.3 Appetite

Appetite can be viewed as a comprehensive term that encompasses a range of parameters related to eating behaviours (King et al., 1997) including the internal driving force for the search, choice, and ingestion of food (de Graaf et al., 2004). Appetite also has been used to collectively describe various sensations associated with food intake (Sørensen et al., 2003) and it has been suggested to include three main components; hunger, satiation and satiety (Mattes et al.). Hunger is a sensation related to the desire to obtain and consume food (Blundell et al., 1996). Satiation is defined as occurring at the termination of an eating occasion when the desire to eat has been satisfied. Satiation can be determined by the amount of food consumed during an eating occasion and the duration of an eating occasion (Blundell et al., 1996). Satiety is the state of an interval between eating occasions where further eating is inhibited as a consequence of a previous eating occasion. The intensity of satiety can be determined either by the inter meal duration or by the amount of food consumed at the subsequent eating occasion (Blundell et al., 1996).

Appetite is a subjective construct and it is hence difficult to quantify. Visual analogue scales can be used to assess the extent to which individuals have a subjective experience of the different dimensions of appetite. In terms of obesity, it is the consequent intake that is of most interest. This can be estimated by methods such as food intake diaries, questionnaires, and biomarkers (Mattes et al., 2005). However, appetite, whilst not a direct marker of subsequent intake, is of interest when studying obesity, because there are associations with intake. Behaviours resulting in differences in appetite may be targets for behaviour modification to improve energy balance regulation (e.g.

different meal patterns), and studies demonstrating differences in subjective appetite can indicate the direction for further work which includes energy intake measurement.

1.3.1 Studying appetite and food intake

In the laboratory setting, an *ad-libitum* meal can be served whilst participants are asked to rate their experience of subjective appetite sensations. Participants may be asked to eat until they feel comfortably full. The food is then re-weighed to calculate the amount and/or energy content of food consumed (Sørensen et al., 2003). Relations between food and reported subjective appetite sensations can then be explored.

It is well documented that palatability has a strong impact on *ad-libitum* food intake (Blundell et al., 2010). Therefore, it is crucial that the experimental foods are similarly liked when studying the effect of particular food properties on appetite. However, the *ad-libitum* food intake differs to a large extent. For instance, it has been shown that people consumed on average 70–80 g from cheese cookies, whereas they had about five times as much of pears with syrup. These findings were not because the differences in liking as the biscuits and pears were about equally liked (Weenen et al., 2005). Cognitive factors play an important role on how much people eat *ad-libitum* from specific food in experimental studies. People gradually learn to estimate satiating effects of various foods and these learning mechanisms control the expectations about the satiating properties of foods (Brunstrom, 2007). Knowledge about the time until the next meal is another cognitive factor that plays a role on *ad-libitum* food intake. It has been shown that people ate less from an *ad-libitum* meal when they knew they had access to food in the next 20 min in comparison with

a situation where the next meal was 2 h ahead. So, people took into account future food availability when deciding on their current eating (De Graaf et al., 1999).

The study of relations between food intake and subjective appetite is more difficult in a free-living environment. The ability to observe the association between appetite and food intake in a free-living environment might be reduced due to difficulties relating to the measurement of food intake (Subar et al., 2003). Furthermore, various factors can disrupt the relationship between appetite and food intake. For example, emotional stress or boredom can lead to overeating (Hill et al., 1991, Yeomans et al., 2004). Moreover, some type of foods, mainly those rich in sugars and fat, are associated with potent rewards that promote eating in the absence of hunger (Volkow et al., 2011). On the other hand, food shortage or social constraints can prevent eating when hungry (Mattes et al., 2005). Indeed, food intake behaviours can be strongly affected by numerous factors such as ambient temperature, food accessibility, nutrient composition, and palatability cues, including taste and smell (Ishii et al., 2003, Perry and Wang, 2012).

1.3.2 Visual analogue scales (VAS)

VAS are a commonly used questionnaire for assessing subjective experiences such as the different dimensions of appetite. The validity and reproducibility of this convenient, quick, and easy tool has been confirmed for a variety of subjective sensations, and is currently considered the gold standard in the field of pain perception (Yarnitsky et al., 1996). VAS validity and reproducibility for assessing appetite have showed inconclusive findings (Flint et al., 2000).

The assessment of motivational behaviour is subjective, meaning that motivational behaviour assessments must be interpreted with care or used concurrently with other methods such as assessments of energy intake to provide insight into behaviours and mechanisms that affect energy balance. Nevertheless, the reliability and validity of VAS are good when used in within-subject designs under controlled conditions, and VAS appear to be sensitive to experimental manipulation (e.g. alterations in diet composition or energy intake) (Stubbs et al., 2000). The VAS remains the only method available for providing a measurement of the entirely subjective appetite.

VAS ratings of 'hunger', 'desire to eat', and 'prospective food consumption' are linked with subsequent food intake. In contrast, ratings of 'satiety' or 'fullness' seem to reflect retrospective food consumption (Hulshof et al., 1993, Barkeling et al., 1995).

1.3.3 Appetite biomarkers

Appetite is physiologically regulated through a complex system which engages numerous hormonal and neuronal signals. Signals that fluctuate considerably within a day, arise largely from the gastro-intestinal tract in response to eating episodes defined as short-term or episodic signals (Blundell et al., 2008). Those episodic signals contribute to the control of meal initiation and termination and include glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and ghrelin (Blundell et al., 2008). Signals that arise from tissue stores (particularly adipose tissue) and fluctuate relatively little within a day, with variation only observed over longer time periods are referred to as long-term or tonic signals (Blundell et al., 2008). Tonic signals portray the magnitude of fat stored in the

body and may contribute to sustaining energy balance (e.g. leptin and adiponectin) (Blundell et al., 2008).

It has been proposed that episodic signals would be associated with a motivational state, whilst tonic signals would be more closely linked to traits for eating (or overeating) (Blundell et al., 2008). In general, both episodic and tonic signals are integrated and provide information to the hypothalamus which controls appetite and thus body weight maintenance (Figure 1.2).

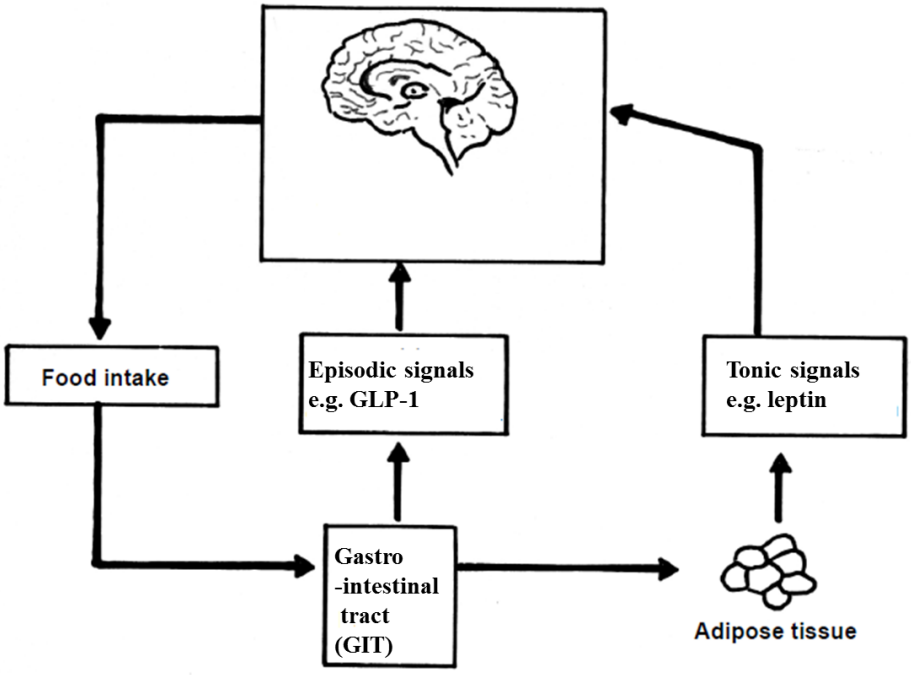

Figure 1.2 Integrated episodic and tonic signals in appetite and food intake.

Adopted from (Konturek et al., 2005).

The concentrations of these hormones could therefore be considered biomarkers for appetite studies, or may contribute to explaining differences in appetite following differences in eating behaviour. The next section discusses some of these biomarkers in more details.

1.3.3.1 GLP-1

GLP-1 is a gut peptide hormone released by the endocrine L-cells of the distal ileum and colon in response to nutrient intake (Baggio and Drucker, 2007) (Figure 1.3). The infusion of GLP-1 has been shown to suppress appetite and reduce food intake (Flint et al., 1998, Gutzwiller et al., 1999, Naslund et al., 1999). The infusion of GLP-1 reduced subsequent food intake does-dependently in both normal-weight and obese individuals (Gutzwiller et al., 1999, Verdich et al., 2001). It has been reported that postprandial GLP-1 secretion declined in obese individuals (Naslund et al., 1999). The infusion of GLP-1 before a meal resulted in a 15% decrease in energy intake at that meal as well as 0.5 kg lost over a five-day period in obese individuals (Näslund et al., 2004). Moreover, GLP-1 is involved in insulin biosynthesis (MacDonald et al., 2002) and has been reported to normalise glucose in individuals with poorly controlled type 2 diabetes (Nauck et al., 1993).

1.3.3.2 Peptide YY (PYY)

PYY is another peptide hormone that is released postprandially by the endocrine L-cell of the distal ileum and colon in its active form (PYY 3-36) (Karra et al., 2009) (Figure 1.3). In normal-weight individuals, the infusion of PYY has been demonstrated to decrease appetite and 24 h food intake (Batterham et al., 2002). It has been reported that obese individuals had lower

fasting PYY concentrations compared with normal-weight (Batterham et al., 2003). However, infusion of PYY resulted in an inhibition of appetite and food intake over 24 h (Batterham et al., 2003). Infusion of PYY has also been associated with reduced durations of eating as well as subjective hunger ratings (VAS) (Degen et al., 2005).

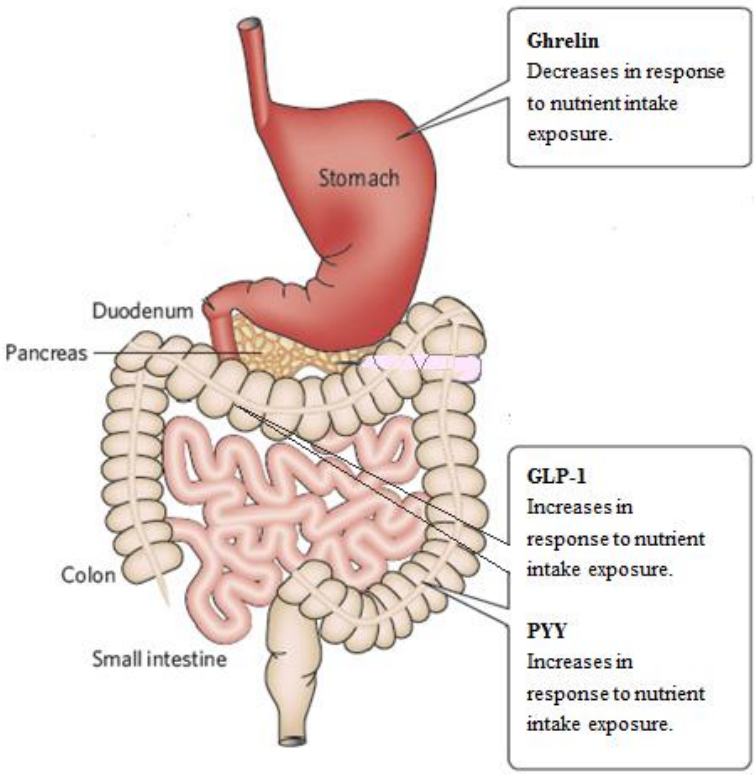

Figure 1.3 Summary of the gastrointestinal tract hormones.
Adopted from (Murphy and Bloom, 2006).

1.3.3.3 Ghrelin

Ghrelin is a 28-amino acid peptide hormone, which is synthesised and secreted mainly by oxyntic cells in the stomach (Kojima et al., 1999) (Figure 1.3). Ghrelin is the only known gastrointestinal hormone identified as an appetite stimulant so far (Buss et al., 2014). Unlike GLP-1 and PYY, ghrelin concentrations increase in the fasting state (Cummings et al., 2001) and are suppressed rather than stimulated by food intake (Wren et al., 2001, Cummings et al., 2004). Carbohydrate and protein are more potent suppressors of ghrelin than fat at equal loads (Foster-Schubert et al., 2008). The amount of energy consumed in a meal shows a dose-dependent association with postprandial suppression of ghrelin levels (Callahan et al., 2004), which suggests that large meals would lead to a greater reduction in subjective hunger than smaller ones.

Several studies showed that ghrelin concentrations are related to feeling of hunger as assessed by VAS (Wren et al., 2001, Blom et al., 2005, Druce et al., 2005). Along with the pre-prandial increase in ghrelin concentrations and its association with hunger ratings, it has been suggested that it plays a role in meal initiation, opposed to within meal satiation (Cummings et al., 2004).

Recently, it has been suggested that ghrelin is involved not only in the regulation of food intake, but also in hedonic eating, and may be crucial for the experience of food-induced reward (Buss et al., 2014). In response to palatable food, under conditions of satiety, ghrelin was found to increase rather than decrease, suggesting that ghrelin's signalling might drive hedonic food intake in the absence of an energy deficit (Monteleone et al., 2013).

Ghrelin concentrations have been observed to be significantly lower in obese compared with normal-weight individuals (McLaughlin et al., 2004, Druce et

al., 2005). However, it has been suggested that obesity may induce ghrelin resistance, and association between ghrelin and eating behaviour observed in normal-weight may not apply to obese individuals (Andrews, 2011).

1.3.3.4 Leptin

Leptin is a peptide released mainly by white adipose tissue (Jéquier, 2002). In the positive energy balance state, leptin concentrations increase substantially leading to suppressing appetite and food intake (Jéquier, 2002). On the other hand, leptin concentrations decline during the state of negative energy balance (Keim et al., 1998). Leptin concentrations are positively correlated with the fat stores in the body (Jéquier, 2002), however, the appetite suppressing action might be weak in obese individuals as they are likely to be leptin resistant (Blundell et al., 2008, Carlson et al., 2009). Leptin resistance has been found to be related to the development of insulin resistance in obese and type 2 diabetic patients (Lafontan and Viguerie, 2006).

1.3.3.5 Adiponectin

Adiponectin is a peptide released exclusively by adipose tissue, (Chandran et al., 2003). In contrast to leptin, adiponectin concentrations, paradoxically, are negatively correlated with the fat stores, with obese having a lower concentration than normal-weight human (Chandran et al., 2003). Low concentrations of adiponectin have been found to be associated with hyperinsulinaemia and the degree of insulin resistance (Weyer et al., 2001) and suggested a reliable biological marker for insulin resistance in type 2 diabetic patients (Chandran et al., 2003). Adiponectin administration has been shown to decrease blood glucose concentrations and enhance insulin sensitivity (Lihn

et al., 2005). These results suggest a possible role for adiponectin to alter glucose or insulin levels to indirectly affect appetite regulation as the reduction in blood glucose levels precedes food requests (Campfield et al., 1996). Furthermore, a significant relationship between fasting adiponectin concentrations and TFEQ scores of disinhibition has been reported (Blundell et al., 2008).

1.4 Menstrual cycle and energy balance

The menstrual cycle is a repeated, normal condition that occurs in females of reproductive age which is characterized by fluctuations in female sex-steroid hormones. In the majority of females, its length varies from 25 to 30 days, with an average of 28 days (Buffenstein et al., 1995). The menstrual cycle can be divided into two distinct phases, follicular and luteal phases, separated by ovulation (Buffenstein et al., 1995). The follicular phase starts on the first day of menstruation and ends with ovulation and the luteal phase starts immediately after ovulation (Davidsen et al., 2007).

Considerable evidence has accumulated to suggest that energy intake decreases during the follicular phase in relation to the increase in estrogen concentrations compared with the luteal phase which is characterized by an increase in progesterone concentrations. (Dye and Blundell, 1997, Brennan et al., 2009, Tucci et al., 2010, McNeil and Doucet, 2012). It has been reported that during ovulation, following the peak in estrogen concentrations, energy intake is at its lowest point (Buffenstein et al., 1995, Dye and Blundell, 1997, Davidsen et al., 2007). The reason behind the reduction in food intake during the follicular phase remains unclear but might involve changes in energy expenditure. It has been observed that total energy expenditure decreased during the follicular

compared with luteal phase (Buffenstein et al., 1995, Dye and Blundell, 1997, Davidsen et al., 2007). However, despite these variations in energy intake and expenditure across the menstrual cycle, no significant changes in body weight or fat percentage have been reported (Buffenstein et al., 1995, Dye and Blundell, 1997).

Appetite hormones may also vary in relation to the different phases of the menstrual cycle. It has been demonstrated that plasma GLP-1 concentrations were lower during the follicular phase compared with the luteal phase (Brennan et al., 2009). Furthermore, hunger ratings were lower during the follicular compared with the luteal phase (Brennan et al., 2009). On the other hand, it has been reported that plasma ghrelin and adiponectin concentrations remain unchanged across the menstrual cycle (Dafopoulos et al., 2009). Therefore, based on the previous evidence, it is important to consider the menstrual cycle phase in studies evaluating energy intake, energy expenditure and appetite in female participants.

1.5 Insulin resistance

Insulin resistance as defined by Berson & Yalow (1970) is ‘a state in which greater-than-normal amounts of insulin are required to elicit a quantitatively normal glucose response to a meal’. Insulin resistance has a potential role in the development of cardiovascular diseases which is the major health concern (Pacini and Mari, 2007). It is also associated with a wide range of diseases including diabetes, hyperlipidaemia and hypertension (Reaven, 1995). Excess body fat increases the risk of insulin resistance hence is of interest in the study of obesity.

Insulin is a hormone made by the β-cells of the pancreatic islets of Langerhans and is considered the main regulator of glucose homeostasis. Insulin controls the stimulation of glucose uptake by muscles and adipose tissue and the inhibition of the production of hepatic glucose (Pacini and Mari, 2007).

Various methods have been developed for quantifying insulin resistance and the hyperinsulinaemic euglycaemic clamp has been referred to as the criterion method. The hyperinsulinaemic euglycaemic clamp was originally developed by Andres (1966) and further developed by DeFronzo (1979). This method is performed after an overnight fast by intravenous infusion of a fixed-rate of insulin, to create a hyperinsulinaemic plateau of plasma insulin concentrations, while glucose is infused at a variable rate in order to achieve and maintain blood glucose concentration constant. Once a steady state has been reached, the rate of glucose infusion required to maintain blood glucose concentration constant, during the time of the clamp, reflects directly the amount of glucose taken up into metabolic tissues. The higher rates of glucose required to maintain blood glucose within the normal range indicates a lower degree of insulin resistance. Frequent blood sampling is required over the clamp period to determine blood glucose in order to maintain a steady fasting level (DeFronzo et al., 1979). Frequent blood sampling as well as time taken and resources required render this method impractical to screen populations or in large epidemiological research (Wallace and Matthews, 2002).

Due to the impracticality of hyperinsulinaemic euglycaemic clamp, several mathematical models for the estimation of insulin resistance have been developed. The homeostatic model assessment of fasting insulin resistance (HOMA-IR) that was developed by Matthews (Matthews et al., 1985) is the

most common one and has been widely used as an index to assess insulin resistance in research. The index is based upon the interactions between insulin and glucose dynamics, which is then used to estimate fasting steady-state insulin and glucose concentrations (Patarrão et al., 2014). The HOMA-IR is calculated using the following equation:

$$\textbf{HOMA-IR} = [Ib \times Gb / k]$$

Where Ib is fasting insulin, Gb is fasting glucose and k is a constant to scale HOMA-IR.

The k factor is normalized to be 22.5, as the normal fasting plasma insulin and glucose obtained from a healthy individual (without insulin resistance) are 5 mU/L and 4.5 mmol/L, respectively. Hence, HOMA-IR index equals 1 in an individual with normal insulin sensitivity (Matthews et al., 1985).

For the most accurate estimation using the HOMA-IR index, the average of three samples for the fasting insulin (taken at 5-min intervals) should be calculated due to the fact that the insulin secretion is pulsatile (Wallace et al., 2004). However, in actual practice only a single sample is often taken (Trout et al., 2007).

Assessments of insulin resistance from HOMA-IR have demonstrated good correlations with estimates from the euglycaemic clamp (Matthews et al., 1985, Bonora et al., 2000). (Matthews et al., 1985, Bonora et al., 2000).

1.6 Review of previous literature relating to meal pattern and health

Meal pattern refers to the frequency (i.e. number of eating occasions) and circadian distribution of eating throughout the course of a day. Eating

occasions refer to meals or snacks, depending on the type and quantity of food consumed as well as the time of the day at which it is consumed (Bellisle, 2014). However, in the studies described in this thesis, the author has used the term ‘meal’ to describe all eating occasions of food or drink (except water) with an interval of at least 1 h before the next occasion.

1.6.1 Irregular meal pattern

An ‘irregular’ meal pattern refers to an erratic consumption of food resulting in notable daily variation in meal patterns.

A body of evidence has shown that irregular meal patterns have become more prevalent across the world over the past few decades, particularly among industrialized countries. Dietary surveys in Sweden assessing meal patterns among adolescents have shown that irregular meal patterns were relatively common among this group (Höglund et al., 1998). Specifically, breakfast was not eaten regularly by 20% of boys and 32% of girls; however, more than 50% of boys and girls did eat free school lunch regularly. Further epidemiological studies in the Nordic countries have reported that irregular meal patterns are common, and have especially increased among adolescents aged 13–18 y (Samuelson, 2000). Kagamimori and colleagues (1999) investigated what risk factors are attributable to obesity-related lifestyles among obese Japanese children, and reported that the irregular eating was more prevalent among obese children than normal-weight children. Furthermore, Murata (2000) mentioned that a sedentary lifestyle and irregular intake of meals were contributing factors to the recent increase in obesity rates and related health problems in Japanese children.

The energy intake trends in the U.S. over the past few decades have indicated a dramatic increase in the number of meals eaten outside of regular meals at home and a decrease in time spent in meal preparation at home (Harnack et al., 2000). Similarly, the U.S. has seen notable changes in dietary behaviours relating to greater energy intake, such as increased irregular snacking, eating away from home, and larger portion sizes of foods and drinks (Briefel and Johnson, 2004)..

Given the evidence to suggest that there is both a greater opportunity to follow an irregular meal pattern and some evidence to suggest that it is becoming more common, it is important to consider the health consequences and the potential role in the concurrent increase in obesity and deterioration in metabolic health.

1.6.2 Meal patterns and general health outcomes

Meal patterns have been studied for over four decades, during which time the relationship between meal patterns and health outcomes has been much debated. It must be noted that the key variable in these earlier studies was meal frequency, with the majority of studies comparing low with high meal frequencies; however, these meal frequencies were generally regular over the study periods. To date, very few studies have evaluated the impact of daily variations in meal frequency (irregular meal pattern). The following literature review includes a consideration of the effects of irregular meal pattern and the more traditional meal frequency studies, as the latter may have relevance to the focus of this thesis, namely the potential impact of irregular meal patterns.

1.6.2.1 Effect of meal pattern on body weight

The majority of meal pattern studies have occurred since the 1960s. Fabry et al. (1964) were the first to report that meal frequency was inversely associated with body weight. They demonstrated in a cohort of 379 males aged 60–64 years that significant excessive body weight was more common among individuals who reported eating three meals or less per day than those who reported eating at least five meals per day. Numerous studies have subsequently assessed the impact of meal frequency on body weight, and either reported an inverse association or failed to demonstrate any relationship between these two variables. For instance, Whybrow and Kirk (1997) found a negative correlation between meal frequency and BMI among 44 females (BMI 20-30 kg/m^2) based on 7-day weighted food diaries, while Drummond (1998), in a cross-sectional study based on unweighted 7-day food diaries, demonstrated a negative relationship between meal frequency and body weight in 48 males (BMI 18-30 kg/m^2) but not in 47 females (BMI 18-30 kg/m^2). Although under-reporters were excluded in these two studies, inconsistent definition of the meal as well as the interval between every occasion may account for the difference in results of these studies. Whybrow and Kirk defined the meal as any eating occasion (including drinks) providing at least 50 kcal, but the interval between eating occasions to be counted as a single or two occasions was not reported; Drummond defined the meal as any eating occasion when food was consumed within 15 min of each other, but not drinks consumed in the absence of food (except milk if excess of 0.5 pint). A study using 12 repeated telephone-administered 24-h dietary recalls over three months compared meal frequency between normal-weight and obese males and

concluded that normal-weight males had a significantly higher meal frequency than obese; however, when the analysis was confined to subjects with valid dietary records, no difference in meal frequency between the two groups was detected (Andersson and Rossner, 1996).

More recent cross-sectional surveys conducted in Europe observed an inverse association between the number of daily meals and BMI (Toschke et al., 2005) and body fat mass (Barba et al., 2006) in children. In the study by Toschke et al (2005), the assessment of meal frequency was based on responses to a single question asking parents "How many meals/day does your child consume?" which may not adequately characterize the children's habitual meal frequency. In Barba et al (2006) study, the meal frequency was investigated by a parental questionnaire referring to the eating frequency of the last 12 months; thus, the findings might be influenced by the recall bias.

Franko et al (2008) found that the higher meal frequency (three or more meals per day), assessed by multiple 3-day food diaries taken over several years, was related to lower BMI among adolescent girls. However, the authors did not indicate any attempt to exclude under-reporters. Mota et al. (2008) supported these findings by showing that a higher daily meal frequency (assessed by a questionnaire) related to a greater likelihood of adolescents having a BMI in the normal range; specifically, they found that, compared to overweight and obese adolescents, a lower proportion of normal-weight adolescents consumed fewer than three meals per day. Additionally, breakfast was skipped significantly more often by obese than by normal-weight subjects.

An intervention study over a period of a one year (1966) found no such effect of meal frequency on body fat deposition in normal-weight children (boys 6–

11 y and girls 6–10 y) living in three different schools. Children consumed three, five, seven meals/day in the first, second and third schools, respectively. The study used parallel groups, not a crossover design, and the children were just closely supervised during term time. Therefore, the differences between the groups' baselines as well as low compliance during holidays make these findings less reliable. Another intervention study (Wadhwa et al., 1973) on six normal-weight males reported an inverse association between meal frequency and body weight. All participants consumed three isoenergetic meals/day in the first week; then three participants consumed the same amount of energy as two meals/day and the other three consumed the same amount of energy as eight meals/day over a further two phases of four weeks each. No randomised design was used in the study and the sample size was too small for the finding to be reliable. More recently, Cameron et al. (2010) conducted an intervention study on obese individuals consuming either three or six meals/day under conditions of similar energy restriction for eight weeks. Cameron et al. concluded that meal frequency has no effect on body weight loss.

In the above literature, however, the absence of a clear definition of a meal, different methodology used to assess meal frequency and problems with the protocol design such as including under-reporters may contribute to some contradictory results. Several characteristics such as body weight, BMI and fat percentage were used in relationship with meal frequency, which may also lead to the inconsistent results.

A cross-sectional study (Sierra-Johnson et al., 2008) of 3,607 adults from Sweden, showed that irregular meal patterns were significantly associated with greater body weight and BMI in comparison with regular meal patterns. In this

study, identifying the regular and irregular eaters was based on their response to the following question: 'Do you eat regular breakfast, lunch and dinner or evening meal each day?' with a 4-point forced choice scale (never, sometimes, usually, or always). Regular eaters were those who answered usually or always, whilst, the answers sometimes or never were assumed to reflect the irregular eaters. However, the main criticism of this study is that the meal pattern as assessed by one question, with specified meals, might not give reliable and valid data.

Previous randomized, crossover design studies in Nottingham, UK (Farshchi et al., 2004a, Farshchi et al., 2005) investigated the impact of irregular meal pattern on energy metabolism and body weight in normal-weight and obese females, reported no significant differences in body weight following a two-week period of irregular (varying between 3 to 9 meals/day) compared with regular meal patterns (6 meals/day). It could be argued that a two-week period was not long enough to produce significant changes in body weight.

1.6.2.2 Effect of meal pattern on total energy expenditure

The influence of meal frequency on TEE over a 24-h period seems almost negligible.

Dallosso et al. (1982) failed to demonstrate significant differences in TEE between two isoenergetic diets of similar composition (one given in two meals and the other in six meals per day) over a two-week period in young and normal-weight males. Furthermore, there was no discernible difference in TEE between two hypocaloric diets (one given as a single meal per day and the other as five meals per day) given over a two-week period (Wolfram et al., 1987). Comparing isocaloric intake with exactly the same foods but in different

frequencies (either two or seven meals per day over 2-day intervals) yielded no differences in the TEE in healthy individuals (Verboeket-van de Venne and Westerterp, 1991). Taylor and Garrow (2001) reported that energy expenditure at night was significantly greater after consuming two meals in contrast to consuming six meals during the waking day, with no significant differences in TEE; this pattern was observed for both overweight and obese females. TEE was also not different between two isoenergetic diets (3 or 14 meals per day) comprising 15% protein, 30% fat, and 55% carbohydrates of energy being consumed by healthy normal-weight males (Munsters and Saris, 2012).

1.6.2.3 Effect of meal pattern on TEF

Studies on the influence of meal frequency on TEF following test meal consumption have provided contradictory results.

Belko and Barbieri (1987) indicated that meal frequency has no effect on TEF in healthy, weight-stable males after comparing TEF assessed for 10 h following two large meals (with each meal providing 50% of the participant's daily energy requirement) and that assessed following four small meals (each meal providing 25% of the participant's daily energy requirement), with all meals comprising the same natural food items and providing the same macronutrients. Likewise, Kinabo and Durnin (1990) noted no significant differences among healthy normal-weight females in TEF values (for 6 h) between one large meal containing 1200 kcal and two smaller meals each containing 600 kcal. More recently, Smeet and Westerterp-Plantenga (2008) found no differences between eating two or three meals/day on TEF (measured over 36 h) in normal-weight females in energy balance.

Conversely, a higher TEF (measured for 5 h) was reported following consumption of one large meal (taken in 10 min) compared with six small meals (taken 30-min apart for 150 min) with the same total caloric content as the large meal in healthy normal-weight females (Tai et al., 1991). A later study supported these findings by reporting a greater TEF (measured at 6 h) after one large meal compared to three small consecutive meals in obese children (Molnar, 1992). However, LeBlanc et al (1993) found the opposite results: they reported that four smaller meals produced greater post-prandial thermogenesis (measured for 4 h) than did one large meal in a small sample of six healthy adults individuals.

The study by Farshchi et al (Farshchi et al., 2004a) concerning eating regularity, reported a significant decrease in TEF assessed for 3 h following a milkshake test drink (the percentages of total energy from the macronutrients were 50 % carbohydrate, 35 % fat and 15 % protein), in healthy normal-weight females following a two-week period of irregular compared with regular meal patterns. A subsequent study confirmed this effect in obese females (Farshchi et al., 2005).

1.6.2.4 Effect of meal pattern on energy intake and appetite

Several studies have assessed the impact of meal pattern on energy intake as an influencing factor on body weight. Most of these studies reported that meal frequency and irregularity are positively associated with greater energy intake.

In an epidemiological study (Edelstein et al., 1992) of 2,034 adults, those who reported eating more frequently (i.e. four meals and more) during the day had a higher energy intake than did those who ate infrequently (i.e. 1–2 meals per day). Westerterp-Plantenga et al. (1994) conducted a study on women

classified as ‘nibblers’ and ‘gorgers’, subjects who reported at least five eating occasions per day were considered ‘nibblers’ and subjects who reported 2–4 eating occasions per day without snacking were considered ‘gorgers’. Both nibblers and gorgers were offered five normal-energy lunches (days 1, 2, 3, 6, and 7) and two similar energy-reduced lunches (days 4 and 5) within a week. During this week, they were asked to consume their habitual breakfast, *ad-libitum* snacks, and the meals provided. The authors concluded that following the energy-reduced meals, nibblers, but not gorgers, compensated by increasing their energy intake.

A later cross-sectional study (Drummond et al., 1998) suggested a positive association between eating frequency and total energy intake among females, but not males. However, positive relationships between the proportion of energy obtained from carbohydrates and meal frequency in both females and males were reported. In a randomised crossover study (Yates et al., 1998), normal-weight males were given a snack either 30 min or 90 min before lunch and evening meals over a 14-day period, separated by a wash-out period. Snacking led to a decrease in energy intake at subsequent meals. Moreover, 90-min pre-meal snacking resulted in a greater reduction in energy consumed at subsequent meals than did 30-min pre-meal snacking. However, daily energy intake increased slightly in both interventions overall, although this increase was less than the energy intake of the consumed snacks.

Speechly and Buffenstein (1999) suggested that eating smaller and more frequent meals can enhance control of appetite. They noted that people who consumed their breakfast as one large meal consumed a greater amount of food

in the subsequent *ad-libitum* lunch than those who consumed their breakfast as five small hourly meals.

A study (Arnold et al., 1993) investigating healthy subjects using a crossover design, failed to detect a difference in total energy intake between two diets, with either three meals per day or 9 meals per day, over two weeks. Total energy intake was also not affected by meal frequency in an experimental study comparing two meals with six meals per day (Taylor and Garrow, 2001).

Regarding regularity of meal patterns, the mean energy intake recorded over 3 days appears to be higher among individuals with an irregular meal pattern compared with those with a regular meal pattern, but no effects of meal patterns on appetite were observed (Farshchi et al., 2004a, Farshchi et al., 2005). However, their use of energy intake measurements for only three days as representative of an entire 14-day intervention period may have resulted in inaccurate results.

1.6.2.5 Effect of meal pattern on carbohydrate metabolism

Fabry et al. (1964) suggested that glucose intolerance was more common among individuals who consumed no more than three meals per day compared with those who reported five or more meals per day. Young et al. (1972) supported this finding, demonstrating a reduction in glucose tolerance among individuals considered 'gorgers' compared with those considered 'nibblers'. Later studies (Wadhwa et al., 1973, Edelstein et al., 1992) showed that a higher meal frequency was associated with lower fasting blood glucose concentrations. However, two studies (Jenkins et al., 1989, Arnold et al., 1993) found that blood glucose and serum insulin (either fasting or following a test meal) were not affected by meal frequency. Most recently, Munsters and Saris

(2012) found that consuming three meals per day led to lower blood glucose concentrations throughout the day compared with consuming 14 meals per day. Sierra-Johnson et al (2008) found no significant association between irregular meal patterns and fasting glucose concentrations, but did note a negative relation between irregular meal patterns and insulin resistance as assessed using the homeostatic model assessment index (HOMA).

The study by Farshchi et al (2004b) on normal-weight females indicated that irregular meal patterns were associated with a degree of insulin resistance, thereby leading to greater insulin response after a test drink. The other study by the same group (Farshchi et al., 2005) on obese females showed that those who had irregular meal patterns demonstrated higher insulin responses after a test drink than did those who had regular meal patterns. However, neither study demonstrated differences in glucose response to a test drink between the two meal patterns.

1.6.2.6 Effect of meal pattern on lipids

The earliest study concerning the effects of meal pattern on lipids (Gwinup et al., 1963) showed that consuming 10 meals per day produced a decrease in lipid concentrations compared with consuming three meals per day; however, these results cannot be considered representative of the general population, as only five males completed this intervention. A following study by Fabry (1964) showed that individuals who reported five meals or more per day were less hypercholesterolaemic than were less-frequent eaters (those who ate three meals or less per day). These results parallel those of Young et al. (Young et al., 1972), Jenkins et al. (Jenkins et al., 1989) and Arnold et al. (Arnold et al., 1993), who all noted that total and low-density-lipoprotein (LDL) cholesterol

concentrations were negatively associated with meal frequency during the day. Another study expanded on these findings, noting that total and LDL-cholesterol concentrations were significantly lower in people who ate smaller and more frequent meals despite their higher intake of calories, dietary cholesterol, and total and saturated fats (Edelstein et al., 1992).

Data obtained from free-living populations (Titan et al., 2001) confirmed the potential benefits of increased meal frequency on total and LDL-cholesterol.

Only one earlier study (Irwin and Feeley, 1967) found the opposite association between meal frequency and total and LDL-cholesterol: namely, Irwin reported increased cholesterol concentration among individuals who ate six meals per day in comparison to those who ate three meals per day.

Meal frequency appears to have no effect on high-density-lipoprotein (HDL) cholesterol or triglycerides (Young et al., 1972, Jenkins et al., 1989, Arnold et al., 1993).

Furthermore, some studies on 'healthy obese participants' involved in a weight reduction diet showed no relationship between meal frequency and any cholesterol concentrations (Bortz et al., 1966, Finkelstein and Fryer, 1971). Arnold et al. (1994) studied this association in free-living hypercholesterolaemic individuals instructed to consume their normal food as three or nine meals per day over a 30-day period. Notably, Arnold did not observe significant differences in total, LDL-, or HDL-cholesterol or triglycerides.

Sierra-Johnson et al. (2008) reported that total and LDL-cholesterol did not differ between regular and irregular eaters, although irregular eaters had significantly higher triglyceride levels.

Previous studies by Farshchi et al. (Farshchi et al., 2004b, Farshchi et al., 2005) found that an irregular meal pattern was associated with higher total and LDL-cholesterol, but not HDL-cholesterol or triglycerides.

1.6.3 General limitations in meal pattern studies

One of the main limitations of these previous studies is the inadequacy and high diversity of definitions of key variables, as well as the lack of standardized methodologies used in this field. For instance, some studies provided no definition of 'meal', whereas others used widely variant definitions, making it hard to compare the findings. Similarly, some studies used body weight to classify the degree of obesity, whereas others used BMI or body fat percentage. Although energy intake is a key factor affecting body weight, some previous studies did not estimate it, despite its relevance to their findings. As such, it is difficult to make any conclusions regarding the relative role of energy intake in previous findings.

Other limitations that explain apparently contradictory findings include widely varying numbers and types of meals as well as the length of intervals between meals, and differences in participants' characteristics. Furthermore, confounding variables such as menstrual cycle in women, physical activity, or previous habitual food intake were uncontrolled in these studies. A number of studies used questionnaire-based investigations, which can be subject to recall bias. Small sample sizes and a lack of power calculation were common in these studies. Consequently, all of these limitations could be reasons for some of the inconclusive results in this field.

1.7 Summary

Potential erosion of traditional meal patterns, greater consumption of meals outside the home, facilitate a shift toward marked inter-daily variation in meal frequency and have coincided with increases in obesity rates in Western societies. Therefore, the question of whether irregular eating might be driving dysregulation of energy balance and metabolic health issues is of great interest. Investigating the impacts of irregular meal pattern on obesity and related health problems while controlling for excess energy intake may be useful for developing effective strategies for preventing obesity and associated diseases.

Most previous studies have investigated the impact of meal frequency on metabolism, and, taken together, have yielded mainly inconclusive results. There is very little research on the relationship between the regularity of daily meal patterns and metabolic health outcomes. The one previous intervention study inter-daily regularity of intake was limited to measuring the impact of a self-selected intervention menu and only investigated the impact of the intervention period on a test drink. Nor was any attempt made to establish whether activity patterns were comparable during the intervention periods. Furthermore, the energy intake was not controlled. Participants were asked to consume their habitual diet distributed in a certain number of meals per day without arrangements of the time of eating.

The purpose of the present project was to determine the potential impact of an irregular meal pattern, having provided an intervention menu and monitoring activity during a 14-day intervention period among healthy normal-weight and insulin resistant overweight/obese females, on the following variables:

- TEF;
- Blood glucose (in response to a test meal) and interstitial glucose concentrations during the intervention period;
- Insulin concentrations in response to a test meal;
- HOMA-IR;
- Fasting lipid profiles (total, LDL, HDL-cholesterol and triglycerides);
- Appetite hormones and
- Reported appetite during the intervention period, in response to a test drink and *ad-libitum* food intake following a test meal.

Figure 1.4 represents the summary of the project with more details regarding the measured valuables.

Study	Chapter No	Measured variables
Normal-weight study (n=11)	Chapter 4	• Anthropometric • TEF • Free-living energy expenditure • GLP-1, PYY and ghrelin • Subjective appetite ratings (VAS) • *ad-libitum* intake
	Chapter 5	• Interstitial glucose concentrations • Serum lipids • Circulating blood glucose concentrations • Serum insulin concentrations • HOMA-IR
Overweight/obese with insulin resistance study (n=9)	Chapter 6	• Anthropometric • TEF • Free-living energy expenditure • Adiponectin, leptin GLP-1, PYY and ghrelin, • Subjective appetite ratings (VAS) • *ad-libitum* intake
	Chapter 7	• Interstitial glucose concentrations • Serum lipids • Circulating blood glucose concentrations • Serum insulin concentrations • HOMA-IR

Figure 1.4 Project summary.

CHAPTER 2 : GENERAL METHODS

Studies 1 (chapter 4 and 5) and 2 (Chapter 6 and 7) presented in this thesis shared a common study design, and methodological procedures. Therefore, to avoid repetition, shared components have been described in this chapter. Methods specific to individual studies are detailed in the relevant chapters.

2.1 Ethical approvals

All studies were run from the David Greenfield Human Physiology Unit, School of Life Sciences, Queen's Medical Centre, the University of Nottingham. All lab visits took place within the unit. The studies were approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee.

2.2 Recruitment and inclusion criteria

Participants were recruited from the student and staff populations of the University of Nottingham via poster advertisements and/or from the general public by an advertisement in a local newsletter which outlined key inclusion criteria (female, healthy and 18-45 years old). Potential participants expressed an interest via phone or email. The study protocol was explained verbally, either over the phone or in person, and then a written information sheet was provided to the participants to read. Participants who remained interested in participating were then invited to attend a screening visit to ensure they met with the inclusion criteria of each study. The following inclusion criteria were used:

- Female,

- Aged 18-45 years,
- Healthy, no history of serious disease as assessed by a medical screening questionnaire (Appendix 3),
- Not currently taking any medication except for the oral contraceptive pill,
- BMI 18.5 - 25 kg/m^2 (Study 1: Chapter 4 and 5) or 28 – 40 kg/m^2 (Study 2: Chapter 6 and 7),
- Insulin resistant, HOMA ≥ 1.5 (Study 2: Chapter 6 and 7),
- Non-smokers,
- Non-high alcohol consumers (< 2-3 units/day),
- Not currently pregnant/lactating,
- With regular menstrual cycles,
- Not currently dieting/seeking to lose weight,
- Weight stable during the last 3 months (self-reported weight change < ± 2 kg),
- Low score for clinical symptoms of depression (Section 2.3.2.1),
- Low score for eating disorder (Section 2.3.2.2) and
- No self-reported allergy or intolerance to any of the foods provided during each study.

2.3 Screening protocol

During the screening visit, which took up to 1 h, the study was explained verbally to participants and they were given a chance to ask questions. It was emphasised that participants should only take part if they were willing to follow all the study instructions. Written, informed consent was obtained by

completion of the standard University of Nottingham consent form (Appendix 1). Participants were made aware that they could withdraw from the study at any point without being required to provide an explanation. Height and body weight were measured (Section 2.3.1), then participants were asked to complete the screening questionnaires (Section 2.3.2). Blood samples were taken for routine tests to confirm their general health (Section 2.3.3). Eligible participants were then asked to complete a weighed 7-day food diary (Section 2.4) during a specified period and asked to return the diary to the investigator before commencing the study.

2.3.1 Height and weight measurements

Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Germany) according to the NHANES Anthropometry Procedures Manual as defined by the CDC (CDC, 2007)

Body weight was measured using an electronic scale to the nearest 0.1 kg (Seca, Germany) whilst participants were wearing light clothing with no shoes and with an empty bladder. Substantial accessories and items in the pockets were removed before the measurement (CDC, 2007).

Body Mass Index (BMI) was calculated from measured height and weight using the standard formula (Keys et al., 1972):

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

2.3.2 Questionnaires

During the screening visit participants were asked to complete the following questionnaires:

- Participant Information (Appendix 2)
- Medical Questionnaire (Appendix 3)
- Beck Depression Inventory (Appendix 4; Section 2.3.2.1)
- Eating Attitudes Test-26 (Appendix 5; Section 2.3.2.2)
- International Physical Activity Questionnaire (Appendix 6; Section 2.3.2.3), (NB- this questionnaire was not used for screening).

2.3.2.1 Beck Depression Inventory

The Beck Depression Inventory (BDI) is a self-rating scale intended to assess the severity of depressive symptoms (Beck et al., 1961). It is recognised to be amongst the most commonly used instrument for assessing depression throughout the world (Richter et al., 1998). The BDI is comprised of 21 items. Each item corresponds to a symptom of depression and respondents rate each item using a four-point scale ranging from zero to three.

Some items have more than one statement marked with the same score. These equivalent statements are labeled a, and b (for example, 2a and 2b). Total score of 0-9 is considered minimal depression, 10-18 is mild depression, 19-29 is moderate depression, and 30-63 is severe depression. Participants who scored ≥ 10 on BDI were excluded from taking part to avoid the potentially confounding impact of depression on appetite and body weight (Paykel, 1977, Weissenburger et al., 1986).

2.3.2.2 Eating Attitudes Test-26

The Eating Attitude Test (EAT) is a self-report instrument originally developed by Garner and Garfinkel (1979) for measuring eating behaviour and attitude frequently observed in anorexia nervosa patients. It has proved useful in

identifying eating disorders in the general population and became the most widely used instrument in a variety of cultures (Garner et al., 1982, Nasser, 1997, Garfinkel and Newman, 2001, Pereira et al., 2008).

The EAT is comprised of 40 items (EAT-40). A responds is given for each item on a 6-point forced choice likert scale. A score of 3 is given for extreme eating disordered responses, a score of 2 for the adjacent, less extreme responses and a score of 1 for the next alternative. The other three remaining alternatives answers are given no score. An overall score of higher than 30 is considered to be an indicator of an eating disorder (Garner and Garfinkel, 1979).

A 26-item version (EAT-26) was developed subsequently (Garner et al., 1982) by performing a factor analysis of EAT-40. Fourteen items were eliminated as they did not load significantly on the three factors obtained, in order to enable an easier and faster instrument. These three factors were: Dieting (relating to avoidance of fattening foods and the preoccupation with being thinner), Bulimia and Food Preoccupation (relating to thoughts about food and indications of bulimia), and Oral Control (relating to self-control of eating and perceived pressure from others to gain weight). EAT-26 is well correlated with EAT-40 (r=0.98) and demonstrates sufficient validity and reliability (Garner et al., 1982). It is scored by using the same scale as the original 40-item, but with a score of 20 as the cut-off point.

2.3.2.3 International Physical Activity Questionnaire

The international physical activity (IPAQ) is a self-reported measure of physical activity levels over the previous 7 days. IPAQ has been developed and validated for use among 18 to 65 years old adults in diverse settings (Craig et

al., 2003). It has become one of the most commonly used physical activity questionnaires (van Poppel et al., 2010).

IPAQ monitors physical activity by recording three specific types of activity which are vigorous-intensity, moderate-intensity and walking. An overall physical activity score is the sum of the frequency (days) and duration (min) of each type of activity. Three levels of physical activity are proposed as the final classification: low, moderate and high. Participants completed the IPAQ during the screening visit and the information subsequently was used to estimate the energy prescription given to each participant (see Section 2.6.1, estimation of energy requirement).

2.3.3 Routine blood tests

During the screening visit, a fasting blood sample was withdrawn from participants by an experienced research nurse. Approximately 10 ml of blood was drawn from the cubital vein for routine blood tests (blood glucose, full blood count and urea and electrolytes) to confirm participants were healthy. Insulin was also determined in Study 2 in order to enable assessment of insulin resistance.

The blood samples for the full blood count, urea and electrolytes and insulin were analysed by the Clinical Chemistry and Haematology Laboratories in the Queen's Medical Centre, University of Nottingham. Blood glucose was measured immediately using a HemoCue device (HemoCue AB, Angelholm, Sweden). Insulin resistance was assessed by using HOMA-IR index (Matthews et al., 1985):

HOMA-IR = [fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5]

Abnormal results were reviewed by a qualified doctor who confirmed a participant's eligibility to take part in the studies described in this thesis.

2.4 Food diaries

Participants were given a weighed 7-day food diary which included a record of the time of eating (Appendix 7) to complete. Food diaries were used to characterize habitual macronutrient intake and food preferences. A 7-day period of recording was chosen to avoid bias being introduced by differences between days of the week (e.g. weekend vs. weekdays) and to provide a reasonable representation of their habitual diet (Black et al., 1991).

Participants were supplied with a digital scale (Salter Aquatronic Electronic Digital Kitchen Scales, Salter housewares LTD, Tonbridge, UK). Written and oral instructions explaining how to weigh and record all food and drink consumed over 7 days were provided. Participants were instructed to keep their habitual diet and return the diaries to the experimenter before commencing the study.

Data from the diaries were subsequently converted to daily energy intake and macronutrient values using food analysis software (Microdiet v 1.2, Downlee Systems Ltd, Chapel-En-le-Frith UK).

Daily energy intake and macronutrients composition were calculated from each day for each participant. Averages were then calculated for the 7 days of recording with individual data then combined to provide group daily energy intake and macronutrient composition data.

2.5 Main study protocol

Studies 1 and 2 had a randomised, crossover design with two 14-day intervention periods, separated by a wash-out period of 14 days. Participants consumed their habitual diet during the wash out period which was included to avoid interaction between the two interventions. The study protocol is outlined in Figure 2.1.

Participants were free living except that during each intervention period, they were required to consume food provided by the investigator. The meal pattern intervention period consisted of consuming: 1. a regular meal pattern (consuming the same number of meals every day), or 2. an irregular meal pattern (consuming a different number of meals every day). The order in which participants received the meal patterns was randomised at the point of entering the study, using an on-line randomisation tool (Randomization.com).

Participants attended the laboratory pre and post each intervention period, for a total of 4 visits. Each laboratory visit lasted up to 5 h. In order to avoid the potential impact on outcome measures of the stage in the menstrual cycle (Solomon et al., 1982, Dye and Blundell, 1997, Davidsen et al., 2007, McNeil and Doucet, 2012), the start of each intervention was identical for each participant in terms of menstrual period (days 1-10).

2.6 Dietary intervention periods

Each participant was provided with, free of charge, all their food during each of the intervention periods. An individual had identical foods during each of the intervention periods and differences between participant food provision were minimised, but were sometimes necessary to meet the different energy requirements of participants. The food was supplied in a 4 day cycle of menus

consisting of a variety of items commonly consumed in the British diet (e.g. cereal, yogurt, pasta, fruits, etc.). An example of one day menu (2050 kcal) is shown in Table 2.1. The menu was designed to cover participants' energy requirement for weight maintenance (Section 2.6.1).

The macronutrient composition of the diet, as a percentage of total energy for the day, was approximately 50% carbohydrates, 35 % fat and 15 % protein. These macronutrient percentages were based on the Report of the Panel on Dietary Reference Values of COMA (Department.of.Health, 1991).

Food was weighed (TE4101 digital scales, Sartorius, Germany), packaged and labelled by the experimenter before being given to the participants. All participants declared an intention to consume the entire amount of food supplied. However, they were asked to record any left-over food in the diary provided.

Participants were reassured that the amount of food provided was designed to ensure a stable body weight over the course of the study. Participants were strictly instructed not to consume any other food or drink (except water) outside the prescribed menus. In addition, they were instructed to refrain from alcohol, limit caffeine-containing drinks to two cups of tea per day (without sugar/milk), and avoid the use of table salt, dressing sauces or condiments. They were advised to maintain their normal physical activity and not enrol in any new physical activity/exercise programme other than that reported at the beginning of the study (screening visit).

Following the design of previous studies in Nottingham (Farshchi et al., 2004a), the number of meals during the regular meal pattern was 6 meals/day whereas the irregular meal pattern ranged from 3 to 9 meals/day. It was

arranged in such a way as to achieve an average figure of 6 meals/day during the 14-day period (i.e. 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, 6 meals per day). Participants were asked to eat their meals at specific times between 08:00 am and 21:00, during both interventions, to remove the potentially confounding impact of time of eating. The only deviation from this instruction was that when they had 3 meals/day, during the irregular period, their last meal was at 18:00 instead of 21:00 in order to allow participants to eat with their families. The meals time table (Table 2.2) was driven from the actual times at which the participants ate the meals in the previous studies (Farshchi et al., 2004a, Farshchi et al., 2004b).

2.6.1 Estimation of energy requirement for the intervention menu

The energy requirement of an individual is the amount of energy intake from food required to balance energy expenditure to keep body weight constant (Macdonald, 2000). When body weight is constant over a prolonged period, an individual is likely to be in energy balance (Macdonald, 2000).

All participants confirmed that they were in energy balance (weight constant during the last 3 months). Measurement of energy expenditure of healthy individuals in energy balance gives a direct estimate of the energy requirement. In order to estimate energy expenditure for each participant, BMR was multiplied by PAL.

2.6.1.1 BMR calculation

BMR (kcal/day) was derived from Henry weight and height equations (Henry, 2005) for women aged 18-30 and 30-60 years. The choice of the equation was determined by participant's age:

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

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

2.6.1.2 PAL calculation

Physical activity was estimated by using IPAQ (Section 2.3.2.3). The level ascribed by the IPAQ was then translated to a PAL level using the Committee on Medical Aspects of Food Policy (COMA) classifications (Department.of.Health, 1991) (IPAQ score low = non active, moderate = moderately active, high = very active) and taking into account occupational activity which was classified according to COMA as light, moderate or heavy.

2.7 Measurements made during the intervention periods

2.7.1 Energy expenditure assessment

Participants wore a SenseWear™ Armband (SWA), (Model MF-SW, BodyMedia Inc, Pittsburgh, PA, USA) to assess their physical activity pattern and energy expenditure continuously during the two intervention periods. The armband was worn over the left triceps muscle, halfway between the acromion process of the scapula and the elbow. Participants were instructed to wear it continuously, including while sleeping and to remove it only for brief periods for bathing, showering or swimming.

2.7.1.1 SWA

SWA has been developed to estimate energy expenditure and physical activity level in free-living individuals. The SWA device is based on multiple sensors

which continuously collect a variety of physiological data including skin temperature, near body temperature, heat flux, body movement and galvanic skin response (BodyMedia). A sensitive electronic thermometer measures the surface temperature of the skin. Near body temperature measures the air temperature around the device to reflect the changes in the environment temperature. Heat flux measures the amount of heat dissipating from the body by using the difference between the skin temperature and the near body temperature. Motion is measured by a 3-axis accelerometer. Galvanic skin response is measured by the electrical conductivity of the skin. The skin's conductivity is affected by sweat gland activity from physical activity and emotional stimuli. All these data combined with the subject's demographic characteristics (age, gender, height and weight) and incorporated into proprietary equations developed by the manufacturer (SenseWear Professional Software, version 7, BodyMedia, Pittsburgh, PA) to estimate energy expenditure (expressed as kcal/min) and physical activity level displayed as metabolic equivalence (METs) (BodyMedia).

The advantages of SWA are that it is portable, easy to use, can be worn comfortably on the arm in virtually all environments (except water). It is also a non-invasive approach and stored data minute by minute over several days or even weeks. A validation study for SWA was conducted and described in Chapter 3.

2.7.2 Appetite assessment

Subjective appetite ratings were assessed by using paper based visual analogue scales (VAS) with words anchored at each end of a 100-mm horizontal line that expressed the most positive and the most negative rating for a question.

Each VAS consisted of 5 questions on a single page. The questions were in the form 'How (rating) do you feel?' and the ratings were 'hungry', 'satisfied', 'full', 'much of a desire to eat' and 'much of thinking to eat' (Flint et al., 2000) (Appendix 8). Participants were instructed to place a vertical mark through the horizontal line describing their current feeling. Quantification was made by measuring the distance (mm) on the horizontal line from the positive rating to the negative rating, providing a score between 0 to100 mm. A plastic ruler was used to measure to the closest mm where the placed mark intersected the horizontal line.

Participants were provided with a booklet (consisting of several sets VAS) in which to record subjective appetite before and after each single meal on days 7 and 14 during both intervention periods, when they were consuming 6 meals/day on each intervention period.

2.7.3 Glucose monitoring

The Medtronic Minimed system gold™ (Northridge, CA, USA) Continuous Glucose Monitoring (CGM) was used to continuously monitor glucose values in interstitial fluid. It was used with the first three participants in Study 1. For the remaining participants in Studies 1 and 2, a new model (iPro™2) was used. CGM was placed subcutaneously over the participant's anterior abdominal wall on day 6 and removed on day 10 (in Study 1) or day 13 (in Study 2) of each intervention period. Finger prick glucose readings were taken four times a day, by the participants, using a portable monitor (Accu-Chek Aviva System, Roche Diagnostics, Switzerland) to calibrate the CGM. Prior to use, all 4 portable monitors used in the study were validated against the glucose oxidase

method (YSI inc, Yellow Spring, USA) in a separate study described in Chapter 3.

A 24 h contact number was available for any queries or if problems arose. Data from CGM were downloaded and glucose profiles were evaluated based on the data collected on day 7, 8 and 9 in Study 1, and on day 7, 8, 9, 10, 11 and 12 in Study 2. The data were analysed per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00). Postprandial iAUC for 90 min was also analysed following each meal in which the same type and amount of food was consumed in regular and irregular periods.

Intra-day glycaemic variability was computed by an approach described by McDonnell et al (2005) specifically for CGM data, known as continuous overlapping net glycaemic action (CONGA-n) (McDonnell et al., 2005). CONGA-n is calculated as the standard deviation of the summed differences between a one hour current observation period and a one hour observation period n hours previously. CONGA-1 was calculated in the morning (current observation from 9:00-10:00) and night (current observation from 22:00-23:00). CONGA-1 indicated intra-day glycaemic variability based on one hour time periods.

2.7.3.1 CGM

CGM works through an electrochemical reaction with glucose in an individual's interstitial fluid. A disposable subcutaneous sensor incorporating glucose oxidase enzyme is used to convert interstitial glucose into electronic signals. The sensor sends the electronic signals continuously to a portable monitor which has a memory to store these signals. The monitor samples the signals once every ten seconds and records the mean signals every five

minutes, leading to 288 values per 24 h. The sensor can stay in place for three (Minimed system gold™ sensors) to six days (iPro™2 sensors).

Recording data can be uploaded into an online software (CareLink iPro™ Therapy Management Software for Diabetes). At the time of uploaded, the finger prick glucose values need to be entered for retrospective calibration of stored electronic signals which will be then converted to glucose concentrations (See Figure 2.2 for an example of the CGM data for 24 h). The accuracy of glucose readings obtained by CGM depends upon the calibration. The precision at calibration can be affected by the time lag of interstitial glucose and blood glucose which has been estimated to be between 4 and 10 min (Boyne et al., 2003, Steil et al., 2005). The imprecision of the monitor used to calibrate the CGM can also affect the accuracy of the measures done by the CGM.

CGM range is between 2.2 and 22.2 mmol/L and any glucose values below or above this range will not be shown in downloaded data.

2.8 Laboratory visit protocol

Participants were asked to attend each laboratory visit at 8:00 am following a minimum 12 h overnight fast and were required to take no exercise other than walking related to carrying out their normal activities of daily living for 48 h before the laboratory visit. The purpose of the laboratory visit was to assess the impact of regular and irregular meal pattern on metabolic variables, subjective appetite and *ad-libitum* food intake.

Immediately after arrival, weight and the other anthropometry measurements were taken (Section 2.8.1). Participants then rested in a semi-supine position for 20 min in a temperature-controlled (23-24 °C) room. Resting energy

expenditure (REE) was measured for 20 min (Section 2.8.2). A cannula was then inserted into a dorsal hand vein for subsequent blood sampling (Section 2.8.4). After fasting blood samples had been obtained, a test drink (Section 2.8.3) was consumed and postprandial measurements made for a further 3 h. An *ad-libitum* test meal was then offered as lunch (Section 2.8.5). Subjective appetite ratings (Section 2.8.6) were assessed fasted, 3 h following the test drink and 1 h following the *ad-libitum* test meal. The laboratory visit protocol is outlined in Figure 2.3.

2.8.1 Anthropometric measurements

2.8.1.1 Height

Height was measured during the first visit (see details in Section 2.3.1).

2.8.1.2 Body weight

Participants were weighed in the fasted state wearing similar light clothes on each visit (see details in Section 2.3.1). The same scale was used throughout all study visits.

2.8.1.3 BMI

BMI was calculated using the standard formula (see details in Section 2.3.1).

2.8.1.4 Circumference measurements

Waist circumference was measured to the nearest 0.1 cm in a horizontal plane, a parallel level to the floor, at a point midway between the lower margin of the last rib and the top of the iliac crest, while the participant was standing with feet about 25–30 cm apart (WHO, 2008).

Hip circumference was measured to the nearest 0.1 cm in a horizontal plane, a parallel level to the floor, at the point yielding the maximum circumference over the buttocks (WHO, 2008).

Both measurements were taken using a non-elastic measuring tape wrapped snugly, but not to the point that the tape was constricting. They were made by the same experimenter throughout all study visits to limit any variability.

In order to determine the waist to hip ratio, waist circumference was divided by hip circumference (WHO, 2008).

2.8.1.5 Body composition

Skinfold thickness measurements were made in order to assess participant's body composition (Durnin and Womersley, 1974). The measurements were made at four sites, at the triceps, biceps, subscapular and suprailiac on the right side of the body whilst the participant stood in a relaxed condition.

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

The pincer type callipers (John Bull British Indicators) were used at the selected sites, where a fold of skin and subcutaneous fat were grasped firmly with the thumb and forefingers, pulling it away from the muscle tissue

underneath. The jaws of the calliper were applied at a constant pressure, at right angles to the pinch and about 1cm below. A reading was obtained within 2 seconds of applying the force. The measurements were made in three cycles, so allowing the skin and subcutaneous fat to recover. An average of the three readings for each site was calculated. The sum of the four skinfolds (biceps + triceps + subscapular + suprailiac) was used to determine body density using (Durnin and Womersley, 1974) equation:

Women aged 16-68 density x 10^3 = - 0.0717 x log $\sum$ (skinfold) + 1.1567

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

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

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

The same callipers were used throughout all study visits by the same investigator.

2.8.2 Energy expenditure measurement

Following the anthropometry measurements, participants rested semi-supine in a hospital bed and were instructed to make themselves comfortable. After a 20 min rest, a perspex ventilated canopy was placed over the participant's head, with the surrounding pliable plastic sheet secured in place. Air for respiration passes through the canopy's holes, whilst expired and room air in the canopy

were extracted through a tube connecting the canopy to the indirect calorimetry system (GEM system; Europa Scientific Ltd, England).

REE was measured in the fasted state for 20 min. TEF was then measured for periods of 15 min at 30 min intervals during the 3 h following the milkshake consumption. During the measurements, participants were asked to breathe normally, relax and remain still. In the intervals between the measurements, they also rested on the bed, but they were allowed to read.

2.8.2.1 Indirect calorimetry

Indirect calorimetry is a widely used technique for measuring energy expenditure. This technique depends upon measurement of respiratory gas exchange. An open-circuit flow-through canopy, with a mass flow meter, mixing chamber and a vacuum pump, was used to draw room air over the participants' face at a rate of 50-60 l/min. This is considered to be the most convenient way for measuring energy expenditure in human studies at rest (Fellows and Macdonald, 1985). The system was connected to a computer, and data from the mass flow meter and gas analysers were used to calculate the volume of oxygen and carbon dioxide using the software provided by the manufacturer. The respiratory gas exchange data were then converted to energy expenditure by using the Weir equation (Weir, 1949):

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

The Weir equation was based on measurement of rate of airflow in a ventilated canopy and the changes in percentage O_2 concentrations between inspired and

expired air. STPD is the standard temperature and pressure of dry air. This equation did not require protein utilization to be measured. 0.2087 is a factor based upon protein representing about 12.5% of total energy intake. The error in energy expenditure measurement is less than 0.5 % for 0-25 % of protein intake from total energy.

2.8.2.2 Indirect calorimetry calibration

The indirect calorimetry system was turned on for half an hour prior to use, to warm up. Two cylinders of pressurised gas of known composition were used to calibrate the gas analysers in the indirect calorimetry system before the start of the experiment. Room air was measured at the start and during each 15 min measurement period to ensure the gas composition passing the canopy is known and stable.

Before commencement of the study, a series of alcohol calibrations were made to ensure valid values were being obtained using the indirect calorimeter. A known amount of alcohol (ethanol) was burnt under a special canopy for each 20 min run. This calibration was repeated monthly and the results reflected that the calorimeter was operating correctly in view of alcohol respiratory exchange ratio being very near the expected value of 0.667 and % recovery being above 95 %.

2.8.3 Test drink consumption

The standardized test drink (vanilla flavour milkshake) was served at room temperature (19 ºC) in an open glass as a breakfast. Participants were instructed to drink it over a period of 10 min. The milkshake test drink comprised 50 % of energy as carbohydrate, 35 % as fat, and 15 % as protein. The test drink

contained skimmed milk (Sainsbury's, London, UK), Build-up (Nestle SA, Lausanne, Switzerland), Polycal (Nutricia Clinical Care, Trowbridge, UK) and double cream (Sainsbury's, London, UK). The volume of 10 kcal/kg body weight was provided to the normal-weight participants. For the obese participants, the volume of 10 kcal/kg healthy body weight (equivalent to a BMI of 22.5 kg/m^2) was provided as the test drink. This gave a volume of the test drink that would be tolerated by participants. A BMI of 22.5 kg/m^2 was selected following the precedent of the calculation of the DRV for energy by Scientific Advisory Committee on Nutrition (SACN, 2011b).

In order to achieve a stable body weight, an individual needs to have about 35 kcal/kg body weight. The test drink was designed to provide approximately one third of this value as a large breakfast to stimulate a reasonably large metabolic response which would return to the baseline 3 h postprandially (Table 2.3).

2.8.4 Blood sampling and processing

Whilst the participant rested semi-supine, their hand was placed into a heated ventilated perspex box (50–55 ºC) (Department of Medical Physics, Nottingham, UK) for 20 min to arterialise the venous blood. A 20 G cannula (pink Venflon) was then inserted into a dorsal hand vein under local anaesthetic (1 % lignocaine: B.Braun Melsungen AG, Melsungen, Germany) for subsequent blood sampling. The hand was kept in the heated box to allow arterialised venous blood sampling (McGuire et al., 1976). A 0.9 % saline infusion (Baxter Healthcare Ltd. Thetford, UK) was started at an approximate rate of 125 ml/h to keep the cannula patent.

Blood samples were drawn from a 3-way tap. The first 2 mL of each sample was discarded to avoid contamination with the saline.

Two blood samples were taken with a 5 min interval in the fasted state just before ingestion of the test drink to assess the mean of fasting blood glucose, serum total, HDL, LDL-cholesterol, triacylglycerol, insulin, plasma Glucagon-Like Peptide-1 (GLP-1), ghrelin, Peptide YY (PYY), adiponectin and leptin (the last two variables were assessed just in Study 2). After the test drink ingestion, blood samples were taken every 15 min for 3 h to assess blood glucose and every 30 min for 3 h to assess serum insulin, plasma GLP-1, ghrelin and PYY. The arterialized venous blood gives a reasonable estimate of the actual arterial value (Liu et al., 1992).

Blood was dispensed into EDTA tubes and serum separating tubes (allowed to clot for 30 min at room temperature before centrifugation). EDTA tubes containing either 20 μl dipeptidyl peptidase IV (DPP-IV) inhibitor (Millipore, Billerica, MA, USA) for GLP-1 measurement or 50 μl aprotinin (Nordic Pharma, Reading, UK) for ghrelin and PYY measurements.

All samples were centrifuged (5702 R, Eppendorf, Germany) for 10 min at 3000 r.p.m at 4 ºC. The supernatant was transferred into plastic labelled tubes and kept at -80 ºC until further analysis. The analyses were carried out at the University of Nottingham by research technicians.

2.8.5 *Ad-libitum* test meal

A pasta-based test meal (providing 167 kcal/100 g with 13, 34 and 53 % energy provided by protein, fat and carbohydrate, respectively) was served at lunchtime to assess *ad-libitum* food intake. This meal had a homogeneous nature, so energy intake could be assessed from the weight of food consumed. The meal consisted of 125 g of dried pasta (Sainsbury's, London, UK) cooked on the day of the study (in a full power 900 W microwave) for 13 min and

stirred mid period. The pasta was then drained, cooled rapidly using cold water and then mixed with 40 g of cheddar cheese (Sainsbury's), 15 g of olive oil (Sainsbury's), and 170 g of tomato and basil pasta sauce (Dolmio, Mars food, UK). The mixture was then chilled until required and heated in the microwave for 2 min before being served to the participants. Participants were given portions of ~500 g and instructed to consume as much as they wanted until they felt 'comfortably full'. The plate of pasta was continually topped up, when it was approximately ¾ empty (Figure 2.4). This ensured that there was always ample hot food available to participants and they were not cued to stop eating by having emptied their plate. Any left-over was removed and energy intake was calculated from the weight of food consumed. Duration and speed (g/min) of eating were also calculated.

2.8.6 Subjective appetite ratings

VAS was presented on single A4 paper sheets (Appendix 8). Participants completed the VAS just before, after and then every 30 min after consumption of the test drink for 3h. Further VAS were completed before and immediately after consuming the lunch test meal, and then at 15, 30, 45 and 60 min. To avoid previous VAS scores impacting on the next set of scores, each VAS was taken from the participant before the next one was provided. During this period of time, participants were asked to stay in the laboratory, but they were free to read.

2.9 Blood samples analysis

2.9.1 GLP-1

Plasma samples from whole blood collected in EDTA coated tube containing DPP-IV inhibitor were defrosted. GLP-1 concentrations were then measured using an ELISA kit (Linco Research, St Charles, MO, USA). The intra-assay coefficient of variation was 5%. All samples were assayed in one assay to avoid inter-assay variation.

2.9.2 PYY

Plasma samples from whole blood collected in EDTA coated tube containing aprotinin were defrosted. PYY concentrations were then measured with commercially available radioimmuno assays (Millipore, Billerica, MA, USA). The intra-assay coefficient of variation was 10%. All samples were assayed in one assay to avoid inter-assay variation.

2.9.3 Ghrelin

Plasma samples from whole blood collected in EDTA coated tube containing aprotinin were defrosted. Plasma total ghrelin concentrations were then measured with commercially available radioimmuno assays (Millipore, Billerica, MA, USA). The intra-assay coefficient of variation was 8%. All samples were assayed in one assay to avoid inter-assay variation.

2.9.4 Adiponectin

Serum samples were defrosted and serum adiponectin concentrations were measured with commercially available radioimmuno assays (Millipore,

Billerica, MA, USA). The intra-assay coefficient of variation was 9%. All samples were assayed in one assay to avoid inter-assay variation.

2.9.5 Leptin

Serum samples were defrosted and serum leptin concentrations were measured with commercially available radioimmuno assays (Millipore, Billerica, MA, USA). The intra-assay coefficient of variation was 9%. All samples were assayed in one assay to avoid inter-assay variation.

2.9.6 Lipids

Serum samples were defrosted and serum total, HDL, LDL-Cholesterol and triacylglycerol levels were quantified by an enzymatic photometric method (HORIBA ABX, Montpellier, France). The intra-assay coefficient of variation for total, HDL, LDL-Cholesterol and triacylglycerol were < 2.5%, < 4.0%, < 4.0% and < 3.0, respectively. All samples were assayed in one assay to avoid inter-assay variation.

2.9.7 Glucose

Blood glucose concentrations were measured immediately using a HemoCue® 201^{+} device (HemoCue AB, Angelholm, Sweden). The HemoCue principle is based upon a glucose dehydrogenase technique and comprises a small dedicated analyser and a disposable micro cuvette. The analytical principle was described by (Banauch et al., 1974). A small amount of blood was placed on a small chamber in the cuvette. The cuvette then was wiped clean and placed into the cuvette holder. Light is passed light through the sample and the absorbance of the coloured formazan is determined at 660nm using a two-wavelength

technique to compensate for turbidity. Glucose concentrations (mmol/L) are then displayed after few seconds on the LCD display. The recommended measurement range for the HemoCue is 0–22.2 mmol/L.

2.9.8 Insulin

Serum samples were defrosted and serum insulin concentrations were measured with commercially available radioimmuno assays (Millipore, Billerica, MA, USA). Insulin sensitivity was calculated using the homeostatic model assessment (HOMA model) (Matthews et al., 1985). The intra-assay coefficient of variation was 10%. All samples were assayed in one assay to avoid inter-assay variation.

2.10 Statistical analyses

SPSS software (version 21 for windows; SPSS) was used for data entry and analysis. All data are presented as means ± standard deviation (SD), unless otherwise stated. Data were tested for normality with the Kolmogorov-Smirnov test to inform whether parametric or non-parametric analysis should be used.

Values for the incremental area under the curve (iAUC) of TEF, appetite ratings, glucose, insulin and gut hormones responses were calculated using delta values relative to the baseline. Values above baseline were considered positive, and below baseline negative. Area was calculated using the trapezoid rule.

Comparisons of the baseline data (pre interventions) were carried out using Student's paired t test (two-tailed).

Two-way repeated measure ANOVA (Factor 1: meal pattern, two levels: regular and irregular meal pattern; Factor 2: visit, two levels: pre and post the

14 days intervention) was conducted to assess the impact of the two week meal pattern intervention on a range of dependant variables (e.g. iAUC for postprandial glucose). Where an interaction was identified, simple main effects were explored by pairwise comparisons. Where no interaction was identified main effects were reported and if significant main effects were found, pairwise comparisons were made for the effect of meal pattern or the effect of visit. Differences were considered significant at $P < 0.05$ for all statistical tests.

Table 2.1 Example of a 2050 kcal menu

Meal No	Meal Time	Meal	Energy /kcal	CHO %	Pro %	Fat %
1	8.00	All Bran 40 g Milk 200 ml + Sugar 1 tbsp. Oatcake 1 piece + Butter 5 g	412	53	13	34
2	11.00	Muller drinking yogurt 2 pots Waffle 1 piece	238	58	12	30
3	12.30	Jacket potato 1 piece Cheddar cheese 40 g Banana 1 piece	451	55	14	31
4	15.30	Custard 1 pot Apple 1 piece	222	55	11	34
5	18.00	Beef lasagne 1 pack Orange juice 1 cartoon	510	54	19	27
6	21.00	Milk 200 ml + Sugar 1 tsp. Rich tea biscuits 3 pieces	216	44	14	42
Total Daily			**2049**	**53**	**14**	**33**

Table 2.2 Meals time table

	Number of eating occasion (daily meals)						
	3	**4**	**5**	**6**	**7**	**8**	**9**
Time (24 h basis)	08:00	08:00	08:00	08:00	08:00	08:00	08:00
							09:30
			11:00	11:00	11:00	11:00	11:00
	12:30	12:30	12:30	12:30	12:30	12:30	12:30
						14:00	14:00
				15:30	15:30	15:30	15:30
	18:00	18:00	18:00	18:00	18:00	18:00	18:00
					19:30	19:30	19:30
		21:00	21:00	21:00	21:00	21:00	21:00

Table 2.3 Milkshake test drink recipes

- Recipe for the milkshake test drink with 449 kcal used for participants weighted 40-49.9 kg:

Ingredients	Amount	CHO	Pro	Fat	Energy
Skimmed milk	220 ml	11 g	7.5 g	0.22 g	76.4 kcal
Build up	38 g	23.9 g	8.8 g	0.3 g	133.8 kcal
Double cream	35 g	0.9 g	0.6 g	16.7 g	155.2 kcal
Polycal	34 ml	21 g	-	-	83.6 kcal
Total weight		56.8 g	16.9 g	17.3 g	-
Total energy		226.9 kcal	66.9 kcal	155.2 kcal	449 kcal
% energy		50.5 %	15 %	34.5 %	-

- Recipe for the milkshake test drink with 547 kcal used for participants weighted 50-59.9 kg:
- ±

Ingredients	Amount	CHO	Pro	Fat	Energy
Skimmed milk	250 ml	12.5 g	8.5 g	0.3 g	86 kcal
Build up	50 g	31.5 g	11.6 g	0.4 g	174.4 kcal
Double cream	43 g	1.2 g	0.7 g	26.6 g	193.5 kcal
Polycal	38 ml	23.5 g	-	-	93.1 kcal
Total weight		68.7 g	20.8 g	21.2 g	-
Total energy		274.7 kcal	81.2 kcal	191.1 kcal	547 kcal
% energy		50.2 %	15 %	34.8 %	-

- Recipe for the milkshake test drink with 649.6 kcal used for participants weighted 60-69.9 kg:

Ingredients	Amount	CHO	Pro	Fat	Energy
Skimmed milk	250 ml	12.5 g	8.5 g	0.3 g	86 kcal
Build up	65 g	40.9 g	15 g	0.5 g	227 kcal
Double cream	51 g	1.4 g	0.9 g	24.4 g	229.3 kcal
Polycal	43 ml	26.6 g	-	-	105.1 kcal
Total weight		81.45 g	24.4 g	25.2 g	-
Total energy		324.8 kcal	95.5 kcal	229.3 kcal	649.6 kcal
% energy		50 %	15 %	35 %	-

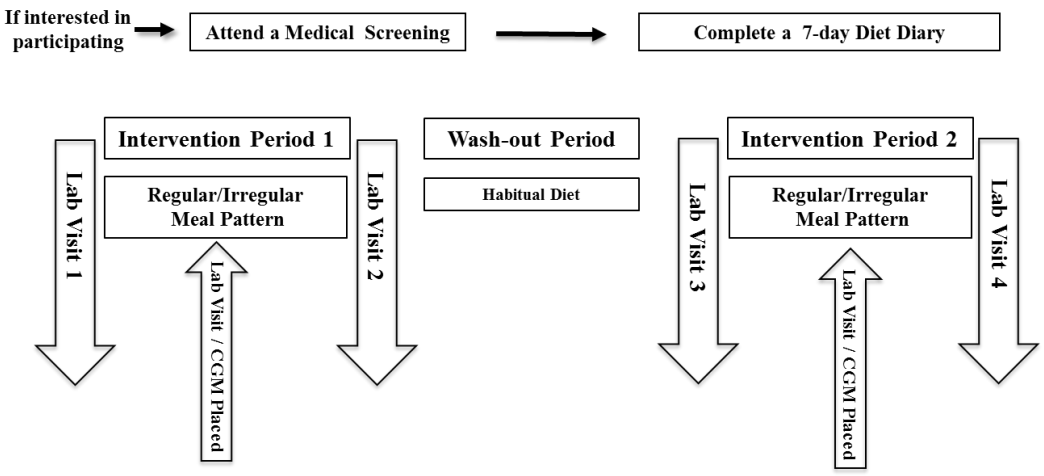

Figure 2.1 Study protocol.

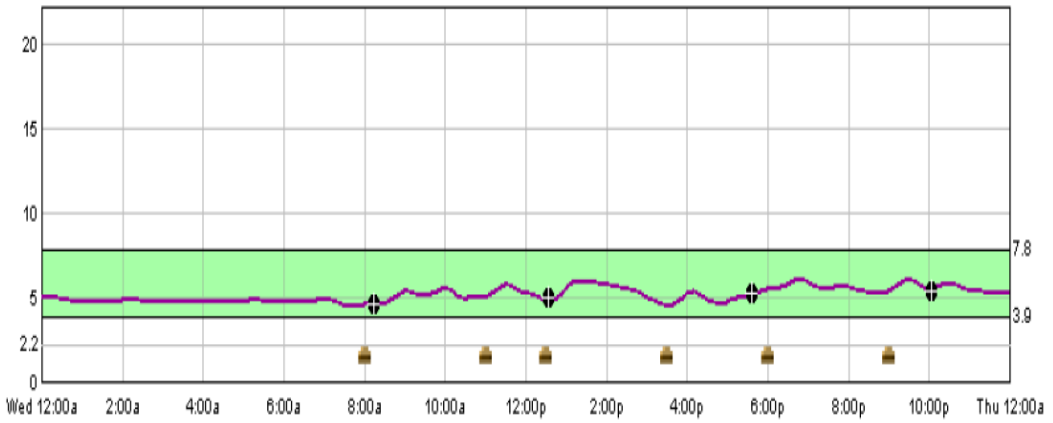

Figure 2.2 Example of 24 h of CGM data

Time of the day is shown on the X axis and glucose in mmol/L is on the Y axis. The purple line shows the continual glucose concentrations, the crosses show the blood glucose concentrations taken by the participant and the brown squares depict the meals consumed.

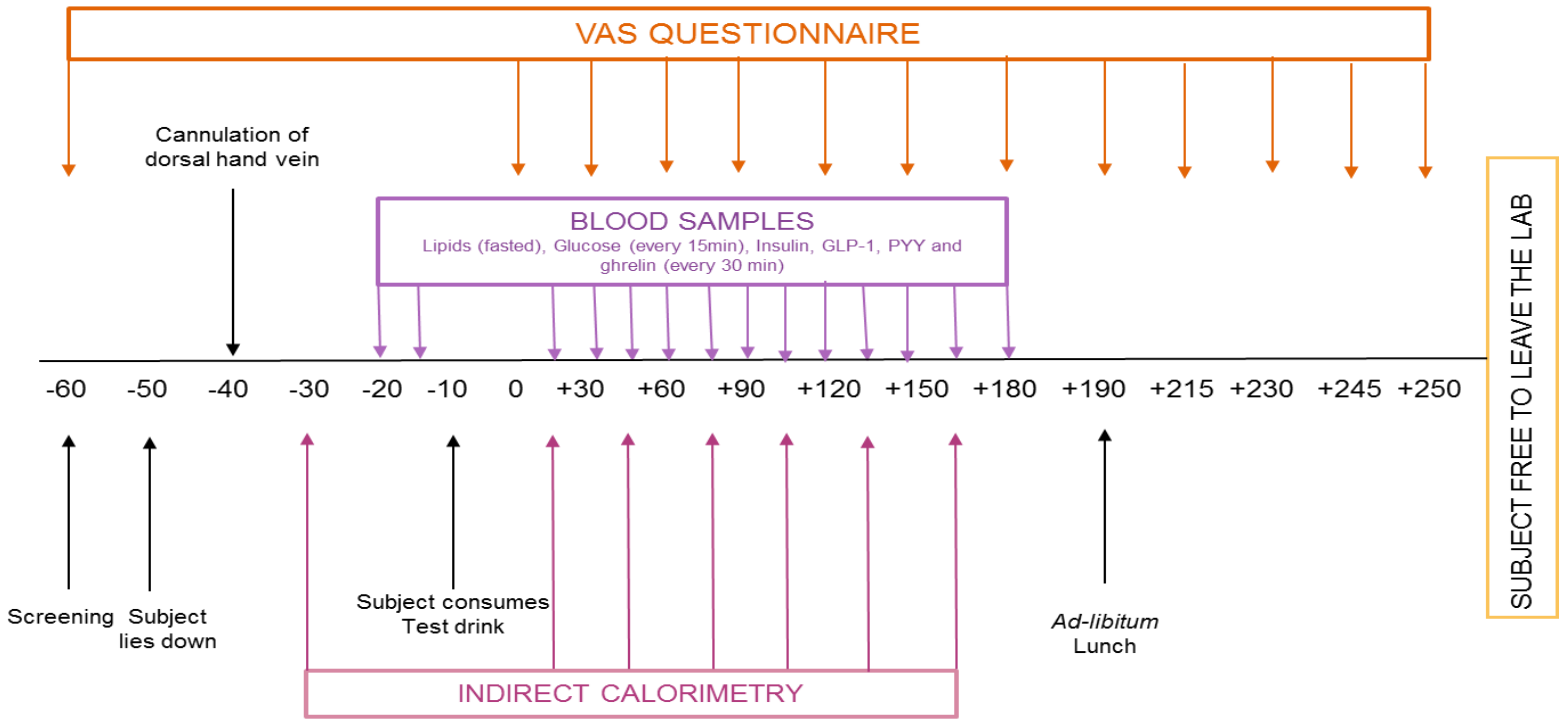

Figure 2.3 Laboratory visit protocol.

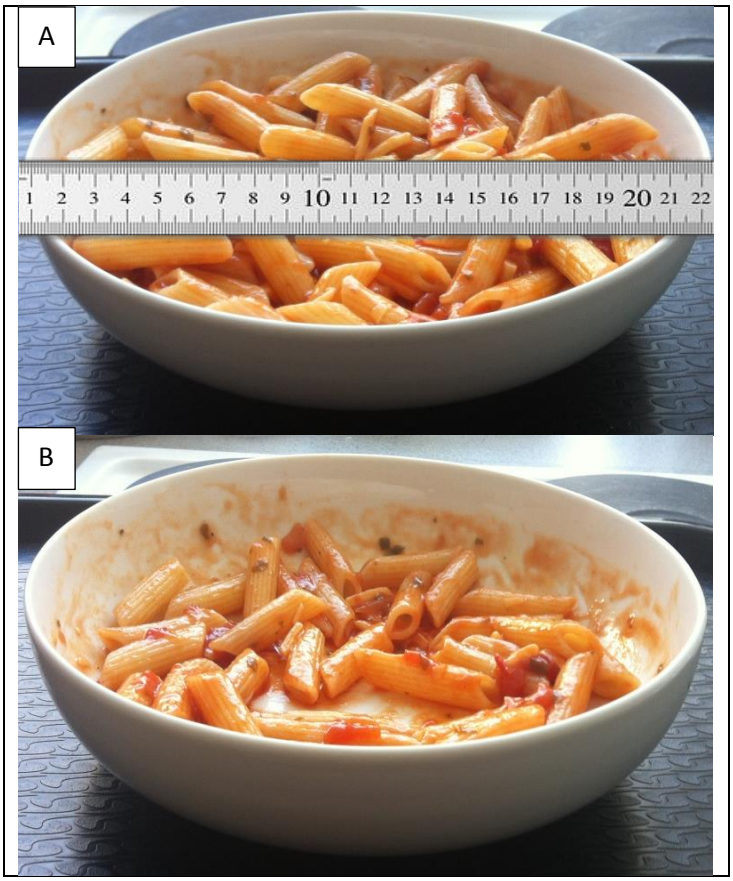

Figure 2.4 *Ad-libitum* pasta test meal.

(A) Full plate of pasta.

(B) ¾ empty plate of pasta (when it was refilled).

CHAPTER 3 : VALIDATION STUDIES OF THE SENSEWEAR ARMBAND AND THE GLUCOSE POCKET ANALYSERS

The SWA and the glucose pocket analysers, used in this thesis, were validated in separate studies and all protocols were approved by the Research Ethics Committee of the University of Nottingham, Faculty of Medicine and Health Sciences.

3.1 Validation of the SWA

The SWA (Model MF-SW, BodyMedia Inc, Pittsburgh, PA, USA, Section 2.7.1.1) was evaluated for accuracy, for estimating energy expenditure in female adults, against the indirect calorimetry (GEMNutrition Ltd, Cheshire, UK) as the criterion method. Furthermore, to assess whether the position of the device on the subject's body and movement of the limb on which the device is placed are important in the estimation of energy expenditure, two SWA devices were used. One was worn on an upper arm (stationary throughout the intervention) and one was worn on an ankle (protocol included leg raises).

3.1.1 Participants and protocol

Nine healthy females, aged 20 to 35 years were recruited by posters placed around the University of Nottingham campus, inviting participation. Inclusion criteria included being a non-smoker, a non-high alcohol consumer (< 2-3 units/day), on no regular medication other than the oral contraceptive pill, neither pregnant nor lactating, with no self-reported history of chronic disease or any other serious medical condition. Written informed consent was obtained from each participant prior to enrolment in the study. Descriptive

characteristics of the participants are presented as mean ± SD in Table 3.1.

Participants attended the David Greenfield Human Physiology Unit for a screening visit, which took up to 30 min. Height and body weight were measured and BMI was calculated according to the procedure explained in Section 2.3.1. Participants were then asked to complete a questionnaire concerning their general health.

After the screening visit, participants were invited to attend the laboratory on a subsequent day after an overnight fasting (12 h). Upon entering the laboratory, participants were required to lie on a bed in a room maintained at 23-24 ºC. The SWA device was placed on the participant's left arm (over the triceps muscle, halfway between the acromion process of the scapula and the elbow) as recommended by the manufacturer and another on the left ankle (5 cm above the talocrural joint). The two devices were in place for a period of 5 to 10 min before data collection to allow for adaptation to skin temperature. They were fully charged and the participant's demographic characteristics including date of birth, gender, height and weight were programmed into the SWA software before each visit. The indirect calorimetry system was turned on for half an hour prior to use, to warm up. The details of using and calibrating indirect calorimetry were explained in detail in Section 2.8.2.2. Room air was measured during each run.

Participants were instructed to lie beneath the ventilated hood perspex canopy in a comfortable position, during the measurement phase, whilst they simultaneously wore two SWA (one on an upper arm and one round an ankle). They were asked to rest in the supine position and make minimal movements, other than that which was prescribed, remain awake and breathe normally for

the duration of the measurements.

Baseline measurements of energy expenditure using the indirect calorimetry were made during an initial 30 min period and during the exercise challenge for an hour. The protocol for energy expenditure measurements is shown in Figure 3.1. During the exercise challenge period, participants were required to lie still (with straight or bent knee), except when performing the exercises. A 2 kg weight was placed around each ankle and the knees were bent (to support the subject's back during the exercise). Then, they were asked to perform extended lower leg raises (of alternate legs) to approximately 90 degrees from the bed (Figure 3.2) at a rate of fifteen times per min (with the frequency regulated by view of a timer). Each minute of exercise was followed by a minute of rest, and this two-minute cycle was repeated five times over a 10 min period. The exercise phase was then followed by 10 min rest. The 20 min programme was repeated three times per hour, with SWA devices and indirect calorimetry system making measurements throughout. Energy expenditure was computed at 1-min intervals for both indirect calorimetry and SWA. The exact start and stop time of each indirect calorimetry measurement was recorded to synchronize with SWA estimate.

At the end of this session, participants were provided with lunch and were free to leave the laboratory.

3.1.2 Statistical Analysis

Data collected SWA devices were downloaded with SenseWear Professional software (version 7; BodyMedia, Pittsburgh, PA). Subsequently, the downloaded SWA data as well as the indirect calorimetry data were imported to Microsoft Excel (2010) for data analysis purposes. Statistical analysis was

carried out with GraphPad (Prism) (version 5.00 for Windows, GraphPad Software, San Diego California USA). If not stated otherwise, results are expressed as mean ± SD.

To validate the SWA, Pearson correlation coefficients were calculated and a simple linear regression was performed to compare between energy expenditure obtained by indirect calorimetry (EE_{IC}) and energy expenditure obtained by SWA, placed on the arm ($EE_{SWA,arm}$) during the baseline and exercise phases. The relationship between $EE_{SWA,arm}$ and energy expenditure obtained by the other SWA device, placed on the ankle ($EE_{SWA,ankle}$) during the two phases was also assessed by using the same analyses. Correlations were classified weak if $r < 0.5$, moderate if $r \geq 0.5$ to < 0.8, strong if $r \geq 0.8$ and perfect if $r = 1$(Zou et al., 2003). Statistical significance for all tests was set at the $P < 0.05$ level.

The Bland-Altman (1999) approach was constructed to analyse the limits of agreement between EE_{IC} versus $EE_{SWA,arm}$. The difference between the two methods was plotted against the mean value for the two methods in order that the limits of agreement can be calculated and plotted. Limits of agreement were derived from the mean difference between the two methods ± 1.96 SD of the differences.

3.1.3 Accuracy of the SWA

Comparison of mean EE_{IC}, $EE_{SWA,arm}$ and $EE_{SWA,ankle}$ averaged across the 9 participants during the baseline and exercise phases is shown in Figure 3.3. Energy expenditure, as expected, was higher during the exercise phase than was seen in the baseline phase when estimated using the criterion method, indirect calorimetry.

The SWA measurements for resting energy expenditure of healthy participants were, comparable in the two locations, but higher than those measured with indirect calorimetry ($EE_{SWA,ankle}$ 1.10 ±0.10, $EE_{SWA,arm}$ 1.09 ± 0.09 vs. EE_{IC} 0.85 ±0.08 kcal/min). The SWA measurements of energy expenditure during exercise were comparable by location, but lower than those measured by indirect calorimetry ($EE_{SWA,ankle}$ 1.07 ± 0.09, $EE_{SWA,arm}$ 1.05 ± 0.09, vs. EE_{IC} 1.17 ± 1.14 kcal/min).

Pearson correlation coefficients showed no significant correlation between EE_{IC} and $EE_{SWA,arm}$ during both baseline ($r = 0.51$; $P > 0.05$) and exercise ($r = 0.59$; $P > 0.05$) phases (Figure 3.4). Pearson correlation coefficient indicated a strong correlation between the $EE_{SWA,arm}$ and $EE_{SWA,ankle}$ during the baseline phase, when neither device was exposed to movement ($r = 0.89$; $P < 0.001$) and exercise phase, when the ankle, but not arm device was exposed to movement ($r = 0.89$; $P < 0.001$) (Figure 3.5).

Bland Altman plots were used to illustrate the level of agreement between EE_{IC} versus $EE_{SWA,arm}$ (Figure 3.6) and $EE_{SWA,arm}$ versus $EE_{SWA,ankle}$ (Figure 3.7) during the two phases. The x-axis represents the mean of the results of two methods, while the y-axis shows the differences between the two methods.

As shown in Figure 3.6, the bias was calculated as the mean of energy expenditure value measured by indirect calorimetry minus the mean of energy expenditure value estimated by the SWA. Low agreement was observed between the two methods. In the Bland-Altman plot for the baseline (Figure 3.6, A), 89% of the values (8 of 9) were within the limit of agreement, and 100% of the values were within the limit of agreement for the exercise phase (Figure 3.6, B).

Bland-Altman plots show good agreement between $EE_{SWA,arm}$ and $EE_{SWA,ankle}$ (Figure 3.7). Bland-Altman plot for the baseline (Figure 3.7, A) displays that all but one of the values were within the limit of agreement, and all the values were within the limit of agreement for the exercise phase (Figure 3.7, B).

Resting energy expenditure estimation by the two SWA devices, at rest, showed good agreement, in common with during light exercise. The latter was despite the fact that the SWA arm sensor was stationary throughout whereas the sensor located on the ankle was subjected to some movement. It thus seems that body movement at the attachment position does not play a major role in energy expenditure estimation despite a body movement sensor being incorporated in to the device. The SWA does not appear to be able to register small increases in energy expenditure with low level exercise, irrespective of whether the limb on which the device is located is involved in the movement. It is likely that the individual characteristics including age, sex, weight and height contribute substantially in estimating energy expenditure made by SWA, and this device is not sensitive to the body movement or the small, short term changes in energy expenditure. The development of exercise-specific algorithms by the manufacturer is warranted to improve the accuracy of SWA of estimating energy expenditure for a period of exercise.

The findings for assessing energy expenditure using the SWA were inconclusive (Fruin and Rankin, 2004, Papazoglou et al., 2006, Malavolti et al., 2007, Bertoli et al., 2008). The observed underestimation or overestimation in the energy expenditure may arise from the variances between physical activity form and level, the size and nature of the participants including age, gender,

and BMI. The differences in SWA version and indirect calorimetry system may also have a major role.

In conclusion, the SWA appears to be unable to detect the small difference in energy expenditure between the resting and exercising period, and neither location nor whether the device location was subjected to movement appeared to have a major impact on the accuracy of the measurement, compared with indirect calorimetry. However, further investigations are required to evaluate the reliability and reproducibility of SWA in different activities, free-living environment and in diverse populations before conclusions can be made about this device.

3.2 Validation of the glucose pocket analysers

Prior to use, all 4 glucose pocket analysers (Accu-Chek Aviva System, Roche Diagnostics, Switzerland) used in this thesis were evaluated for accuracy against the YellowSprings™ glucose analyser (YSI) (Yellow Springs Inc. Ohio. USA). A hyperinsulinaemic euglycaemic clamp (DeFronzo et al., 1979) was performed to generate arterialised venous blood samples which spanned the hypoglycaemic and euglycaemic range.

3.2.1 Participants and protocol

Before commencing the study, written informed consent was obtained from a healthy female. The participant visited the laboratory after an overnight fasting (12 h). Upon arrival, height and body weight were measured as described in Section 2.3.1. Body surface area was calculated, from these measures, using the formula by Mosteller (Mosteller, 1987):

Body Surface Area = √ (Height (cm) x Weight (kg) / 3600)

Following the measurements, the participant rested semi-supine and electrodes were placed on the participant's chest and 3-lead ECG monitoring was carried out throughout the study. The hand was placed into a hot air-warmed Perspex box (50–55 ºC) (Department of Medical Physics, Nottingham, UK) for 15 min, to arterialise the venous blood, prior to the insertion of a pink cannula into a dorsal hand vein under local anaesthesia (1% lignocaine: B.Braun Melsungen AG. Melsungen, Germany). The cannula was kept patent using a 0.9% saline infusion (Baxter Healthcare Ltd. Thetford, UK). The hand was placed back into the warmer, where it remained during the clamp period. A second cannula was inserted (under local anaesthesia) antegrade into a forearm vein for infusion of 0.5 IU/ml Actrapid insulin (Novo Nordisk A/S. Bagsværd, Denmark) and 20 % glucose (Baxter Healthcare Ltd. Thetford, UK) solutions. The pocket glucose analysers were switched on and their calibration checked using the manufacturer's test strip.

A baseline blood sample (0.5 ml) was withdrawn from the arterialised venous line and determined with both YSI and the pocket glucose analysers. All readings were recorded. Following the baseline sample, the 60 mIU/m^2/min hyperinsulinaemic clamp was started. Arterialised venous blood samples (0.5 ml each) were taken at 5-min intervals to determine glucose using the two analysers as before. The 20% glucose infusion kept the blood glucose at the desired concentration. Euglycaemia was maintained for 60 min, and then the blood glucose was allowed to drop gradually to 2.5mmol/L during the following 20 min. This hypoglycaemia was maintained for 20 min (to generate 5 samples) before the blood glucose was increased to a concentration > 6

mmol/L. The insulin infusion was terminated after 2 h and the participant was provided with a high carbohydrate meal. The 20 % glucose infusion continued as required, with infusion rate titrated as before to maintain euglycaemia. Blood sampling continued until the blood glucose was stable without the need for the 20% glucose infusion. By that time, all cannulae and equipment were removed and the participant was free to leave the laboratory.

3.2.2 Statistical analysis

Data derived from YSI and glucose pocket analysers were coded and analysed using GraphPad GraphPad (Prism) (version 5.00 for Windows, GraphPad Software, San Diego California USA).

Correlation analysis of glucose concentration recorded by each pocket analyser against corresponding values from the YSI is expressed as the Pearson correlation coefficients. Statistical significance for all tests was set at the $P < 0.05$ level.

Bland and Altman plots were followed for glucose concentration obtained from each pocket analyser compared with corresponding values from the YSI. Limits of agreement were derived from the mean difference between YSI and pocket analysers ± 1.96 SD of the differences.

3.2.3 Accuracy of the glucose pocket analysers

Four Accu-Chek glucose pocket analysers were assessed against YSI as a criterion' method. Correlation analyses between YSI versus individual analysers (Figure 3.8) were strong and significant. Pearson correlation coefficient and significant level of YSI versus Accu-check, 1, 2, 3 and 4 were $r= 0.970$ ($P < 0.0001$), $r = 0.959$ ($P < 0.0001$), $r = 0.967$ ($P < 0.0001$) and

r=0.962 (P < 0.0001), respectively. Bland Altman plots were also used to show the level of agreement between YSI and individual analysers (Figure 3.9). The bias was calculated as the mean of the glucose concentration obtained with pocket analysers minus the mean of the glucose concentration estimated by YSI and ranged from 0.21 – 0.25. Bland Altman plots showed an acceptable agreement between the reference method and each of the pocket analysers. Bland-Altman plots displayed that all, but two of the reading were within the limit of agreement. The accuracy of the pocket analysers is important, as the pocket analyser readings were used in this thesis to calibrate the CGM. Hence any inaccuracy in the pocket analysers would affect the accuracy of glucose readings obtained by CGM.

In conclusion, the current study has shown that the measurement of blood glucose with the pocket analysers has a strong correlation and an acceptable agreement with the determination by the YSI in a broad range of euglycaemia, and in hypoglycaemia. The accuracy of the pocket analysers was deemed to be adequate for this thesis. Finally, it should be mentioned that the very small sample size used in this validation study is acknowledged.

Table 3.1 Participants' characteristics*

Age (years)	29.0 ± 3.0
Weight (kg)	65.9 ± 12.2
Height (cm)	158.2 ± 6.6
BMI (kg/m^2)	26.5 ± 6.0

*mean ± SD

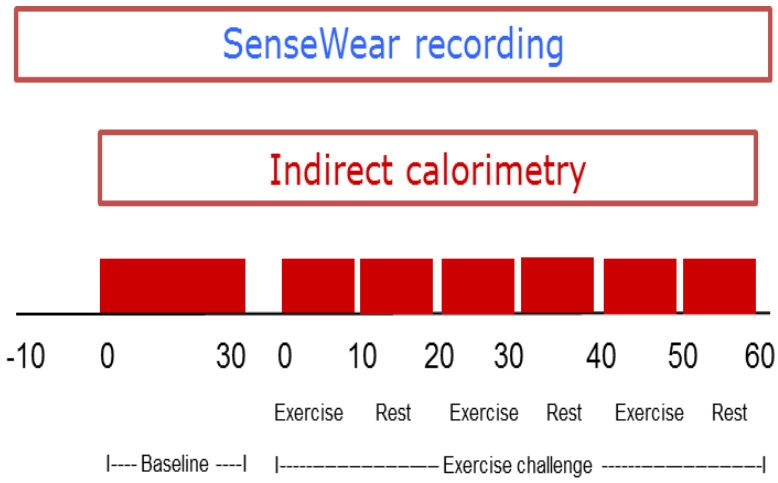

Figure 3.1Experimental protocol.

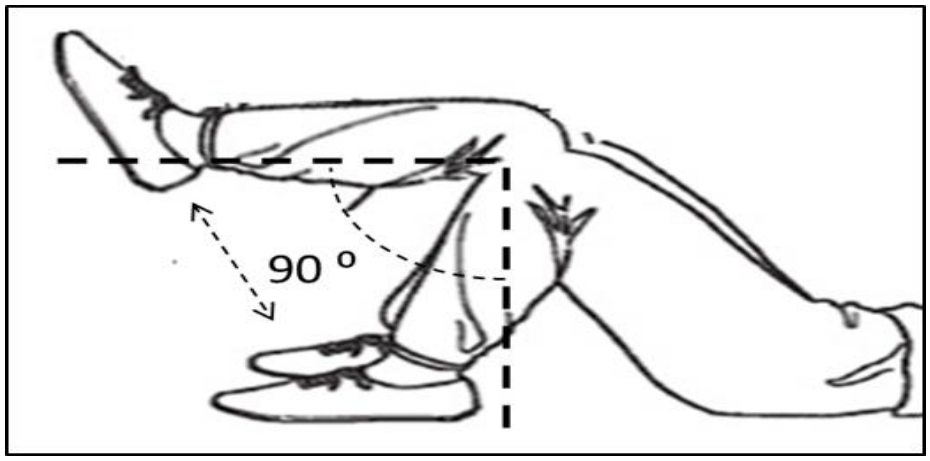

Figure 3.2 Leg rises diagram.

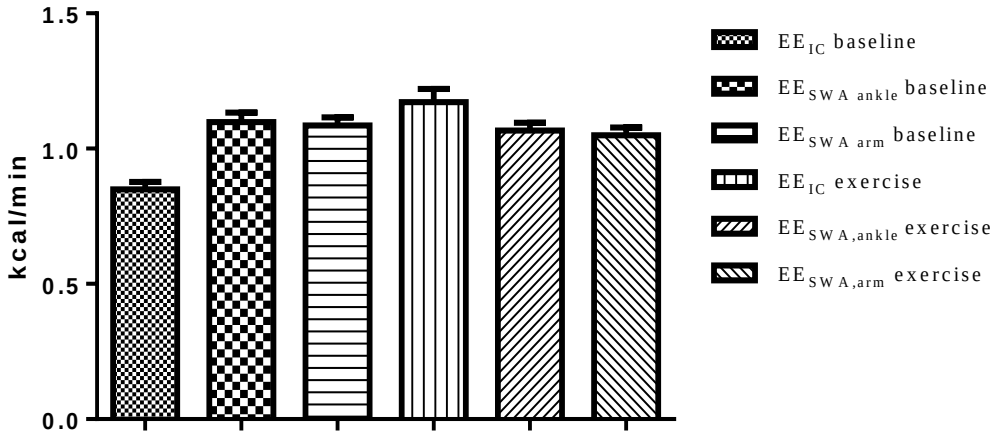

Figure 3.3 Mean (±SEM) EE_{IC}, $EE_{SWA,ankle}$ and $EE_{SWA,arm}$ during baseline and exercise phases.

n=9

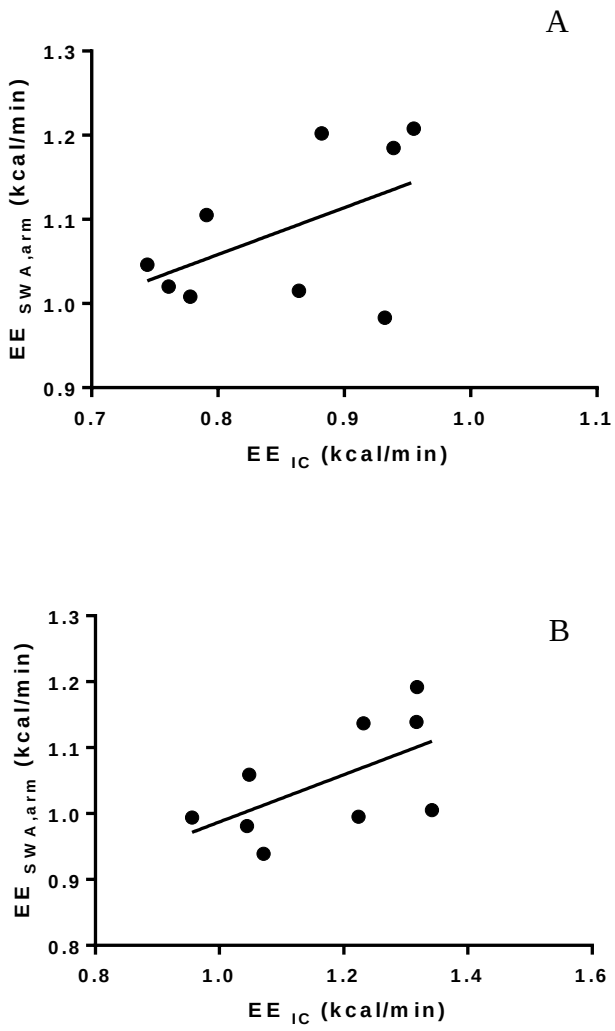

Figure 3.4 Linear correlation between EE_{IC} and $EE_{SWA,arm}$ during the baseline (A: r = 0.51; P > 0.05) and exercise phases (B: r = 0.59; P > 0.05).

n=9

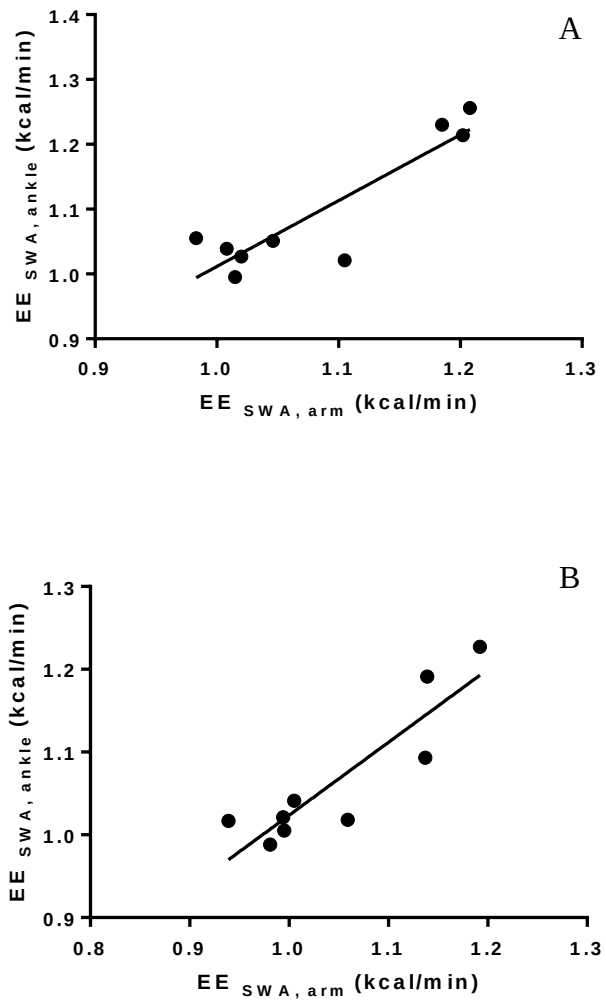

Figure 3.5 Linear correlation between $EE_{SWA,arm}$ and $EE_{SWA,ankle}$ during the baseline (A: r = 0.89; P < 0.001) and exercise phases (B: r = 0.89; P < 0.001).

n=9

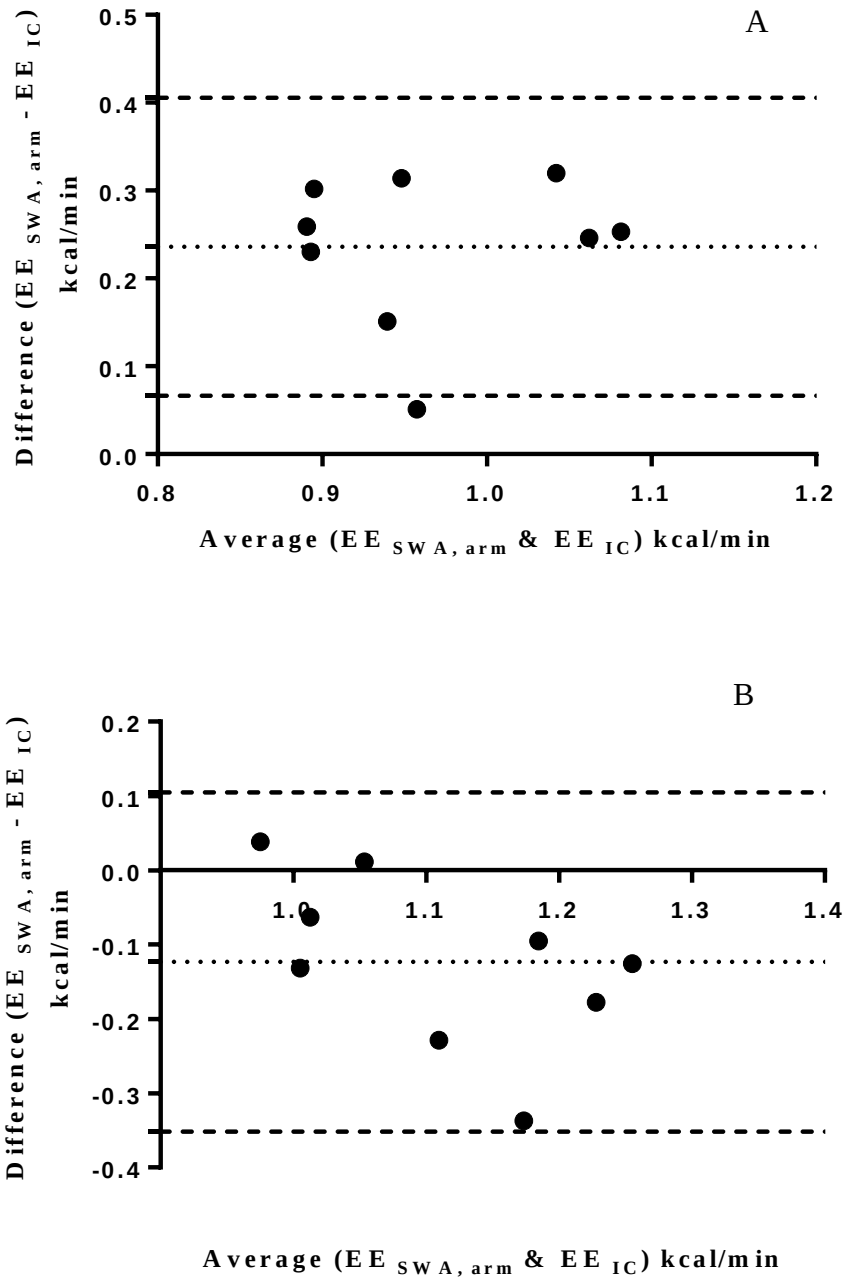

Figure 3.6 Bland-Altman plot of bias between measured $EE_{SWA,arm}$ and EE_{IC} during the baseline (A) and exercise (B) phases. The dotted line represents the mean bias of the two methods and the dashed lines represent the limits of agreement.

n=9

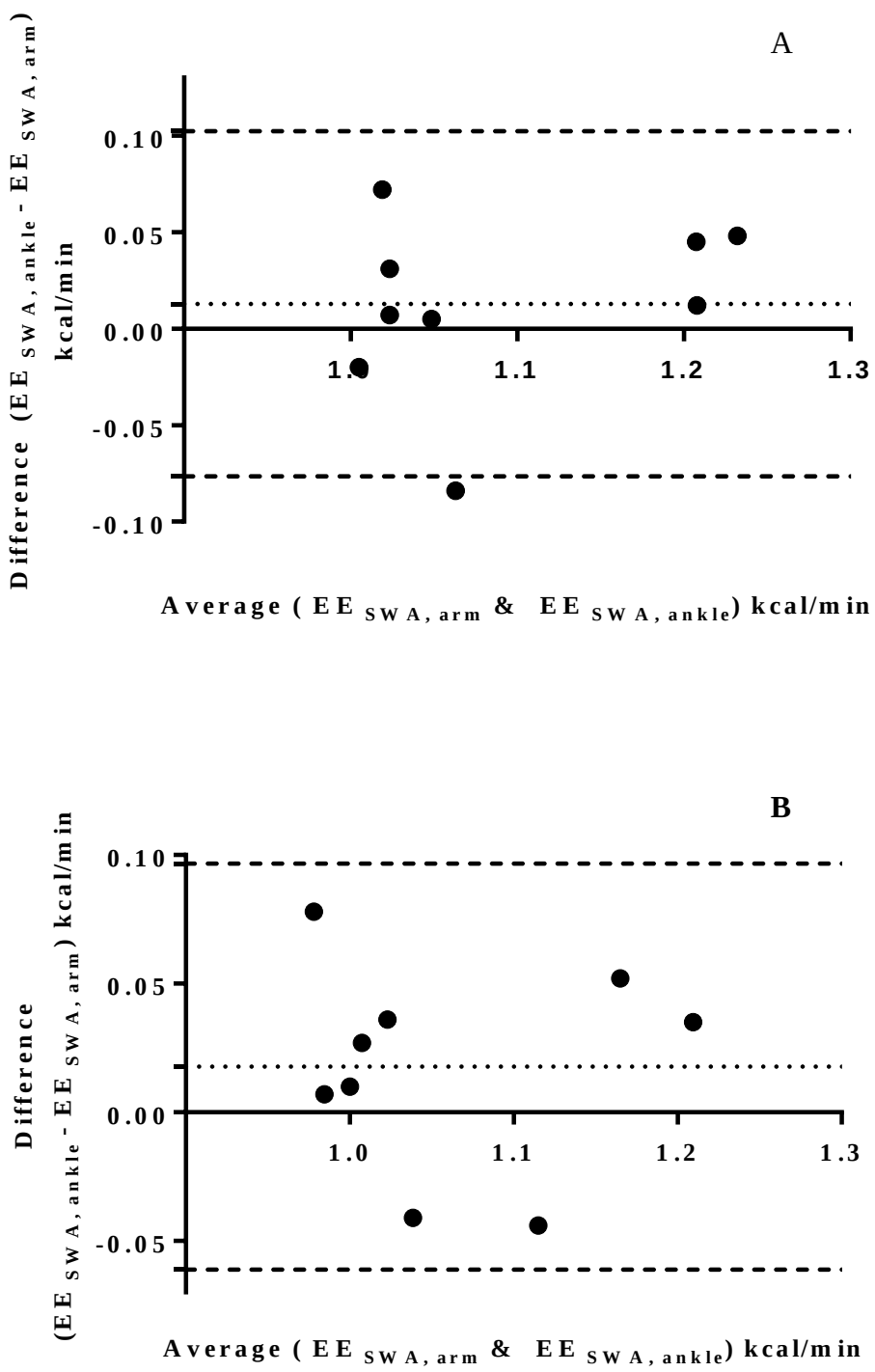

Figure 3.7 Bland-Altman plot of bias between measured $EE_{SWA,arm}$ and $EE_{SWA,ankle}$ during the baseline (A) and exercise (B) phases. The dotted line represents the mean bias of the two methods and the dashed lines represent the limits of agreement.

n=9

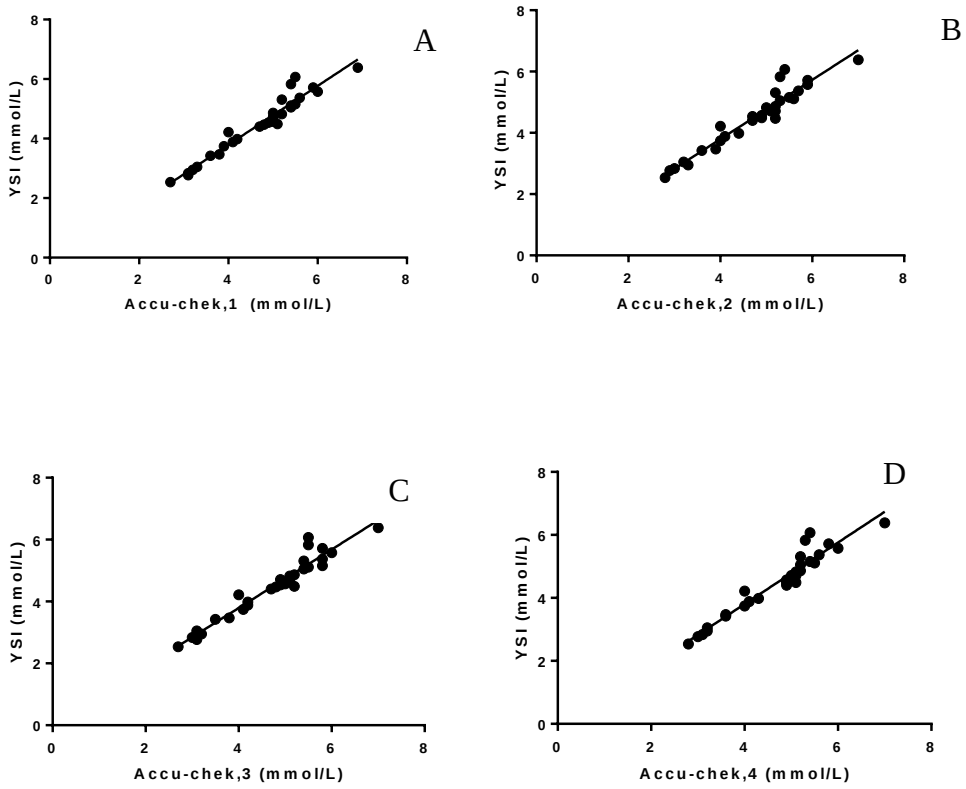

Figure 3.8 Linear correlation between YSI and glucose pocket analysers, Accu-chek 1 (A: r= 0.970; P < 0.0001), Accu-chek 2 (B: r = 0.959; P < 0.0001), Accu-chek 3 (C: r = 0.967; P < 0.0001) and Accu-chek 4 (D: r=0.962; P < 0.0001).

n=1

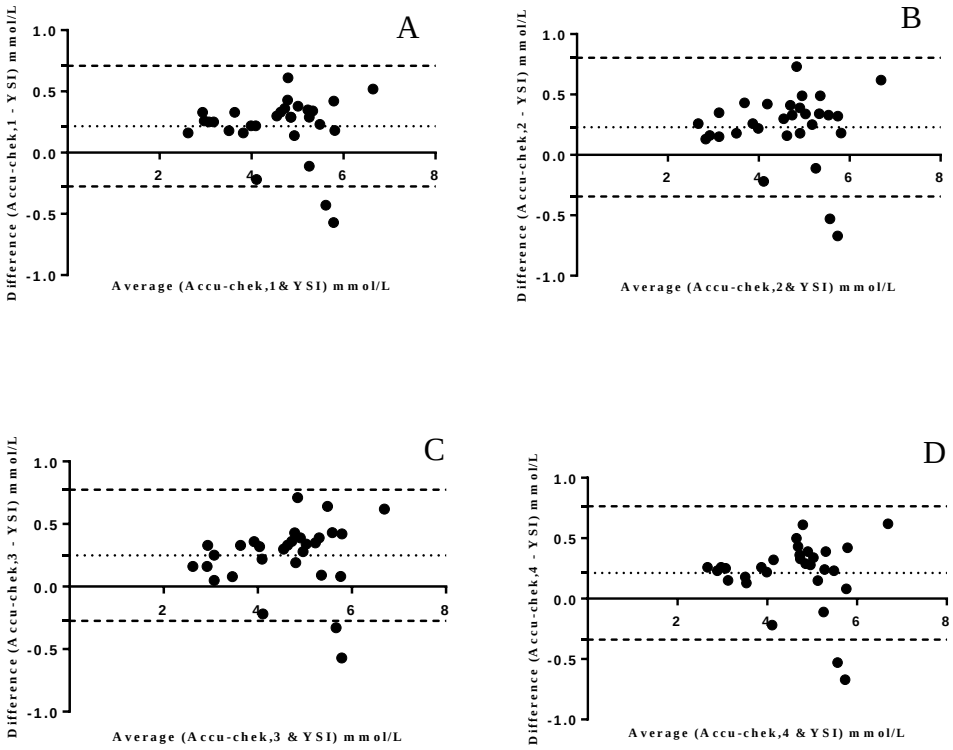

Figure 3.9 Bland-Altman plot of bias between blood glucose obtained with glucose pocket analysers versus YSI, Accu-chek 1 (A), Accu-chek 2 (B), Accu-chek 3 (C) and Accu-chek 4 (D). The dotted line represents the mean bias of the two methods and the dashed lines represent the limits of agreement.

n=1

CHAPTER 4 : INFLUENCE OF THE IRREGULAR MEAL PATTERN ON THE THERMIC EFFECT OF FOOD AND APPETITE REGULATION IN HEALTHY NORMAL-WEIGHT FEMALES

4.1 Introduction

The rapid increase in the prevalence of obesity over the past few decades has occurred whilst genetic susceptibility has remained stable, suggesting that changes in lifestyle and the environment may be contributory factors (SACN, 2011a). There is some evidence that irregular meal patterns have become more prevalent (Höglund et al., 1998, Samuelson, 2000, Bellisle et al., 2003). An irregular meal pattern might affect energy balance and, in consequence, the control of body weight. However, studies which have evaluated the impact of an irregular meal pattern on energy metabolism, in adults, are few. A previous study by Farshchi and colleagues (Farshchi et al., 2004a) has reported a significant decrease in TEF in normal-weight females following a two-week period of irregular compared with a regular meal pattern. A subsequent study has confirmed this effect in obese females (Farshchi et al., 2005). However, food intake in those studies was self-selected, the eating time was not specified and free living energy expenditure was not monitored.

The aim of this study was to investigate, in healthy normal-weight females the effect of an irregular meal pattern on the TEF. The impact of meal pattern on gut hormones, subjective appetite ratings and *ad-libitum* food intake of a test meal were also investigated. The study was undertaken under conditions that were more controlled than previously (food was provided and energy expenditure was estimated, under free-living conditions using SWA).

To the best of the author's knowledge, no studies using a design similar to this are available in the literature.

4.2 Methods

4.2.1 Sample size

Using one sample t-test model, power calculated at the level of 0.8 (Lenth, 2006). Primary endpoint = difference in TEF (kcal/min) over 3 h. Results obtained from the previous study (Farshchi et al., 2004a) indicated that the iAUC TEF after a regular meal pattern was 0.74 ± 0.37 kJ/min and after an irregular meal pattern was 0.39 ± 0.26 kJ/min. Therefore, with a cross-over design, eleven participants in each group would be required to detect a difference in TEF with the power of 80% at the significance level of 0.05.

4.2.2 Participants

The study (ethical approval reference: J14082012 BMS) was conducted on eleven healthy normal-weight (mean body weight 58.5 ± 6.6 kg; mean BMI 22.0 ± 1.5 kg/m^2) females aged 18-40 years (mean 24.1 ± 4.9 years). Details of participant recruitment and inclusion criteria were explained in Section 2.2.

The study was registered at clinical Trials.gov with the identification number: NCT02052076.

4.2.3 Study protocol

The design of the study protocol was explained in details in Section 2.5 and outlined in Figure 2.1. In summary; the study followed a randomized crossover design with two, 14-day intervention periods, separated by a wash-out period

of 14 days. In period 1, participants followed either a regular (6 meals/day) or an irregular meal pattern (3-9 meals/day). In period 2, participants followed the alternative meal pattern to that followed in period 1. Participants were asked to visit the laboratory at the start and end of each period after an overnight fast.

4.2.4 Dietary intervention periods

In the two intervention periods, identical foods were provided to a participant in amounts designed to keep body weight stable. In order to avoid monotony and boredom during the intervention period, the food was supplied in a 4 day cycle of menus. Menus were designed for 1900 kcal/day, 2050 kcal/day and 2350 kcal/day to meet the different estimated energy requirements of participants. Energy requirements were calculated for each participant (Section 2.6.1), and then participants were assigned the menus with the closest energy requirement (± 100 kcal).

The procedures for dietary intervention were explained in details in Section 2.6.

4.2.5 Measurements made during the intervention periods

4.2.5.1 Energy expenditure assessment during the intervention period

SWA was used to assess participants' physical activity pattern and energy expenditure continuously during the intervention periods as explained in Section 2.7.1

4.2.5.2 Appetite assessment during the intervention period

The protocol for the appetite assessment during the intervention periods was described in Section 2.7.2

4.2.6 Laboratory visit protocol

The details of the laboratory visit protocol were explained in details in Section 2.8 and displayed in Figure 2.3. In this visit, anthropometric measurements, REE, TEF (following the test drink consumption), subjective appetite, gut hormones and *ad-libitum* intake were determined in order to examine the effect of regular and irregular meal pattern on these variables. Lipids, glucose and insulin were also determined and their results are presented in Chapter 5.

4.2.6.1 Anthropometric measurements

Anthropometric measurements that were taken in this study include: weight, height, waist circumference, hip circumference, waist to hip ratio and body composition. Procedures for anthropometric measurements were explained in Section 2.8.1.

4.2.6.2 Energy expenditure measurement

Energy expenditure was measured by indirect calorimetry system during the laboratory visit. The protocol of energy expenditure measurement was explained in Section 2.8.2.

4.2.6.3 Test drink consumption

Participants were provided a milkshake test drink as breakfast. The volume of consumed milkshake was based upon the participant's weight (an average of

10 kcal/kg body weight). The formula and the composition of the milkshake were explained in Section 2.8.3.

4.2.6.4 Blood sampling and processing

Blood variables including GLP-1, PYY and ghrelin were determined at each laboratory visit. The procedures for blood sampling and processing were explained in Section 2.8.4.

4.2.6.5 *Ad-libitum* test meal

A pasta-based test meal was given as a lunch. The energy composition and recipe were explained in Section 2.8.5.

4.2.6.6 Subjective appetite ratings

Participants were asked to complete the VAS during the lab visit to assess subjective appetite. The protocol was explained in Section 2.8.6.

4.2.7 Blood samples analysis

Blood samples analysis for GLP-1, PYY and ghrelin was explained in Section 2.9.

4.3 Statistical analysis

All data are reported as mean ± SD, unless otherwise indicated. The details of the statistical methods were explained in Section 2.10.

4.4 Results

All participants completed the study, although blood samples could not be performed on one participant due to problems associated with vein cannulation.

Five participants were scheduled to start with the regular meal pattern and six others with the irregular one. Participant flow in this study is represented in Figure 4.1.

4.4.1 Anthropometric measurements

There were no significant differences in bodyweight, body composition, or other anthropometric measurements at the pre-intervention visits between the two meal patterns. There were no significant changes across the study visits either for body weight, body composition or anthropometric measurements (Table 4.1).

4.4.2 Energy Intake

Self-reported daily energy intake before the start of the study (2081 ± 214 kcal/day) was similar to the estimated energy requirement for weight maintenance (2104 ± 204 kcal/day). However self-reported carbohydrate percentage (47 ± 4.1%) was significantly lower, and self-reported fat percentage (38 ± 3.7%) was significantly higher than the prescribed intervention diet (53 ± 0.9% carbohydrate and 33 ± 0.9% fat) (paired T-test, $p < 0.01$). Self-reported protein percentage did not show significant differences compared with the prescribed diet (14 ± 2.5 vs 14 ± 0.7%, respectively).

During the study, food intake was designed to be the same by type, and quantity, and hence provide the same amount of energy in the regular and irregular intervention periods. Food diaries over each period indicated that 97.6 ± 5.9% and 99.7 ± 1.0% of the energy given was consumed in the regular and irregular intervention periods, respectively.

Food diaries did not show any significant differences in energy intake between the two intervention periods (2043 ± 248 kcal/day regular and 2098 ± 195 kcal/day irregular intervention periods). The composition of consumed foods also did not differ significantly between the two intervention periods (being 53 ± 0.9% carbohydrate, 14 ± 0.4% protein and 33 ± 0.8% fat in regular and 53 ± 0.3% carbohydrate, 14 ± 0.5% protein and 33 ± 0.7% fat in irregular intervention periods).

4.4.3 Free-living energy expenditure

On average, the SWA device was worn 96.8 ± 5.5 % and 95.1 ± 7.7 % of the regular and irregular intervention periods, respectively. Mean values of total estimated energy expenditure obtained by the armband during the intervention period for both regular and irregular meal patterns did not yield significant differences (2241± 360 kcal/day and 2305 ± 399 kcal/day for regular and irregular intervention periods, respectively. The mean of the physical activity level during the regular and irregular intervention periods did not show a significant difference (1.60 ± 0.2 and 1.64 ± 0.2 METs for regular and irregular intervention periods, respectively).

SWA data for estimated energy expenditure showed a trend of higher energy expenditure compared with energy intake (2043 ± 248 kcal/day for energy intake vs. 2241 ± 360 kcal/day for energy expenditure, paired T-test, $p = 0.06$) during the regular intervention period. Estimated energy expenditure was significantly higher compared with energy intake (2098 ± 195 kcal/day energy intake vs. 2305 ± 399 kcal/day energy expenditure, paired T- test, $p = 0.03$) during the irregular intervention period.

4.4.4 Energy expenditure (indirect calorimetry data)

Fasting REE was not significantly different at the pre-intervention visits. There was also no meal pattern by visit interaction, or main effect of meal pattern or visit for fasting REE (1167 ± 134, 1207 ± 89, 1183 ± 171 and 1188 ± 149 kcal/day in pre, post-regular and pre, post-irregular visits, respectively).

REE increased above the fasting values, after the test drink, at all visits. The overall TEF for the 3 h postprandial period is shown in Figure 4.2. There was no significant difference in overall 3 h TEF at the pre-intervention visits. There was a significant interaction between meal pattern and visit for the 3 h TEF (ANOVA, $p < 0.05$). TEF post-regular visit was increased significantly compared with pre-regular visit (paired T-test, $p < 0.01$) unlike in the irregular visits, where there was no significant difference between pre and post-intervention visits. TEF post-regular visit was 11.1 ± 15.8 kcal (34.6 ± 48.3 %) higher than post irregular visit (paired T-test, $p < 0.05$).

4.4.5 Blood variables

4.4.5.1 GLP-1

Fasting plasma GLP-1 concentrations did not show significant differences pre-interventions (Figure 4.3). Fasting plasma GLP-1 concentrations did not show a significant meal pattern by visit interaction or main effect of meal pattern, but there was a significant main effect of visit (ANOVA, $p < 0.05$) on fasting plasma GLP-1 concentrations.

Mean fasting plasma GLP-1 concentrations decreased approximately by 16% and 20% post-regular and irregular visits, respectively compared with pre-intervention visits.

Following consumption of the test drink, plasma GLP-1 concentrations increased in all visits (Figure 4.3). iAUC for plasma GLP-1 concentrations (Figure 4.4), showed no significant interaction between meal pattern and visit, or main effects for meal pattern or visit.

4.4.5.2 PYY

Figure 4.5 shows fasting plasma PYY concentrations over the study visits. There were no significant differences pre-intervention visits. Fasting plasma PYY concentrations showed no significant meal pattern by visit interaction or main effect of meal pattern. However, there was a significant main effect of visit (ANOVA, $p < 0.05$). Mean fasting plasma PYY concentrations decreased about 9% and 23% post-regular and irregular visits, respectively compared with pre-intervention visits.

Plasma PYY concentrations increased rapidly above the fasting values after consuming the test drink and remained at a plateau until the last sampling time point in all visits (Figure 4.5). iAUC for the 3 h postprandial period in all visits (Figure 4.6) showed no significant interaction between meal pattern and visit or main effect for meal pattern. However, there was a significant main effect of visit (ANOVA, $p < 0.05$). Mean iAUC for plasma PYY concentrations increased approximately by 57% post-regular compared with pre-regular visit, and by 70% post-irregular compared with pre-irregular visit.

4.4.5.3 Ghrelin

Figure 4.7 shows the results of plasma ghrelin concentrations over the study. There were no significant differences in plasma ghrelin concentrations pre-

intervention visits. No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting plasma ghrelin.

Following consumption of the test drink, plasma ghrelin concentrations declined in all visits (Figure 4.7). iAUC for plasma ghrelin (Figure 4.8) showed no significant interaction between meal pattern and visits, or main effects for meal pattern or visit.

4.4.6 Subjective appetite ratings

4.4.6.1 Responses to the test drink

There were no significant differences between the pre-intervention visits for any of the subjective appetite ratings collected in the fasting state or following the test drink (Table 4.2).

There was also no meal pattern by visit interaction, or main effect of meal pattern or visit for fasting VAS ratings (Table 4.2).

The assessments of hunger ratings for the 3 h postprandial period in all visits (Table 4.2) showed no significant interaction between meal pattern and visit or main effect for meal pattern, but a significant main effect of visit (ANOVA, $p < 0.05$).The response for the other VAS ratings did not show significant differences between the intervention periods (Table 4.2).

4.4.6.2 Responses to the *ad-libitum* test meal

The responses (for hunger, fullness, satiety, desire to eat and prospective food consumption) for the 1 h postprandial period did not differ significantly between the pre-intervention visits (Table 4.2).

The responses in all visits (Table 4.2) showed no significant interaction between meal pattern and visit or main effect for meal pattern or visit.

4.4.6.3 Responses to the meal pattern

Subjective appetite ratings were assessed pre and post-meals during day 7 and 14, when 6 meals/day were consumed in both regular and irregular intervention periods. On day 7, comparison of mean pre-meal ratings (average of the 6 pre-meal ratings on the day) did not show significant differences between the intervention periods (Table 4.3). However, mean post-meal ratings showed significant differences in hunger and fullness between the intervention periods. Higher post-meal ratings for hunger and lower for fullness were observed in the irregular compared with the regular intervention period (paired T- test < 0.05). The mean differences between pre and post-meal ratings for prospective food consumption ratings was lower in the regular intervention than was seen in the irregular one (Table 4.5), but did not reach a significant level (p=0.08, paired T-test). The mean differences between pre and post-meal ratings for the other VAS ratings did not show significant differences between the two intervention periods (Table 4.5).

On the final day of intervention (day 14), the ratings of pre-meals hunger (Table 4.4) was significantly greater in the irregular compared with the regular intervention period ($p < 0.05$, paired T-test). Furthermore, the ratings of post-meal hunger were significantly greater in the irregular period ($p < 0.05$, paired T-test). The pre and post-meal values for the other VAS appetite ratings did not show significant differences between the two intervention periods (Table 4.4). Comparison of the mean differences between pre and post-meal ratings did not

show significant differences between the two intervention periods for any of VAS ratings (Table 4.5).

4.4.7 Intake at the *ad-libitum* test meal

There was no significant difference between participants' energy intake at the *ad-libitum* test meal pre-intervention visits. There was no meal pattern by visit interaction or main effect of meal pattern or visit for participants' energy intake across the study visits (778.8 ± 272.8, 745.7 ± 214.7, 722.4 ± 324.0 and 764.3 ± 246.6 kcal in pre and post-regular and irregular visits, respectively).

The duration of eating and speed of consuming the *ad-libitum* test meal were not significantly different pre-intervention visits. The duration of eating did not show a significant interaction between the meal pattern and visit or main effect of meal pattern or visit (9.6 ± 3.9, 9.8 ± 3.8, 9.5 ± 3.1 and 9.1 ± 2.3 min in pre and post-regular and irregular visits, respectively). Speed of eating also showed no significant difference between the two intervention periods (51.1 ± 13.2, 47.9 ± 10.1, 45.1 ± 13.4 and 50.6 ± 11.1 g/min in pre and post-regular and irregular visits, respectively).

4.5 Discussion

The present study aimed to investigate the effect of an irregular meal pattern, compared with a regular meal pattern, on TEF, appetite and anthropometric measurements, in healthy normal-weight females. Participants were requested to consume 6 meals/day in the regular intervention period and from 3 to 9 meals/day, (average of 6 meals/day), in the irregular intervention period.

A regular meal pattern, with carefully controlled energy intake, food type and composition, time of food consumption and stage in menstrual cycle (Solomon

et al., 1982, Dye and Blundell, 1997, Davidsen et al., 2007, McNeil and Doucet, 2012) was shown to result in greater TEF following a test drink (mean of 11.1 ± 15.8 kcals more over 3 h) compared with an irregular meal pattern. These findings are consistent with those of a previous study (Farshchi et al., 2004a), in which total energy intake was not controlled as carefully during the two week intervention periods.

The current study also has an indication, from the SWA device, that participants had complied with the request to undertake similar levels of activity during the intervention periods. An attempt was made to limit differences in other components of energy expenditure, such as physical activity in order to reduce the potentially confounding effect on the key outcomes of interest (e.g. appetite). Clearly, if meal pattern influences physical activity, this study design would not have been appropriate to detect this.

Research on the effects of meal pattern on metabolism started in the 1960s and focused on meal frequency (Fabry et al., 1964, Fabry et al., 1968). Several studies attempted a further evaluation of the impact of meal frequency on energy balance and body weight.

It seems that there is no relationship between meal frequency and total energy expenditure (Dallosso et al., 1982, Wolfram et al., 1987, Verboeket-van de Venne and Westerterp, 1991), whilst the findings of studies investigating the effect of meal frequency on the TEF have suggested contradictory results. Some of these studies indicated that meal frequency appears to have no effect on energy expenditure (Belko and Barbieri, 1987, Kinabo and Durnin, 1990). On the other hand, other studies recorded a higher total TEF following consumption of one large meal compared with several small meals, providing

the same total energy (Tai et al., 1991, Molnar, 1992). However, one of the main criticisms of these studies is uncontrolled confounding effects such as menstrual cycle in women, or previous habitual food intake.

The present study was designed specifically to investigate the consequences of irregularity, as opposed to comparing differences in frequency of two regularly consumed patterns. Thus participants were required to consume 6 meals/day on the day prior to the final laboratory visit on both interventions, having either consumed 6 meals every day for two weeks, or an irregular number of meals over two weeks. Providing 6 meals before each final test day was in order to eliminate an acute effect of the meal frequency on the day immediately preceding the laboratory visit. However, it is possible that the differences seen might not be a direct effect of the irregularity of the meal pattern, but a consequence of a longer term carry over effect of days with either a higher or lower number of meals per day than the 6 meals provided in the regular meal pattern intervention.

It has been reported that impaired thermogenesis is associated with insulin resistance (Ravussin et al., 1983, Ravussin et al., 1985). To examine this association between impaired TEF and insulin resistance due to the irregular meal pattern, blood samples were collected feasted and following the test drink as explained in Chapter 5. The findings demonstrated a degree of insulin resistance post-irregular visit and these findings discussed further in Chapter 5.

The results of the earlier study (Farshchi et al., 2004a), in which food was self-selected, suggest the possibility of overeating as a consequence of consuming meals irregularly. In the present study food diaries indicate that, as intended, reported food intake was not significantly different between interventions.

Subjective appetite sensations can thus be compared without differences in energy intake or macronutrient content potentially confounding the results. Interestingly subjective appetite differences were noted whilst the subjects were free living, rather than in response to the test drink or meal. Greater post-meal ratings for hunger and lower for fullness on day 7 (6 meals/day on both interventions), during the irregular meal pattern period suggest a reduction in the satiation experienced with the irregular meal pattern. Additionally, greater pre-meal and post-meal ratings for hunger were observed on the final day of the irregular meal pattern when again 6 meals were consumed in both interventions. These findings indicated that after 14 days of irregular eating, the overall hunger had increased, which would not be surprising as the participants exposed to a longer irregular intervention.

The mean energy intake of pasta consumed at the *ad-libitum* test meal in the laboratory was decreased approximately by 4% post-regular visit and increased approximately by 5% post-irregular visit. The difference was not statistically significant potentially because the study was insufficiently powered for this component. However nor were the differences in subjective appetite seen following both the test drink and the *ad-libitum* meal.

Ghrelin is known to stimulate appetite and is related to subjective hunger ratings in the absence of food cues (Wren et al., 2000, Wren et al., 2001, Blom et al., 2005). Neither the fasting ratings for hunger during the laboratory visits nor the fasting plasma ghrelin concentrations showed any differences.

GLP-1 and PYY are inversely associated with subjective appetite and energy intake (Naslund et al., 1999, Batterham et al., 2002). No meal pattern effect was found but the present study demonstrated that fasting plasma GLP-1 and

PYY concentrations were lower following the intervention periods compared with before the intervention periods. An explanation might be that in the day prior to the post regular and irregular visits participants consumed 6 meals/day with the same food type and composition, whilst the food that was self-selected and the number of meals in the day prior to the pre-intervention periods was unknown. The 7 day food record indicated that habitual diet contained a lower percentage of carbohydrate and a higher percentage of fat. Another explanation might be that the potential effects of the phases of the menstrual cycle on GLP-1 (Brennan et al., 2009) and PYY. The present study started in the early part of the follicular phase, therefore when the participants attended for second visit they were at the third week of the cycle.

The differences observed in PYY in response to the test drink were consistent with the differences in VAS hunger responses. Following the test drink, PYY responses increased, whilst the hunger responses decreased post-intervention visits compared with the pre-intervention visits. These findings confirm the inverse relationship between PYY and subjective appetite (Batterham et al., 2002)

Free-living energy expenditure, assessed with SWA did not show significant differences between the regular and irregular intervention periods. SWA mean energy expenditure was higher than energy intake approximately by 10 % in both regular and irregular intervention periods. The use of SWA is suitable for energy expenditure estimated in a free-living environment. However, several studies (Bertoli et al., 2008, Elbelt et al., 2010) reported an overestimation of energy expenditure measured by SWA when compared with indirect calorimetry.

Despite a significantly greater TEF after the regular meal pattern, the regular meal pattern did not produce any statistically significant changes either in body weight or in other anthropometric measurements. This may have been due to the short time period of the study suggesting that a longer study would be warranted.

In conclusion, the findings from the present study have indicated that consuming a regular meal pattern for a 14-day period produced a greater TEF compared with an irregular meal pattern. Differences in subjective appetite ratings indicate that reduced appetite may be experienced with a regular meal pattern compared with an irregular meal pattern which might impact upon compliance with energy restricted diets. These desirable effects potentially could contribute beneficially to weight control and obesity management among the population adopting a regular, consistent, meal pattern. It would be of interest in future works to study overweight and obese individuals in the same protocol to make the findings applicable to a broader population and to conduct longer term studies.

Table 4.1 Participants' characteristics over the study*

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Body weight (kg)	58.7 ± 6.1	58.3 ± 6.2	58.6 ± 6.6	58.2 ± 6.1
BMI (kg/m^2)	22.0 ± 2.0	21.8 ± 1.9	21.9 ± 1.9	21.8 ± 2.0
Body fat (%)	22.2 ± 3.0	22.1 ± 3.6	22.3 ± 3.5	22.7 ± 3.8
Waist (cm)	69.5 ± 5.5	69.5 ± 5.1	70.5 ± 5.7	69.9 ± 5.1
Waist/hip	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6

*mean ± SD, n=11

There were no significant differences in participants' characteristics across the study (ANOVA).

Table 4.2 Fasting and iAUC for subjective appetite ratings following the test drink and the *ad-libitum* meal over the study*

		Regular meal pattern		Irregular meal pattern	
		Pre	**Post**	**Pre**	**Post**
Hunger	**Fasting** (mm)	53 ± 30	61 ± 28	50 ± 28	66 ± 31
	Test drink [1](mm over 3h)	-1533 ± 6725	-4526 ± 4832	-2214± 5261	-4525 ± 4778
	***Ad-libitum* meal** (mm over 1h)	-3172 ± 1272	-3002 ± 1491	-2906 ± 1428	-2977 ± 1863
Satiety	**Fasting** (mm)	24 ± 24	27 ± 22	20 ± 17	19 ± 19
	Test drink (mm over 3h)	5408 ± 6397	4939 ± 5286	5950 ± 3577	5866 ± 3535
	***Ad-libitum* meal** (mm over 1h)	3199 ± 1246	3562 ± 1636	3196 ± 2139	3773 ± 1709
Fullness	**Fasting** (mm)	17 ± 17	26 ± 25	17 ± 22	17 ± 17
	Test drink (mm over 3h)	6530 ± 3951	5072 ± 4348	5974 ± 3465	5945 ± 3594
	***Ad-libitum* meal** (mm over 1h)	3356 ± 1611	3712 ± 1649	3416 ± 1881	3983 ± 1512
Desire to eat	**Fasting** (mm)	57 ± 26	59 ± 31	50 ± 25	65 ± 27
	Test drink (mm over 3h)	-2584 ± 5791	-3490 ± 5837	-2295 ± 5389	-4403 ± 4005
	***Ad-libitum* meal** (mm over 1h)	-3124 ± 1457	-3323 ± 1676	-2683 ± 1951	-3042 ± 2067
Prospective food consumption	**Fasting** (mm)	63 ± 22	63 ± 21	58 ± 17	64 ± 26
	Test drink (mm over 3h)	-3060 ± 4749	-3454 ± 3034	-2897 ± 3662	-3934 ± 4476
	***Ad-libitum* meal** (mm over 1h)	-3107 ± 1473	-3164 ± 1470	-2672 ± 1605	-3020 ± 1741

*mean ± SD, n=11

[1] There was a significant main effect of visit on hunger responses to the test drink (ANOVA, $p < 0.05$).

Table 4.3 Comparison of mean appetite ratings (all day points combined) on day 7 of regular and irregular meal patterns*

mm	Regular meal pattern		Irregular meal pattern	
	Pre-meals	**Post-meals**	**Pre-meals**	**Post-meals**
Hunger	46.5± 10.2	14.5± 7.0 [1]	48.8± 10.0	23.4 ±6.0 [1]
Satiety	42.2± 12.0	74.9 ±5.1	40.4± 13.1	74.6 ±5.8
Fullness	39.5± 12.2	80.6 ±4.4 [2]	40.2± 13.0	73.6 ±5.3 [2]
Desire to eat	51.8± 10.2	22.3 ±7.1	49.6± 9.8	26.0 ±6.0
Prospective food consumption	56.5± 7.7	24.9 ±8.3	54.4± 8.3	29.9 ±8.1

*mean ± SD, n=11

[1] There was a significant difference in post-meals hunger ratings between the two intervention periods (paired T-test, $p < 0.05$).

[2] There was a significant difference in post-meals fullness ratings between the two intervention periods (paired T-test, $p < 0.05$).

Table 4.4 Comparison of mean appetite ratings (all day points combined) on day 14 of regular and irregular meal patterns *

mm	Regular meal pattern		Irregular meal pattern	
	Pre-meals	**Post-meals**	**Pre-meals**	**Post-meals**
Hunger	51.0± 11.5 [1]	18.9± 4.5 [2]	58.0± 8.7 [1]	22.8± 5.0 [2]
Satiety	40.7± 7.4	77.2± 2.6	44.0± 13.3	75.3± 4.7
Fullness	44.6± 13.1	75.6± 3.5	37.2± 9.0	76.0± 3.6
Desire to eat	51.3± 11.9	26.5± 4.3	58.2± 5.9	24.9± 3.9
Prospective food consumption	58.6± 9.3	30.9± 4.5	55.6± 9.3	27.9± 3.3

*mean ± SD, n=11

[1] There was a significant difference in pre-meals hunger ratings between the two intervention periods (paired T-test, $p < 0.05$).

[2] There was a significant difference in post-meals hunger ratings between the two intervention periods (paired T-test, $p < 0.05$).

Table 4.5 Mean differences between subjective appetite ratings before and after the meal on day 7 and 14 of regular and irregular meal patterns, when 6 meals were consumed*

mm	Regular meal pattern		Irregular meal pattern	
	Day 7	Day 14	Day 7	Day 14
Hunger	-32.1 ± 25.4	-38.2 ± 19.9	-24.7 ± 14.4	-30.9 ± 17.8
Satiety	32.7 ± 20.0	38.3 ± 16.0	35.2 ± 18.4	32.6 ± 16.0
Fullness	39.1 ± 16.9	38.5 ±16.2	36.2 ± 16.8	32.5 ± 13.2
Desire to eat	-30.1 ± 22.5	-33.5 ± 19.1	-25.3 ± 14.8	-26.2 ± 16.9
Prospective food consumption	-31.7 ± 15.5	-30.8± 17.2	-25.9 ± 10.9	-26.9 ± 12.7

*mean ± SD, n=11

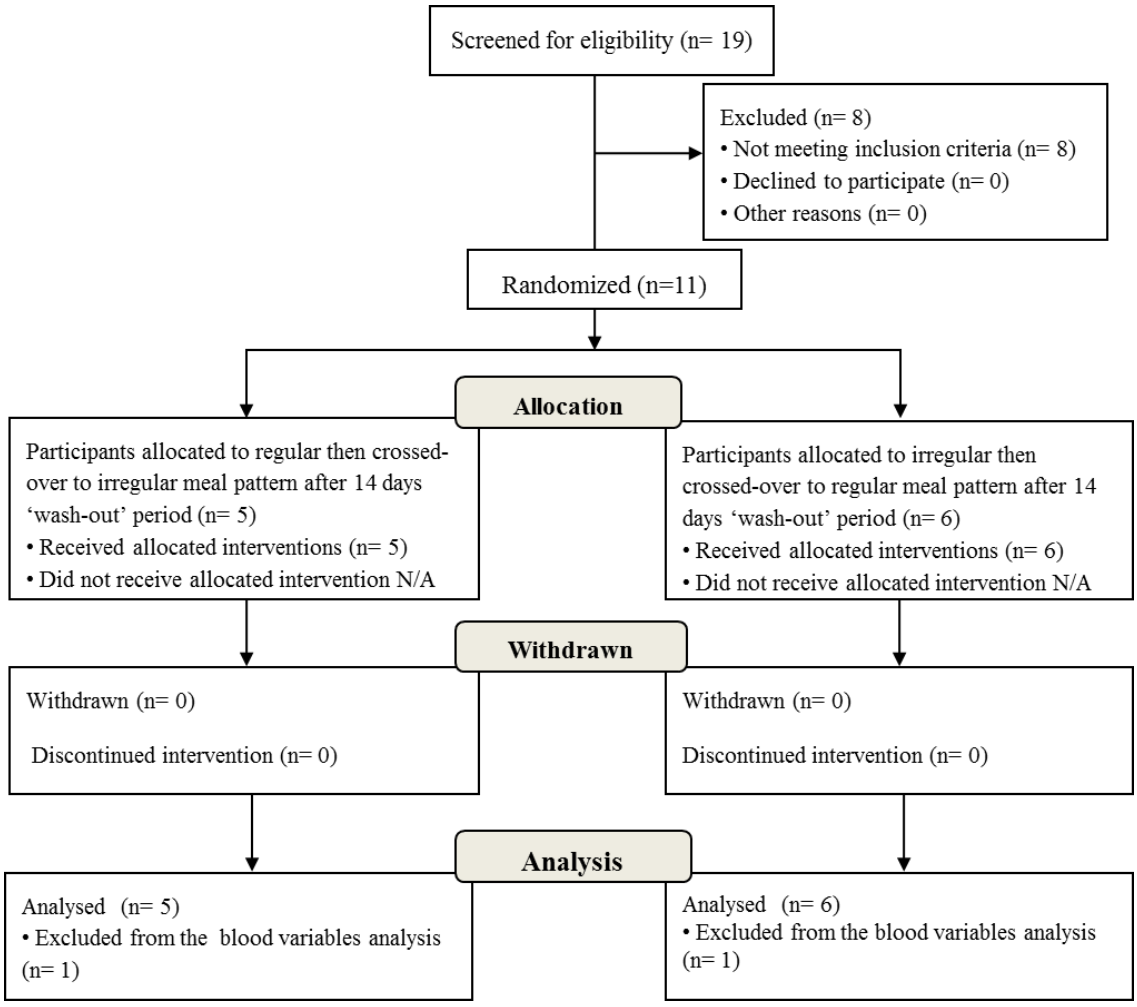

Figure 4.1Study participant flow diagram.

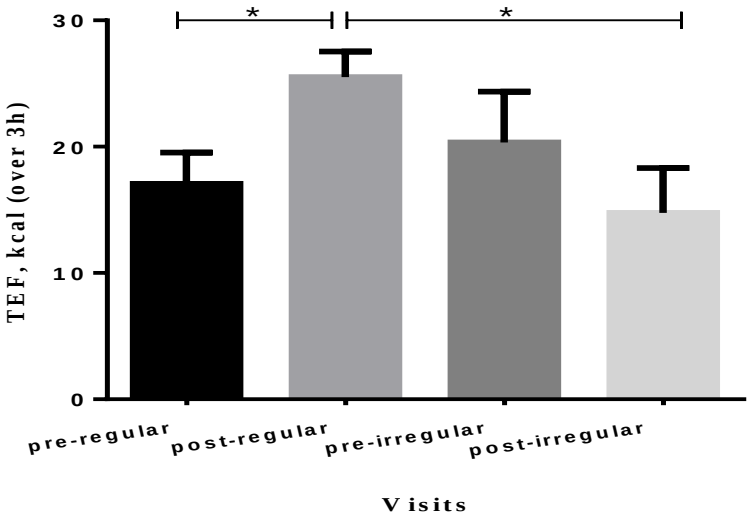

Figure 4.2 Mean (± SEM) iAUC for TEF in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the tr apezoidal method.

n=11

* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (ANOVA, $p < 0.05$). iAUC for TEF was significantly higher post-regular compared with post-irregular meal pattern (paired T-test, $p < 0.05$). iAUC for TEF was significantly higher post-regular compared with pre-regular meal pattern (paired T-test, $p < 0.05$).

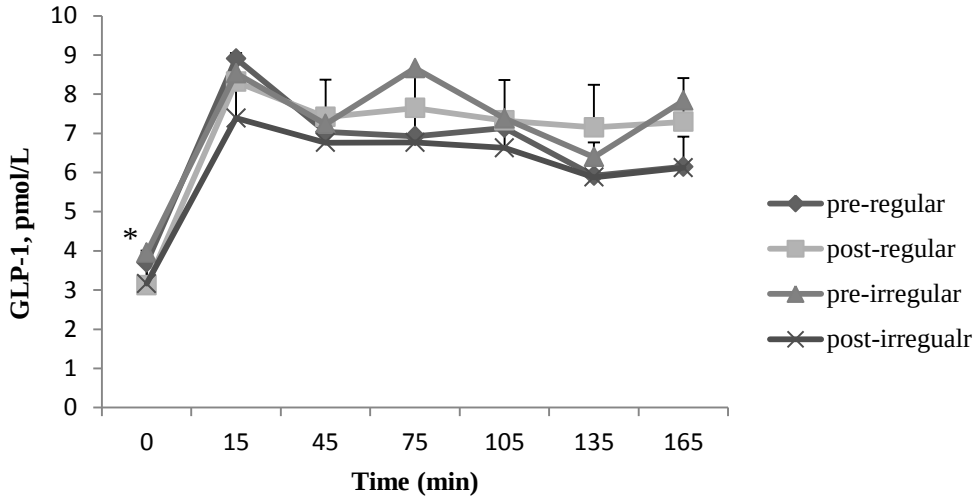

Figure 4.3 Mean (± SEM) plasma GLP-1 concentrations in healthy females at baseline, and after the test drink consumption, pre and post-regular and irregular meal patterns.

n=10. For clarity, SEM values only presented for post-regular and irregular profiles.

*There was a significant main effect of visit on fasting plasma GLP-1 concentrations (ANOVA, $p < 0.05$).

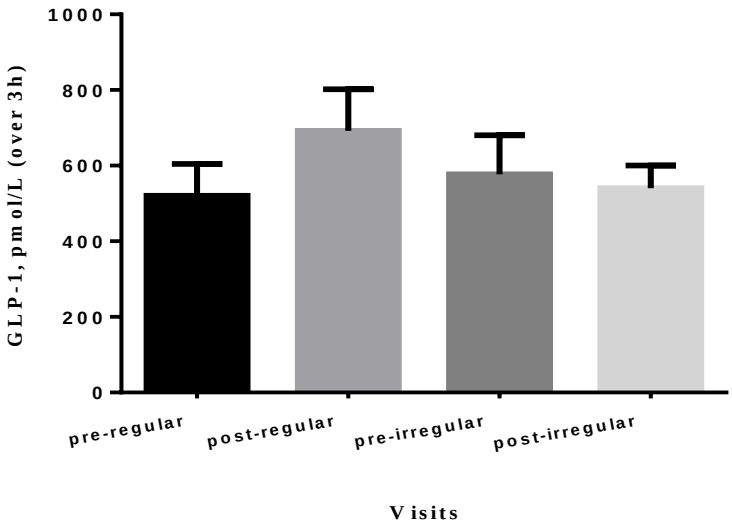

Figure 4.4 Mean (± SEM) iAUC plasma GLP-1 concentrations in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=10

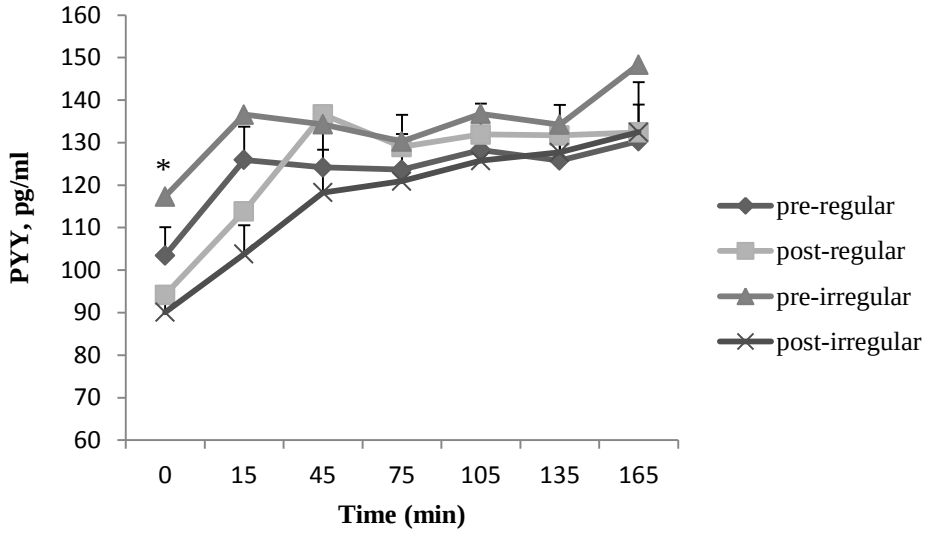

Figure 4.5 Mean (± SEM) plasma PYY concentrations in healthy females at baseline, and after the test drink consumption, pre and post-regular and irregular meal patterns.

n=10. For clarity, SEM values only presented for post-regular and irregular profiles.

*There was a significant main effect of visit on fasting plasma PYY concentrations (ANOVA, $p < 0.05$).

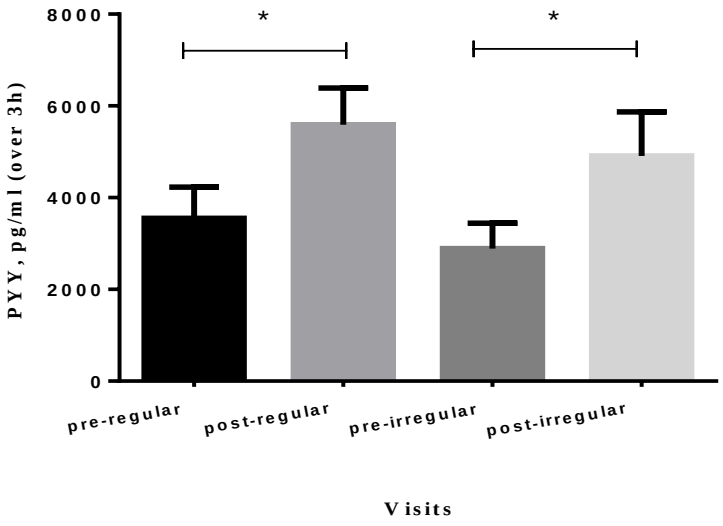

Figure 4.6 Mean (± SEM) iAUC plasma PYY concentrations in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=10

* There was a significant main effect of visit on iAUC plasma PYY concentrations (ANOVA, $p < 0.05$).

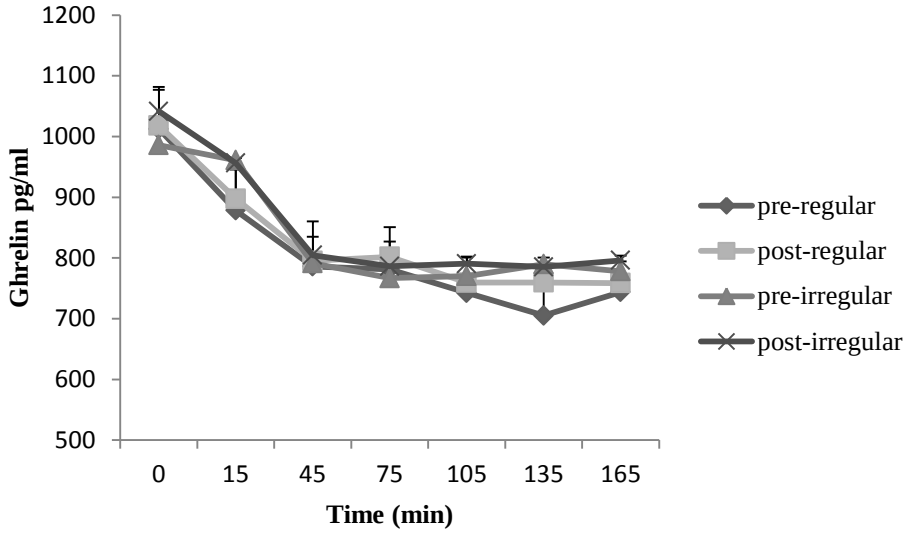

Figure 4.7 Mean (± SEM) plasma ghrelin concentrations in healthy females at baseline, and after the test drink consumption, pre and post-regular and irregular meal patterns.

n=10. For clarity, SEM values only presented for post-regular and irregular profiles.

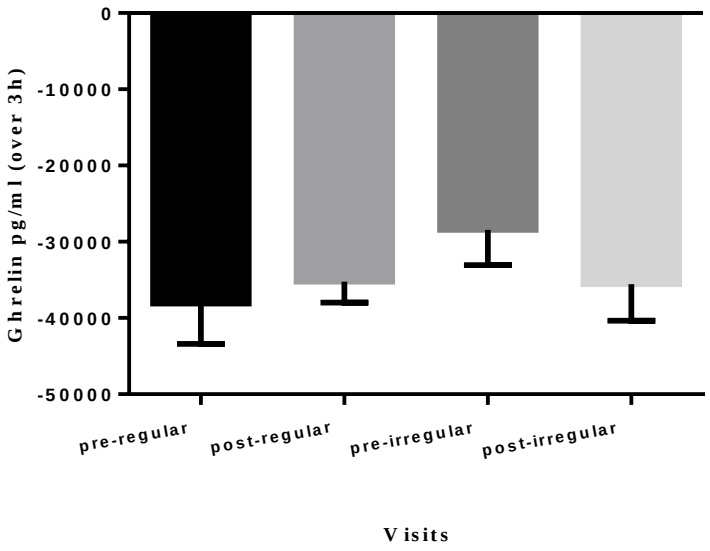

Figure 4.8 Mean (± SEM) iAUC plasma ghrelin concentrations in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=10

CHAPTER 5 : INFLUENCE OF THE IRREGULAR MEAL PATTERN ON CIRCULATING LIPIDS, GLUCOSE AND INSULIN IN HEALTHY NORMAL-WEIGHT FEMALES

5.1 Introduction

Obesity and associated diseases (diabetes and cardiovascular disease) currently pose major challenges to health in developed and developing countries. A healthy lifestyle plays a vital role in minimizing the risk of these diseases by reducing cardiometabolic risk factors. Identifying novel strategies that reduce cardiometabolic risk is imperative. Dietary composition affects metabolism and hence health outcomes. However, other aspects of diet, such as meal pattern (e.g. the frequency), may also be important. The influence of meal frequency on health including cardiometabolic risk has long been a matter of debate. An early study by Fabry and colleagues (1964) showed an inverse relationship between meal frequency and hypercholesterolemia and glucose tolerance. Reduced levels of total and low-density lipoprotein (LDL) cholesterol when meal frequency is increased were also reported (Jenkins et al., 1989, Edelstein et al., 1992, Arnold et al., 1993, Titan et al., 2001). However, other studies failed to find significant effects of meal frequency on cholesterol (Bortz et al., 1966, Jordan and Novascone, 1989). There has been recent interest in the possible role of between day variations in frequency. An intervention study (Farshchi et al., 2004b) has shown that irregular eating (different numbers of meals on each day) was associated with potentially deleterious alterations in lipids and carbohydrate metabolism. There were greater fasting levels of total and LDL cholesterol, a decrease in fasting insulin sensitivity and a greater insulin response to a test meal following a two-week period of an irregular

meal pattern in contrast to a regular meal pattern in normal-weight females. The author is not aware of any published studies in which a regular meal pattern is compared with an irregular one whilst measuring glucose continuously in a free-living environment.

The aim of the present study was therefore to assess the potential impacts of meal pattern on serum lipids, glucose and insulin response to a test drink, and free-living sub-cutaneous glucose profile amongst healthy normal-weight females. Food was provided to match energy intake in the two meal patterns.

5.2 Methods

5.2.1 Sample size

Data reported in this chapter are secondary endpoints. The sample size of this study reflects that which was calculated from the overall TEF as a primary endpoint for the study (Chapter 4). Sample size calculation for the primary endpoint (TEF) was explained in Section 4.2.1.

5.2.2 Participants

The study presented in this chapter was undertaken on the same participants of Chapter 4 at the same time, but in order that the outcomes could be described and discussed clearly, they have been presented separately.

Eleven healthy normal-weight (BMI 18.5 and 25 kg/m^2) females aged 18-40 years (mean 24.1 ± 4.9 years) participated in the study (ethical approval reference: J14082012 BMS; ClinicalTrials.gov ID: NCT02052076). All participants met the inclusion criteria explained in Section 2.2.

5.2.3 Study protocol

The design of the study protocol was explained in details in Section 2.5 and outlined in Figure 2.1.

5.2.4 Measurements made during the intervention periods

5.2.4.1 Glucose monitoring

CGM was used to measure glucose concentrations under free-living conditions for 3 consecutive days in each period (day 7, 8 and 9). CGM data per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00) were analysed for each day of the three days. CONGA-1 was also calculated in the morning (current observation from 9:00-10:00) and night (current observation from 22:00-23:00) for each day.

On day 7 (6 meals/day in both regular and irregular interventions) postprandial iAUC for 90 min was analysed following each meal (breakfast, mid-morning snack, lunch, afternoon snack, dinner and night snack). On day 8 (6 meals/day vs. 5 meals/day in regular and irregular interventions, respectively) participants consumed the same type, amount and energy content of food at breakfast, mid-morning snack and night snack in both intervention periods. However, the afternoon snack was omitted during the irregular period and the food distributed between lunch and dinner. On day 9 (6 meal/day vs. 9 meals/day in regular and irregular periods, respectively), the same type, amount and energy content of food at lunch, dinner and night snack were consumed. The breakfast was divided into two meals during the irregular period. The mid-morning and afternoon snacks were also divided into two small meals in order to achieve 9 meals/day. Therefore, postprandial iAUC on day 8 and 9 was analysed

following each meal in which the same type and amount of food was consumed in regular and irregular periods (i.e. day 8: Breakfast, mid-morning and night snacks; day 9: Lunch, dinner and night snack). Glucose monitoring procedure is described in details in Section 2.7.3

5.2.5 Laboratory visit protocol

The protocol of the laboratory visit was explained in details in Section 2.8 and displayed in Figure 2.3. Briefly, in this visit blood was sampled before and for 3 h following the consumption of a milkshake test drink to determine fasting lipids, glucose and insulin concentrations.

5.2.6 Blood samples analysis

Blood samples analysis for lipids, glucose and insulin was explained in Section 2.9.

5.3 Statistical analysis

All data are reported as mean ± SD, unless otherwise indicated. The details of the statistical methods were explained in Section 2.10.

5.4 Results

All participants completed the study, although blood samples could not be performed on one participant due to problems associated with vein cannulation. Five participants were scheduled to start with the regular meal pattern and six others with the irregular one. Participant flow in this study is represented in Figure 4.1.

5.4.1 Free-living CGM

Eleven participants collected CGM data in each intervention period. Of these, two were excluded, as inadequate data were obtained. For the remaining nine participants, CGM data on day 7, 8 and 9 were analysed.

Mean 24 h, max, min and iAUC for glucose concentrations (Table 5.1) showed no significant differences between the two intervention periods. There were also no significant differences in the day period and the night period between the two intervention periods for these variables.

Glycemic variability (CONGA-1) showed also no significant differences between the two intervention periods (Table 5.1). On day 7 (6 meals in both interventions), postprandial (breakfast +90 min) iAUC analysis showed a significantly higher glucose concentration in the irregular meal pattern intervention compared with the regular meal pattern intervention (Table 5.2). (paired T-test, $p < 0.05$). On day 9 (6 meal/day vs. 9 meals/day in regular and irregular periods, respectively), postprandial (Lunch +90 min) iAUC analysis in the irregular intervention was significantly higher compared with the regular intervention (Table 5.2) (paired T-test, $p < 0.05$). Moreover, on day 9, postprandial (dinner +90 min) iAUC was significantly higher in the irregular meal pattern intervention period than was seen in the regular one (Table 5.2) (paired T-test, $p < 0.05$). No differences appeared in the other postprandial iAUC analysis (Table 5.2).

5.4.2 Serum lipids

The results for fasting serum lipids are shown in Table 5.3. There were no significant differences between the pre-intervention visits.

There were no significant interactions for meal pattern by visit for serum total, LDL, HDL-cholesterol and serum triglycerides across the study. There were also no main effects of meal pattern or visit.

5.4.3 Blood glucose

5.4.3.1 Fasting values

Table 5.3 shows the results of fasting blood glucose in all visits. There were no significant differences at the pre-intervention visits. No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting blood glucose across the study.

5.4.3.2 Response to the test drink

Blood glucose responses to the test drink pre and post both regular and irregular intervention visits are shown in Figure 5.1. Blood glucose concentrations reached a maximum level 30 and 45 min after the test drink and remained above fasting levels at the last sampling time-point (180 min after the test drink) in all visits. There were no significant differences at the pre-intervention visits in the peak values of blood glucose. The peak values (Table 5.3) did not show a significant interaction for meal pattern by visit or main effects for these two factors.

Blood glucose iAUC response to the test drink (Figure 5.2) showed no significant differences at the pre-intervention visits. A significant interaction between meal pattern and visit were observed in iAUC responses (ANOVA, $p < 0.05$). A larger area was seen at post-irregular visit compared with post-regular visit (245.2 ± 110.6 and 200.3 ± 88.7 mmol/L in 3 h, respectively),

(paired T-test, $p < 0.05$). Post-irregular visit, blood glucose iAUC was also significantly higher than pre-irregular visit (173.3 ± 60.3 mmol/L in 3 h), (paired T-test, $p < 0.05$).

5.4.4 Serum insulin

5.4.4.1 Fasting values

Table 5.3 shows the results of fasting serum insulin in all visits. There were no significant differences at the pre-intervention visits. No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting serum insulin across the study.

5.4.4.2 Response to the test drink

Figure 5.3 shows serum insulin responses to the test drink at the pre and post-intervention visits. Serum insulin concentrations increased rapidly after consuming the test drink in all visits. Following peak values (15 and 45 min after the test drink ingestion); concentrations declined to some extent but remained above fasting values for the reminder of the sampling period.

The peak values of insulin (Table 5.3) did not show significant differences between the pre-intervention visits. There was no significant meal pattern by visit interaction or main effects of meal pattern or visit in the peak values.

Serum insulin iAUC were not significantly different in the pre-intervention visits. There was no significant interaction between meal pattern and visit on iAUC for serum insulin nor were there significant main effects for meal pattern or visit (Figure 5.4).

5.4.5 HOMA-IR

HOMA-IR values (Table 5.3) did not show significant differences between pre-regular and pre-irregular visits. There was no significant interaction for meal pattern by visit for HOMA-IR. There were also no main effects of meal pattern or visit.

5.5 Discussion

The aim of this study was to investigate the impact of regular and irregular meal patterns on fasting serum lipids, glucose and insulin profiles under isoenergetic well-controlled conditions. Participants were requested to consume 6 meals/day in the regular intervention period and from 3 to 9 meals/day (average of 6 meals/day) in the irregular intervention period. Each participant consumed the same type and amount of food regardless of whether they followed the regular or irregular meal pattern, and the body weight of all participants was stable throughout the two intervention periods.

This study establishes that in healthy normal-weight females, postprandial glucose concentration (during 3 h following the test drink consumption) is lower after a 14-day period of a regular meal pattern compared with an irregular meal pattern. At day 7 of the interventions in free-living conditions, iAUC for postprandial glucose (for 90 min after breakfast) was lower in the regular compared with the irregular intervention period when the same number of meals was consumed. Similar results were seen for iAUC for postprandial glucose after lunch and after dinner in which the same type, amount and energy content of the meals were consumed at day 9 of the interventions. iAUC for postprandial glucose (for 90 min after lunch) and (for 90 min after dinner) were lower in the regular compared with the irregular intervention period.

To the author's knowledge, this is the first study that has evaluated the effect of consistency of meal pattern on glucose concentrations using continuous sub-cutaneous glucose monitoring in free-living participants.

An interest in meal frequency developed in the 1960s (Fabry et al., 1964). Several studies attempted to evaluate the impact of meal frequency on cardiometabolic risk factors, with inconclusive results (Jenkins et al., 1989, Jordan and Novascone, 1989, Bortz et al., 1966, Arnold et al., 1993, Solomon et al., 2008). In these meal frequency studies, however investigators compared a range of meal frequencies, but were not testing the impact of between day consistency.

The current study found significant differences in iAUC for glucose response to the test drink between the two intervention periods, but did not find differences in measured insulin response. In order to eliminate an acute effect of the meal frequency immediately preceding the laboratory visit, participants consumed the same number of meal (6 meals/day) on the day prior to post-intervention visits. Potentially, the differences seen might be consequences of a longer carry over effect of either those meal patterns above, or below, the 6 meals provided in the regular meal pattern intervention, rather than a direct effect of the irregularity of the meal pattern.

Munsters and Saris (2012) showed that 3 meals/day led to lower blood glucose concentrations throughout the day (AUC) compared with 14 meals/day. In that study, blood was sampled fasted, 30 min following the breakfast ingestion and subsequently every 1 h until 21:30. Unfortunately the blood samples in this study were drawn from an antecubital vein and the validity relative to arterial or arterialised venous is unclear.

In the earlier study (Farshchi et al., 2004b) with the same study design as the current study, except that the prescribed diet was not provided to the participants, normal weight females showed no significant differences in glucose response to the test drink. However, a significant increase was observed in AUC of insulin responses to the test drink after the irregular compared with the regular meal pattern. In this study, mean energy intake recorded over 3 days (when 6 meals/day were consumed during the regular period; 6, 3 and 9 meals/day were consumed during the irregular period) was higher during the irregular meal pattern compared with the regular meal pattern which may have led to an increased insulin response to the test drink in irregular meal pattern intervention period.

Several factors regulate postprandial glucose responses including insulin secretion. The current study demonstrates that whilst the blood glucose responses to the test drink were lower after the regular intervention period compared with the irregular one, such meal patterns had no effect on insulin responses when the test drink was consumed. These findings suggest that the regular meal pattern improved insulin sensitivity. Therefore, the glucose disposal was more efficient after the regular intervention period compared with the irregular one.

Measurement of glucose in a free-living environment using CGM in this study has provided novel information. On day 7 of the interventions, when 6 meals/day were consumed during the regular and irregular periods, a higher postprandial (after breakfast) iAUC was found in the irregular period compared with the regular one. Although the meal frequency had been different the day before, the last meal had been identical on both interventions. On day 8 (6

meals/day vs 5 meals/day in regular and irregular interventions, respectively) participants consumed the same type and amount of food at breakfast, the mid-morning snack and the night snack. No significant differences were observed between the regular and irregular periods for any of the identical meals. Standardization of the participants' diet the day before (day 7, 6 meals/day were consumed in both interventions) could explain this result, and prior to the night snack, there had been a difference in the immediately preceding meals (dinner and lunch). Future work should consider how quickly such adaptation to meal pattern occurs.

On day 9 (6 meals/day vs 9 meals/day in regular and irregular interventions respectively), the same type and amount of food were consumed at lunch, dinner and the night snack. Postprandial (after lunch and after dinner) iAUC on day 9 in the irregular intervention (9 meals/day) were higher compared with day 9 in the regular intervention (6 meals/day) even though these meals were identical.

The 24 h, day and night iAUC for glucose data on day 7, 8 and 9 of the regular period were not significantly different from the corresponding days during the irregular period. Munsters and Saris (2012) illustrated non-significant differences in 24 iAUC for glucose (from CGM data) between 14 meals/day and 3 meal/day.

Fasting triglyceride and HDL cholesterol concentrations showed no significant differences between the two meal patterns in the present study. This is in agreement with the previous study in normal-weight females (Farshchi et al., 2004b). On the other hand, the observations relating to fasting total and LDL cholesterol are not consistent with those of the previous study (Farshchi et al.,

2004b). Although the participants in the current study were similar to those in the previous study with respect to age, BMI and body fat, their ethnicities might be different which may explain the differences in these findings. Another factor may have contributed to the differences between the current findings and those reported previously is that the same type and amount of food was consumed in both intervention periods in the present study, while the food was self-selected in the previous study, which may mean the type and amount of food consumed varied between the two interventions.

In conclusion, a 14-day period of irregular eating appears to produce a degree of insulin resistance which may indicate a deleterious effect on health. These findings suggest that a regular meal pattern could be a lifestyle factor that may promote an individual's health. Further studies are warranted, particularly in overweight and obese individuals with and without type 2 diabetes.

Table 5.1 Analyses of the CGM data compared between the two meal pattern interventions*

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)
Fasting	4.7±0.8	4.9±0.4	4.9±0.4	5.0±0.6	4.9±0.6	5.1±0.4
Mean 24 h	5.2±0.5	5.3±0.4	5.4±0.6	5.2±0.4	5.2±0.4	5.5±0.3
Mean day h**	5.3±0.7	5.4±0.5	5.5±0.5	5.3±0.4	5.3±0.4	5.6±0.3
Mean night h**	4.9±0.3	5.2±0.6	5.2±0.8	4.9±0.6	5.0±0.4	5.1±0.5
Max 24 h	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.4	7.2±0.8	7.9±1.2
Max day h	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.3	7.2±0.8	7.9±1.2
Max night h	5.5±0.4	5.8±0.8	5.9±0.7	5.8±1.0	5.5±0.5	5.8±0.6
Min 24 h	4.1±0.8	4.3±0.5	4.1±0.5	3.8±0.4	3.9±0.5	4.1±0.5
Min day h	4.1±0.8	4.3±0.5	4.1±0.6	4.3±0.4	4.1±0.6	4.3±0.4
Min night h	4.5±0.4	4.7±0.6	4.8±0.8	4.2±0.6	4.4±0.6	4.5±0.6
iAUC 24h	566.9±935.2	464.8±756.9	625.7±633.4	473.2±760.0	659.3±834.9	969.0±808.8
iAUC day h	553.3±723.0	376.7±610.4	515.0±591.7	500.8±547.1	629.9±637.6	850.5±685.5
iAUC night h	-95.0±226.8	-74.1±169.4	-186.4±209.8	-69.5±138.4	-75.5±199.7	-136.2±145.9
CONGA-1(9:00-10:00)	0.67±0.6	0.68±0.4	1.13±0.8	1.14±0.7	0.59±0.3	0.72±0.3
CONGA-1 (22:00-23:00)	0.38±0.22	0.36±0.1	0.60±0.4	0.32±0.2	0.32±0.2	0.52±0.2

*mean ± SD, n=9. ** Day h (7:00-midnight), Night h (midnight-7:00).

Table 5.2 Analyses of the CGM data (postprandial, meal +90 min, iAUC) compared between the two meal pattern interventions*

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)
iAUC-breakfast +90	50.3±54.4 [1]	56.3±52.0	-	95.7±70.8 [1]	66.6±42.2	-
iAUC-mid-morning snack +90	25.3±29.3	29.9±40.4	-	31.8±42.3	43.2±25.9	-
iAUC-lunch +90	34.6±40.0	-	51.4±43.9 [2]	21.5±45.0	-	102.8±74.7 [2]
iAUC-afternoon snack +90	36.8±61.0	-	-	41.7±43.1	-	-
iAUC-dinner +90	46.0±58.9	-	50.5±43.3 [3]	56.3±53.0	-	90.3±54.7 [3]
iAUC-night snack +90	17.2±21.7	25.3±26.7	9.4±45.0	35.7±32.1	21.3±33.0	23.1±21.9

*mean ± SD, n=9.
[1, 2, 3] There were significant differences between the two intervention periods (paired T-test, $p < 0.05$).

Table 5.3 Fasting blood measurements and peak postprandial glucose and insulin concentrations over the study*

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Serum cholesterol				
Total (mmol/L)	4.22 ± 1.13	4.34 ± 1.07	4.14 ± 1.25	4.15 ± 0.92
LDL (mmol/L)	2.48 ± 1.01	2.60 ± 1.04	2.44 ± 0.97	2.48 ± 0.82
HDL (mmol/L)	1.41 ± 0.21	1.39 ± 0.23	1.31 ± 0.30	1.31 ± 0.24
Serum triglycerides (mmol/L)	0.74 ± 0.23	0.80 ± 0.31	0.81 ± 0.55	0.83 ± 0.32
Blood glucose (mmol/L)	4.6 ± 0.40	4.4 ± 0.24	4.5 ± 0.52	4.3 ± 0.55
Serum insulin (mIU/L)	9.64 ± 2.87	8.97 ± 2.55	10.28 ± 4.14	8.52 ± 2.95
HOMA IR	1.98 ± 0.96	1.77 ± 0.52	2.04 ± 0.91	1.60 ± 0.57
Glucose Peak (mmol/L)	7.4 ± 0.57	6.7 ± 0.65	6.8 ± 0.55	6.9 ± 0.80
Insulin peak (mIU/L)	83.1 ± 46.49	83.1 ± 54.94	103.8 ± 78.41	71.6 ± 32.25

*mean ± SD, n=10

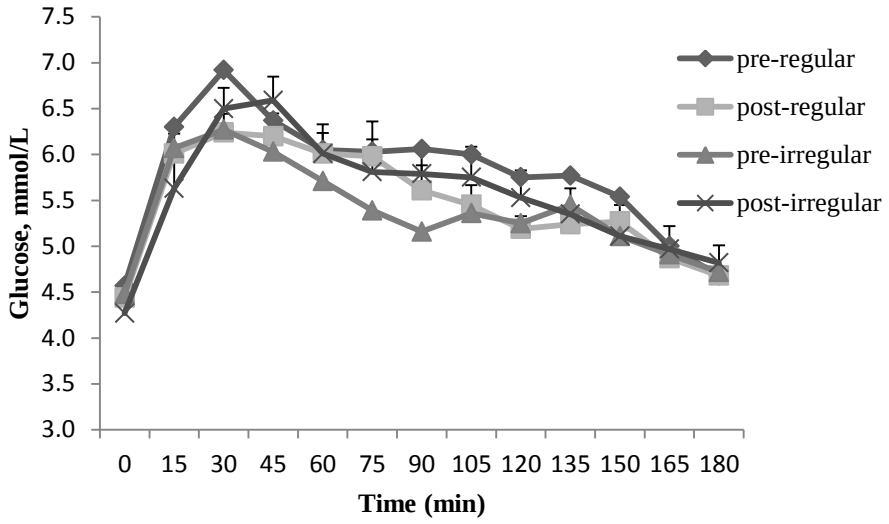

Figure 5.1 Mean (±SEM) blood glucose concentrations in healthy females at the visits pre and post-regular and irregular meal patterns.

n=10. For clarity, SEM values only presented for post-regular and irregular profiles.

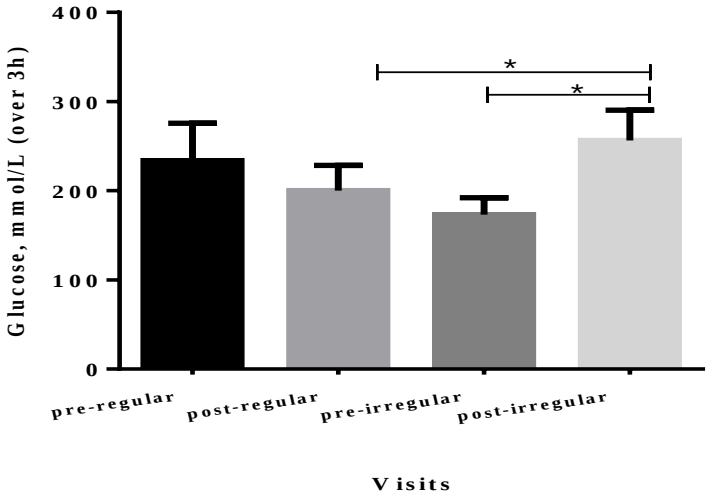

Figure 5.2 Mean (±SEM) iAUC for blood glucose concentrations in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=10.

* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (ANOVA, $p < 0.05$). iAUC for blood glucose concentration was significantly lower post-regular compared with post-irregular meal pattern (paired T-test, $p < 0.05$). iAUC for blood glucose concentration was significantly higher post-irregular compared with pre-irregular meal pattern (paired T-test, $p < 0.05$).

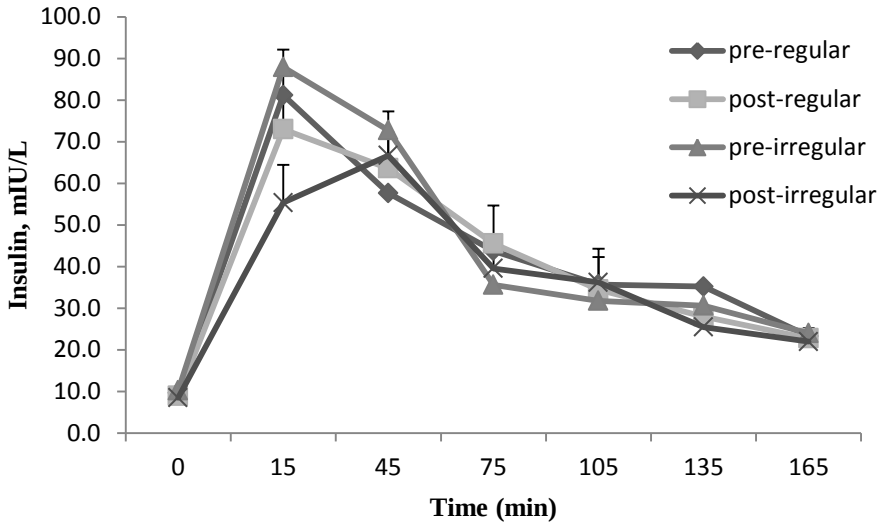

Figure 5.3 Mean (± SEM) serum insulin concentrations in healthy females at baseline, and after the test drink consumption, pre and post-regular and irregular meal patterns.

n=10. For clarity, SEM values only presented for post-regular and irregular profiles.

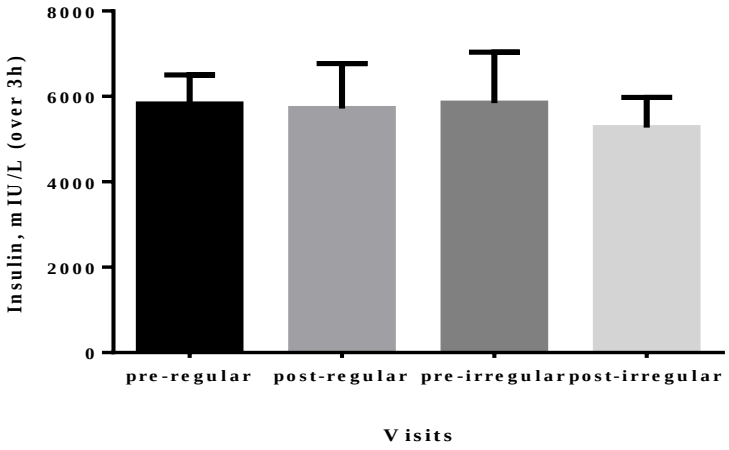

Figure 5.4 Mean (±SEM) iAUC for serum insulin concentrations in healthy females in the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=10

CHAPTER 6 : INFLUENCE OF THE IRREGULAR MEAL PATTERN ON THE THERMIC EFFECT OF FOOD AND APPETITE REGULATION IN OBESE, INSULIN RESISTANT FEMALES

6.1 Introduction

The growing prevalence of obesity and related diseases represents a major public health concern (Ali et al., 2008). A previous study in normal-weight participants (Farshchi et al., 2004a) suggested that meal regularity was associated with potentially beneficial impacts on energy balance. These impacts seemed to be more pronounced in obese participants (Farshchi et al., 2005). Farshchi et al. (2005) showed that an irregular meal pattern, with self-selected food, over a two-week period was associated with lower TEF in a group of ten obese females compared with a regular meal pattern. However, the impact of an isoenergetic diet of the regular or irregular meal pattern on the TEF and appetite hormones, with consideration of free-living energy expenditure, was not studied.

This study aimed to ascertain whether the regularity of a meal pattern over two weeks affects the thermogenic response to a test breakfast, appetite hormones, subjective appetite ratings and *ad-libitum* food intake of a test lunch in obese with insulin resistance females on an isoenergetic diet. During the two intervention periods, identical food was provided and SWA was used to monitor free-living energy expenditure.

6.2 Methods

6.2.1 Sample size

The primary end point of normal-weight females study described in this thesis was differences in TEF (kcal/min) over 180 min between regular and irregular meal patterns. The iAUC TEF after the regular meal pattern was 25.8 ± 6.8 kcal and after the irregular meal pattern was 14.8 ± 11.7 kcal. Using one sample T-test model, and statistical power at the level of 0.8 (Lenth, 2006), the number of participants with a cross-over design was 10 to achieve adequate statistical power.

6.2.2 Participants

Ten overweight and obese females aged 18-45 years (mean 36.2 ± 6.0 years), with insulin resistance (mean HOMA 2.8 ± 0.9) participated in this study (ethical approval reference: A16012014 SoL) according to the inclusion criteria described in Section 2.2. Participants were required to have a BMI in the range of 28-40 kg/m^2. The majority had a BMI within the range 30-40 kg/m^2 whilst one participant was in the overweight category (25-29.9 kg/m^2), therefore, the term obese females was used in this study to describe both the obese and the one overweight participant (mean body weight 85.6 ± 13.1; mean BMI 33.3 ± 3.1).

This study was registered with ClinicalTrials.gov, identification number: NCT02582606.

6.2.3 Study protocol

A more detailed description of the study protocol was described in Section 2.5 and outlined in Figure 2.1. Briefly, in a randomized crossover study, the participants underwent two 14-day intervention periods (with a 14-day wash-out period). In period 1, participants consumed either a regular (6 meals/day) or an irregular (between 3 and 9 meals/day) meal pattern. In period 2, participants followed the alternative meal pattern. Participants were asked to visit the laboratory at the start and end of each period after an overnight fast.

6.2.4 Dietary intervention periods

During the regular and irregular meal pattern interventions, identical foods were provided in amounts designed to keep body weight constant over the study periods. The food was supplied in a 4 day cycle of menus in order to avoid monotony and boredom during the 14-day period. Menus were designed for 2050 kcal/day, 2350 kcal/day, 2550 kcal/day and 2900 kcal/day to meet the different estimated energy requirements of participants. Having estimated participants' energy requirements were calculated (Section 2.6.1); they were assigned the menus with the closest energy requirement (± 100 kcal).

The procedures for dietary intervention were explained in details in Section 2.6

6.2.5 Measurements made during the intervention periods

6.2.5.1 Energy expenditure assessment during the intervention period

During the two intervention periods, participants' physical activity pattern and energy expenditure continuously were monitored by using SWA as explained in Section 2.7.1.

6.2.5.2 Appetite assessment during the intervention period

The protocol for the appetite assessment during the intervention periods was explained in Section2.7.2.

6.2.6 Laboratory visit protocol

The protocol for the laboratory visit was explained in detail in Section 2.8 and outlined in Figure 2.3. In this visit, anthropometric measurements, REE, TEF (following the test drink consumption), subjective appetite, appetite hormones and *ad-libitum* intake were determined in order to examine the effect of regular and irregular meal pattern on these variables. Lipids, glucose and insulin were also determined and their results are presented in Chapter 7.

6.2.6.1 Anthropometric measurements

On arrival at the laboratory, anthropometric variables including weight, height, waist circumference, hip circumference, waist to hip ratio and body composition were carried out. Procedures for anthropometric measurements were explained in Section 2.8.1.

6.2.6.2 Energy expenditure measurement

Energy expenditure was measured with an indirect calorimetry system during the laboratory visit. The protocol of energy expenditure measurement was explained in Section 2.8.2.

6.2.6.3 Test drink consumption

A milkshake test drink was served as breakfast. The volume of consumed milkshake was based on the participant's healthy body weight (an average of

10 kcal/kg healthy body weight). Healthy body weight was calculated at a BMI of 22.5 kg/m^2, as weight (kg) = 22.5 x height^2 (m).

The formula and the composition of the milkshake were explained in Section 2.8.3.

6.2.6.4 Blood sampling and processing

Circulating adiponectin, leptin, GLP-1, PYY and ghrelin were determined at each laboratory visit as explained in Section 2.8.4

6.2.6.5 *Ad-libitum* Test Meal

At lunch time, an a*d-libitum* pasta test meal was served to the participant. The energy composition and recipe for the pasta were explained in Section 2.8.5

6.2.6.6 Subjective Appetite Ratings

Participants were asked to complete the VAS during the lab visit to assess subjective appetite. The protocol was explained in section 2.8.6.

6.2.7 Blood samples analysis

Blood samples analysis for adiponectin, leptin, GLP-1, PYY and ghrelin was explained in Section 2.9

6.3 Statistical Analysis

All data are reported as mean ± SD, unless otherwise stated. The details of the statistical methods were explained in Section 2.10.

6.4 Results

Five participants were scheduled to start with the regular meal pattern and five others with the irregular one. However, one participant withdrew from the study after she completed the irregular period due to lack of time. Therefore, of ten obese females recruited, nine participants completed the study Participant flow in this study is represented in Figure 6.1.

6.4.1 Anthropometric measurements

Anthropometric measurements of the participants are presented in Table 6.1. Pre-intervention visits showed no significant differences in bodyweight, nor did bodyweight change across the two intervention periods. Body composition, waist circumference and waist-to-hip ratio did not show significant differences, either at the pre-intervention visits or across the two intervention periods.

6.4.2 Energy Intake

Self-reported daily energy intake before the start of the study (1921 ± 386 kcal/day) was significantly lower than the estimated energy requirement for weight maintenance (2473 ± 340 kcal/day) (paired T-test, $p < 0.05$).

Carbohydrate percentage showed significant differences between self-reported (47 ± 6.5 %) and prescribed intervention diet (53 ± 0.2 %) (paired T-test, $p < 0.05$). Self-reported fat percentage (36 ± 4.7 %) tended to be higher than fat percentage in the prescribed intervention diet (33 ± 0.2 %), but did not reach the significant level (paired T-test, $p = 0.08$). However, there were no significant differences between self-reported protein percentage (16 ± 3.6 %) and protein percentage in the prescribed intervention diet (14 ± 0.3 %).

Food diaries provided during the two intervention periods showed that 99 ± 1.4 % and 99 ± 1.0 % of the energy from the food provided was consumed in the regular and irregular intervention periods, respectively. There were no significant differences between energy intake during the regular intervention period (2470 ± 356 kcal/day) and the irregular one (2475 ± 355 kcal/day), nor were significant differences seen in the food composition consumed in the regular intervention period (53 ± 0.3 % carbohydrate, 33 ± 0.3 % fat and 14 ± 0.4 % protein) compared with the irregular intervention period (53 ± 0.8 % carbohydrate, 33 ± 0.6 % fat and 14 ± 0.3 % protein).

6.4.3 Free-living energy expenditure

In both the regular and irregular intervention periods, the mean of time spent wearing the SWA device was 96.83 ± 4.25 and 95.58 ± 5.80%, respectively. There were no significant differences in the mean values of total energy expenditure, measured by SWA, (2542 ± 451 and 2461 ± 299 kcal/day for regular and irregular intervention periods, respectively). There were also no significant differences in the mean for physical activity level between the two intervention periods (1.22 ±0.07 and 1.23 ± 0.09 METs for regular and irregular intervention periods respectively).

Energy expenditure obtained by SWA during the regular intervention period (2542 ± 451 kcal/day) was not significantly different from energy intake consumed in the same period (2470 ± 356 kcal/day). In the irregular intervention period, there were also no significant differences between energy expenditure obtained by SWA (2461 ± 299 kcal/day) and energy intake consumed (2475 ± 355 kcal/day).

6.4.4 Energy expenditure (indirect calorimetry data)

There were no significant differences between fasting REE at the pre-intervention visits. In addition, fasting REE did not show a significant meal pattern* visit interaction or main effect of meal pattern or visit (1411.2 ± 186.8, 1324.0 ± 128.6, 1374.5 ± 128.6 and 1354.1 ± 242.26 kcal/day in pre, post-regular and pre and post-irregular visits, respectively).

Following the test drink consumption, REE increased above the fasting values at all visits. Figure 6.2 shows the overall TEF for the 3 h postprandial period and there were no significant differences between the pre-intervention visits. However, comparison of the overall TEF values across the study showed a significant meal pattern by visit interaction (ANOVA; $p < 0.05$). TEF rose after the regular intervention period and fell after the irregular meal pattern. Furthermore, TEF was significantly lower post-irregular intervention period compared with the post-regular one (paired T-test, $p < 0.05$). TEF post-regular visit was 5.0 ± 6.5 kcal higher than the post-irregular visit (paired T-test $p < 0.05$).

6.4.5 Blood variables

6.4.5.1 Adiponectin

Fasting serum adiponectin concentrations were not significantly different between the pre-intervention visits.

No significant meal pattern by visit interaction or main effects for meal pattern or visit in fasting serum adiponectin concentrations was observed across the study visits (7.67 ± 1.99, 7.19 ± 2.64, 7.94 ± 2.26, 7.61 ± 1.96 µg/mL in pre and post-regular and pre and post-irregular visits, respectively).

6.4.5.2 Leptin

There were no significant differences in fasting serum leptin concentrations at the pre-intervention visits.

Furthermore, no significant interaction between meal pattern and visit, or main effects for meal pattern or visit were seen in fasting serum leptin concentrations over the study visits (56.70 ± 24.58, 49.26 ± 15.64, 50.10 ± 21.88 and 49.23 ± 21.97 µg/L in pre and post-regular and pre and post-irregular visits, respectively).

6.4.5.3 GLP-1

Fasting plasma GLP-1 concentrations showed no significant differences between the pre-interventions (Figure 6.3). There was no significant meal pattern by visit interaction for fasting plasma GLP-1 concentrations. A significant main effect of visit (ANOVA, $p < 0.05$) was seen in fasting plasma GLP-1 concentrations. Mean fasting plasma GLP-1 concentrations increased by 5% and 28 % post-regular and post-irregular visits, respectively, compared with pre-intervention visits.

A dramatic increase in plasma GLP-1 concentrations occurred after the test drink during all visits. Following peak values, plasma GLP-1 concentrations declined but remained above the fasting values until the last sampling time point in all visits (Figure 6.2). There was a significant interaction between meal pattern and visits in iAUC for plasma GLP-1 concentrations across the study (ANOVA; $p < 0.05$) (Figure 6.4), Plasma GLP-1 concentrations iAUC tended to increase post-regular visit compared with pre-regular visit but did not reach significance (paired T-test $p = 0.09$), unlike at the irregular visits, where there was no significant difference between pre and post-intervention visits. Plasma

GLP-1 concentrations post-regular visit were significantly higher than post-irregular visit (paired T-test $p < 0.05$).

6.4.5.4 PYY

There were no significant differences in fasting plasma PYY concentrations at the pre-intervention visits (Figure 6.5). Fasting plasma PYY concentrations did not show a significant meal pattern by visit interaction or main effect of meal pattern or visit.

Following consumption of the test drink, plasma PYY concentrations increased rapidly above the fasting values and remained at a plateau until the last sampling time point during all visits (Figure 6.5). iAUC for plasma PYY concentrations (Figure 6.6) showed no significant interaction between meal pattern and visits, or main effect on meal pattern or visit.

6.4.5.5 Ghrelin

Figure 6.7 shows the concentrations of plasma ghrelin at all study visits. There were no significant differences in fasting values at the pre-intervention visits. No significant meal pattern by visit interaction or main effects for meal pattern or visit in fasting plasma ghrelin concentrations was observed across the study visits.

Following the test drink consumption, plasma ghrelin concentrations decreased gradually during all visits (Figure 6.7). iAUC for plasma ghrelin (Figure 6.8) showed a trend for an interaction between meal pattern and visits, but did not reach significance (ANOVA, $p = 0.08$). No significant main effect of meal pattern or visit was observed in plasma ghrelin iAUC.

6.4.6 Subjective appetite ratings

Fasting VAS ratings (hunger, satiety, fullness, desire to eat and prospective food consumption) were not significantly different between the pre-intervention visits, nor were there significant meal pattern by visit interactions or main effects of meal pattern or visit (Table 6.2).

6.4.6.1 Responses to the test drink

iAUC responses for VAS ratings over the 3 h postprandial period did not elicit significant differences between pre-regular and pre-irregular intervention visits. iAUC responses did not show significant differences across the study visits, either (Table 6.2).

6.4.6.2 Responses to the *ad-libitum* test meal

There were no significant differences in iAUC responses for VAS ratings over the 1 h postprandial period between pre-intervention visits. There was also no significant meal pattern by visit interactions or main effect of meal pattern or visit with iAUC responses (Table 6.2).

6.4.6.3 Responses to the meal pattern during the intervention period

Participants completed the VAS before and after each meal on day 7 and 14 (6 meals/day) of each intervention period.

On day 7, comparison of mean pre-meal for all VAS ratings (average of the 6 pre-meal ratings on the day) did not demonstrate significant differences between the regular and irregular intervention periods (Table 6.3). No significant differences were seen in the mean of post-meal VAS ratings (average of the 6 post-meal ratings on the day) between the regular and

irregular intervention periods. The mean of the differences between pre and post-meal with respect to the VAS ratings did not demonstrate significant differences between the two intervention periods (Table 6.5).

On the final day (day 14), the pre-meal values for the VAS ratings were not significantly different between the regular and irregular intervention periods (Table 6.4). Post-meal values for the VAS ratings also did not show significant differences between the regular and irregular intervention periods (Table 6.4). Comparison of the mean of the differences between pre and post-meal ratings did not indicate that any significant differences existed between the intervention periods for any of the VAS ratings (Table 6.5).

6.4.7 Intake at the *ad-libitum* test meal

Participants' energy intake during the *ad-libitum* test meal, duration of eating and speed of consuming meal did not exhibit significant differences between the pre-intervention visits.

There was no meal pattern by visit interaction or main effect of meal pattern or visit for participants' energy intake across the study visits (854.1 ± 271.5, 858.0 ± 215.9, 823.1 ± 273.3 and 900.4 ± 215.7 kcal in pre and post-regular and irregular visits, respectively).

The duration of eating did not show a significant interaction between the meal pattern and visit or main effect of meal pattern or visit (12.1 ± 5.7, 12.3 ± 7.4, 11.3 ± 5.9 and 11.0 ± 3.6 min in pre and post-regular and irregular visits, respectively). There was also no significant interaction between the meal pattern and visit or main effect of meal pattern or visit on speed of eating (46.2 ± 12.4, 48.2 ± 15.2, 46.9 ± 11.5 and 51.8 ± 14.4 g/min in pre and post-regular and irregular visits, respectively).

6.5 Discussion

The aim of this study was to investigate the effects of consuming regular and irregular meal pattern on TEF response to a test meal, appetite hormones, subjective appetite ratings and *ad-libitum* food intake of a test meal in obese, insulin resistant females on an isoenergetic diet.

The current study found that a 14-day period of consuming of regular meal pattern resulted in a higher TEF measured for 3 h following a breakfast, milkshake test drink. A higher iAUC of plasma GLP-1 concentrations, following the test drink, was also detected in response to the regular compared with the irregular intervention period. Fasting plasma GLP-1 concentrations were higher after the intervention periods than before. Furthermore, there was a tendency for ghrelin to decrease in response to the test drink post-regular intervention period.

The present study emphasizes that an irregular meal pattern is associated with a lower TEF. This might contribute to the development of obesity over the long term. These findings are in accordance with findings from the earlier study of obese females used similar protocol, but the food consumed was self-selected (Farshchi et al., 2005).

Despite increased TEF post-regular intervention period, there was not a statistically significant change in body weight or the other anthropometric measurements. This would be expected because of the short duration of the intervention. A similar observation has been reported in the earlier study in obese females (Farshchi et al., 2005).

Obesity might be associated with a reduction of the postprandial GLP-1 response (Ranganath et al., 1996, Ranganath et al., 1999). Increases in GLP-1

concentrations have been shown to suppress subjective appetite and reduce subsequent energy intake in humans (Naslund et al., 1999). In the present study, although fasting plasma GLP-1 concentrations were higher after both intervention periods, the regular meal pattern produced higher plasma GLP-1 concentrations in response to the test drink suggesting a potential impact on appetite suppression, which would favour obesity management. However, no significant differences were observed in *ad-libitum* food intake across the study and this may reflect insufficient power to demonstrate a reduction in energy intake associated with the difference in GLP-1.

GLP-1 concentrations can be affected by the phases of the menstrual cycle (Brennan et al., 2009). Participants started the meal pattern interventions in the same phase of the menstrual cycle which may be part of the explanation that fasting plasma GLP-1 concentrations were higher post-interventions compared with pre-interventions.

Unlike many other gut hormones, ghrelin concentrations increase in the fasted state and are suppressed rather than stimulated by food intake (Cummings et al., 2001). Ghrelin has been recognised as a gut hormone that stimulates appetite and food intake in humans (Wren et al., 2001). In the current study, plasma ghrelin concentrations tended to decrease post-regular intervention but did not reach significant levels, potentially because the study was insufficiently powered for this outcome. This finding suggests that, although the reduction did not reach significant level, a regular meal pattern has a potential role in appetite suppression and hence might facilitate a reduction in food intake.

The present study however failed to produce any significant differences in VAS ratings between the two intervention periods either in the laboratory or free living conditions.

No significant differences were seen in fasting adiponectin, leptin, PYY and PYY responses. This might be due to a lack of statistical power.

There is consistent evidence that obese individuals may selectively under-report snacks (Heitmann and Lissner, 1995, Drummond et al., 1998, Goris et al., 2000). In the current study, despite instructions to maintain a normal food intake, food diaries of habitual diet before the start of the study showed a significant lower mean energy intake than the estimated energy requirement for weight maintenance. Stable body weight was successfully maintained during the two intervention periods which suggests that the methods of estimating energy requirement used in the current study were valid and that providing subjects' weight was stable pre-intervention, they had under reported their habitual energy intake.

Energy expenditure measurements obtained with the SWA during the two intervention periods indicated a compliance with requested instructions to maintain similar levels of activity during the regular and irregular intervention periods. These instructions were intended to reduce the potentially confounding effect of physical activity level on the key outcomes of interest.

Energy expenditure as measured by SWA during the regular and irregular intervention periods was not different from the energy intake consumed in the two interventions. Although a poor agreement between SWA and indirect calorimetry has been reported (Papazoglou et al., 2006, Bertoli et al., 2008),

Mackey and colleagues (2011) showed that the SWA accurately estimated free-living energy expenditure.

In summary, in obese females with insulin resistance, a regular meal pattern is associated with a greater TEF and postprandial GLP-1. This demonstrates that the constancy of daily meal pattern may be a contributory factor to weight control. However, this is an aspect which merits further longer term investigations.

Table 6.1 Participants' characteristics over the study*

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Body weight (kg)	86.5 ± 13.6	86.1 ± 13.5	85.3 ± 12.9	85.7 ±13.4
BMI (kg/m^2)	33.7 ±3.3	33.5 ±3.3	33.2 ±3.1	33.3 ±3.2
Body fat (%)	40.8 ±7.7	40.9 ±8.8	40.9 ±8.3	41.2 ±8.3
Waist (cm)	91.4 ±11.7	90.6 ±11.9	90.9 ±11.6	91.1 ±11.6
Waist/hip	0.78 ±0.1	0.78 ±0.1	0.78 ±0.1	0.78 ±0.1

*mean ± SD, n=9

Table 6.2 Fasting and iAUC for subjective appetite ratings following the test drink and the *ad-libitum* meal over the study *

		Regular meal pattern		Irregular meal pattern	
		Pre	**Post**	**Pre**	**Post**
Hunger	**Fasting** (mm)	42 ± 29	62 ± 23	50 ± 34	56 ± 20
	Test drink (mm over 3h)	-1129±3562	-3979±3907	-1015±7335	-973±3146
	***Ad-libitum* meal** (mm over 1h)	-2950±1587	-3125±1323	-3077±1641	-4236±1172
Satiety	**Fasting** (mm)	20 ± 13	24 ± 19	17 ± 18	27 ± 18
	Test drink (mm over 3h)	5022±4329	3832±2848	5383±5776	2733±2878
	***Ad-libitum* meal** (mm over 1h)	3022±1651	3939±1275	3289±1573	3581±1940
Fullness	**Fasting** (mm)	24 ± 22	24 ± 19	17 ± 12	18 ± 15
	Test drink (mm over 3h)	4114±5292	3677±3132	4962±4337	3074±3125
	***Ad-libitum* meal** (mm over 1h)	3360±1550	4219±901	3838±1293	3760±1115
Desire to eat	**Fasting** (mm)	39 ± 24	48 ± 20	52 ± 32	54 ± 22
	Test drink (mm over 3h)	-263±4243	-561±5325	-411±7005	-813±4725
	***Ad-libitum* meal** (mm over 1h)	-3161±1602	-3691±1155	-3161±1455	-3663±1139
Prospective food consumption	**Fasting** (mm)	55 ± 21	57 ± 18	57 ± 25	53 ± 17
	Test drink (mm over 3h)	-2712±5418	-2529±5401	-1176±5803	-818±2647
	***Ad-libitum* meal** (mm over 1h)	-3219±1235	-3342±1064	-3219±1317	-4037±1146

*mean ± SD, n=9

Table 6.3 Comparison of mean appetite ratings (all day points combined) on day 7 of regular and irregular intervention periods*

mm	Regular meal pattern		Irregular meal pattern	
	Pre-meals	**Post-meals**	**Pre-meals**	**Post-meals**
Hunger	51.2 ± 16.3	17.8 ± 16.1	49.4 ± 13.6	15.7 ± 15.3
Satiety	43.4 ± 12.6	79.9 ± 14.8	45.5 ± 14.4	81.0 ± 17.6
Fullness	38.1 ± 14.3	78.4 ± 13.6	41.4 ± 14.6	81.1 ± 16.5
Desire to eat	56.3 ± 19.1	25.7 ± 24.8	50.3 ± 15.0	21.6 ± 19.1
Prospective food consumption	55.4 ± 15.3	24.9 ± 23.8	51.4 ± 15.9	20.4 ± 20.8

*mean ± SD, n=9

Table 6.4 Comparison of mean appetite ratings (all day points combined) on day 14 of regular and irregular intervention periods*

mm	Regular meal pattern		Irregular meal pattern	
	Pre-meals	**Post-meals**	**Pre-meals**	**Post-meals**
Hunger	51.4 ± 19.8	23.8 ± 18.6	50.2 ± 9.2	16.0 ± 14.5
Satiety	39.3 ± 14.3	76.2 ± 17.1	43.9 ± 7.0	80.8 ± 12.5
Fullness	41.4 ± 17.3	73.0 ± 15.5	42.7 ± 8.7	79.6 ± 12.6
Desire to eat	57.3 ± 15.6	19.7 ± 18.9	52.4 ± 12.8	20.0 ± 15.8
Prospective food consumption	55.7 ± 15.9	19.3 ± 19.6	53.2 ± 11.4	21.5 ± 15.3

*mean ± SD, n=9

Table 6.5 Mean differences between subjective appetite ratings before and after the meal on day 7 and 14 of regular and irregular intervention periods when 6 meals were consumed*

mm	Regular meal pattern		Irregular meal pattern	
	Day 7	Day 14	Day 7	Day 14
Hunger	-33.9 ± 14.9	-27.4 ± 14.4	-34.5 ± 16.3	-34.1 ± 16.1
Satiety	37.6 ± 12.1	37.3 ± 13.5	36.3 ± 10.2	36.9 ± 13.6
Fullness	41.3 ± 14.0	32.3 ± 19.6	40.7 ± 14.4	36.9 ± 13.8
Desire to eat	-30.9 ± 19.7	-37.8 ± 14.8	-28.7 ± 19.1	-32.4 ± 17.7
Prospective food consumption	-30.9 ± 18.4	-36.5± 16.1	-31.8 ± 19.6	-31.7 ± 15.3

*mean ± SD, n=9

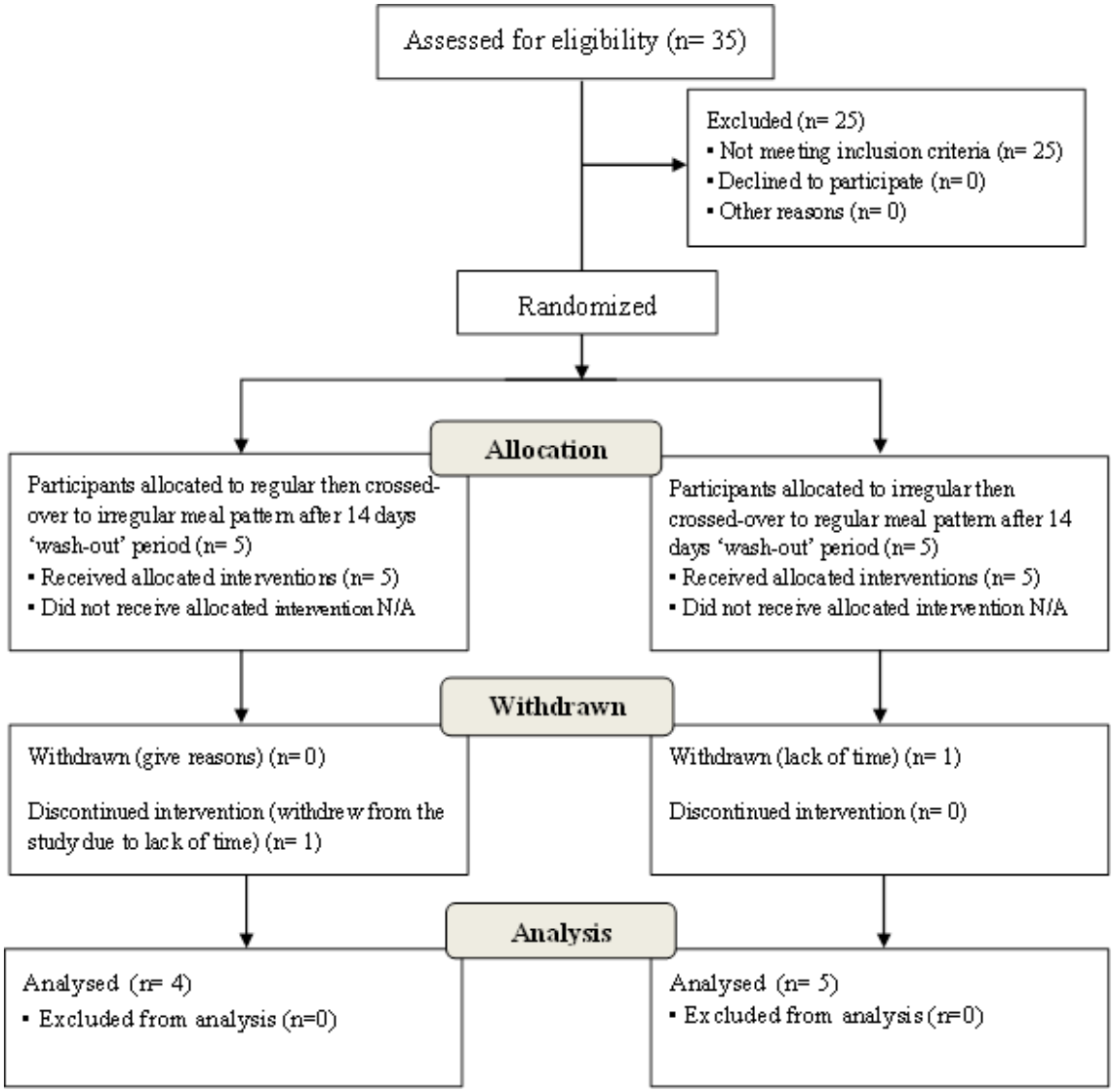

Figure 6.1 Study participant flow diagram

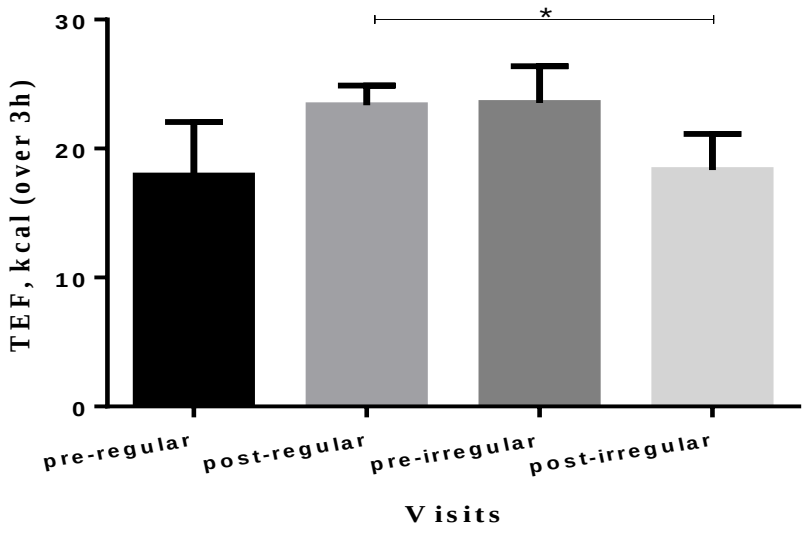

Figure 6.2 Mean (±SEM) iAUC for TEF in obese females at the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9.

* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (ANOVA; $p < 0.05$). iAUC for TEF was significantly higher post-regular compared with post-irregular meal pattern (paired T-test $p < 0.05$).

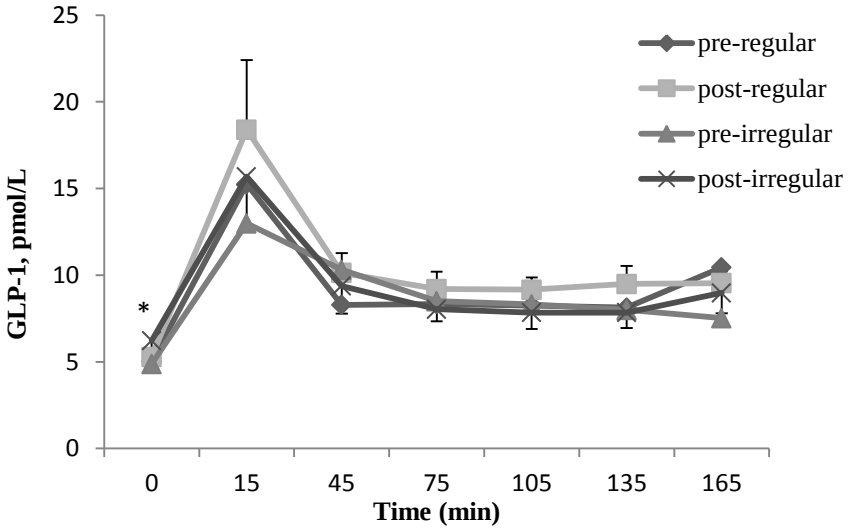

Figure 6.3 Mean (± SEM) plasma GLP-1 concentrations in obese females at baseline and after the test drink consumption, pre and post-regular and irregular meals.

n=9. For clarity, SEM values only presented for post-regular and irregular profiles.

*There was a significant main effect of visit on fasting plasma GLP-1 concentrations (ANOVA, $p < 0.05$).

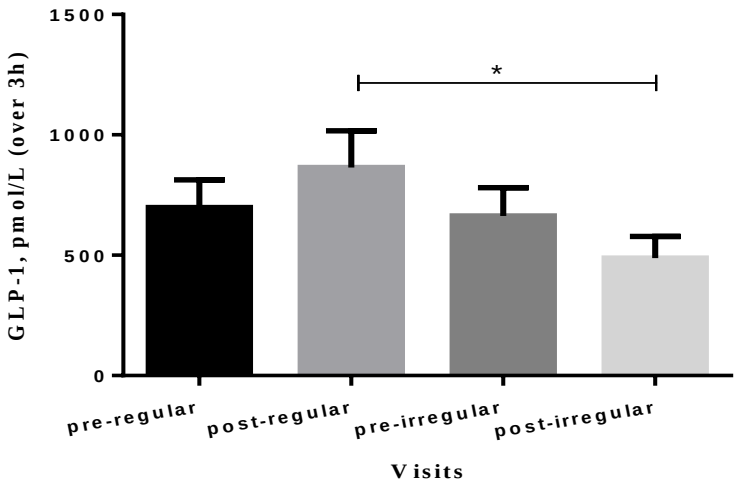

Figure 6.4 Mean (± SEM) iAUC plasma GLP-1 concentrations in obese females at visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9.

* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (ANOVA; $p < 0.05$). iAUC plasma GLP-1 concentration was significantly higher post-regular compared with post-irregular meal pattern (paired T-test $p < 0.05$).

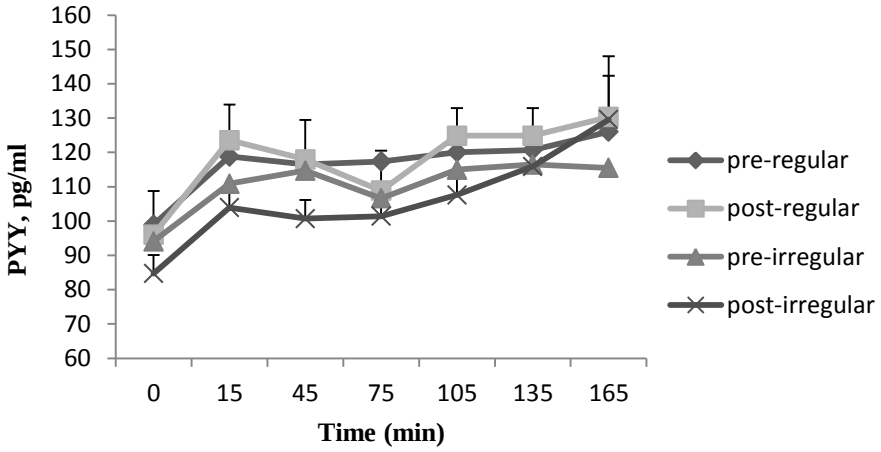

Figure 6.5 Mean (± SEM) plasma PYY concentrations in obese females at baseline and after the test drink consumption pre and post-regular and irregular meal patterns.

n=9. For clarity, SEM values only presented for post-regular and irregular profiles.

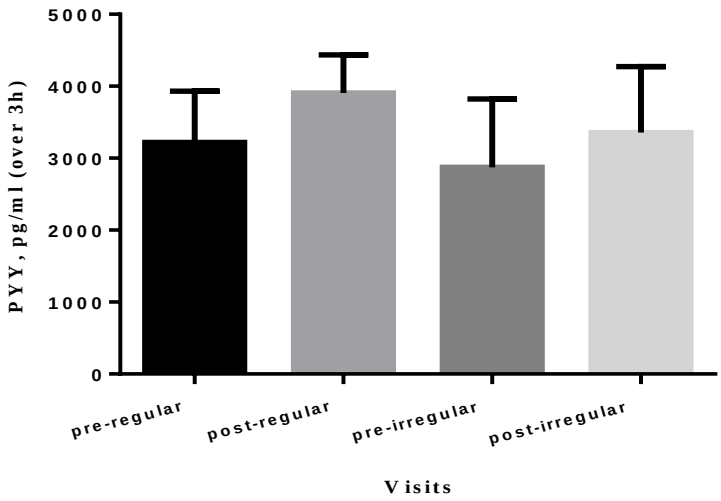

Figure 6.6 Mean (± SEM) iAUC plasma PYY concentrations in obese females at visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9.

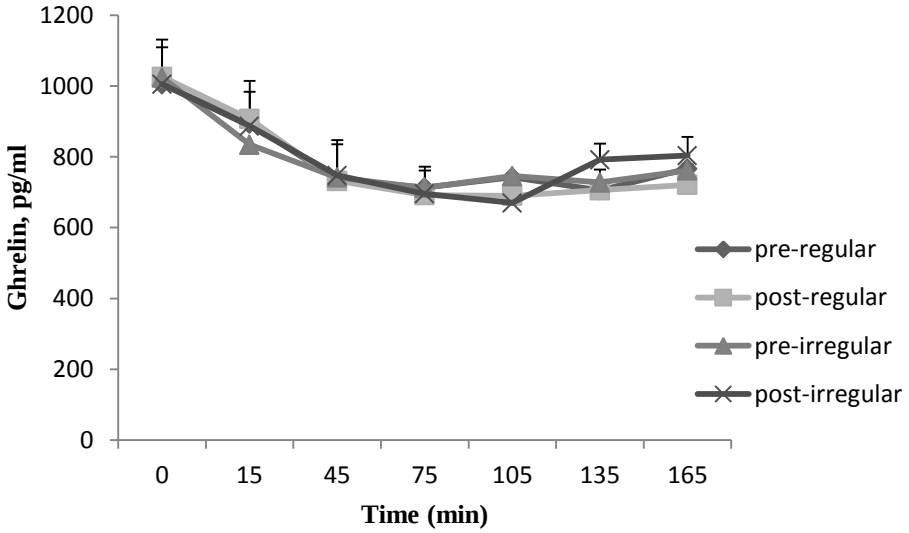

Figure 6.7 Mean (± SEM) plasma ghrelin concentrations in obese females at baseline and after the test drink consumption pre and post-regular and irregular meal patterns.

n=9. For clarity, SEM values only presented for post-regular and irregular profiles.

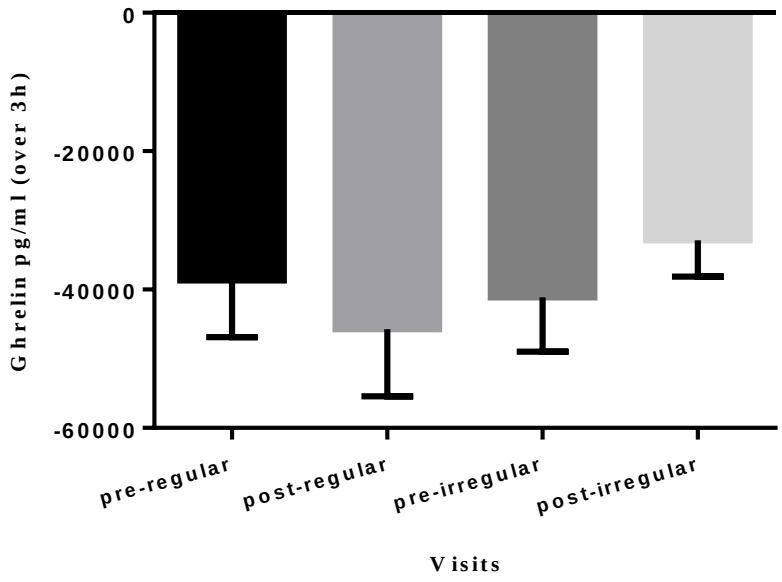

Figure 6.8 Mean (± SEM) iAUC plasma ghrelin concentrations in obese females at visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9.

CHAPTER 7 : INFLUENCE OF THE IRREGULAR MEAL PATTERN ON CIRCULATING LIPIDS, GLUCOSE AND INSULIN IN OBESE, INSULIN RESISTANT FEMALES

7.1 Introduction

Obesity is an increasingly common problem and is associated with both an increase in cardiometabolic risk factors and disease (Wang et al., 2011). Some aspects of diet can play a crucial role in the development of both obesity and cardiometabolic risk factors including insulin resistance, dyslipoproteinemia and hyperglycemia. It has been suggested that irregular meal pattern may have potentially deleterious effects on carbohydrate metabolism and lipid profiles (Farshchi et al., 2004b). Furthermore, my previous study on normal-weight females showed an improvement in the glucose uptake following the regular intervention. Obese individuals, in whom there is an increased risk of or already evidence of metabolic disturbance, are an important group to study. A greater insulin response to a test drink was observed in obese females following an irregular meal pattern compared with a regular one (Farshchi et al., 2005). However, the diet was self-selected and glucose was only measured when fasting and in response to a test drink. Therefore, in order to test the hypothesis that a regular meal pattern may improve insulin sensitivity, whereas an irregular meal pattern may reduce insulin sensitivity, in obese individuals with insulin resistance the following study was undertaken. In contrast to the previous work (Farshchi et al., 2005) food was provided to match energy intake in the two meal patterns, and glucose was monitored continuously during the intervention period, as opposed to just testing glucose response to a test drink.

This study aimed to ascertain the effects of irregular meal patterns on circulating lipids, glucose and insulin with a controlled energy intake in obese, insulin resistant females.

7.2 Methods

7.2.1 Sample size

Data reported in this chapter are secondary endpoints. The sample size of this study reflects that which was calculated from the overall TEF as a primary endpoint (Chapter 6) for the study. Sample size calculation for the primary endpoint (TEF) was explained in Section 6.2.1.

7.2.2 Participants

This chapter and Chapter 6 describe the same study (ethical approval reference: A16012014 SoL; ClinicalTrials.gov ID: NCT02582606), but different outcomes (lipid, glucose and insulin) are reported in this chapter.

Ten overweight and obese females (mean body weight 85.6 ± 13.1; mean BMI 33.3 ± 3.1).aged 18-45 years (mean 36.2 ± 6.0 years) with insulin resistance (mean HOMA 2.8 ± 0.9) participated in this study according to the inclusion criteria described in Section 2.2.

7.2.3 Study protocol

The design of the study protocol was explained in details in Section 2.5 and outlined in Figure 2.1.

7.2.4 Measurements made during the intervention periods

7.2.4.1 Glucose monitoring

Glucose concentrations were monitored under free-living conditions using the CGM for 6 consecutive days (day 7, 8, 9, 10, 11 and 12) in each period.

The data obtained were analysed per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00). CONGA-1 was also calculated in the morning (current observation from 9:00-10:00) and night (current observation from 22:00-23:00). Furthermore, postprandial (meal + 90) iAUC was analysed following each meal on day 7 (6 meals/day in both regular and irregular periods). Postprandial (meal + 90) iAUC on day 8 (6 meals/day vs 5 meals/day in regular and irregular interventions, respectively), day 9 (6 meal/day vs. 9 meals/day in regular and irregular periods, respectively) and day 10 (6 meals/day vs. 8 meals/day in regular and irregular periods, respectively) was analysed following each meal in which the same type and amount of food was consumed in both regular and irregular interventions (i.e. day 8: Breakfast, mid-morning and night snacks; day 9: Lunch, dinner and night snack; day 10: Breakfast, lunch and night snack). However, on day 11 (6 meals/day vs. 3 meals/day in regular and irregular periods, respectively) and 12 (6 meals/day vs. 4 meals/day in regular and irregular periods, respectively), postprandial (meal + 90) iAUC was not calculated for any meals because there was no identical meals (type and amount of food) consumed during those days of the regular and irregular periods. Glucose monitoring procedure was described in details in Section 2.7.3.

7.2.5 Laboratory visit protocol

The protocol for the laboratory visit was explained in details in Section 2.8 and displayed in Figure 2.3. Briefly, in this visit blood was sampled before and for 3 h following the consumption of a milkshake test drink to determine serum lipid, blood glucose and serum insulin concentrations.

7.2.6 Blood samples analysis

Blood sample analysis for lipids, glucose and insulin was explained in Section 2.9.

7.3 Statistical analysis

All data are reported as mean ± SD, unless otherwise indicated. The details of the statistical methods were explained in Section 2.10.

7.4 Results

Five participants were scheduled to start with the regular meal pattern and five others with the irregular one. However, one participant withdrew from the study after she completed the irregular period due to lack of time. Figure 6.1 shows the participant flow in this study.

7.4.1 Free-living CGM

Nine participants collected CGM data on day 7, 8, 9, 10, 11 and 12 of each intervention period. One of these failed, providing inadequate data, therefore data from eight participants were used for analysis.

The 24 h mean, minimum, maximum and iAUC values for glucose concentrations during the two intervention periods are shown in Table 7.1 and Table 7.2. No significant differences were observed between the regular and

irregular intervention periods for any of these values. There were also no significant differences in the mean, minimum, maximum and iAUC values during the day (7:00–midnight) or night (midnight-7:00) between the two intervention periods (Table 7.1 and 7.2). Glycemic variability (CONGA-1) showed also no significant differences between the regular and irregular interventions (Table 7.1 and 7.2).

Postprandial (meal +90 min) iAUC analyses on day 7, 8, 9 and 10 are shown in Table 7.3. The values during the regular intervention period did not differ significantly from the values during the irregular intervention period.

7.4.2 Serum lipids

Fasting serum lipid concentrations from all visits are shown in Table 7.4. There were no significant differences at the pre-intervention visits in any serum lipids variables.

Fasting serum total and LDL-cholesterol showed no significant interaction between meal pattern and visit, or main effect of meal pattern or visit over the study visits. There was no significant meal pattern by visit interaction or main effect of meal pattern for fasting serum HDL-cholesterol and triglycerides. However, a significant main effect of visit (ANOVA, $p < 0.05$) was seen in these variables. Mean fasting serum HDL-cholesterol concentrations decreased approximately by 11 % and 3% post-regular and post-irregular visits, respectively, compared with pre-intervention visits, whilst mean fasting serum triglycerides concentrations increased approximately by 19 % and 23% post-regular and post-irregular visits, respectively, compared with pre-intervention visits, with no significant differences between the meal patterns.

7.4.3 Blood glucose

7.4.3.1 Fasting values

The pre-intervention fasting blood glucose concentrations did not differ significantly between the two meal patterns. Fasting blood glucose did not show a significant meal pattern by visit interaction or main effect of meal pattern or visit (Table 7.4).

7.4.3.2 Response to the test drink

There were no significant differences in peak values and iAUC for blood glucose between pre-regular and pre-irregular intervention visits.

Blood glucose concentrations increased above the fasting values after the test drink and reached a maximum level between 30 and 45 min at all visits (Figure 7.1). Following the peak values, the concentration diminished towards fasting concentrations at all visits. There was no significant interaction between meal pattern and visit or main effect of meal pattern or visit in blood glucose peak values.

Blood glucose iAUC over 3 h following test drink consumption (Figure 7.2) did not exhibit a significant interaction between meal pattern and visit or main effect of these two variables.

7.4.4 Serum insulin

7.4.4.1 Fasting values

There were no significant differences in fasting concentrations for serum insulin between pre-regular and pre-irregular intervention visits.

There was no significant interaction for meal pattern by visit for fasting serum insulin concentrations. There was also no main effect of meal pattern or visit (Table 7.4).

7.4.4.2 Response to the test drink

The pre-intervention peak values and iAUC for serum insulin did not differ significantly between the two meal patterns.

Following the test drink, serum insulin concentrations increased and following peak values, these concentrations gradually dropped for the reminder of the sampling period at all visits (Figure 7.3). No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in peak values across the study visits. iAUC for serum insulin at all visits is shown in Figure 7.4. iAUC for serum insulin demonstrated no significant interaction between meal pattern and visit or main effect of visit. However a significant main effect of meal pattern was observed.

7.4.5 HOMA-IR

HOMA-IR values from all visits are shown in Table 7.4. HOMA-IR was not significantly different at the pre-intervention visits. There was also no meal pattern by visit interaction, or main effect of meal pattern or visit for HOMA-IR.

7.5 Discussion

The aim of this study was to investigate the effects of an irregular meal pattern on lipid profiles, glucose and insulin in obese females with insulin resistance.

The study did not show significant differences in insulin resistance, as assessed by using HOMA-IR index in a group of nine obese, insulin resistant females. These findings are in accordance with the findings from the earlier study by Farshchi and colleagues (2005). Farshchi et al. (2005) found no significant differences in HOMA-IR between a 14-day period of regular and irregular interventions (food was self-selected during the two interventions) on ten obese but otherwise healthy females.

Whilst there was no significant interaction between meal pattern and visit or main effect of visit on iAUC insulin responses, there was a significant main effect of meal pattern. No significant differences in iAUC insulin responses between the pre-intervention visits were observed. However, iAUC insulin responses were higher after the regular intervention than after the irregular one. Food diaries provided during the two interventions showed high compliance with the prescribed diet in both regular and irregular interventions. There were no significant differences between energy intake during the regular and the irregular intervention periods, nor were there significant differences between the two inventions in regard to food composition. Furthermore, iAUC of glucose responses to the test drink did not show significant differences in regular and irregular intervention periods.

The earlier study on obese females (Farshchi et al., 2005), reported a higher measured insulin response after the irregular intervention compared with the regular one. A possible explanation for this finding might be that the mean energy intake recorded over three days was significantly higher during the irregular meal pattern than it was during the regular meal pattern.

However, a comparison of macronutrient composition between food diaries recorded during the regular intervention and food diaries recorded during the irregular intervention did not yield any significant differences (Farshchi et al., 2005).

Regarding fasting serum insulin, the present study indicated no difference across the study. A similar observation was reported in the previous study (Farshchi et al., 2005).

The present study reported no significant difference in fasting blood glucose, glucose peak and glucose responses to the test drink. These findings are in agreement with the findings of the earlier study (Farshchi et al., 2005).

Measurements of glucose in a free-living environment, using CGM, on day 7, 8, 9, 10, 11 and 12 of the regular period were not different from the corresponding days during the irregular period.

No significant differences furthermore appeared in fasting serum total or LDL-cholesterol over the study and these findings are not consistent with those of the previous study (Farshchi et al., 2005). Farshchi et al. (2005) reported that the irregular meal pattern was associated with lower fasting serum total and LDL-cholesterol compared with the regular one. Part of the explanation may be that the higher energy intake during the irregular intervention compared with the regular intervention (Farshchi et al., 2005). In the present study the same type and amount of food was consumed in both intervention periods, whilst the food was self-selected in the previous study, which may mean the type and amount of food consumed varied between the two interventions.

No meal pattern effect was found on fasting serum HDL-cholesterol and triglycerides concentrations, but fasting serum HDL-cholesterol concentrations

were lower and fasting serum triglycerides concentrations were higher following the intervention periods compared with before. This might have been due to the differences in carbohydrate percentage between self-reported habitual diet and consumed intervention diet. Moreover, it might be that in the day prior to post-regular and irregular visits participants consumed the same food type and composition, whilst the food that was self-selected in the day prior to the pre-intervention visits may have differed.

It should be mentioned that all the above variables (lipids, glucose and insulin) are secondary outcomes and the main study was not powered for them. Therefore, a lack of power for these variables might be a part of the explanation of the non-significant findings.

In conclusion, the findings from the present study have indicated that consuming either a regular or irregular meal pattern for a 14-day period did not result in a detectable difference in insulin sensitivity in obese, insulin resistant females. Therefore, these findings provide no support for the hypothesis that a regular meal pattern may improve insulin sensitivity, whereas an irregular meal pattern may reduce insulin sensitivity in obese females with insulin resistance. However it would be of interest to determine these effects in further long term studies not only in females but also in males and with type 2 diabetes patients to produce more reliable and relevant findings to for public health.

Table 7.1 Analyses of the CGM data compared between the two meal pattern interventions on day 7, 8 and 9 *

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)
Fasting	5.3±1.5	5.7±0.6	5.7±0.6	5.1±0.5	5.6±0.5	5.5±0.7
Mean 24 h	5.6±0.5	5.6±0.4	5.9±0.6	5.6±0.6	5.7±0.7	6.2±0.6
Mean day h	5.6±0.6	5.7±0.4	6.1±0.6	5.6±0.5	5.8±0.8	6.2±0.7
Mean night h	5.6±0.6	5.6±0.6	5.5±0.7	5.6±0.6	5.5±0.7	5.8±0.5
Max 24 h	7.8±1.8	7.7±1.7	8.3±1.7	7.1±0.7	7.7±1.4	8.1±1.3
Max day h	7.8±1.8	7.9±1.9	8.3±1.7	7.0±0.7	7.6±1.4	8.1±1.3
Max night h	6.1±0.9	6.2±0.7	6.2±1.2	6.2±0.7	6.2±0.7	6.7±0.7
Min 24 h	4.0±0.8	4.1±0.9	4.5±0.4	4.3±0.6	4.6±0.6	4.8±0.4
Min day h	4.0±0.8	4.0±0.9	4.8±0.3	4.3±0.6	4.7±0.6	4.9±0.4
Min night h	5.0±0.6	4.9±0.4	4.8±0.6	5.0±0.7	5.0±.09	5.4±0.5
iAUC 24h	744.8±686.3	301.9±235.6	603.3±367.9	994.9±778.5	355.3±211.5	759.9±798.8
iAUC day h	550.0±454.5	269.1±234.2	618.0±352.2	685.9±597.2	335.5±278.7	661.1±532.5
iAUC night h	-96.6±140.2	-107.7±176.9	-41.3±60.3	-53.0±197.9	-180.7±174.0	-90.5±185.9
CONGA-1(9:00-10:00)	0.89±1.2	0.72±0.6	0.83±1.1	0.83±0.6	0.53±0.3	0.69±0.6
CONGA-1 (22:00-23:00)	0.29±0.2	0.48±0.3	0.46±0.3	0.39±0.3	0.49±0.2	0.44±0.3

***mean ± SD, n=8**

Table 7.2 Analyses of the CGM data compared between the two meal pattern interventions on day 10, 11 and 12 *

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 10 (6 meals)	Day 11 (6 meals)	Day 12 (6 meals)	Day 10 (8 meals)	Day 11 (3 meals)	Day 12 (4 meals)
Fasting	5.3±0.7	5.7±0.6	5.9±0.9	5.5±0.5	5.6±0.6	5.6±0.5
Mean 24 h	5.9±0.6	6.3±0.6	6.1±0.7	5.9±0.5	6.0±0.3	5.9±0.7
Mean day h	5.9±0.6	6.4±0.6	6.2±0.6	5.9±0.4	6.0±0.3	5.9±0.7
Mean night h	5.7±0.7	6.1±0.9	5.9±0.8	5.7±0.6	5.8±0.2	5.8±0.7
Max 24 h	8.2±1.4	7.7±1.4	8.6±1.6	7.9±1.4	8.2±1.7	8.2±1.9
Max day h	8.2±1.4	8.1±1.3	8.6±1.6	7.9±1.4	8.2±1.7	8.2±1.9
Max night h	6.3±1.1	7.1±1.2	6.4±0.9	6.4±1.0	6.4±0.4	6.4±1.0
Min 24 h	4.2±0.3	4.9±0.2	4.3±0.6	4.5±0.7	4.6±0.7	4.5±0.7
Min day h	4.2±0.3	5.0±0.2	4.4±0.7	4.7±0.8	4.7±0.7	4.4±0.6
Min night h	5.2±0.6	5.5±0.9	5.4±0.5	5.1±0.6	5.3±0.2	5.3±0.7
iAUC 24h	797.6±982.8	684.6±770.8	356.2±432.4	624.5±266.1	624.6±433.1	363.4±482.6
iAUC day h	670.2±738.3	548.6±601.9	342.5±408.8	490.1±186.6	514.3±313.7	301.2±344.0
iAUC night h	-118.5±345.4	-180.7±142.5	-91.7±112.8	-193.7±311.2	-162.9±211.1	-48.0±90.9
CONGA-1(9:00-10:00)	0.89±0.6	0.82±0.9	0.48±0.7	0.64±0.5	0.78±1.1	0.72±1.1
CONGA-1 (22:00-23:00)	0.39±0.3	0.27±0.2	0.99±0.7	0.54±0.3	0.80±1.0	0.59±0.6

*mean ± SD, n=8

Table 7.3 Analyses of the CGM data (postprandial, meal +90 min, iAUC) compared between the two meal pattern interventions on day 7, 8, 9 and 10 *

Glucose (mmol/L)	Regular meal pattern				Irregular meal pattern			
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 10 (8 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)	Day 10 (8 meals)
iAUC-breakfast +90	75.9±98.9	64.7±89.8	-	88.9±60.9	77.2±46.1	61.3±20.0	-	68.0±41.5
iAUC-mid-morning snack +90	47.9±31.1	38.7±31.0	-	-	40.8±23.2	54.8±44.2	-	-
iAUC-lunch +90	46.7±52.5	-	83.9±114.3	36.9±18.1	45.5±39.3	-	91.2±58.5	54.1±16.5
iAUC-afternoon snack +90	34.1±27.2	-	-	-	30.9±11.6	-	-	-
iAUC-dinner +90	49.8±49.7	-	78.1±72.2	-	38.1±27.6	-	104.3±84.4	-
iAUC-night snack +90	35.5±22.6	58.2±45.0	36.4±20.3	80.33±44.8	36.1±26.3	45.9±28.4	67.4±49.5	44.3±27.8

*mean ± SD, n=8

Table 7.4 Fasting blood measurements and peak postprandial glucose and insulin concentrations over the study *

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Serum cholesterol (mmol/L)				
Total	4.33 ± 0.47	4.30 ± 0.38	4.25 ± 0.39	4.44 ± 0.38
LDL	2.49 ± 0.46	2.55 ± 0.44	2.47 ± 0.30	2.69 ± 0.38
HDL[1]	1.35 ± 0.23	1.22 ± 0.24	1.31 ± 0.20	1.27 ± 0.22
Serum triglycerides[2] **(mmol/L)**	0.98 ± 0.41	1.17 ± 0.38	0.86 ± 0.25	1.12 ± 0.31
Blood glucose (mmol/L)	4.77 ± 0.4	4.66 ± 0.3	4.90 ± 0.5	4.70 ± 0.4
Serum insulin (mIU/L)	16.4 ± 6.3	15.6 ± 7.7	18.0 ± 9.7	17.0 ± 7.7
HOMA IR	3.5 ± 1.5	3.3 ± 1.7	4.1 ± 2.8	3.6 ± 1.6
Glucose Peak (mmol/L)	7.39 ± 1.3	7.40 ± 1.4	7.49 ± 1.2	7.48 ± 1.8
Insulin peak (mIU/L)	144.0 ± 52.1	140.4 ±46.3	150.7 ± 58.0	138.5 ± 44.5

*mean ± SD, n=9

[1, 2]A significant main effect of visit was observed with HDL and triglyceride concentrations ($p < 0.05$, ANOVA).

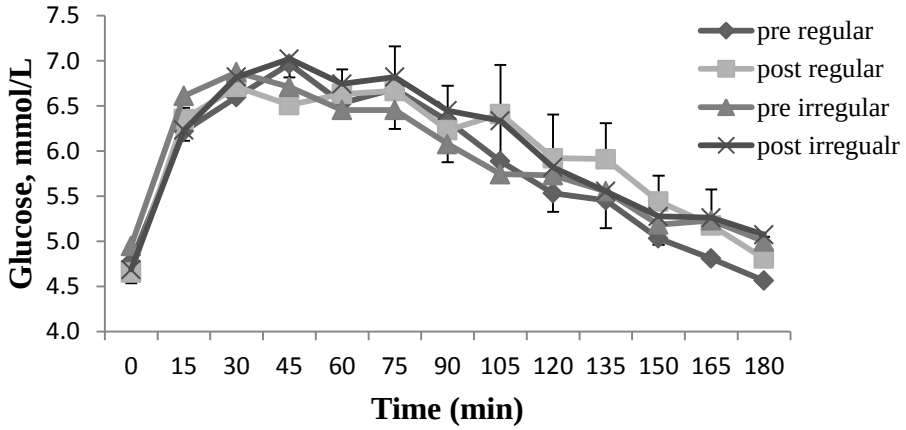

Figure 7.1 Mean (±SEM) blood glucose concentrations in obese females at the visits pre and post-regular and irregular meal patterns.

n=9. For clarity, SEM values only presented for post-regular and irregular profiles.

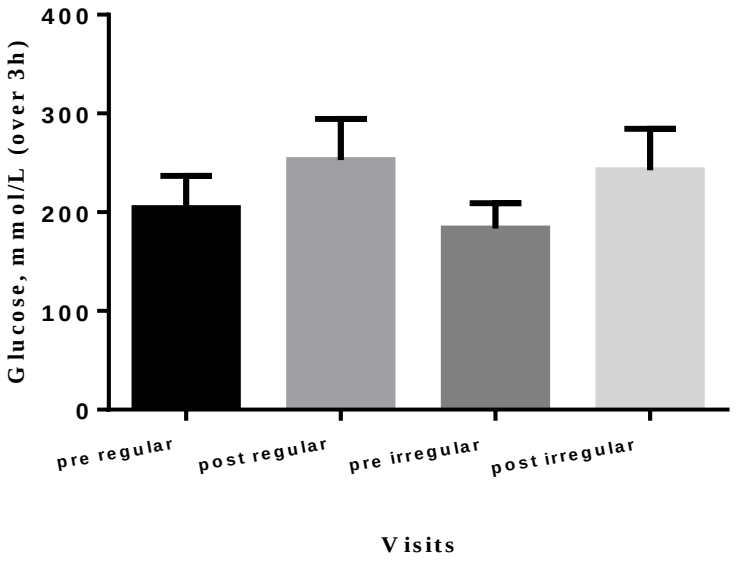

Figure 7.2 Mean (±SEM) iAUC for blood glucose concentrations in obese females at the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9.

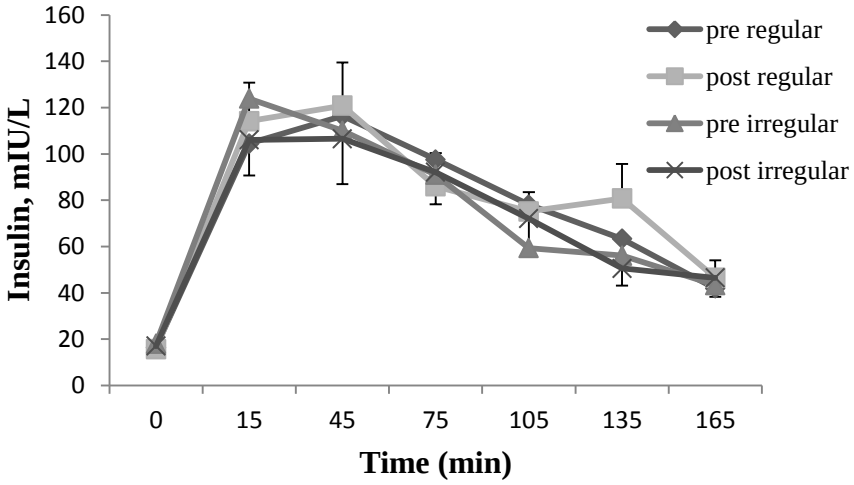

Figure 7.3 Mean (±SEM) serum insulin concentrations in obese females at baseline and after the test drink consumption pre and post-regular and irregular meal patterns.

n=9. For clarity, SEM values only presented for post-regular and irregular profiles.

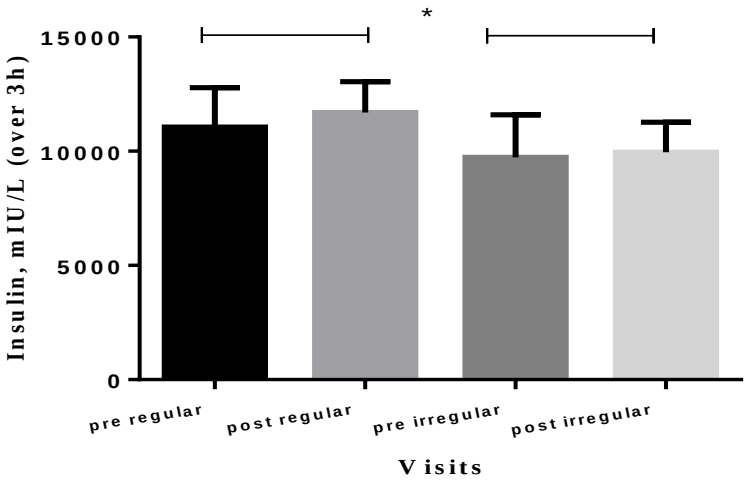

Figure 7.4 Mean (±SEM) iAUC for serum insulin concentrations in obese females at the visits pre and post-regular and irregular meal patterns, measured by the trapezoidal method.

n=9

* There was a significant main effect of meal pattern on iAUC for serum insulin concentrations (ANOVA, $p < 0.05$).

CHAPTER 8 : GENERAL DISCUSSION

The overall aim of this thesis was to investigate, in crossover design, in healthy normal-weight (Chapter 4 and 5) and obese, insulin resistant (Chapter 6 and 7) females the effects of an irregular versus a regular meal pattern on the TEF. The impact of meal pattern regularity on circulating glucose, insulin, lipids, appetite hormones, subjective appetite ratings and *ad-libitum* food intake at a test meal, were also investigated.

Participants were requested to consume 6 meals/day and from 3 to 9 meals/day, (average of 6 meals/day), in the regular and irregular intervention periods, respectively. The study was designed to provide menus that varied only in the number of meals, not the food items between the two periods. Reported compliance in both normal-weight and obese was high regarding both adhering to the assigned prescribed diets with respect to consuming the foods at the appropriate time and abstaining from other food or drink. No significant differences were observed in energy intake or food composition between the two meal patterns. This gave the opportunity to compare the effects of irregular meal pattern without differences in energy intake acting as a confounding factor.

8.1 Main findings

8.1.1 TEF

The results of the studies described in this thesis, establish that, after a 14-day period of an irregular meal pattern involving healthy, normal-weight females (Chapters 4 and 5) and obese, insulin resistant females (Chapters 6 and 7), the

TEF derived from a test drink (comprised of 50% of energy as carbohydrates, 35% as fat and 15% as protein) was significantly lower in both normal-weight and obese females compared with after the same period of a regular meal pattern. These findings are in agreement with earlier studies of normal-weight and obese females and used similar protocol, though the food consumed was self-selected (Farshchi et al., 2004a, Farshchi et al., 2005).

In my study of normal-weight females and in the Farshchi et al. studies (Farshchi et al., 2004a, Farshchi et al., 2005) concerning normal-weight and obese but otherwise healthy, females, the irregular meal pattern seems to produce a degree of insulin resistance compared with the regular meal pattern. There is evidence that insulin resistance is associated with blunted TEF (Ravussin et al., 1985) which could be the mechanism behind the impaired TEF that was observed after the irregular meal pattern. However, this finding does not necessarily pose as a plausible assumption in obese females with insulin resistance. In my study concerning obese with insulin resistance, although the TEF was lower after the irregular intervention than it was after the regular intervention, a higher degree of insulin resistance was observed after the regular intervention. These findings are contradictory to the hypothesis that an irregular meal pattern has a negative impact on insulin resistance, leading to a lower TEF.

Therefore, further investigations are warranted to explore the actual mechanism behind these observations and to better understand the effects of meal pattern on TEF and whether there are different mechanisms in the normal-weight and the obese.

Some studies reported that TEF was significantly lower in obese compared with normal-weight individuals (Pittet et al., 1976, Kaplan and Leveille, 1976, Bessard et al., 1983), but not others (Felig et al., 1983, Nair et al., 1983). In my normal-weight and obese studies, the TEF response to the test drink post regular meal pattern was lower in obese (23.3 ± 4.6 kcal over 3h) compare with normal-weight participants (25.8 ± 6.8 kcal over 3h) but it was not significant. In contrast, the TEF response to the test drink post irregular meal pattern was higher in obese (18.3 ± 8.4 kcal over 3h) compared with normal-weight participants (14.8 ± 11.7 kcal over 3h) but also it was not significant. The difference in TEF response between post regular and irregular meal pattern was higher in normal-weight than was seen in obese participants (11.1 ± 15.8 kcal and 5.0 ± 6.5 kcal in normal-weight and obese females, respectively). Accordingly, it seems that the regular meal pattern would be more beneficial for normal-weight individuals in terms of obesity management.

No significant differences in body weight or the other anthropometric measurements were discovered over the course of the study of both normal-weight and obese. However, these findings were not surprising as the same energy intake was consumed in both intervention periods. Additionally, fourteen days might not be long enough of a time period to bring about significant energy balance differences to result in a detectable change in body weight or in other anthropometric measurements with the particular sample size and equipment used.

In this protocol, participants were required to undertake the same level of activity with both meal patterns. Data obtained from the SWA device showed a similar pattern of activity. It would thus not be possible, by design, to explore

whether the regularity of the meal pattern has any impact on the activity. It should be noted that although the validation of SWA presented in this thesis showed no significant correlation between SWA and the indirect calorimetry, the reproducibility of the SWA has not been validated. However the similar patterns of activity would suggest that participants complied with the request to behave similarly during the two arms of the study.

The higher TEF that was seen after the regular meal pattern may produce a notable reduction in body weight over the long term. Therefore, a longer intervention study would help to explore more of the effects of eating regularity on energy expenditure and body weight.

8.1.2 Carbohydrate metabolism

The measurements of blood glucose following the test drink with the normal-weight participants showed a lower postprandial iAUC after the regular intervention than it did in the irregular intervention. However, both intervention periods showed similar insulin profiles. Farashi et al. (Farshchi et al., 2004b, Farshchi et al., 2005) reported that lower insulin concentrations were associated with a similar level of blood glucose concentrations with a regular compared with an irregular meal pattern in both normal-weight and obese participants. All of these findings lead to the proposal that the regular meal pattern improved insulin sensitivity.

In a free-living environment with the normal-weight participants, data obtained from CGM showed a higher postprandial (after breakfast) iAUC on day 7 during the irregular period compared with the regular one. Postprandial (after lunch and after dinner) iAUC on day 9 in the irregular intervention (when 6 meals were consumed on both protocols) were higher compared with day 9 in

the regular intervention. These results further support that regular meal pattern may improve insulin sensitivity and lead to better control of glucose concentrations. There is no study evaluating the effects of meal patterns on glucose in a free-living environment.

The normal-weight participants demonstrated high compliance with the prescribed menus in the regular and irregular meal pattern interventions. The two meal patterns did not show any significant differences in energy intake and food composition. However, earlier studies (Farshchi et al., 2004a, Farshchi et al., 2005), in which food was self-selected, suggest the possibility of overeating as a consequence of consuming meals irregularly. Thus, the differences in glucose that the two interventions exhibited in my study of normal-weight participants occurred without differences in energy intake or macronutrient content potentially confounding the results.

It is recommended for future studies to include a euglycaemic insulin clamp, to explore the pathophysiological mechanisms underlying the inverse association between an irregular meal pattern and the glucose disposal rate.

In regard to the obese study, participants also showed good compliance with the prescribed menus in both interventions with no significant differences in energy intake or food composition between the regular and irregular interventions. However, a main effect of meal pattern was observes in iAUC insulin responses to the test drink with higher responses after the regular than irregular intervention period, in obese study. The regular intervention period had a higher iAUC insulin response compared with the irregular intervention period.

There is some evidence that adiponectin concentrations are lower in obese individuals and are inversely associated with insulin resistance (Weyer et al., 2001, Chandran et al., 2003). Leptin resistance has also been found to be related to the development of insulin resistance in obese and type 2 diabetic patients (Lafontan and Viguerie, 2006). However, no significant differences were found in the adiponectin and leptin concentrations in the obese study.

GLP-1 infusion has been shown to enhance insulin sensitivity study in individuals with type 2 diabetes (Zander et al., 2002). However despite this evidence, iAUC GLP-1 and iAUC insulin responses were higher after the regular intervention than the irregular one in obese insulin resistant females.

A lack of power could partly explain these findings, as the measurements of the insulin concentrations and appetite hormones were a secondary outcome in the studies presented in this thesis.

8.1.3 Lipid profiles

The fasting total and LDL-cholesterol concentrations were not affected by either intervention in the normal-weight and obese studies. In contrast, Farshchi et al. (Farshchi et al., 2004b, Farshchi et al., 2005) reported that the regular meal pattern was associated with lower fasting total and LDL-cholesterol concentrations in normal-weight and obese participants. In these previous studies, the higher energy intake reported during the irregular intervention could potentially explain these findings. However, in my studies, the same type and amount of food was consumed during the regular and irregular intervention periods. Yet, food was self-selected in the previous studies, which may mean the type and amount of food consumed varied between the two interventions.

In my obese study, lower fasting HDL-cholesterol and higher triglyceride concentrations were observed after both regular and irregular interventions compared with before interventions. In contrast, no significant differences were observed in the fasting HDL-cholesterol and triglyceride concentrations in my normal-weight study and the earlier studies in both normal-weight (Farshchi et al., 2004b) and obese participants (Farshchi et al., 2005). These findings suggest that the differences noted between the two studies are likely to be linked with insulin resistance.

An inverse relationship between insulin resistance and HDL-cholesterol concentration has been reported (Laws and Reaven, 1992). It has been also documented that hypertriglyceridemia is one of the metabolic abnormalities associated with insulin resistance (Laws et al., 1989, Reaven, 2005). A decline in HDL-cholesterol concentrations following an ingestion of low-fat/high-CHO regimes might occur in insulin resistant individuals (Reaven, 2005). Moreover, the low-fat/high-carbohydrate regimes can lead to raised hepatic synthesis and secretion of VLDL-triglyceride, consequentially higher fasting triglyceride concentrations (Abbasi et al., 2000, McLaughlin et al., 2000, Reaven, 2005).

Despite both normal-weight and obese participants consuming similar types of food and macronutrient compositions, the obese participants were insulin resistant. The higher carbohydrate and lower fat percentages in the prescribed diet compared with their habitual diet could be the reason behind the reduction in fasting HDL-cholesterol and the increase in triglyceride concentrations following the regular and irregular meal patterns. It would be worth investigating the effects of regular and irregular meal patterns in combination

with high fat/low carbohydrate and/or hypoenergetic diets on dyslipidaemia in obese insulin resistant individuals.

It should be mentioned that both normal-weight and obese participants were instructed to maintain a normal food intake, food diaries of habitual diet before the start of the study. However, obese, but not normal-weight participants, showed a significantly lower mean energy intake than the estimated energy requirement for weight maintenance. It has been documented that the 7-day food diary is a reliable technique for estimating daily energy intake in free-living individuals (Krantzler et al., 1982). However, this method has been shown to underestimate energy intake in obese individuals (Schoeller et al., 1990). Furthermore, there is consistent evidence that obese individuals may selectively under-report snacks (Heitmann and Lissner, 1995, Drummond et al., 1998, Goris et al., 2000)

8.1.4 Appetite regulation

GLP-1 has been shown to act as an appetite suppressant (Naslund et al., 1999). However, in the obese study, fasting subjective appetite ratings did not differ across the study although fasting GLP-1 concentrations increased post-interventions compared with pre-interventions. Likewise, in the normal-weight study, the reduction in fasting GLP-1 concentrations post-interventions compared with pre-interventions had no effects on the fasting subjective appetite ratings. The potential effects of the phases of the menstrual cycle on GLP-1(Brennan et al., 2009) might explain the differences in fasting GLP-1 concentrations that were exhibited in the post-interventions and pre-interventions of the two studies. Another potential explanation may be that in the day prior to the post-intervention visits, 6 meals/ day with the same food

type and composition were consumed, whilst in the day prior to the pre-intervention visits, the food that was self-selected and the meals number and composition were unknown. The 7 day food record would suggest that habitual diet contained a lower percentage of carbohydrate and a higher percentage of fat.

The regular meal pattern followed by obese participants was able to produce a higher iAUC for GLP-1 responses compared with the irregular meal one and no such effects in normal-weight study. However, there were no significant differences in the subjective appetite ratings across the obese study.

There were no significant differences in GLP-1 responses to the test drink between normal-weight and obese participants across the study visits. These findings are in accordance with those of a previous study by (Ahweyevu et al., 2008). Ahweyevu et al. (2008) reported that GLP-1 responses to multiple meals of different energy level were similar between normal-weight and obese participants.

PYY has been shown to decrease appetite and food intake (Batterham et al., 2002). In my normal-weight study, subjective ratings of hunger following the test drink were lower after the regular and irregular interventions than before interventions. These findings could be explained by the higher iAUC for PYY concentrations that were observed after the regular and irregular interventions than before the interventions in this study. Fasting plasma and iAUC PYY concentrations did not differ significantly across the obese study. Similarly, subjective appetite ratings did not show significant differences between the interventions in the obese study. Fasting PYY concentration was found to be lower in obese individuals compared with normal-weight (Batterham et al.,

2003). However, in my studies, both fasting PYY and PYY responses to the test drink appeared not to be different between normal-weight and obese participants,

It has been reported that increased GLP-1 and PYY concentrations are associated with a reduction in subsequent food intake (Hameed et al., 2009). However, these findings were not supported by the studies of normal-weight and obese females presented in this thesis. The study on obese females was not able to show that energy intake at the *ad-libitum* lunchtime test meal was reduced with increasing concentrations of GLP-1 post-regular intervention. Likewise, the higher PYY concentrations that were seen post-interventions had no effect on the energy intake at the *ad-libitum* meal in the normal-weight study. The disparity between these results may reflect an insufficient power for these components in the current studies.

In terms of fasting ghrelin, the associated results did not show significant differences in either normal-weight or obese study. iAUC ghrelin showed also no significant differences, although there was a trend for higher iAUC responses after the regular intervention and lower responses after the irregular intervention in the obese study.

Ghrelin has been shown to be related to subjective hunger ratings in the absence of food cues (Wren et al., 2000, Wren et al., 2001, Blom et al., 2005). Neither the fasting ratings for hunger during the laboratory visits nor the fasting plasma ghrelin concentrations showed any differences in both normal-weight and obese studies. However, in the normal weight study, the responses of hunger ratings (after the test drink) were lower post-intervention visits compared with pre-intervention visits with no differences observed in the

ghrelin response. Several studies observed that ghrelin concentrations were lower in obese compared with normal-weight individuals (McLaughlin et al., 2004, Druce et al., 2005). However, no significant differences in fasting ghrelin and ghrelin responses to the test drink were observed between normal-weight and obese participants.

In free-living participants, the mean of the pre-meals (on day 7 and 14) and post-meals (on day 14) ratings for hunger was lower during the regular meal pattern. These findings were inconsistent with those in the study of obese females in which no differences in appetite ratings were observed between the two interventions neither in the laboratory nor in the free-living environment.

The differences in gut hormone results and subjective appetite ratings between studies of normal-weight and obese females may have occurred due to the differences in participants' characteristics. Further investigations, with adequate sample sizes are warranted to establish the mechanism of the gut hormones and subsequence food intake in response to a regular and an irregular meal pattern.

8.2 Strengths and limitations

Studies presented in this thesis used the same basic protocol of those earlier studies (Farshchi et al., 2004a, Farshchi et al., 2004b, Farshchi et al., 2005). However, improvements on the protocol were made in order to avoid the previous limitations.

One of the main limitations was the food intake in those earlier studies was self-selected and the energy intake was not well controlled. Participants were asked to consume their habitual diet distributed on a certain number of meals per day without instruction on the time of eating. Moreover, energy

expenditure and physical activity level under free living condition were not monitored.

The current protocol was undertaken under conditions that were more controlled than previously. Firstly, in the two intervention periods, identical foods were provided in amounts designed to keep body weight stable. Therefore, the same food in type and amount was consumed in the regular and irregular meal pattern so that the only difference between the two interventions was the pattern. All foods provided were selected to be easy for participants to comply with at home or outside the home.

Secondly, energy expenditure is another component of the energy balance equation, and therefore deserves specific attention. Participants were asked to undertake comparable levels of activity during the two intervention periods. To be sure of full compliance with these instructions, free-living energy expenditure was assessed during the intervention periods using SWA in the improved protocol.

Further information on free-living glucose profiles was also obtained using CGM. *Ad-libitum* energy intake was assessed and further VAS subjective appetite ratings were obtained. Appetite hormones that were determined in the new protocol have provided more reliable and useful data which would support the VAS results.

In common with the previous studies, to reduce the potentially confounding effect of the menstrual cycle on the key outcomes of interest (Solomon et al., 1982, Buffenstein et al., 1995, Davidsen et al., 2007, McNeil and Doucet, 2012), the start of each intervention period was similar with regard to the stage of menstrual cycle for each participant. However this did mean that the second

visit within each arm was undertaken at a different phase in the menstrual cycle hence could potentially explain why a main effect of time is found with some variables.

In order to eliminate an acute effect of the meal frequency on the day immediately preceding the laboratory visit, participants were required to consume 6 meals/day on the day prior to the final laboratory visit on both interventions.

Body weight stability across the studies presented in this thesis suggests that the methods of estimating energy requirement used in the current study were valid in both normal-weight and obese individuals.

Despite the attempt to overcome the limitations in the previous studies, this thesis should be considered within the context of certain limitations. In the normal-weight and obese studies described in this thesis, participants selected were female and the majority of them were younger than 40 years. Hence, the thesis outcomes and conclusions may not be generalizable to males or older individuals.

The relatively small sample size used in the study detailed in Chapter 6 and 7 of this thesis is acknowledged. It is possible that differences smaller than those identified as detectable by the power calculation may have escaped detection for some variables. The studies were powered for TEF as this was the primary outcome measure of interest.

Participants were required to fast overnight and take no exercise other than walking related to carrying out their normal activities of daily living for 48 h before each laboratory visit. Although free-living food intake was not

standardised the day before the pre-intervention laboratory visits, there were no differences in the measurements variables between these two visits.

8.3 Conclusion

Adopting a regular meal pattern may be a potential objective for body weight and appetite control, hence obesity prevention in normal-weight individuals and management for those who are obese, as well as for subsequent morbidity such as cardiovascular disease and type 2 diabetes. A regular meal pattern also seems to improve insulin sensitivity in normal-weight individuals which may indicate a beneficial impact on insulin resistance and the risk reduction of cardiovascular pathology.

These findings suggest that a regular meal pattern appears to be a lifestyle factor that may promote an individual's health. However, further investigations are needed to gain a better understanding of the meal pattern and the variations in macronutrient content that are most effective to reduce dyslipidaemia and hyperinsulinemia especially in individuals with insulin resistance.

Overall, further controlled and longitudinal studies of both sexes that evaluate the impact of promoting the regular meal pattern are extremely desirable to strengthen the evidence that currently exists. These studies should continue to explore the feasibility and the effects of interventions.

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Appendix 1: Healthy Volunteer's Consent Form

University of Nottingham,

School of Biomedical Sciences

David Greenfield Physiology Unit, Queen's Medical Centre

Title of Project:

Name of Investigators:

Healthy Volunteer's Consent Form

Please read this form and sign it once the above named or their designated representative, has explained fully the aims and procedures of the study to you

- I voluntarily agree to take part in this study.
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached.
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.
- I agree to the above investigators contacting my general practitioner [and teaching or university authority if appropriate] to make known my participation in the study where relevant.
- I agree to comply with the reasonable instructions of the supervising investigator and will notify him immediately of any unexpected unusual symptoms or deterioration of health.
- I authorise the investigators to disclose the results of my participation in the study but not my name.
- 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.
- I authorise the investigators to disclose to me any abnormal test results.
- I understand that I can ask for further instructions or explanations at any time.
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.
- I confirm that I have disclosed relevant medical information before the study.
- I shall receive an inconvenience allowance of £…. 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.

- 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 venepuncture >50ml, endoscopy) or exposure to ionising radiation.
- I confirm that I have not been exposed to more than 5 mSv of ionising radiation in the last 12 months.

Name: ……..

Address: …….

Telephone number: ……

Signature: ……. **Date:** …….

I confirm that I have fully explained the purpose of the study and what is involved to:

…….

I have given the above named a copy of this form together with the information sheet.

Investigators Name:…….

Investigators Signature: …….. **Date**: ……

Study Volunteer Number: (……..)

Appendix 2: Participant Information

Screening Visit Date: ____/____/_____ **Subject code**: _____

Personal details

Name _____ Ethnicity _____

Contact details mobile _____ e-mail _____

Date of birth ____/____/19_____ Age _____yrs.

Occupation: _____

How many hours a week do you work out of the home? ____ h

The following sections will be completed by the investigator. Thank you

Health screening

Weight _____kg _____lb.

Height _____cm _____feet 'inches.

BMI _____ kg/m^2

Blood glucose _____mmol/L Date of last period ____/____/_____

Weight stable in the last 3 months? □ Yes □ No

Health status: □ Poor □ OK □ Good □ Excellent

Activity level: _____ BDI value: _____ EAT scores: _____

Dietary analysis (Food diary)

Energy (Kcals) _____ Protein (% total energy) _____

CHO (% total energy) _____ Fat (% total energy) _____

Energy requirements

BMR estimated (Henry equation): _____

PAL from IPAQ information: _____

Estimated daily energy intake requirement BMR * PAL = _____ (Kcals)

Recruited? ☐ **Yes** ☐ **No**

Appendix 3: Medical Screening Questionnaire

Subject code ……

Name: …….. **Date completed:** ……/……/……
Date of birth: ……/……/…… **Age** …….. yrs

Please answer the following questions to the best of your knowledge. If you are unsure about any question, please ask the investigator.
Thank you…

Are you currently taking any medication? □ Yes □ No
If the answer is yes, please give details:
……..
……

Are you currently taking any dietary supplements i.e. multivitamins, cod liver oil, etc?
□ Yes □ No
If the answer is yes, please give details:
……..
……

Have you been diagnosed with any of the following health problems:

Breathing problems i.e Asthma, exercise induced bronchospasm □ Yes □ No
Hay fever □ Yes □ No
Cardiovascular disease i.e. Angina, heart attack, stroke, or hypertension □ Yes □ No
Metabolic disease ie diabetes, hypothyroidism, hypercholesterolemia □ Yes □ No
Epilepsy or nervous disorder □ Yes □ No

Please detail any other medical condition/s
……
……
If you answered '*yes*' to any of the above, please give details:
……
……

Have you suffered from any other medical problem lasting longer than a few days? (eg gastrointestinal problems) □ Yes □ No
If the answer is yes, please give details:
……..
……

Can you confirm that you have regular periods □ Yes □ No
Please tell us the date of your last period :
……..
……

Do you smoke tobacco/cigarettes at the present time? □ Yes □ No

If the answer is yes, please give details:

……..……

..……...

If not, have you ever smoked? □ Never □ Gave up ……. years ago

On average, how many of the following alcohol containing drinks do you consume in a week?

None …….

125ml (Pub measure) glass red wine…….

125ml (Pub measure) glass white wine…….

Bottles of wine (750ml): in addition to any wine intake recorded above…….

Single 'shot' (35ml) of spirits…….

Bottle (330ml) of lager…….

Pint of lager (5%)…….

Half Pint of lager (5%)…….

Pint of Bitter…….

Half Pint of Bitter…….

'Alco-pops'…….

Other …….

Please give details of 'other' drinks:

……..……

..……...

Has your body weight been stable during the last 3 months? □ Yes □ No

If the answer is no, please indicate whether your weight has increased or decreased over the last 3 months and please state the amount (even if only approximately)

…… Kg □ Gain □ Loss

Have you ever dieted in the past in order to lose weight? □ Yes □ No

If yes please give the last time:

……..

……

How much weight did you lose?

……..

……

Do you suffer from any food allergies or intolerance? ie Celiac disease, lactose intolerance. □ Yes □ No

If the answer is yes, please give details:

……..

……

Are you on a medically prescribed diet? ie diabetic, gluten-free, etc. □ Yes □ No

If the answer is yes, please give details:

……..

……

Are you currently, or have you ever followed a high protein diet such as Atkins or South Beach diet? □ Yes □ No

Would you consider yourself a vegetarian? ie eat no meat or fish, but eat dairy products. □ Yes □ No

Would you consider yourself a vegan? ie eat no meat or fish or dairy products. □ Yes □ No

Using the following codes, please assign the number that describes your liking for the foods listed below.

1. Dislike it very much, I wouldn't eat it
2. Mildly dislike this food, but I would eat it
3. Neither like or dislike it
4. Mildly like this food
5. Like this food very much

A vanilla flavoured milk shake type drink ___ A vegetarian pasta meal ___

Appendix 4: Beck Depression Inventory (Beck et al., 1961)

Please read each group of statements carefully and pick out the one statement that best describes the way you have been feeling during the past two weeks, including today. Circle the number besides the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group.

A. Mood

0 I do not feel sad
1 I feel blue or sad
2a I am blue or sad all the time and I can't snap out of it
2b I am so sad or unhappy that it is very painful
3 I am so sad or unhappy that I can't stand it

B. Pessimism

0 I am not particularly pessimistic or discouraged about the future
1a I feel discouraged about the future
2a I feel I have nothing to look forward to
2b I feel that I won't ever get over my troubles
3 I feel that the future is hopeless and that things cannot improve

C. Sense of Failure

0 I do not feel like a failure
1 I feel I have failed more than the average person
2a I feel I have accomplished very little that is worthwhile or that means anything
2b As I look back on my life all I can see is a lot of failures
3 I feel I am a complete failure as a person (parent, husband, wife)

D. Lack of Satisfaction

0 I am not particularly dissatisfied
la I feel bored most of the time
lb I don't enjoy things the way I used to
2 I don't get satisfaction out of anything any more
3 I am dissatisfied with everything

E. Guilty Feeling

0 I don't feel particularly guilty
1 1 feel bad or unworthy a good part of the time
2a I feel quite guilty
2a I feel bad or unworthy practically all the time now
3 I feel as though I am very bad or worthless

F. Sense of Punishment

0 I don't feel I am being punished
1 I have a feeling that something bad may happen to me
2 I feel I am being punished or will be punished
3a I feel I deserve to be punished
3b I want to be punished

G. Self-Hate

0 I don't feel disappointed in myself
1a I am disappointed in myself
1b I don't like myself
2 1 am disgusted with myself
3 I hate myself

H. Self- Accusations

0 I don't feel I am any worse than anybody else
1 I am very critical of myself for weaknesses or mistakes
2a I blame myself for everything that goes wrong
2b I feel I have many bad faults

I. Self- Punitive Wishes

0 I don't have any thoughts of harming myself
1 I have thoughts of harming myself but I would not carry them out
2a I feel I would be better off dead
2b I have definite plans about committing suicide
2c I feel my family would be better off if 1 were dead
3 I would kill myself if I could

J. Crying Spells

0 I don’t cry any more than usual
1 I cry more now than I used to
2 I cry all the time now. I can't stop it
3 I used to be able to cry but now I can't cry at all even though 1 want to

K. Irritability

0 I am no more irritated now than I ever am
1 I get annoyed or irritated more easily than I used to
2 I feel irritated all the time
3 I don't get irritated at all at the things that used to irritate me

L. Social Withdrawal

0 I have not lost interest in other people
1 I am less interested in other people now than I used to be
2 I have lost most of my interest in other people and have little feeling for them
3 I have lost all my interest in other people and don't care about them at all

M. Indecisiveness

0 I make decisions about as well as ever
1 I am less sure of myself now and try to put off making decisions
2 I can't make decisions any more without help
3 I can't make any decisions at all any more

N. Body Image

0 I don't feel I look any worse than I used to
1 I am worried that I am looking old or unattractive
2 I feel that there are permanent changes in my appearance and they make me look unattractive
3 I feel that I am ugly or repulsive looking

O. Work Inhibition

0 I can work about as well as before
1a It takes extra effort to get started at doing something
lb I don't work as well as I used to
2 I have to push myself very hard to do anything
3 I cannot do any work at all

P. Sleep Disturbance

0 I can sleep as well as usual
1 I wake up more tired in the morning than I used to
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
3 I wake up early every day and can't get more than 5 hours sleep

Q. Fatigability

0 I don't get any more tired than usual
1 I get tired more easily than I used to
2 I get tired from doing anything
3 I get too tired to do anything

R. Loss of Appetite

0 My appetite is no worse than usual
1 My appetite is not as good as it used to be
2 My appetite is much worse now
3 I have no appetite at all any more

S. Weight Loss

0 I haven't lost much weight, if any, lately
1 I have lost more than 5 pounds
2 I have lost more than 10 pounds
3 I have lost more than 15 pounds

T. Somatic Preoccupation

0 I am no more concerned about my health than usual
1 I am concerned about aches and pains or upset stomach or constipation or other unpleasant feelings in my body
2 I am so concerned with how I feel or what I feel that it's hard to think of much else
3 I am completely absorbed in what I feel

U. Loss of Libido

0 I have not noticed any recent change in my interest in sex
1 I am less interested in sex than I used to be
2 I am much less interested in sex now
3 I have lost interest in sex completely

Thank you…

All information provided will be kept strictly confidential and used only for the purpose of this study

Appendix 5: Eating Attitudes Test-26 **(Garner et al., 1982)**

Please choose one response by marking a check to the right for each of the following statements:		**Always**	**Very often**	**Often**	**Sometimes**	**Rarely**	**Never**
1	Am terrified about being overweight						
2	Avoid eating when I am hungry						
3	Find myself preoccupied with food						
4	Have gone on eating binges where I feel that I may not be able to stop.						
5	Cut my food into small pieces.						
6	Aware of the calorie content of foods that I eat						
7	Particularly avoid food with a high carbohydrate content (i.e. bread, rice, potatoes, etc.)						
8	Feel that others would prefer if I ate more.						
9	Vomit after I have eaten.						
10	Feel extremely guilty after eating.						
11	Am occupied with a desire to be thinner						
12	Think about burning up calories when I exercise						
13	Other people think that I am too thin.						
14	Am preoccupied with the thought of having fat on my body						
15	Take longer than others to eat my meals						
16	Avoid foods with sugar in them.						
17	Eat diet foods.						
18	Feel that food controls my life						
19	Display self-control around food.						
20	Feel that others pressure me to eat						
21	Give too much time and thought to food						
22	Feel uncomfortable after eating sweets						
23	Engage in dieting behaviour.						
24	Like my stomach to be empty.						
25	Have the impulse to vomit after meals.						
26	Enjoy trying new rich foods.						

Appendix 6: International Physical Activity Questionnaire
(Craig et al., 2003)

Subject code_____

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives.

The questions will ask you about the time you spent being physically active in **the last 7 days**.

Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

VIGOROUS ACTIVITIES

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   □ No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   ____hours per day _____minutes per day □ Don't know/Not sure

MODERATE ACTIVITIES

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do **not** include walking.

   _____ days per week

   □ No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing moderate physical activities on one of those days?

____hours per day _____minutes per day □ Don't know/Not sure

WALKING

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

□ No walking → **Skip to question 7**

6. How much time did you usually spend walking on one of those days?

____hours per day _____minutes per day □ Don't know/Not sure

SITTING

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

_____ hours per day _____ minutes per day □ Don't know/Not sure

Appendix 7: Weighted Food Diary 7 days

University of Nottingham

School of Life Sciences

Weighted Food Diary 7 days

Name: ……..

Date start: ……../……/……

Instruction Sheet for Food Diary

Complete 7 days of food records

- Please keep your normal diet and your usual eating habits.
- Write down the day and date using the format provided below each time you start a new day of recording.
- Start a new page for each day and use as many pages as you need.
- Record the time and location each time you eat or drink.
- Record exactly what you have to eat or drink (each item on a separate line).
- Describe the food and beverages you consume specifically in as much detail as possible. Like cooking method (fried, stewed, grilled etc.), type of food (white or brown bread, full cream or skimmed milk, fatty or lean meat etc.) and any additions (oils, sugar, sauces etc.).
- For combined foods/homemade dishes include a description of ingredients with the amount for the whole recipe, the cooking method, the number of people the recipe serves and how much of the whole recipe you have eaten. Use the recipe section to write this down.
- Write down the brand name if there are any, e.g. Nestle, Hovis, supermarket own brands etc.
- Please keep food labels /wrappers from any manufactured foods that you eat to help us get accurate information about the product.
- Diet records are only reliable with accurate measures.
  - Measure your food in grams (g) and drinks in milliliters (ml) with the food scale and the standard glass provided.
  - For manufactured foods/drinks, quantity can be identified using weights from labels.
  - Give the number of items, e.g. 2 cups of tea, 4 fish fingers 1 slice of bread, 2 eggs, etc.
  - Only record the amount actually eaten.
- Please record your eating as you go, not from memory at the end of the day.
- Carry your food diary wherever you go, and write down as you eat.
- Take your scales with you to weigh food eaten away from home, but if you are unable do this, it will be necessary to estimate the food portion size in household measures (cup, teaspoon, tablespoons etc.)
- Don't forget coffee/tea with cream/sugar, spreads (butter, margarine, jam etc.), gum and sweets, crisps and nuts, beer or other alcoholic drinks.
- After each day of recording you will be prompted to tell us whether this was a typical day and the reasons if it was not.
- Overleaf you can see examples that show you how we would like you to record your food and drink.

Thank you for your time…

Examples

Date 11/09/2012		**Day** Monday		
Time & Meal/ Snack	**Where**	**Food/Drink Description**	**Portion Size**	**Brand Name**
6.30 am	Bedroom	Instant coffee, decaffeinated	200 ml	Nescafe
		Milk (fresh, semi-skimmed)	30 ml	Tesco's own
		Sugar white	30 g	Silverspoon
7.30 am	Kitchen	Toast, white medium sliced	40 g (2 slices)	Hovis
		Light spread	5 g	Flora
		Instant coffee with milk and sugar	As above	As above
		Apple with skin (small)	67 g	-
10.00 am	work	Digestive biscuit-chocolate coated on one side	30 g (2 pieces)	McVities
		Unsweetened orange juice	250 ml (1 cartoon)	tropicana
12.45 pm	work	Soup - minestrone	200 ml	Don't know
		Spaghetti, wholemeal	120 g	Sainsbury's own
		Bolognese sauce (see recipe)	65 g	-
		Courgettes (whole medium), Fried in corn oil.	100 g	-

Examples

Write in recipes or ingredients of made up dishes or take away dishes			
NAME OF DISH Bolognese sauce		**SERVES 4**	
Ingredients	**Amount**	**Ingredients**	**Amount**
Co-op low fat beef mince	500 g	Garlic	3 cloves
Onion	1 medium	Sweet red pepper	1 medium
Napoli chopped tomatoes	400 g tin	Tesco tomato puree	1 tablespoon
Tesco olive oil	1 tablespoon	Mixed herbs	1 dessertspoon
Brief description of cooking method: Fry onion & garlic in oil, add mince and fry till brown. Add pepper, tomatoes & herbs. Simmer for 30 mins.			

Date ____/____/_____		Day _____		
Time & Meal/ Snack	**Where**	**Food/Drink Description**	**Portion Size**	**Brand Name**
Did you finish all the food and drink that you recorded? If no, please go back to your record and make a note of any leftover ……				
Was this a typical day? If not, why? ……....				

Write in recipes or ingredients of made up dishes or take away dishes			
NAME OF DISH ……..		**SERVES ……**	
Ingredients	**Amount**	**Ingredients**	**Amount**
Brief description of cooking method:			

Appendix 8: VAS

Subject code (….) Date ___/___/_____

Visit number (….) Time point (……..)

Please make a vertical mark through the horizontal line to show how you feel at the moment. Left and right extremes represent minimum and maximum values respectively.

	How hungry do you feel?	
I am not hungry at all	_____	I have never been more hungry
	How satisfied do you feel?	
I am completely empty	_____	I cannot eat another bite
	How full do you feel?	
Not at all	_____	Totally full
	How strong is your desire to eat?	
Very weak	_____	Very strong
	How much do you think you can eat?	
Nothing at all	_____	A lot