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INTEGRATING COMPONENTS OF ENERGY INTAKE IN IMPAIRED GLUCOSE TOLERANT AND TYPE 2 DIABETIC POPULATIONS

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A thesis submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

QUEEN MARGARET UNIVERSITY

2008

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ABBREVIATIONS

A	absorbance
ANOVA	analysis of variance
ARC	arcuate nucleus (of the hypothalamus)
ADP	adenosine di-phosphate
ATP	adenosine tri-phosphate
AUC	area under the curve
BBB	blood brain barrier
BMI	body mass index
BMR	basal metabolic rate
BP	blood pressure
BW	body weight
CCK	cholecystokinin
CHO	carbohydrate
cm	centimetre
CNS	central nervous system
CO_2	carbon dioxide
CRP	c-reactive protein
CVD	dardiovascular disease
d	day
DIT	diet induced thermogenesis
EDTA	ethylenediaminetetraacetic acid
EE	energy expenditure
EI	energy intake
FFA	free fatty acids
g	gram
G-6-P	gluconate-6-phosphate
GI	gastrointestinal

GIP	gastric inhibitory polypeptide/glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide 1
GLUT4	glucose transporters
GP	general practitioner
GPASS	General Practitioner Administration System for Scotland
H^+	hydrogen
H_2O	water
HED	high energy density
ht	height
IGT	impaired glucose tolerant
IL-1	interleukin-1
IL-6	interleukin-6
k/cal	kilocalorie
kg	kilogram
l	litre
LCPUFA	long chain polyunsaturated fatty acid
LED	low energy density
LHA	lateral hypothalamic area
LMS	labelled magnitude scale
LPCT	Lothian Primary Care Trust
LUHT	Lothian University Hospital Trust
m	metre
mg	milligram
mins	minutes
ml	millilitre
mm	millimetre
MM	millimolar
mmol/l	millimole per litre
mol	mole

MUFA	monounsaturated fatty acid
N	nitrogen
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide hydrogen
NO_x	nitric oxide
NPRQ	non-protein respiratory quotient
NPY	neuropeptide Y
NSP	non-starch polysaccharide
NTS	nucleus of the tractus solitarius
Nu	urinary nitrogen
O_2	oxygen
PAL	physical activity level
PC	prospective consumption
PROP	6-n-propylthiouracil
PTC	phenythiocarbamide
PUFA	polyunsaturated fatty acid
PVN	paraventricular nucleus
QMU	Queen Margaret University
R&D	research & development
RMR	resting metabolic rate
RQ	respiratory quotient
SEM	standard error of the mean
SFA	saturated fatty acid
TEE	total energy expenditure
TMB	tetramethylbenzidine
TNF	tumor necrosis factor
µl	microlitre
VAS	visual analogue scales
VCO_2	volume of carbon dioxide production
VO_2	volume of oxygen consumption

VMH	ventromedial hypothalamic nucleus
WGH	Western General Hospital
WHO	World Health Organisation
WHR	waist to hip ratio
wt	weight
WTCRF	Wellcome Trust Clinical Research Facility, Edinburgh
yrs	years

DECLARATION

I declare that the work contained in this thesis is original. I have been solely responsible for the organisation and day-to-day running of the study contained herein, as well as all aspects of data collection and the analysis of the results, unless otherwise referenced.

Jill Sommerville

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank those people who have been invaluable to this thesis in terms of their support, encouragement, understanding and guidance over the years.

Firstly, I would like to thank my primary advisor Dr Isobel Davidson for her expert knowledge, inspiration and continued support throughout, I am truly indebted. I would also like to thank all the people who helped me throughout my PhD studies. In particular Dr Casey Stewart and Dr John McKnight who both were an integral part of the process of patient recruitment required for my study and thanks to Dr Ewan Crawford who offered me guidance on patient recruitment and who was the independent advisor to my patients. Thanks to everyone at the Wellcome Trust Clinical Research Facility for the use of their fantastic resources to ensure that participants had a 'pleasurable' experience during the study.

Lastly, I would like to thank my family and friends for the continuous support, patience and motivation given to me during my studies.

ABSTRACT

Objective   During feeding there is an integrated 'whole body' response which endeavours to maintain energy homeostasis. The integrated response consists of sensory, postingestive, postabsorptive and cognitive feedback which exerts control over ingestive behaviour. It is accepted that when an imbalance in this integrated response occurs and may promote an increased fat mass and ultimately can lead to obesity which is known to play an important role in the development of IGT and type 2 diabetes. This study investigated the integrated responses of a test meal to determine any differences between IGT, type 2 diabetics and controls in their integrated response mechanisms. This knowledge may be important in both predicting the onset of these diseases and in the treatment of them.

Research Design and Methods IGT and type 2 diabetics with a BMI greater than 30 and were recruited together with a group of healthy controls. The study assessed habitual energy intakes and energy expenditure in all groups. All participants' height, weight, BMI and WHR were measured. A taste test assessed the sensory component of food intake. The metabolic response and parallel changes in appetite to the meal were recorded at baseline and at 15, 30, 60, 90 and 120 minutes.

Results   Control participants had significantly lower weight ($p<0.01$), BMI ($p<0.01$), waist ($p<0.01$) and hip ($p<0.01$) measurements compared to IGT and the type 2 diabetic groups. Habitual diet diaries indicated a lower sugar intake in the type 2 diabetic group compared with IGT and control groups. Percentage protein intake was significantly lower in control participants (14.4%, $p<0.05$) compared to IGT (17.2%) and type 2 diabetics (18.5%).   Activity diaries highlighted an indication of increased strenuous/physical activity in the control participants compared to IGT participants however, this was not statistically significant. The control group showed greater sensitivity to PROP followed by type 2 diabetics and then IGT participants ($p<0.05$). Throughout the study the control participants rated themselves the most hungry compared to IGT ($p<0.05$) and type 2 diabetics ($p<0.01$) respectively and controls were also the least satiated ($p<0.05$). There was no difference in fullness ratings. Control participants rated prospective consumption the highest compared to IGT and then type 2 diabetics ($p<0.05$) respectively. The differences in EE measured by calorimetry when normalised for body weight indicated that IGT ($p<0.01$) and type 2 diabetic participants ($p<0.01$) had significantly lower EE than control participants. CHO oxidation rates were significantly lower in IGT and type 2 diabetics ($p<0.05$). Investigating the blood parameters showed no differences in plasma ghrelin responses, that IGT participants had the highest overall plasma glucose ($p<0.01$) and insulin ($p<0.05$) responses.

Conclusions   It is clear that there are subtle differences in the pathways of energy balance in IGT and type 2 diabetics compared to controls; including sensitivity to taste, subjective feelings of appetite, EE, oxidation rates and differing blood parameters. Taste appears to be an important contributor to the sensory control of food intake and is associated with an increased sugar intake. Furthermore, differences between IGT and type 2 diabetics demonstrate that the degree of management of the disease can influence the effectiveness of the metabolic pathways controlling food intake. It is not clear which component is the most influential in the control of food intake and it is likely that the synergistic effects are what potentiate the diseases and make them difficult to combat.

CHAPTER 1 - INTRODUCTION

1.1   FOOD INTAKE AND ENERGY BALANCE

Food or energy intake is regulated by the balance of physiological mechanisms responsible for promoting and inhibiting intake. This process of energy intake is dictated by energy requirements i.e. metabolism and physical activity which regulates energy expenditure. Energy balance is thus achieved when energy intake equals energy expenditure. Energy intake, a result of feeding behaviour, is regulated by the constant circulation of neural and hormonal (neuro-endocrine) signals and the changes in signalling as a consequence of intake. Such signals are received decoded and acted on by appropriate tissues resulting in energy homeostasis. The physiological systems involved include gastrointestinal, cardiovascular, brain, and musculoskeletal and are responsible for the maintenance of this complex underplay of communication. Such co-ordination should ensure an appropriate supply of nutrients and the regulation of the episodic pattern of eating.

The motivation to feed is crucial in the homeostatic control of feeding behaviour and is regulated centrally by two key areas, the brain stem and the arcuate nucleus (ARC) of the hypothalamus (Park and Bloom 2005). The ARC receives input from the periphery; including incretins and hormones released during and after feeding, such as leptin, ghrelin, cholecystokinin (CCK), neuropeptide Y (NPY) and insulin; and other parts of the brain. Within the ARC there are two interconnecting collections of neurons, one having a positive influence and one having a negative influence, which

project to the paraventricular nucleus (PVN) where the regulation of food intake and energy expenditure is controlled. The homeostatic control of feeding behaviour should be sensitive enough to ensure that energy intake meets expenditure and maintains energy balance. In disease states where neuro-endocrine regulation is disrupted then energy homeostasis fails. This includes disease states such as IGT and type 2 diabetes. In a similar manner to positive and negative balance of inputs maintaining energy homeostasis Blundell et al (2001) described appetite as a balance between excitatory and inhibitory processes. The excitatory processes being 'hunger' which arises from bodily energy needs and the inhibitory process being 'fullness' which arises from postingestive physiological processing of foods consumed and results in satiety; the process in which further food intake is inhibited. Both excitatory and inhibitory processes can be modulated by neuro-endocrine signals arising from the body's energy stores. The need to seek food arises by metabolic processing and is given direction through specific sensory systems, such as smell and taste and is driven by metabolic demand (Freidman 1995).

Current trends indicate that control of energy intake and levels of energy expenditure can significantly diverge so that chronic high levels of imbalance between these mechanisms can occur; which is either recognised as a result of low physical activity levels; increased energy intake; or a combination of the two (McGough 2001). The resultant energy imbalance indicates that dysfunction of mechanisms that dictate food/energy intake are not tightly coupled with energy expenditure and therefore promote expansion of fat mass leading to overweight and obesity (Stubbs 1998). Increased fat mass and adipose tissue, in recent years, has been implicated in promoting an inflammatory response (Chandalia and Abate 2007). The process of

increased fat mass results in decreasing concentrations of long chain polyunsaturated fatty acids (LCPUFA) and has a negative feedback control on inflammation (Das 2007). Under normal conditions LCPUFA help to promote the mechanisms responsible for regulating food intake by potentiating insulin action and shifting leptin action. When the actions of insulin and leptin are dysfunctional they contribute to the development of metabolic syndrome by promoting the inflammation process. Markers of inflammation are elevated in subjects with obesity, insulin resistance, type 2 diabetes and IGT intimating that metabolic syndrome is a low-grade inflammatory condition (Das 2007). Food intake is such a key element in the prevention and treatment of IGT and type 2 diabetes because, as discussed earlier, an imbalance can lead to obesity therefore, it is important to understand how the inflammatory process is influenced by poor dietary choices and chronic overeating. Research therefore centers on the energy intake side of the energy balance equation (energy balance = energy intake (EI) − energy expenditure (EE)) because of the many factors/mechanisms which influence food intake. However, a significant point to remember is that exercise is anti-inflammatory in nature suggesting that symptoms of the metabolic syndrome can be counteracted.

1.2   THE INFLUENCE OF TASTE IN THE CONTROL OF FOOD INTAKE

One of the key mechanisms responsible for the promotion of energy intake and encouraging food ingestion is taste. Promotion of food intake occurs due to the palatability of food (Yeomans et al. 2005) and the sensitivity of the mouth in relaying the attributes of the palatable food. Palatability increases appetite and therefore food consumption, whereas satiety limits consumption by reducing meal size or by

delaying the time of the next meal (Drewnowski 1998). Measures of palatability include the perceived pleasantness of a given food, intent to eat, and the amount of food consumed (Drewnowski 1997b). Finally, sensory-specific satiety was specifically defined as reduced palatability of the just-consumed food relative to other foods (Rolls 1985). Due to the opposing effects of palatability and satiety, the most palatable foods are by definition the least satiating, and vice versa. Therefore, taste can either increase food intake or decrease it depending on the palatability of the food type. Furthermore, the palatability of one food being consumed can decrease while simultaneously the palatability of another food increases. For example, Snoek et al (2004) reported that while eating sausages, the liking for sausages strongly declined at the same time that the liking for other savoury foods declined to a lesser degree, and the liking for sweet foods increased. The decrease in liking of a particular food is also associated with reduced intake of that particular food in a subsequent meal (Snoek et al. 2004). Some researchers have argued that the palatability of sugar and fat overrides normal satiety signals, thereby leading to overconsumption and weight gain (Green and Blundell 1996). Furthermore, Drewnowski and Levine (2003) found that a continued high fat diet affects the circuitry in the brain involving appetite, reward (opiate) and energy metabolism. Sensory specific satiety is said to occur straight after consumption of that food, therefore before any opportunity for digestion and absorption of the food and so it is specific to the sensory aspects of the food i.e. texture, colour and flavour (Rolls 1997). The manipulations that occur in feeding behaviour as a result of palatability, sensory specific satiety and taste may also affect energy expenditure by negatively changing the efficiency of energy utilisation.

Therefore taste is a fundamental influencing component of food intake. The mechanisms regulating taste suggest physiological, psychological and also genetic factors in the homeostatic control of food intake. Taste sensations serve as an indicator of a food's nutritional value and are important in the development of food preferences as well as driving appetite. Human and animal experiments show that there is a learned association of a food's taste with its postingestive effects including salivary, gastric, pancreatic, and intestinal secretions (Booth 1982; Warwick et al. 1990). These associations permit modulations of food choice and/or meal size, and will therefore initiate fullness, in anticipation of these postingestive effects. Therefore, it is logical to propose that eating behaviour will be directed towards foods which have obvious energy value i.e. sweet or fatty foods (Blundell 1991; Schiffman 1998) which elevate opiate levels, giving a pleasurable effect. In general most humans possess a strong liking for the sweet taste of foods and for fatty texture. The term taste involves many sensory experiences including true taste i.e. the perception the primary tastes of salt, sweet, sour and bitter, retronasal olfaction i.e. the perception of olfactory stimuli from within the oral cavity, and oral somato-sensation which refers to the perception of touch, temperature and pain. The ability to taste sweetness and bitterness varies. The variability is thought to be associated with the density of fungiform papillae on the tongue (Drewnowski 1997). Furthermore, taste can also be influenced by disease states such as exposure to pathogens; trauma and infection; malnutrition; medication; surgical interventions and aging. For example the chorda tympani nerve functions in taste sensation and innervates taste buds of the tongue, and in turn interacts with signals associated with ingestive and digestive activity in the processing area within the dorsal medulla (Bartoshuk 2000). Damage to this nerve may cause poor taste sensitivity on the anterior tongue and may also affect oral

somato-sensations due to inhibited nerve action (Dinehart et al. 2006). Poor taste sensitivity can result in altered food choices.

1.2.1 The Relationship between Taste and Age

It is known that the CNS has a minute to minute dependence on nutrient supply and so can profoundly affect dietary intake. Current theories describe functions of brain receptors for cholecystokinins, opioid-like endorphins, and serotonin that appear to influence eating behaviour and satiety (Selhub et al. 2000). So altered eating behaviour and/or declining appetite, due to taste, may lead to nutritional deficiencies. Epidemiological evidence linking low vitamin status or intake with a decline in neurocognitive function in the elderly was first described by Goodwin et al (1983). These authors showed that healthy elderly subjects who had low blood concentrations or intakes of folate, vitamin B-12, vitamin C, and riboflavin scored poorly on tests of memory and nonverbal abstract thinking. However, the importance of these observations with respect to declining appetite in the elderly is uncertain. Sensory impairments may be associated with decreased appetite in older individuals including, declining olfactory function which may impair flavour perception and lead to a decreased appetite or changes in food choices. Rolls and McDermott (1991) studied the effects of age on sensory-specific satiety in older adults (45-60 years) and the elderly (65-80 years). Rolls hypothesised that sensory specific satiety is attenuated in the elderly which causes them to consume a more mundane diet and therefore to eat less which the hypothesised that the elderly compensated for by adding flavour to their food. Both Fanelli and Stevenhagen (1985) and Brown (1976) found that dietary variety was found to decline in the oldest participants (>65). However, Rolls found

no decline in sensory specific satiety in the older and elderly participants which suggests that the mechanisms underlying taste are not well understood. The relationship between taste and age needs further investigation. However, analysing the literature surrounding the relationship between taste and age gave definition to the age range identifiable for the current study.

1.2.2   The Genetic Sensitivity of Taste

Poor sensitivity to specific taste was identified as 'Taste Blindness' by Fox (1931) who later went on to record the taste qualities of phenythiocarbamide (PTC), a bitter compound with the N-C=S group. Further studies led to the conclusion by Blakeslee & Salmon, (1931) that PTC non-tasting is a Mendelian recessive characteristic i.e. individuals with two recessive alleles (tt) are non tasters and individuals with 1 dominant allele (Tt or tT) and two dominant alleles (TT) are tasters. More recent studies (Reed et al. 1999) suggested that taster status may also influence the phenotype of a person. When an individual has the nontaster phenotype, and therefore a lack of ability to taste, it is necessary to understand if and how this alters food intake and also, importantly how the satiety cascade is affected because this may alter the process of meal cessation. Furthermore, if an individual has a lack of taste which results in a lack of satiety does this lead to a tendency to overeat?

Overall, taste appears to be moderately heritable. However, the genetic and environmental factors that, together, add up to create the human taste phenotype make it difficult to assess heritability. The most common studies are genotype-phenotype correlations for taste-related genes and behaviour. On one hand, the behavioural

assays for sensitivity have been in widespread use since the early 1930's however, results are sensitive to method as shown by Bartoshuk (2000) and the methods used to assess bitter sensitivity vary considerably. The earliest studies on the genetics of taste perception used PTC crystals (Snyder 1931; Blakeslee 1931). Their use was later followed by PTC-impregnated filter paper and PTC solutions in water (Blakeslee and Salmon 1935). In the 1960's, Fischer et al (1963) was the first to substitute 6-n-propylthiouracil (PROP) for PTC because PROP was odorless and less toxic. Three criteria have been considered in the choice of PTC and PROP, the question of odor cues, the degree of separation of nontasters and tasters and the toxicities of the compounds (Lawless 1980). PROP was found to be both odorless and less toxic and although PCT was found to be a better discriminator than PROP concern over the toxicity of PCT has led to increased use of PROP in recent years (Lawless 1980). Taste thresholds are now determined by using a modified up-and-down procedure that involves forced-choice judgments and successive serial dilutions of the bitter compounds. The Prop series traditionally started with solution 14, containing 1.021 g Prop/L (corresponding to 0.006 mol/L), with progressive dilutions down to solution 1 (Kalmus 1971). Current methods call for 15 Prop solutions ranging in concentration from 0.000001 to 0.0032 mol/L that increase in one-quarter log increments on the molar scale (Gent and Bartoshuk 1983). Lawless (1980) also investigated different methodologies (paper tests, forced choice thresholds, recognition thresholds and category ratings) and found that there were high rates of misclassification with paper tests and reported that threshold tests were accurate to a quarter log step and found no difference between ascending and descending threshold tests. New methods are still being developed using PROP, including new methods using PROP filter paper (Zhao 2003) and some methods simply involve tasting a solution and rating the degree of

liking or disliking and the intensity on labeled magnitude scales (Keskitalo et al. 2007). This method is particularly used for children. In adults, threshold tests are the methodology of choice when assessing the detection thresholds of the bitter compound PROP. The current study utilised threshold tests using the up-down procedure. McBurney and Collings (1977) introduced the up-down procedure with forced choice into modern taste psychophysics. Where subjects are given two stimuli (water and a given concentration of the tastant) and asked to choose the one with a taste where the concentration is either increased or decreased after non-detection or detection respectively. The reversal points determine the threshold and a run is ended after two correct choices. A recent study suggests that three correct choices gives adequate reliability of the threshold (Marks and Wheeler 1998) without risking subject fatigue. Therefore, in the current study this up-down method was used with reliability that the correct number of choices to detect a subject's true threshold. If this procedure is used with only one correct choice and the procedure is begun below the subject's true threshold, the 'threshold' that results can be much lower than the subject's true threshold (Bartoshuk 2000).

Fisher et al (1963) considered the behavioural implications of the genetic variation in taste and found associations between PROP tasting and drug sensitivities, personality type, smoking habits and, vitally, food preferences. So far, studies investigating taste genetics have only considered bitterness however other studies have shown bitter and sweet pathways to be analogous (Drewnowski 1997b). Increased sensitivity to PROP was initially linked to the enhanced perception of sweet taste and increased rejection of bitter PROP solutions (Drewnowski 1997b). Increased sensitivity has also been hypothesised to cause a reduction in food intake associated with the perception of

sweet and fatty foods and therefore poor sensitivity has been linked with increased food intake (Drewnowski 1998). As this body of evidence has increased, Bartoshuk (1991; Bartoshuk 2000; Bartoshuk et al. 1994) detected differences between taste perceptions in the tasters of PROP; those who perceived PROP (0.0032M concentration) as moderately bitter as medium tasters and those who perceived PROP as extremely bitter as supertasters (Bartoshuk 1991). From then onward three bitterness taster groups; non-tasters, tasters, and super-tasters were referred to (Bartoshuk 1991).

As discussed earlier the N-C=S group which categorises bitter compounds are also associated with containing phytochemicals or phytonutrients (Drewnowski et al. 1997a). These phytochemicals are plant-based phenols that are present in the everyday diet and are reported to have antioxidant and anticarcinogenic properties. Duffy (2004) studied the variation in oral sensations, dietary behaviours and cardiovascular (CVD) risks. The ability to taste PROP has been linked with the rejection of other bitter compounds and with reduced acceptance of some bitter foods. The most frequently cited example of bitter food rejection is the reported avoidance by PTC tasters of bitter compounds found in raw cruciferous vegetables: cabbage, broccoli and brussel sprouts (Niewind et al. 1988). Further studies show PROP tasters have reduced preferences for grapefruit juice, Japanese green tea, and selected soy products (Akella et al. 1997; Kaminski et al. 2000). Bitter foods contribute a major dietary source of bioactive phytochemicals, some of which are thought to reduce the risk of cancer and coronary heart disease. This leads to the hypothesis that tasters and super-tasters of PROP may have reduced dietary exposure to the bitter but beneficial phytonutrients that are found in fruit and vegetables (Drewnowski and

Gomez-Carneros 2000). As discussed earlier other differences between tasters and non-tasters have also been described. Some concentrations of sucrose, neohesperidin dihydrochalcone and saccharin tasted sweeter to tasters than to non-tasters (Gent and Bartoshuk 1983) while Looy and Weingarten (1992) found that tasters were more apt to dislike the taste of sweet solutions. However, Schiffman et al (1985) found no difference in the sensory evaluations of soft drinks whatever the sweetener between tasters and non-tasters so results have been conflicting. Finally, studies with PROP sensitivity focused on adiposity and a study found that greater PROP bitterness was associated with lower fat preference and lower body mass indices in non-obese individuals (Hutchins et al. 2002). Therefore, Duffy (2004) hypothesised that the variability in taste may explain the differences in preference for foods/beverages and dietary intake and ultimately chronic diseases such as obesity, IGT and type 2 diabetes which are all known risk factors of CVD. It is unknown, however if the PROP effects on adiposity, in the obese, are similar to that of non-obese individuals or if they are overshadowed by the multiple causes of obesity, including dysfunctional physiological and metabolic control mechanisms or environmental influences.

The question, of course then arises about the relationship between PROP tasting and body mass index (BMI, kg/m²). The association between PROP tasting and body weight remains unproven however, some researchers have suggested that PROP tasting may protect against obesity (Tepper and Nurse 1997; Tepper 1998). Duffy et al (1999) found that reduced PROP sensitivity, i.e. lack of taste, resulted in an increased BMI in normal weight persons. In contrast, in overweight and obese individuals an increased sensitivity correlated with an increased BMI. Tepper (1998) found that normal weight non-tasters could not distinguish the fat content of dressings

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(high and low fat) and liked the high-fat dressing more than the low-fat dressing, whereas the two taster groups gave higher ratings of fat-content to the high fat dressing and liked both samples equally. The observed response to fat content may be analogous to the dislike of intensely sweet stimuli by PROP tasters in the sucrose studies by Looy and Weingarten (1992). In general, findings provide indirect evidence that the dietary patterns of PROP tasters may have important implications for weight status, but study findings have been varied therefore require further confirmation. Bartoshuk (2000) investigated the associations between PROP tasting and the liking/disliking for sweet/high fat foods and found a negataive association that was strongest in women. Nevertheless, the association found in women support the hypothesis that supertaster females will eat less high sweet/fat foods compared to a nontaster, due to their dislike of those food types. Long-term this may prevent supertasters from gaining weight. Thus, sensitivity to taste dictates dietary intake. Tepper and Ullrich (1999) found both female and male supertasters were thinner in their sample where restrained eaters were excluded. The evidence discussed suggests that individuals who cannot taste well cannot make food selections based on prior learned associations between taste quality and the metabolic consequences of that food. That is, inappropriate food selections may be made that compromise general health. Therefore it could be suggested that the lack of sensitivity to the fat content of foods may be what leads to positive energy balance. Furthermore, the continual intake of fatty foods will promote a positive energy balance and weight gain. The high energy density and palatability of sweet and fatty foods has been associated with higher energy intake (Drewnowski and Specter 2004). Recent studies have shown that highly palatable, energy dense foods have been associated with reduced satiation and satiety and passive overconsumption of fats and sweets (Drewnowski and Specter

2004). Taste status is therefore important in the integrated control of food intake and energy balance.

1.3 MECHANISMS OF SATIETY

Following ingestion of food, neural and endocrine mechanisms are responsible for the regulation of feeding behaviour. Gastrointestinal chemo-receptors respond to the nutrient products of digestion (sugars, fatty acids and amino acids). In addition, stretch and mechanoreceptors are activated by the presence of food in the stomach and proximal small intestine. Signals from gastrointestinal receptors (mechano and chemo-receptors) are transmitted via vagal afferent nerves to the medulla and hind brain where integration of this visceral input occurs so that the physical and chemical properties of food can affect the short-term regulation of food intake i.e appetite.

Signals arise from every part of the feeding sequence involving location, selection, ingestion, digestion, and the absorption of nutrients, see figure 1.1. These signals can be localised to sensory inputs from the nose and mouth, gastrointestinal signals, circulating factors, metabolic signals, nutrient stores and the postabsorptive sensory capabilities of the liver and the nervous system (Blundell 1992; Stubbs 1999). These subsystems functionally interact to produce a feeding sequence, initiated by hunger and ending with satiety. Hunger can therefore be described as the motivation to seek and consume food which initiates a period of feeding behaviour (Blundell and Halford 2000). Appetite is independent of hunger as it can be considered in the short term as the physiological processes which together with psychological and environmental factors determine the day-to-day episodic pattern of meal and snack consumption, and

in the long term be considered a homeostatic process that maintains the energy balance and ensures energy storage (Blundell and Halford 2000). Satiation is the within-meal process that brings the period of eating to an end, thereby determining meal size whereas satiety is the process in which further food intake is inhibited and therefore determines the time between meals (Blundell and Halford 2000).

As discussed earlier, the sensory properties of sugar and fat make them difficult to resist. Fat content appears to influence palatability, and thus food choices, and consequently affects food consumption (Drewnowski and Rock 1995). It is for this reason that fat is seen to be a weak indicator of satiety. The effects of different nutrients on the capacity to satiate has undergone much investigation and the results have varied. Some studies suggest that CHO is more satiating than fat (Lawton et al. 1998) and others have indicated that fat and CHO are equally satiating, in that changes in the energy content of foods associated with the modification of either the fat or CHO content are followed by accurate energy compensation (Rolls et al. 1991). More recent studies have established that protein is the most satiating nutrient (Paddon-Jones et al. 2008). In substituting other macronutrients for protein, it may have a role in the facilitation of weight loss by promoting satiety and causing a reduction in energy consumption. However, it is often assumed that the initiation of feeding occurs following hunger but meal initiation does not only depend on internal cues but also environmental cues, such as time of the day, social events and food cues, all of which can trigger an eating episode (see figure 1.1).
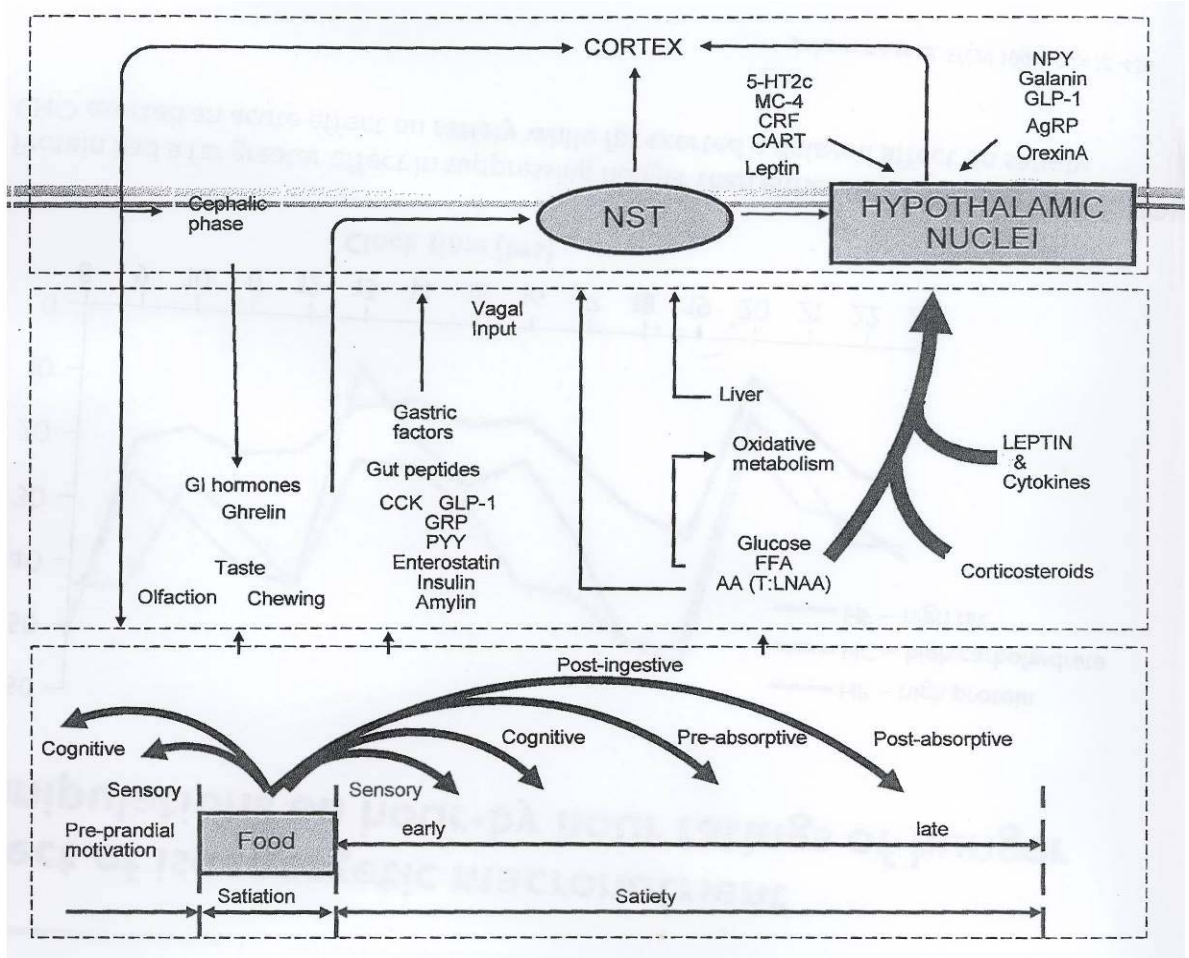
Figure 1.1 The Satiety Cascade. Conceptualisation of the contributions of the cognitive and behavioural events, physiological and metabolic events and the brain interactions to the time course of satiety (Blundell et al. 2005). PVN = paraventricular nucleus; NST = nucleus of the tractus solitarius; CCK = cholecystokinin; FFA = free fatty acids; T:LNAA = tryptophan: large neutral amino acids. **These are the levels of psychological events (hunger perception, cravings, hedonic sensations) and behavioural operations (meals, snacks, energy and macronutrient intakes); the level of peripheral physiology and metabolic events; and the level of neurotransmitter and metabolic interactions in the brain. Appetite reflects the synchronous operation of events and processes in the three levels. Neural events trigger and guide behaviour, but each act of behaviour involves a response in the peripheral physiological system; in turn, these physiological events are translated into brain neurochemical activity. This brain activity represents the strength of motivation to eat and the willingness to refrain from feeding. The lower part of the psychobiological system illustrates the appetite cascade which prompts us to consider the events which stimulate eating and which motivate organisms to seek food, those behavioural actions which actually form the topography of eating, and those processes which follow the termination of eating and which are referred to as postingestive events. Even before food touches the mouth, physiological signals are generated by the sight and smell of food. These events constitute the cephalic phase of appetite. Cephalic-phase responses are generated in many parts of the gastrointestinal tract; their function is to anticipate the ingestion of food. During and immediately after eating, afferent information provides the major control over appetite. It has been noted that afferent information from ingested food acting in the mouth provides primarily positive feedback for eating; that from the stomach and small intestine is primarily negative feedback.**

1.3.1   Gastric Emptying and the effect on Satiety

An important internal mechanism which can influence satiety is gastric emptying. Gastric emptying is the limiting step between meal ingestion and the metabolic uptake of nutrients at the cellular level. As such, its role in the control of eating behaviour has been emphasised in health and disease (Muurahainen et al. 1988). Studies have shown that patients with anorexia nervosa have markedly delayed gastric emptying (Duhois et al. 1979) and obese patients have accelerated emptying (Johansson et al. 1976) also support the existence of such a relationship and emphasise its potential involvement in clinical disorders such as IGT and type 2 diabetes. Emptying of the stomach slows down as its energy content increases (Hunt 1980), and this process might prolong the duration of postprandial satiety, and thus help to regulate energy intake. Gastric emptying has therefore been utilised as an internal physiological biomarker of satiety in research.

Starchy foods are known to elicit different postprandial blood responses of glucose and insulin. Pasta, legumes, and products based on whole-cereal grains are slowly digested foods, whereas potatoes, most breakfast cereals, and conventional bread products elicit high metabolic responses. The glycaemic index (GI) was introduced to classify starchy foods according to their effect on postprandial glycaemia (Jenkins et al. 1981). The GI is defined as the incremental area under the curve (AUC) for blood glucose after ingestion of a test product as a percentage of the corresponding area for a reference product (glucose or white bread). An insulinemic index can be calculated from the corresponding incremental insulin AUCs. In a study of different bread products, postprandial glucose and insulin responses were lower after ingestion of

sourdough bread than after the ingestion of a corresponding yeast bread (Liljeberg 1995). In the same study, it was found that a bread with added sodium propionate also lowered blood glucose and insulin concentrations, despite its neutral pH. In a study by Liljeberg and Björck (1996), the influence of sodium propionate on the gastric emptying rate (GER) was studied by using paracetamol as a marker. As judged from a lowered paracetamol concentration in the blood after ingestion of bread with added sodium propionate, it was concluded that the salt reduced the GER (Liljeberg and Björck 1996). Thus, it was suggested that the bread with added sodium propionate was the mechanism for the lowered metabolic response because there was no evidence of any effects on the rate of starch hydrolysis in similar in vitro experiments. It is known that GERs are influenced by the volume, energy content, and density of the meal and by the particle size of the gastric contents (Horowitz et al. 1994); however, other meal factors are likely to influence the luminal receptors of the small intestine, which control gastric emptying. In the same study by Liljeberg and Björck (1996) satiety was reported to have lasted longer after ingestion of bread with added sodium propionate than after ingestion of the reference bread without sodium propionate. Prolonged post meal satiety usually occurs concomitant with low GERs because the extension of the stomach is one factor that promotes a feeling of satiety.

Along with the influence of differing food items GER is influenced by a number of peripheral biomarkers such as cholecystokinin (CCK), peptide YY (PYY) and glucagon like peptide-1 (GLP-1). The inhibitory effect of GLP-1 on gastric emptying has been reported in both normal subjects (Nauck et al. 1997) and in individuals with diabetes (Gutniak et al. 1996). It has been suggested that obese individuals have attenuated GLP-1 response to meals. Naslund et al (1998) infused obese men with

either saline or GLP-1 to determine the effects on gastric emptying. The results of the study showed decreased rates of gastric emptying and hypothesised that the increased concentration of GLP-1 caused a prolonged effect on mechano and chemo-receptors in the stomach and small intestine, which relay important information to the CNS, via the vagus nerve. Therefore, GLP-1 administered to obese subjects may have a weight-reducing and slimming effect if given over a prolonged period of time. In summary, this study showed that GLP-1 administered to obese subjects induced a prolonged period of postprandial satiety and slower rate of gastric emptying than did saline infusion. This effect of GLP-1 on hunger may be mediated through central mechanisms or vagal afferent pathways together with prolonged gastric emptying. Additional gastrointestinal hormones, particularly CCK (Muurahainen et al. 1988) and peptide YY (PYY) (Lin et al. 1996) are also important in mediating the slowing of gastric emptying induced by small-intestinal nutrients. Previous studies showed that CCK (Muurahainen et al. 1988), and PYY (Allen 1984) slow gastric emptying: CCK (Fraser 1993) by stimulating isolated pyloric pressure wave (IPPWs) frequency and inhibiting distal gastric (antral) contractions and PYY by inhibiting interdigestive migrating contractions in the small intestine (Lin et al. 1996). In summary, the influence of gastric emptying on satiety has been established and therefore food is initially the most crucial component that influences the physiological mechanisms that indirectly control satiety.

1.4   THE AUTONOMIC CONTROL OF FEEDING BEHAVIOUR

The presence of food further stimulates inhibition of eating by maintaining the physiological mechanisms mediating satiety. In order to achieve energy homeostasis

it is necessary to regulate the energy intake with energy expenditure to ensure that energy stores are maintained at an appropriate level. Energy balance is achieved through the integration of periphery signals when feeding commences. The signals involved which influence feeding behaviour are located in multiple brain areas. The hypothalamus plays a key role in this process. Meal-related satiety information is conveyed to the nucleus of the tractus solitarius (NTS) in the medulla, on which converge vagally transmitted signals from the gastro-intestinal tract, including taste, gastric distension and portal vein glucose levels. For example, high blood glucose, i.e. after feeding, facilitates efferent activity of the pancreatic and hepatic branch of the vagus nerve which is mediated by the intestinal peptide cholecystokinin (CCK) (Beglinger and Degen 2004). CCK signals to the NTS via CCK_A receptors on the sensory terminal of the vagus nerve and in turn causes an increase in insulin secretion from the pancreas and glycogen synthesis in the liver and ultimately results in satiation, the cessation of feeding (Fry and Ferguson 2007). Endocrine pathways have been identified which activate the afferent fibres of the vagus nerve, i.e. in the action of ghrelin or influence hypothalamic activity directly, i.e. in the action of leptin. The ventromedial hypothalamic nucleus (VMH) was long considered to be a 'satiety centre'. Stimulation of the VMH inhibits feeding (Williams et al. 2001). Recent studies have shown a high abundance of leptin receptors in neurons of the VMH. The VMH has direct connections with paraventricular neurons and the lateral hypothalamic area (LHA), where the regulation of food intake and energy expenditure is controlled. Low blood glucose (hypoglycaemia) activates efferent activity of the pancreatic, hepatic and adrenal branches of the splanchnic nerve, which results in increased glucagon secretion from the pancreas, release of glucose from the liver and secretion of catecholamines from the adrenal medulla and is the stimulation required

for feeding initiation (Woods and Porte 1974). This process is mediated by neuropeptide Y (NPY), an orexin, which induces feeding. NPY containing neurons originate in the ARC of the hypothalamus and innervate the LHA which has been classically viewed as the 'feeding centre'. Stimulation of the LHA increases food intake and also contains large numbers of glucose-receptive neurons that respond to circulating glucose levels, probably mainly via pathways ascending from the hypothalamus.

Mayer (1953) formulated the 'glucostatic' theory that glucose availability to specific glucose-sensing neurons is an important factor regulating feeding behaviour and ultimately body weight. Glucose is the main metabolic fuel of the brain. Reductions in blood glucose or any blockade of neuronal glucose utilisation powerfully stimulate feeding. Specific parts of the central nervous system (CNS) contain neurons that can detect changes in ambient glucose concentration, for example glucose responsive neurons in the LHA and medulla oblongata (Williams et al. 2001). However, their place in the hierarchy of the CNS system that regulates feeding is uncertain, although they may have some involvement in the modulation of the autonomic efferent activity. It is becoming clear that glucose-sensing neurons may communicate extensively with other appetite-regulating neuronal systems. This suggests that blood glucose and the activity of peripheral glucose sensors directly and indirectly affect feeding behaviour.

There are other peripheral metabolic sensing neurons in the regulation of energy homeostasis and include neural afferents from the hepatic portal vein, gut, and carotid sinus. The messages from these peripheral areas are all conveyed via the vagus and

sympathetic nervous system to the NTS in the medulla and are then relayed to a variety of other brainstem and hypothalamic areas. These are joined by neural inputs from somatic afferents mediating sight, sound, taste, pain and touch and these incoming signals are integrated within clusters of neurons which are also responsive to metabolic and hormonal inputs from the periphery. Therefore the CNS has an important role in the control of food intake.

1.5   THE METABOLIC CONTROL OF FOOD INTAKE

Whilst the physical properties and the endocrine responses which occur due to the presence of food in the gut initiate satiety responses, the consequences of chemical digestion and absorption of nutrients further compounds mechanisms of satiety enabling a metabolic control of food intake.

The gastrointestinal (GI) tract processes ingested food, both mechanically and chemically, into small, absorbable units. Thus carbohydrates (CHO) are processed in the stomach and small intestine into fatty acids and monosaccharides; fats/lipids are transformed into glycerol and fatty acids and finally, proteins are cleaved to amino acids. The digestive end products become absorbed by the body. The entire process of digestion is coordinated by interactions of the enteric nervous system that innervates the walls of the GI tract which cause the release of peptides from specialised endocrine cells into the circulation or to serve as neurotransmitters mediating signals from the enteric nervous system. The GI hormones are synthesised and secreted from the mucosa of the stomach and upper small intestine and their release is mediated by both the cephalic mechanism, via the vagus nerve, and the

intraluminal mechanisms, via the cholinergic system (Vay et al. 1971). These signals are transmitted from the gut to the brain and are interpreted in the hypothalamus, reflecting the load of nutrients ingested. The gut peptides exhibit a response to food intake and some have been examined carefully to determine whether the response is of physiological relevance. For example, there are three main GI peptides which are released following the ingestion of food; gastrin, whose response has been determined to be in gastric acid secretion (Lam et al. 1980); CCK, whose response to a meal is in gallbladder contraction (Byrnes et al. 1981); and secretin, whose response has been associated with pancreatic bicarbonate output to regulate the pH of the duodenal contents (Yamada 1985). The effect that food exerts on GI hormones therefore depends on the macronutrient mix of the meal because separate nutrients elicit different responses to different peptide containing cells (Yamada 1985). CCK, for example, is stimulated by fats (Matzinger et al. 2000), gastrin appears to be stimulated by protein and amino acids (Lichtenberger 1982) and CHO's are known to stimulate other polypeptides such as gastric inhibitory polypeptide (also known as glucose-dependent insulinotropic peptide or GIP) and is a member of the secretin family of hormones (Cataland et al. 1974). Incretins, such as GIP and GLP-1, are a type of GI hormone that cause an increase in the amount of insulin released from the β-cells of the islets of Langerhans in the pancreas. Release of incretins can occur even before the presence of increased blood glucose and therefore insulin is released rapidly following food intake. Incretins slow the rate of absorption of nutrients into the blood stream by slowing gastric emptying and therefore may play a role in reducing food intake (Marks et al. 1991). Other hormones released in response to food include ghrelin, which is produced in both the stomach and the hypothalamus and hormone concentrations decrease when food is consumed. Also, leptin is

produced by adipocytes in response to food intake and has a role in inhibiting food intake (Ahrén and Havel 1999) As a group, the peptides of digestion are satiety signals because most create a sensation of fullness in humans and reduce food intake when administered to humans or animals (Beglinger and Degen 2006) and all play a potential role in the maintenance of energy balance. There are other endocrine regulators of food intake such as cytokines, IL-6 (interleukin-6) and TNF-α (tumor necrosis factor), which also have a role in the inhibition of food intake (Havel 2001) and are associated with infection, inflammation and cancer (Weingarten 1996), glucocorticoids which increase food intake, and probably interact with insulin and leptin in the long-term regulation of energy homeostasis (Havel 2001), and growth hormone which is also associated with both increased and decreased food intake (Havel 2001).

All this said, it is important to understand that food sources are not digested equally. Flatt (1996) hypothesised that fuels are digested according to their storage capacity i.e. alcohol will be metabolised first due to the body having no storage capacity for alcohol, then protein, glucose and finally fat, having the largest storage capacity and is therefore metabolised last. However, fuels are also not metabolised exclusively but are metabolised simultaneously as a 'mixed fuel'. Fuels are then either oxidised or stored (Friedman 1998). Friedman (1995 and 1998) suggested that the partitioning of metabolic fuels causes alterations in energy balance, see figure 1.2.   Neural, endocrine, and biochemical mechanisms determine which fuels are used through which pathways and in which tissues. For example, metabolic fuels may be stored as fat, oxidised for energy or for heat production, or converted to milk to feed offspring. Because mechanisms of fuel partitioning determine whether, and in which tissues,

metabolic fuels are oxidised, shifts in the flux of fuels could affect eating behaviour by altering the metabolic signal that controls food intake. Fuel partitioning indirectly effects food intake by altering the oxidation of fuels in the tissue or cells that generate the signal for appetite control. The liver has an important role in monitoring the changes in fuel metabolism that control food intake. Russel (1963) was the first to propose a role for the liver in feeding behaviour and since then a considerable amount of research has supported the hypothesis that satiety and hunger are influenced by signals elicited in changes in liver metabolism (Novin and VanderWeele 1977; Tordoff & Friedman 1986). Friedman (1998) suggested that this signal is associated with ATP production in the liver i.e. decreases in ATP production stimulate eating behaviour whereas increases in ATP suppresses eating.

Furthermore, Friedman (1998) went on to suggest that under normal conditions there is a balance between fuel storage, mobilisation and utilisation however in obesity the balance changes towards fat storage instead of oxidation and therefore the fuel mix changes and contributes towards further weight gain. What is not clear is why this occurs but the shift from oxidation to storage may be linked with the increased concentration of free fatty acids (FFA) in plasma as a result of higher body fat (DeFronzo et al. 1992). The elevated concentration of FFA attenuate glucose uptake in muscle by inhibiting glucose oxidation and is strongly associated with insulin resistance (Wolfe 2006) which is a hallmark of the metabolic syndrome.

This interplay between the synergistic effects of the peripheral and central control of food intake, taste including palatability and sensory-specific satiety to individual foods, and true fullness to a mixed meal is how our body deals with the physiological

and metabolic consequences of food. Thus, the body arrives at the integrated cognitive response to a meal. All components are necessary but singly each component has a different importance within this response. The importance that each component has on food intake is still to be elucidated.

A

Normal/Steady State

B
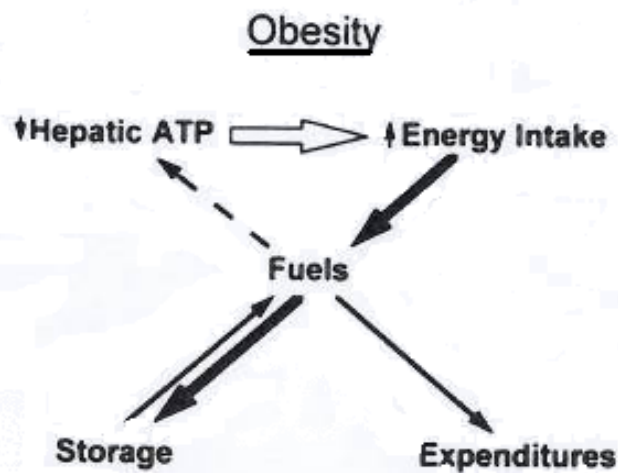
Obesity

Figure 1.2 Fuel Partitioning and Energy Intake (Friedman 1995)
   A) Relationship between fuel partitioning and energy intake control under
      normal/steady state conditions
   B) Relationship between fuel partitioning and energy intake in obesity where
      increased energy intake is associated with increased energy deposition.

1.6   SHORT AND LONG-TERM CONTROL OF FEEDING

The satiety cascade which occurs as a consequence of feeding may suggest that only short term influences, lasting hours and relating to individual meals exist to promote inhibition of eating. However, it is clear that metabolic control over episodic eating and energy intake occurs over significantly longer periods extending to days or months (see figures 1.3 and 1.4). Importantly, together the short and long-term signals interact to regulate energy balance. Therefore if there is dysregulation in the short-term feeding process(es) then it is possible that long-term homeostasis, and thus body weight regulation, may consequently be affected. Inevitably, increased energy intake and positive energy balance results in altered body composition and the change alone may influence feeding behaviour. Hormonal influence may also affect the short and long-term control of food intake.

Short-term Signals Regulating Feeding
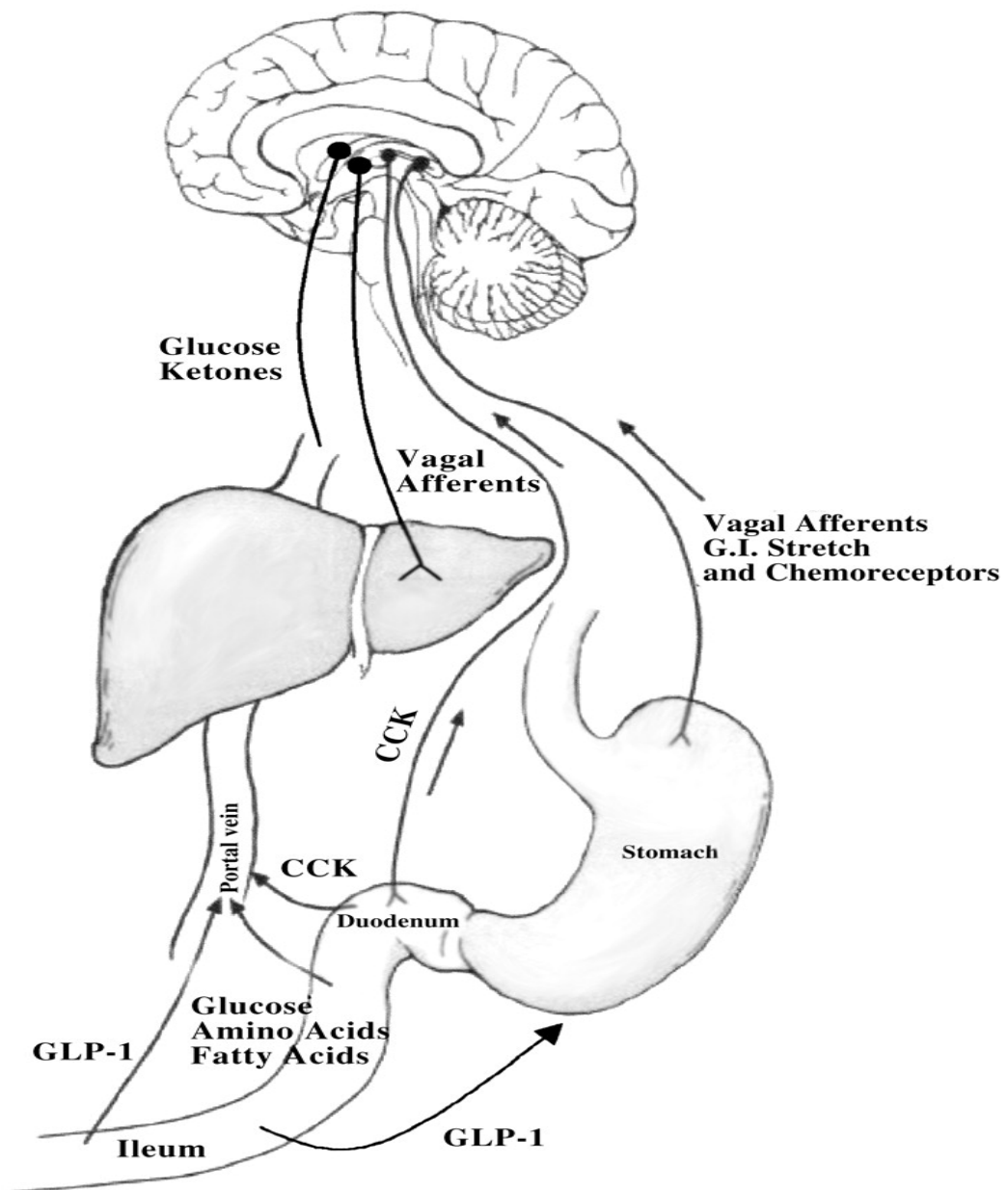
Figure 1.3 Short-Term Signals Regulating Food Intake (Havel 2001)
Signals from the GI tract and the liver are involved in short-term regulation. Afferent signals travel in vagal nerve fibers from stretch receptors and chemoreceptors activated by the presence of nutrients in the stomach and proximal small intestine. Nutrients arriving via the portal vein may also trigger vagal afferent signals from the liver.

Long-Term Signals Regulating Feeding
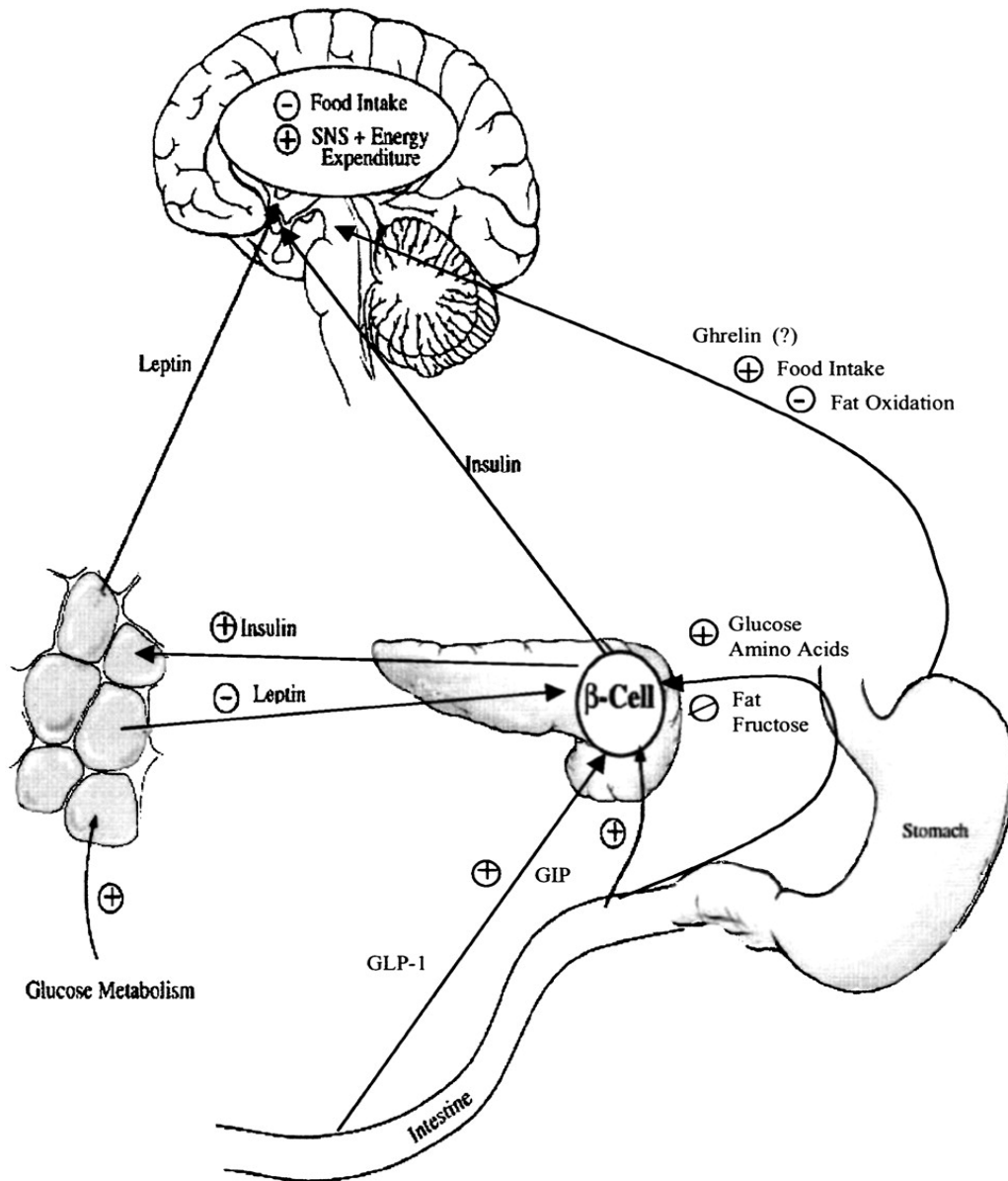
Figure 1.4 Long-Term Signals Regulating Food Intake (Havel 2001)
Insulin and leptin are important long-term regulators of food intake and energy balance. Both act in the CNS to inhibit food intake and to increase energy expenditure, most likely by activating the sympathetic nervous system.

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1.7 THE HORMONAL INFLUENCES OF THE CONTROL OF FOOD INTAKE

1.7.1 Ghrelin

Peripheral factors that participate in the short and long term control of appetite and adiposity can be either anorexigenic and promote reductions in food intake and consequently a reduced body weight or orexigenic and promote feeding and cause an increase in body weight. Ghrelin is a unique hormone in that it activates appetite and promotes food intake whereas all the other known GI hormones uniformly inhibit food intake. Therefore ghrelin concentrations increase during fasting, and are reduced by the presence of food in the stomach. Most GI hormones and their receptors are present in regions of the CNS involved in regulating feeding behaviour and therefore inhibit/promote feeding when administered both peripherally and when administered, at low doses, directly into the brain. (Havel 2001). It is unclear if the primary target for ghrelin and other GI hormones is in the periphery, in the CNS, or in both. It is likely that both peripheral and CNS production and action of GI hormones represent comparable pathways in the regulation of feeding behaviour.

Ghrelin is a novel 28-amino acid peptide hormone that has been recognised as an important regulator of hormone release and feeding promotion and was first identified based on its stimulation of growth hormone secretion via a growth hormone secretagogue receptor in animals and humans (Bowers 2001). The mammalian ghrelin structure has an n'octanoyl modification at its third serine residue which is essential for its activities to stimulate growth hormone secretion from the pituitary

cells. The stomach produces three quarters of the circulating ghrelin and is the principal site of ghrelin synthesis. The gastric fundus, in the stomach, is the most abundant source of ghrelin and produces ten times more of hormone per gram of tissue than does the next richest site, the duodenum (Havel 2001). Ghrelin is produced in the oxyntic mucosa of the stomach by enterendocrine cells. Therefore when food is ingested, there is a rapid reduction in ghrelin levels due to the close proximity of the ghrelin producing cells. Concentrations of ghrelin producing cells decrease throughout the small intestine as the distance from the stomach pylorus increases. In humans, ghrelin levels rise before meals and fall rapidly after the ingestion of a meal (Williams and Cummings 2005) and secretion is finely regulated by several factors. The stimulatory effect of ghrelin on feeding is independent of the stimulation of grown hormone (Yoshihara et al. 2002) which suggests it has an independent role in promoting food intake. During fasting, ghrelin stimulates the ARC neurons that cause an increase of two orexigenic peptides, NPY and agouti related protein which initiate feeding and promotes an increase in body weight. In contrast both leptin and insulin, produced from adipose tissue, exerts its influence on the ARC and inhibit feeding and produce a reduction in body weight. This influence is exerted by suppressing NPY and agouti related protein peptides. Studies involving ghrelin have shown increased stimulation of GI motility, gastric acid secretions and pancreatic exocrine secretion (Mora et al. 2005). The bodily changes that occur as a result of changes in ghrelin concentration are in anticipation of a meal and therefore prepare the GI tract for food processing and transport.

Ghrelin's role is therefore in the regulation of mealtime hunger regulation and meal initiation by influencing postingestive satiety. This hormone is a potent stimulator of

short term food intake and stimulates food intake more effectively than does any known molecule, except NPY (Havel 2001). Ghrelin has been attributed to stimulating appetite and food intake even more in obese individuals than lean (Mora et al. 2005).

1.7.1.1 Ghrelin and Obesity

Individuals with obesity have been shown to have reduced plasma ghrelin levels and individuals with anorexia nervosa have been shown to have increased plasma ghrelin levels which suggests a negative feedback mechanism is in control of energy homeostasis (Shiiya et al. 2002). Fasting ghrelin concentrations in obese and type 2 diabetic patients have been shown to be negatively correlated with BMI, fasting insulin and fasting leptin concentrations within both groups (Shiiya et al. 2002). This may suggest a role for ghrelin in long-term feeding behaviour and body weight. Attenuation of ghrelin has been found in obesity and may be causative of the long-term positive energy balance that leads to obesity. The mechanism of attenuated ghrelin may be linked with macronutrient intake. The link between glucose and ghrelin-producing cells was originated by studies which investigated the short-term response to oral and intravenous administration of glucose and water to normal subjects. The study found that only the glucose administration caused a reduction in subjects' plasma ghrelin concentrations (Shiiya et al. 2002). Similar studies investigated the administration of lipids and high-fat diets and found that they suppress postprandial ghrelin concentrations less effectively than glucose (Monteleone et al. 2003) which further advocates that glucose and insulin may modulate the postprandial ghrelin response (Blom et al. 2005). Therefore, the long

term role of ghrelin may be linked to the long-term fluctuations in glucose and insulin concentrations which become dysfunctional in chronic states such as obesity, IGT and type 2 diabetes when blood glucose and insulin levels are elevated over a long period of time. Therefore, it is not only important to understand the role that circulating ghrelin plays in food intake from meal to meal, it is necessary to understand the factors which regulate plasma ghrelin levels in relation to feeding and its long term role in the maintenance of energy balance and also its possible link in the pathology of obesity and related chronic diseases such as IGT and type 2 diabetes.

1.7.2 Leptin

Although leptin is not directly investigated in this study it is important to discuss this hormone due to the antagonistic effects of leptin to ghrelin. Leptin is a circulating hormone mainly secreted by white adipose tissue and influences body weight homeostasis by altering food intake and energy expenditure. Leptin influences the activity of the hypothalamus by modulating the expression of several neuropeptides to promote satiety. Leptin interacts with both components of the energy balance relationship by promoting satiety and also by having a stimulatory effect on energy expenditure (Campfield et al. 1995). Once released into the bloodstream leptin must gain access to specific regions in the brain, mainly the hypothalamus, involved in the regulation of food intake and energy balance. The discovery of leptin was revolutionary because it confirmed the hormonal link between adipocyte and the brain, therefore an understanding of the biology of leptin offers significant insight into the complex inter-relationships among adipose tissue, the nervous system and peripheral organs. Leptin is also increasingly spoken of as a general 'metabolic'

hormone, in that a rapidly expanding range of processes with which it interacts are being documented. These processes include the inhibition of insulin secretion from the ß-cells of the pancreas (Emillson et al. 1997), the stimulation of glucose utilisation (Kamohara et al. 1997), stimulation of sugar transport across the small intestine (Lostae et al 1998), and actions in the immune system (Cock and Auwerx 2003). So it is clear that leptin has a role in both short and long-term pathways controlling food intake.

1.7.2.1 Leptin and Obesity

Research shows that the effectiveness of the leptin receptor is reduced in obesity, resulting in disruption of the leptin pathway which prevents critical hypothalamic areas from perceiving pertinent cues on energy status (De Fanti et al. 1998). If the hypothalamus does not receive, sense, or properly integrate the nutritional state, a superficial negative energy balance and subsequent energetic efficiency ensue, resulting in obesity. Diet induced obesity in humans is associated with increased leptin levels (Mercer and Speakman 2001). Although leptin levels rise proportionally with adiposity, the increased leptin fails to curtail the progression of obesity (Zhang and Scarpace 2006). Analogous to hyperinsulinemia and insulin resistance, hyperleptinemia has been postulated to be indicative of 'leptin resistance' (Flier 1998). Early studies suggested that leptin crosses the blood brain barrier (BBB) by a saturable transport system (Banks et al. 1996). Research has discussed the possibility that the leptin transport system could be saturated near physiological concentrations in lean individuals, implying that the elevated leptin levels observed in obesity can produce no biological effects because the system is no longer responding (Flier 1998).

Banks et al (1996) also suggested an impaired transport of circulating leptin across the BBB in obese CD-1 mice due to a decreased capacity of the BBB to transport leptin. Human parallels are now recognised with the description of obese subjects with mutations in leptin or the leptin receptor (Montague et al. 1997). Zhang and Scarpace (2006) further postulate that a leptin resistant state disrupts normal energy homeostasis, favours positive energy storage, and thus leads to even greater obesity. Leptin works synergistically with CCK to inhibit feeding. Leptin is an adiposity signal and known for its long-term role in adiposity control and its short term role in modulating meal size (see figure 1.4) according to changes in energy balance and CCK operates as a satiety signal to reduce meal size.

1.7.3   Cholecystokinin (CCK)

Appetite is regulated by a complex system of central and peripheral signals which interact in order to modulate the individual responses to nutrient ingestion. Satiety signals originate from the GI tract during a meal and through the vagus nerve, reach the NTS in the brainstem (see figure 1.3). From the NTS, afferent fibers project to the ARC where satiety signals, namely CCK, are integrated with adiposity signals, namely leptin and insulin, creating a final response to a meal.

CCK is released from endocrine cells localised in the mucosal layer of the proximal small intestine and travels via the portal vein to the liver and via the systemic circulation to the CNS. CCK is also produced in the CNS and is released by hypothalamic neurons during feeding. CCK release is stimulated by dietary fat, amino acids and small peptides released during protein digestion. CCK inhibits food

intake by the activation of CCK_A receptor subtype. Moran et al (1998) demonstrated a defect in the CCK_A receptor in obese rats resulting in reduced CCK. CCK is likely to transmit vagal afferent signals to the medulla in the hindbrain by acting on receptors located in the pylorus and liver. CCK's role in feeding regulation is in energy homeostasis and is to inhibit food intake by inducing postprandial satiety by communicating fullness and thereby decreasing meal size. West et al (1984) showed that CCK function is in short-term satiety by repeatedly administering CCK to rats when meals were initiated. This resulted in reduced meal size. However, the subsequent number of meals eaten increased and after an initial period of adaptation overall energy intake was unaffected and weight loss was minimal.

Schneeman et al (2003) hypothesised that the constituents of food would alter the way in which nutrients are handled in the postprandial period and this in effect would be evident in the hormonal and lipid response to the meal. This study showed that fat, particularly dairy fat, is a more potent stimulator of CCK than a blend of non-dairy fats and found increased CCK concentrations in women. This finding is of interest when considering CCK function(s) and relative interactions with other hormones in the response to eating. For example, CCK has also been shown to slow gastric emptying, thus inhibiting food intake and also to have a role controlling the glycaemic response to a meal by increasing the sensitivity of tissues to insulin (Schwartz et al. 1994) to maintain consistent blood glucose levels. CCK release has also been shown to induce satiety in rats (Burton-Freeman et al. 1997) however, although an association between CCK and satiety has been postulated, studies on humans have been controversial. Some studies showed a strong relationship between CCK release and satiety, using VAS (Burton-Freeman et al. 2002) and others did not (Schneeman

et al. 2003). This suggests that there may be other influences, overriding signals within the food intake regulatory system under different dietary challenges. These other influences may be important when considering obesity, IGT and type 2 diabetes because their habitual diet has been shown to be consistently high in fat content, particularly saturated fat (Walker et al. 1996). This may implicate fat in promoting dysfunction in the CCK pathway which normally inhibits feeding. It is therefore important to know how CCK varies with these conditions and how it interacts in the role of energy homeostasis for the diagnosis and treatment of IGT and type 2 diabetes.

1.7.3.1. CCK and Obesity

Initial studies in obese rats (De Fanti et al. 1998) investigated the hypothesis that the synergistic interaction between leptin and CCK in the regulation of food intake may be dysfunctional. Studies on the fatty Zucker rat showed defective leptin signalling and has been associated with altered long-term dietary cues yet the consequences of this defect on short-term cues, such as CCK remain unclear. One possibility is that the defect in the leptin signalling system seen in obesity, may cause a blunting of CCK release from the PVN and effectively abolish the ability of CCK to inhibit feeding and thus promoting increased meal size. (De Fanti et al. 1998). A recent study by Little et al (2007) concurred with the results of De Fanti et al (1998) and suggested that chronically elevated plasma CCK concentrations, induced by a continual increased consumption of dietary fat, mediate the reduction in sensitivity to the inhibitory effects of CCK on food intake. Human studies have not shown consistent results like those involving rats. For example, the CCK response to a high fat diet via lipid infusions was not affected in humans although elevated postprandial

CCK concentrations were reported. It has therefore been suggested that the increased postprandial plasma CCK response in humans is primarily due to a greater amount of nutrients present in the small intestine to stimulate the localised secretion of CCK which is resultant of more rapid gastric emptying. Therefore, it is still not clear whether CCK has a role in human obesity because some studies have shown that fasting concentrations of CCK have been elevated in obese subjects (Baranowska et al. 2000) suggesting a CCK resistance but this has not been demonstrated in all studies (Lieverse et al. 1994). Any impairment in the functionality of a hormone involved in the control of food intake is pivotal because its influence on other metabolic factors, such as insulin, will also impact on metabolic control and therefore alter the sensitivity of endocrine factors regulating the long term control of energy intake.

1.7.4 Insulin

It was first proposed by Woods and colleagues in the 1970s that insulin is a long term regulator of food intake, energy balance and body adiposity (Woods et al. 1974). Parasympathetic nerves innervating the pancreas are activated as a direct effect of incoming nutrients, especially glucose and amino acids, causing the secretion of insulin from the islet ß-cells of the endocrine pancreas (see figure 1.4). Fat does not induce insulin secretion, although some fatty acids appear to be necessary for the full insulin secretory response to glucose (Havel 2001). Insulin receptors have been identified in a number of brain regions which are known to influence the regulation of feeding including the ARC of hypothalamus. Havel (1999) studied the effects of body adiposity to overall insulin secretion and insulin concentration in the systemic

circulation and found a correlation between fasting insulin levels and the insulin response with body adiposity. In addition to inhibiting food intake, insulin increases sympathetic neural activity and energy expenditure. Thus, insulin can regulate energy balance by inhibiting energy intake and by increasing thermogenesis.

CNS neurons do not produce insulin, however the hormone insulin is transported into the brain via a receptor-mediated mechanism that is saturated at high insulin concentrations and therefore this is not a rapid process. The transport process occurs over a period of hours as circulating insulin concentrations rise and thus is consistent with the theory that insulin is a long-term regulator of feeding behaviour.

1.7.4.1 Insulin and Obesity

After feeding, insulin is preferentially transported into the hypothalamus, compared with other brain areas and so the hypothalamic insulin content is increased. This increase in hypothalamic insulin occurs after a high CHO meal, but does not occur after a high fat meal because there is a smaller circulating level of insulin after a high fat meal. The transport system in the brain which controls circulating insulin levels is thought to be impaired following a high fat meal. It was demonstrated in dogs that chronic consumption of high fat meals impairs brain insulin transport and the impairment is predictive of weight gain (Kaiyala et al. 2000). Together the effects of reduced insulin secretion and reduced insulin transport to the CNS may contribute to the increased energy intake and obesity observed in humans consuming high fat diets. In other words, the intake of dietary fat and therefore the fatty acid composition of tissues may influence insulin sensitivity. This can potentially be changed through

long-term dietary modifications (Mann 2000). Therefore, the protocol of the current study included the assessment of habitual dietary intake to determine if there was differences in content, particularly fat content as it is known to have implications in the functionality of insulin.

Insulin and leptin act in the CNS to inhibit food intake and to increase energy expenditure most likely by activating the sympathetic nervous system. The long-term regulation of food intake is paramount because the damaging effects that long-term increased body weight has on the neuro-endocrine metabolic control markers is considerable and can result in metabolic syndrome, diabetes and other chronic illnesses.

1.8   GLUT-4 TRANSLOCATION

Insulin promotes glucose uptake by muscle and adipose tissue via the stimulation of GLUT-4 transporters from intra-cellular sites to the plasma membrane. A low abundance of the GLUT-4 transporter has also been shown to be present in low abundance in the brain endothelial cells lining the BBB in rats and mice (McCall 1997; Sankar 2002) which facilitates glucose transportation into the CNS in an insulin-independent fashion (Sankar et al. 2002). This corresponds with Mayer's (1953) glucostatic theory which suggests that glucose is the preferred source of energy, especially for the brain and that hunger depends on the rate that glucose crosses the cell membrane and is actually used by the body. Therefore satiety occurs when glucose utilisation is adequate and a low level of glucose utilisation causes hunger, regardless of blood glucose concentration. Mayer (1953) suggested that the

rate of glucose utilisation is monitored by the so called 'satiety centre' or ventromedial hypothalamus (VMH) and that VMH activity increases in response to increased glucose utilisation leading to satiety. Therefore, GLUT-4 mediated transport in the muscle and brain is essential to the maintenance of glucose homeostasis.

Attenuated GLUT-4 translocation and glucose uptake by muscles and fat cells following insulin stimulation represent a prime defect in insulin resistance. In mice, adipose-selective disruption of GLUT-4 leads to secondary insulin resistance in the liver and muscle and results in IGT (Huang et al. 2005). Alteration of GLUT-4, i.e. expression or function, contribute toward the development of insulin resistance and diabetes. Under normal circumstances insulin causes an upregulation of GLUT-4 mRNA in adipocyes but this effect is dependent on glucose concentration (Bilan et al. 1992). The response was seen at low glucose concentrations (5.6mM) but not at high glucose concentrations (25mM) which suggests a suppressing effect of glucose on insulin induced GLUT-4 gene expression (Bilan et al. 1992). Results have been controversial and not all studies concur with this assessment of suppressed GLUT-4 transportation (McCall et al. 1997) in hyperglycaemic states. Bilan et al (1992) suggested that individuals with insulin resistance will demonstrate a lack of effect of insulin in a high glucose environment which may be due to the impaired insulin signalling produced by high glucose i.e. glucotoxic effect. Reducing the glucose response in hyperglycaemic states, such as IGT and type 2 diabetes, is important, therefore GLUT-4 translocation is an important control mechanism in these conditions. In the muscle, glucose transport by GLUT-4 is insulin-stimulated. Bilan et al (1992) showed that glucose transporter function but not expression was impaired

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by high glucose concentration in diabetic rats i.e. high glucose impairs insulin action on transport but not via altered GLUT-4 expression, whereas, long-term exposure to high glucose in combination with high insulin levels produced a reduction in GLUT-4 abundance. Therefore, GLUT-4 therapy has been used as a means to lower plasma glucose in diabetic animals (Dolan et al. 1997). Although research is inconsistent, GLUT-4 has a role in the control of energy balance through the maintenance of blood glucose levels which provide messages of fuel availability to the brain to determine metabolic feeding cues.

1.9   INSULIN RESISTANCE

An accretion of adipose tissue, as in obesity, is associated with insulin resistance (Frayn 2001). Obesity and associated insulin resistance and hyperlipidemia are hallmarks of the metabolic syndrome and are major risk factors for type 2 diabetes. It was in 1961 that the concept of insulin resistance was applied, following the observation that more obese diabetics were resistant to the blood glucose-lowering effect of insulin (Rabinowitz and Zierler 1961). Therefore the body's sensitivity to insulin, as in insulin resistance, has known to be associated with body fat since then. The mechanisms by which an accumulation of fat storage can lead to widespread changes in glucose and lipid metabolism, insulin resistance, and other mechanisms are still unclear. Insulin resistance has two important characteristics 1) decreased cellular responses to insulin or perturbation of the insulin signalling pathways and 2) mutations in the insulin receptor gene which leads to alterations of receptor synthesis, degradation and function, i.e. in binding and alterations in translocation and function of glucose transporters (GLUT4), in severe insulin resistance. Adipose tissue plays an

important role in the development of insulin resistance and increased circulating levels of free fatty acids (FFA) derived from adipocytes are demonstrated in insulin resistant states (Corcoran et al. 2007). Increased FFA have been the most consistently discussed associated aberration in the etiology of obesity and insulin resistance. FFA cause an inhibition of glucose uptake and utilisation in muscle, glycogen synthesis, glycolysis and an increase in heptatic production of glucose (Boden et al. 1994) and an associated glucose-stimulated insulin secretion (Capentier et al. 1999), all of which contribute to maintaining the insulin resistant condition. Insulin resistance may not be the same and may not occur at the same time in all tissues of the body i.e. muscle is resistant very early in the progression of metabolic syndrome whereas adipose tissue is not resistant in the early stages of whole-body insulin resistance (Das 2002). The longer term toxic effect of FFA may effect ß-cells in the pancreas and this may be the element which associates obesity, insulin resistance and the development of type 2 diabetes (Unger 1995).

Associated aberrations of insulin resistance include a propensity to oxidise fat at the expense of glucose due to reduced glucose utilisation (Frayn 2001). Insulin resistance is associated with reduced glucose utilisation at a given insulin concentration and failed activation of adipose tissue lipoprotein lipase by insulin. The partitioning of fatty acids towards oxidative tissues such as muscle is associated with impaired activation of adipose tissue lipoprotein lipase by insulin. This situation leads to the idea that insulin resistance may be seen as an adaptation to increasing fat mass (Frayn 2001; Flatt 1995b). Ultimately, insulin resistance not only affects the metabolism of glucose, but affects lipid metabolism and is also associated with hypertension and endothelial dysfunction.

1.10   NUTRIENT STORAGE

Any energy intake in excess of total energy expenditure is stored, however there is a hierarchy in the extent to which the macronutrients can be stored, as discussed earlier, and this has implications for their metabolic fate, once ingested. Alcohol is a toxic drug which cannot be stored. The storage capacity for protein and carbohydrate is limited and converting these nutrients to a more readily stored form is energetically expensive, therefore they are selectively oxidised. The storage capacity for fat is potentially very large. In some very obese subjects body fat can exceed 40% of total body weight (Stubbs 1998). However, differences between energy stored and energy expended and differences in the composition of the fuel mixture oxidised may lead to important individual differences in the metabolic fate of excess energy under conditions of positive energy balance. A positive balance of protein will lead to rapid metabolism of a high proportion of the amount of protein ingested, the percentage depending on the body's requirements for specific amino acids. Similarly as CHO intake increases, more of it is disposed of by oxidation. Because there is a ceiling on adaptive changes in energy expenditure (EE) an increased oxidation of protein and CHO will lead to a decrease in the oxidation of fat. Conversely, intake of fat does not promote fat oxidation and actually leads to fat storage (Flatt et al. 1988). Diet composition can influence total energy intake and can alter nutrient balance without changing energy expenditure (Tremblay et al. 1989). The fuel mix oxidized can be assessed by RQ (respiratory quotient) and can be measured by indirect calorimetry. The RQ of fat is 0.7, Protein 0.8 and CHO 1.0. An excess of CHO affects energy and nutrient balance differently than that of an excess of fat and Horton et al (1995) found that an excess of fat leads to more body fat accumulation than an equivalent excess of

CHO. Also, an increase in the thermic effect of food was observed with an excess of CHO and fat. An increase in fat content in the diet will cause a lowering of the RQ towards fat oxidation and a reduction in oxidation and promotion of storage (Horton et al 1995). This suggests that an excess intake of fat can lead to a positive energy balance and may pre-dispose an individual to obesity. Flatt's general theory is that the fuel mixture oxidized must match the fuel mixture ingested by seeking to regulate carbohydrate. Hill et al (1994) proposed that some individuals may be at a higher risk of developing obesity than others, characterised by a metabolic and/or a behavioural susceptibility to weight gain. He proposed that a behavioural susceptibility to obesity creates the opportunity for positive energy balance to occur, such as overeating or lack of activity, whereas metabolic susceptibility to obesity determines the metabolic fate of the excess energy when positive energy balance occurs. For example, excess fat leads to more body fat accumulation than does CHO (Horton et al. 1995). Furthermore, individuals, for example, with a high metabolic susceptibility would be even more inclined to accumulate more body fat but less glycogen during periods of positive energy balance than an individual would with a lesser metabolic susceptibility to obesity. This knowledge together with the knowledge that excess fat affects energy and nutrient balances differently than excess CHO does (Horton et al. 1995) provides important information about the potential impact of dietary composition and nutrient storage on body weight regulation and obesity development. Additionally, the implications of long term obesity, such as in type 2 diabetes necessitate investigation.

1.10.1 Thermic Effect of Food

Diet induced thermogenesis (DIT) is the increase in energy expenditure above resting associated with the digestion, absorption and storage of food. DIT accounts for between 5-15% of the total daily EE (Tentolouris et al. 2008). The thermic effect of specific nutrients is highest for protein (20-30%), then CHO (5-10%) and finally fat (0-3%). The central problem in the control of energy balance has always thought to have been in the regulation of energy intake, however animal studies have shown that in excess energy intake the regulation of thermogenesis is important for the control of body fatness (Miller and Payne, 1962). This has then led to a proliferation of research comparing total daily EE or another component of EE such as RMR or DIT. The most significant outcome from this research was that a reduced DIT may contribute to the development or maintenance of obesity. However, studies examining differences in DIT have conflicting results. Some studies show a reduced DIT between lean and obese subjects (Segal et al. 1990) and others show no differences in DIT (D'Allesio et al. 1988; Tentolouris et al. 2008). The conflicting results seem to be due to a number of reasons including different methodologies, test meals, pre-loads, age-related differences and daily fluctuations dependant on the amount and type of nutrients consumed and the type and amount of physical activity (Weststrate 1993). Therefore, the goal of the current study was to assess the DIT induced after a 'normal' mixed nutrient meal and compare the results of IGT, type 2 diabetics and control participants.

1.11   THE PUBLIC HEALTH BURDEN OF CHRONIC DISEASES

1.11.1 Obesity

Obesity was highlighted as a major public health problem in the UK first by a joint department of Health/Medical Research Council report in 1974, and then by the 1983 Royal College of Physicians (London) Report. Currently, in Scotland 44% of adult men are overweight with a body mass index (BMI) of 25.9-29.9 and a further 14% are obese (BMI>30). In women 32% are overweight and of that figure 17% are obese (SIGN, Obesity in Scotland). Thus half of the adult male population and a third of the female population are at risk because they have gained excess weight. Furthermore, about 20% of children are overweight. Overweight children are found particularly in families where both parents are overweight or obese. The younger an individual develops weight gain and obesity, the greater the long-term morbidity.

As demonstrated already body weight is regulated by powerful physiological signals which change appetite and satiety to a far greater extent than is recognised. However, only a small persistent discrepancy between daily intake and energy output is required to induce substantial weight gain over time. Metabolic responses to overeating play only a modest role in buffering changes in energy balance. The metabolic rate relates to the body weight of an individual, but there are substantial differences between individuals. Many factors interact to induce weight gain, including behavioural, physiological, genetic, medical, therapeutic and psychological causes. To try to identify a single factor as the cause of obesity in a patient oversimplifies a complex process.

The two major factors now being shown to be conducive to weight gain in western populations are 1) a reduction in activity levels, which decreases the total energy expenditure per day and 2) the ingestion of high-fat, energy dense foods, which facilitates excess energy intakes (Prentice and Jebb 1995).

1.11.2 Impaired Glucose Tolerance

IGT is conventionally defined as a plasma glucose level of ≥7.8 and<11mmol/L 2 hours after ingestion of 75g glucose (WHO 1999). IGT is a strong predictor of type 2 diabetes and so it is important to clarify the determinants of IGT. A few epidemiological studies have reported an association between greater abdominal obesity, measured by waist circumference or waist-to-hip ratio, and the risk of IGT. Progression from normal glucose tolerance to IGT is mostly due to deterioration of early-phase insulin secretion and to a lesser degree is the contribution of insulin resistance (Suzuki, 2003). Individuals with IGT are at increased risk of developing type 2 diabetes (Yudkin and Coppack 1994). However, IGT may revert to normal glucose tolerance and may not progress inexorably to diabetes. Indeed, a great deal of heterogeneity exists in the rates of progression to diabetes in different populations and is higher in non-white racial/ethnic groups. IGT is not only associated with progression to type 2 diabetes but is independently a risk factor for coronary heart disease (Califf et al. 2008). IGT and type 2 diabetics have a number of common risk factors including obesity, advancing age, gestational diabetes, family history, dyslipidemia and insulin resistance. Thereby arguing that IGT is a precursor of type 2 diabetes, however it has not been quantified what factors contribute to the progression from IGT to type 2 diabetes.

1.11.3 Type 2 Diabetes

In the UK there are approximately 2 million people who are diabetic and, in Scotland diabetes is rapidly becoming a health problem as over 170,000 people are diagnosed as diabetic (Scottish Government 2006). Furthermore, research would indicate that thousands more have the condition but have not yet been diagnosed (Scottish Government 2006). Diabetes UK claim that this figure will double in the next 25 years (Diabetes UK 2008). The Scottish Diet report highlighted obesity as a growing problem of relevance to the high rates of type 2 diabetes, hypertension and hypercholesterolaemia in Scotland (SIGN 1995). In fact 8 out of 10 type 2 diabetics are obese (Diabetes UK 2008). The increased prevalence of childhood obesity is also precipitating type 2 diabetes in children (McGough 2001).

The pathology of type 2 diabetes occurs when ßeta cells within the islets of langerhans, in the pancreas may be reduced in number and in chronic states of type 2 diabetes this may result in fibrosis and deposition of amylin polypeptide within the islets. (Halton et al. 2006). Concurrent with aberrant changes in insulin, as discussed above are other known mechanisms (sensory, physiological and psychological) which are dysfunctional in obesity and type 2 diabetes. It is unclear which of the known mechanisms influence food intake to the greatest degree and this has never been investigated but it is probably due to the implications of the numerous tests that would need to be incorporated into the study design. It is, of course, likely that it is the synergistic effect of these dysfunctional mechanisms which causes the progression of obesity and type 2 diabetes and which makes it so difficult to reverse the damage and effects of these chronic illnesses. History shows, for example, it is difficult for

individuals to lose weight and to maintain weight loss (Tate et al. 2007; Johnstone et al. 2008). This may be an effect of dysfunctional pathways brought about by consistent positive energy balance. The increased knowledge earned by understanding the integrated response to food intake could potentially help in the prediction of obesity, IGT and type 2 diabetes.

1.12   OBESITY AND CHRONIC INFLAMMATION

The presence of inflammation in obesity has clearly been demonstrated (see figure 1.5). The common feature in each investigating article is the inflammation–obesity–insulin resistance connection. Specifically, the study of Ortega Martinez de Victoria et al (2009) comments on factors related to macrophage activation in adipocytes as a source for cytokines for systemic inflammatory effects. In contrast, the study of Haus et al (2009) provides data on systemic effects resulting from inflammatory activation, namely, the relationship of cytokines with circulating lipid intermediates. The evidence is accumulating that adipose tissue plays a major role in the production of cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α, which, in turn increases the production of C-reactive protein (CRP) from the liver. In addition, data from the West of Scotland Coronary Prevention Study (Freeman et al. 2002) showed that C-reactive protein predicted the development of type 2 diabetes in middle-aged men independently of established risk factors. This long-term alteration in production may adversely affect the sensitivity and/or effectiveness of physiological systems controlling long-term energy balance. The long-term effects of this dynamic system are not well documented.

Dietary factors, in particular dietary carbohydrates, have been considered in the aetiology of inflammation. It is thought that dietary carbohydrates characterised by different postprandial insulin responses, may influence plasma cytokine concentrations and gene expression in adipose tissue. This of course implicates glycaemic index in the control of inflammation which mediates the insulin response. Therefore, diets containing a high proportion of high glycaemic index foods (i.e. high in energy dense, fatty foods) are known to produce a heightened insulin response may be implicated in the promotion of inflammation . A hypothesis has been made that ingestion of high glycaemic foods results in initial hyperinsulinaemia and a subsequent transient drop in glycaemia below fasting concentrations. This is thought to activate cortisol and catecholamines, which restore fasting glucose concentrations and increase nonesterified fatty acid (NEFA) concentrations, which reflects increased mobilisation from adipose tissue and stimulation of lipolysis. Sustained increases in plasma FFA can cause insulin resistance (Frayn, 2001). Moreover, increased concentration of TNF-α and IL-6 are associated with insulin resistance (Ruderman, 2006).

Diet is a significant contributor to adiposity and is therefore an important component in the investigation of inflammation. Dietary fat and plasma fatty acid composition, which is at least in part influenced by diet, are related to insulin sensitivity, blood pressure, plasma lipid profile and inflammation. In obese individuals there is a strong association between IL-6 and TNF and obesity (Das 2002; Hotamisligil et al. 1993). Increased CRP is secondary to increased IL-6 secretion. Leptin causes an upregulation in the production of IL-6 and also induces release of IL-1 in CNS. The effects of leptin on food intake and body temperature are mediated by IL-1. Thus

leptin's influence is to further augment the synthesis and release of pro-inflammatory cytokines (i.e. IL-6). The result of elevated pro-inflammatory cytokines in the hypothalamus may damage the specific neurons that sense plasma glucose levels and control the secretion of insulin from the pancreas, affecting glucose metabolism and potentially triggering positive energy balance and obesity. These observations provide the link between an increase in the expression, and the plasma concentration of a proinflammatory cytokines and insulin resistance (see figure 1.5). Subsequently, other metabolic sites may also be involved during the progression of complications i.e. hepatic and skeletal muscle insulin resistance. It has been shown that adipocyte precursors and diverse immune cells such as T lymphocytes and macrohages possess similar potentials in escalating inflammatory cytokine production (Rosen et al. 1989). Thus, it appears that obesity is associated with a 'local' low-grade inflammation characterised by increased macrophage infiltration of adipose tissue and the production of inflammatory cytokines such as IL-6 and TNF which in turn causes an increased CRP concentration. This may lead to endothelial dysfunction and perpetuate insulin resistance and then eventually atherosclerosis and cardiovascular disease (CVD). It is not clear if the inflammatory response is the primary or secondary event that causes these diseases. However, it is clear that study findings have important clinical and public health implications (Martin et al. 1992). Insulin resistance is a major risk factor for type 2 diabetes, coronary heart disease, stroke, and kidney disease. Identifying risk factors for insulin resistance is important in the development of strategies for the prevention and treatment of insulin resistance. Inflammatory markers such as tumor necrosis factor, interleukin-6 and C-reactive protein have been associated with an increased risk of diabetes (Hu et al. 2004). In addition, inflammatory factors such as C-reactive protein and fibrinogen have been

found to play an important role in the pathogenesis of cardiovascular disease (Danesh et al. 2004; Ridker et al. 2000). Prospective epidemiologic studies have indicated that C-reactive protein has additive effects on the risk of cardiovascular disease among patients with insulin resistance (Ridker et al. 2003). Treatment of inflammation may reduce the risk of insulin resistance, diabetes, and related cardiovascular disease.

Generally, the acute inflammatory response causes a reduction in appetite (Black 2006) but with a chronic inflammatory response, as in type 2 diabetes, there appears to be no such change in feeding behaviour. Black (2006) suggested that one reason for this could be because the IL-6 response is not strong enough. It is not known if the long-term increase in cytokine levels causes permanent damage to glucose sensing neurons and the resultant poor insulin control. However, this being the case, damage to glucose sensing neurons may influence glucose uptake from the blood which may have an array of effects including altered ATP levels, altered fuel mix, and therefore changes in storage and utilisation which ultimately may be the reason for further weight gain in already obese individuals.
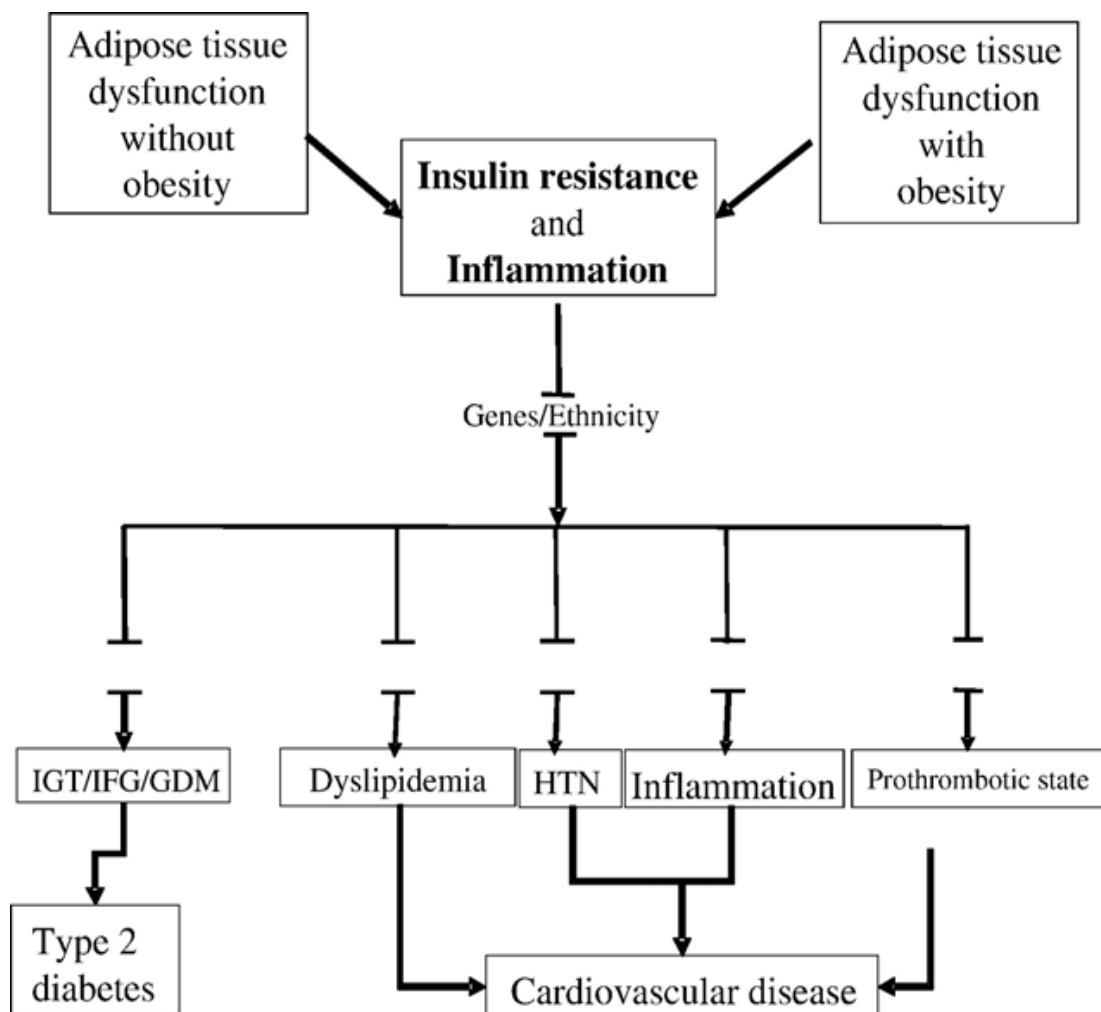
Figure 1.5 Schematic representation of the relationship between adipose tissue and the various components of the metabolic syndrome: the concept of adipose tissue dysfunction, IGT (impaired glucose tolerance, IFT (impaired fasting glucose), GDM (gestational diabetes mellitus), HTN (hypertension) (Chandalia and Abate 2007).

1.13   PHYSICAL ACTIVITY

As discussed in the very first chapter the importance of maintaining energy balance is paramount in leading a healthy lifestyle. The key to this is to match energy intake with energy expenditure. The relationship between physical inactivity and various health problems is well established (Blair et al. 1996; Hardman 1996). The body of evidence over the past half century has led to the consensus that sedentary living leads to coronary artery disease and perhaps to some cancers, stroke, non-insulin-dependent diabetes mellitus and other health problems (Bouchard et al. 1994). Overweight and obesity accentuate all the manifestations of metabolic syndrome and their development may be related to sedentary living; therefore, some discussion of the potential of exercise to influence weight regulation is necessary and it is consequently important to recognise what the differences in physical activity are between the three groups in the current study. Biological mechanisms that contribute to the lower risk associated with activity include improved lipoprotein profile, insulin action, glucose tolerance, lower blood pressure and the loss of total weight and body fat (Williams 2001). Lower death rates associated with regular physical activity are consistent in different populations (Blair et al. 1996; Blair & Connelly 1996). The level of physical activity has been identified as one factor which may exert a beneficial effect on insulin and glucose dynamics. For example, one study of 70-89 yr old men found that insulin concentrations during an oral glucose tolerance test were lowest in men with the highest physical activity levels, undertaken mainly by walking, cycling, and gardening (Feskens et al. 1994). The findings are similar for women: in a prospective study of 87,000 middle-aged women, those taking part in vigorous exercise had only two-thirds the risk for developing type 2 diabetes when compared to

inactive women (Manson et al. 1991). The positive effects following an acute bout of exercise include an enhancement in glucose uptake into skeletal muscle, via the GLUT-4 facilitated diffusion; this is partly an insulin-independent contractile effect, which persists for several hours after the cessation of exercise (Young et al. 1987). In addition, the response of the glucose transport system to insulin is enhanced; usually lasting about 48 hours (Mikines et al. 1988). The enhanced transport system is important for individuals who have dysfunctional glucose and insulin regulation as exercise may help to improve the effectiveness of mechanisms where glucose and insulin are involved, most importantly energy metabolism. Beneficial metabolic changes are correlated with the loss of body weight and body fat due to increasing energy expenditure with exercise. This has been reported to be regardless of exercise intensity (Bouchard et al. 1993). Exercise over months and years, may well stimulate long-term effects on whole-body insulin sensitivity, which are independent of qualitative changes in insulin-mediated transport in muscle, due to body composition changes i.e. decreased adipose tissue and increased muscle mass. The positive effects of exercise are well documented and have consequently resulted in reduced appetite sensations in lean and obese rats (Ramirez et al. 1997. Short and long-term effects of exercise facilitate to improve energy expenditure by acting on the hypothalamus to coordinate the neuro-endocrine and appetite responses to exercise (Engler 2007) and therefore maintain energy balance.

1.14   METHODS FOR MEASURING ENERGY INTAKE AND ENERGY EXPENDITURE

One of the major obstacles in nutritional epidemiology research has been uncertainty about the validity of existing dietary assessment methodologies (Block 1982). The doubly labeled water method for measuring total daily energy expenditure can serve as a reference for assessing the accuracy of conventional dietary intake methodologies (Schoeller 1990). This concept is based on the premise that the measurement of total energy expenditure in free-living individuals can serve as a proxy measure of energy intake when subjects are in energy balance (Poehlman 1992). Studies measuring energy intake have found that self-reported food intakes underestimated habitual energy intakes (Livingstone et al. 1992; Bandini et al. 1990). However, they lend themselves better to large epidemiological studies due to their advantages; such as low cost, ease of completion and they are adaptable to most research settings. Self-reported measurements of energy intake include weighed records, 24-hour recall, food frequency questionnaires. There are other methods of estimating energy intake such as prediction equations which measure energy expenditure and are used on the premise that the measure of EE can serve as a alternative measure of energy intake when individuals are in energy balance (Johnson et al. Although, accuracy is questionable and there is no gold standard method of measuring energy intake in free living subjects, a protocol which validated the 4-day weighed intake (Bingham and Day 1997) made this the method of choice in the current study.

There are many different methodologies for measuring energy expenditure. Measurements of daily energy expenditure (EE) are made to estimate energy

requirements. Measurements are either 1) continuous i.e. direct and indirect calorimetry, 2) factorial i.e. activity diaries, accelerometers, 3) heart rate monitoring and the determination of individual oxygen consumption, 4) doubly labeled water or 5) energy intake in conjunction with changes in body composition. Continuous measurements of EE would give the most reliable results however are not feasible for studies on free living individuals. For this reason the current study utilised factorial methods for the measurement of EE.

1.15   SUMMARY OF THE CURRENT RESEARCH IN FOOD INTAKE

Body weight should be well regulated through biological control of food intake. The regulation of food intake is often seen to be controlled via the brain, as a monitor of peripheral body fat stores via messages such as leptin, insulin and acetylation-stimulation protein (Levitsky 2005), initiating a set of peptide and neuro-endocrinal events that constitute the neural substrates of eating behaviour and energy expenditure. Therefore biologically, it is clear that body weight is not well regulated. There is a failure in one or more of the feedback systems which controls energy balance that leads to the development of obesity. Research into feeding behaviour and body weight control has taken two approaches; appetite and energy balance. In the research of appetite the area measured is normally food intake and is often concerned with the qualitative aspects of eating such as food choice, preferences and the sensory aspects of food, together with the hunger, fullness and the hedonic sensations which accompany eating. Among energy balance research the area measured is usually energy intake and is concerned with the quantitative aspects of eating and with the energy value of food i.e. macronutrient composition of food and

its impact on energy balance. Research on appetite control has been heavily influenced by psychologists and energy balance research has been the spectrum of physiologists, biochemists and nutritionists. These two approaches to research into 'feeding behaviour' conflict when explaining abnormal eating and the consequences for weight gain. It is unclear whether obesity is brought about by enhanced feelings of hunger, ill-judged food choices, weakened satiety, sensorily induced over-consumption, from inadequate oxidation of fuels, or different neuro-endocrine responses to dietary intake. The divergent approach into the investigation of feeding behaviour reveals that the mechanisms controlling feeding are, not only biological but, mutifactorial and complex and are characterised by the on-going interaction between peripheral and central mechanisms that sense and respond to changes in the external environment i.e. nutrient supply and temperature, and to the internal environment i.e. changes in plasma glucose. It seems apparent that the homeostatic control of feeding behaviour is less well designed to cope against an excessive environmental supply of energy and nutrients (Blundell and Stubbs 1997).
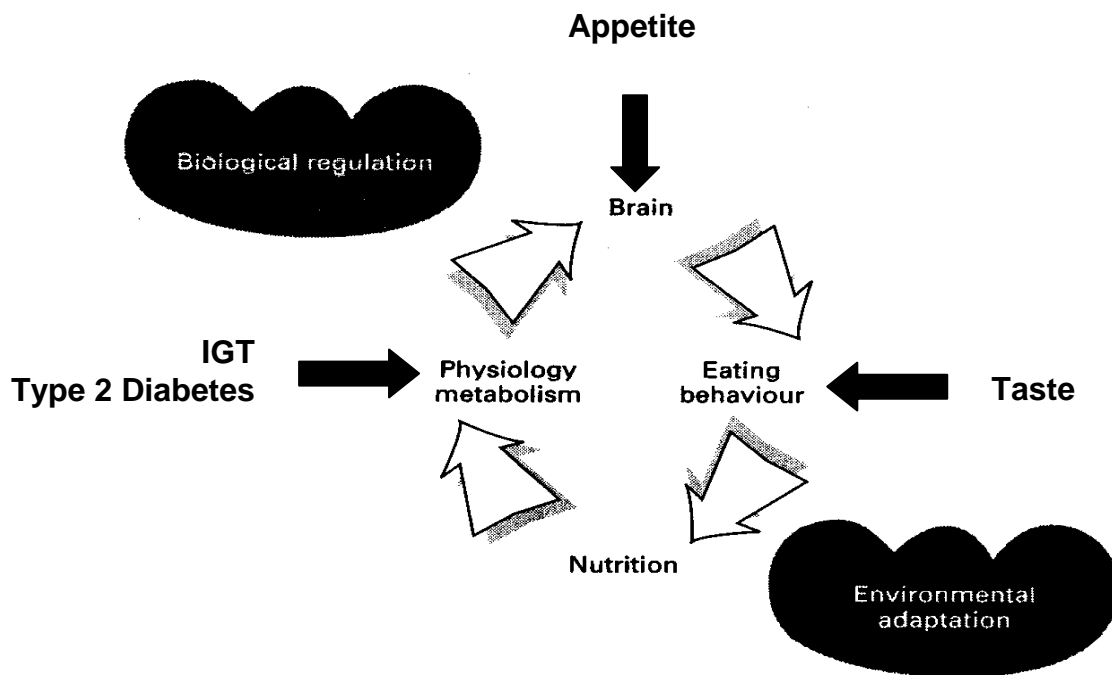
Figure 1.6 Modified schematic diagram illustrating that the pattern of eating behaviour arises from an interaction between biological regulation and environmental adaptation. (Blundell and Tremblay 1995). IGT and Type 2 Diabetes affects the physiology of metabolism, appetite may affect afferent brain signals, and taste sensitivity may affect eating behaviour, all denoting a complex interplay of mechanism controlling the homeostasis of food intake.

1.16   GENERAL AIMS OF THE THESIS

The aim of the study was to investigate the components involved in the metabolic handling of food and how the responses differ between IGT, type 2 diabetic and control participants.

1.16.1 Objectives of the Thesis:

- To determine anthropometric measures in IGT, type 2 diabetics and a healthy control group and compare differences.

- To investigate differences in habitual energy intake and energy expenditure and compare the differences between IGT, type 2 diabetics and control participants.

- To measure taste in IGT, type 2 diabetics and a healthy control group.

- To investigate subjective ratings of appetite including; hunger, satiety, fullness and prospective consumption.

- To measure energy expenditure and diet induced thermogenesis in IGT, type 2 diabetics and a healthy control group.

- To determine fasting and postprandial rates of substrate oxidation in IGT, type 2 diabetics and a healthy control groups.

- To determine the integrated response to a test meal measuring glucose, insulin and the novel hormone ghrelin.

- To identify any associations between taste, appetite, blood profiles and food intake.

CHAPTER 2 - METHODS

2.1   PARTICIPANTS

Participants eligible for recruitment were between the ages of 20 and 70 years in accordance with the inclusion criteria. Impaired glucose tolerant (IGT) and type 2 diabetic participants' blood glucose levels must have been taken within the last 12 months and must have still been within the range for diagnosis of that disease, see the diagnosis range below.   The participants blood glucose concentrations determined whether they were placed into the type 2 diabetic group or the IGT group.   IGT and type 2 diabetic participants were recruited having a body mass index (BMI) of greater than 30, were not taking any antihyperglycemic medication, and were free from any other metabolic disease as per the inclusion criteria.

Table 2.1 Definition and Diagnosis Criteria for IGT and Type 2 Diabetes

	IGT	**Type 2 Diabetes**
Fasting	<7.0 mmol/l	≥7.0 mmol/l
2 Hour/Random	≥7.8 and ≤11.1 mmol/l	≥11.1 mmol/l

WHO 1999

A group of healthy control subjects were also recruited who had no diagnosed metabolic disorder.   Exclusion criteria included patients who were claustrophobic because they had to undergo tests which involved being covered by a hood and patients must have been free from any other metabolic disease.

2.1.1 Recruitment

Patients at the Western General Hospital (WGH) in Edinburgh were recruited by screening patient records to identify their eligibility as type 2 diabetic participants. When they attended their outpatient appointment at the diabetic clinic within the WGH, subjects were then approached and were informed about the study and provided with an information sheet. Participants were given one week to read the study information before they were contacted by telephone where the content of the study was discussed further and participants were recruited if willing. Both appointments for the study were made with the participant at this stage. IGT participants were recruited from the same diabetic clinic although it was also necessary to involve GP practices in Lothian. This was done by sending letters out to GP practices outlining the study and asking for access to their patients (appendix 1). Patients meeting the inclusion criteria were identified by searching on GPASS and then by mailing the study information to those patients on behalf of the practice. Participants completed and sent back a 'patient details' form (appendix 2) to become involved in the study and then contact was made by telephone to discuss the study. At this stage if the participants were willing to be involved in the study both appointments were made. Control participants were recruited by advertisement in Queen Margaret University. All subjects were provided with verbal and written information regarding the study and informed consent (appendix 3) was obtained from all participants. Participants were provided with the name, telephone number and email address of the study's independent advisor. Once recruited, participants were requested to attend 2 sessions with less than 1 month between the initial and second test day. The first session was simply introducing the participants to the

environment, nursing staff and to equipment that would be used on the main study day, particularly the calorimeter to ensure they felt comfortable with it and all the other procedures. Height, weight and BMI were recorded at the first session and subjects were instructed to complete diet and activity diaries to bring along to their second session and finally to complete a 24 hour urine sample to be collected on arrival for their second session. The second session was when the test procedures were carried out. Participants were asked to avoid alcohol for the 24 hours prior to the study days to ensure a normal hydration status and were also asked to avoid exercise the day before the study days to ensure a normal metabolic status. Participants were also instructed to fast for 12 hours prior to commencing the second study day (water was allowed during this period) to allow for fasting data to be collected.

2.1.2 Experimental Protocol
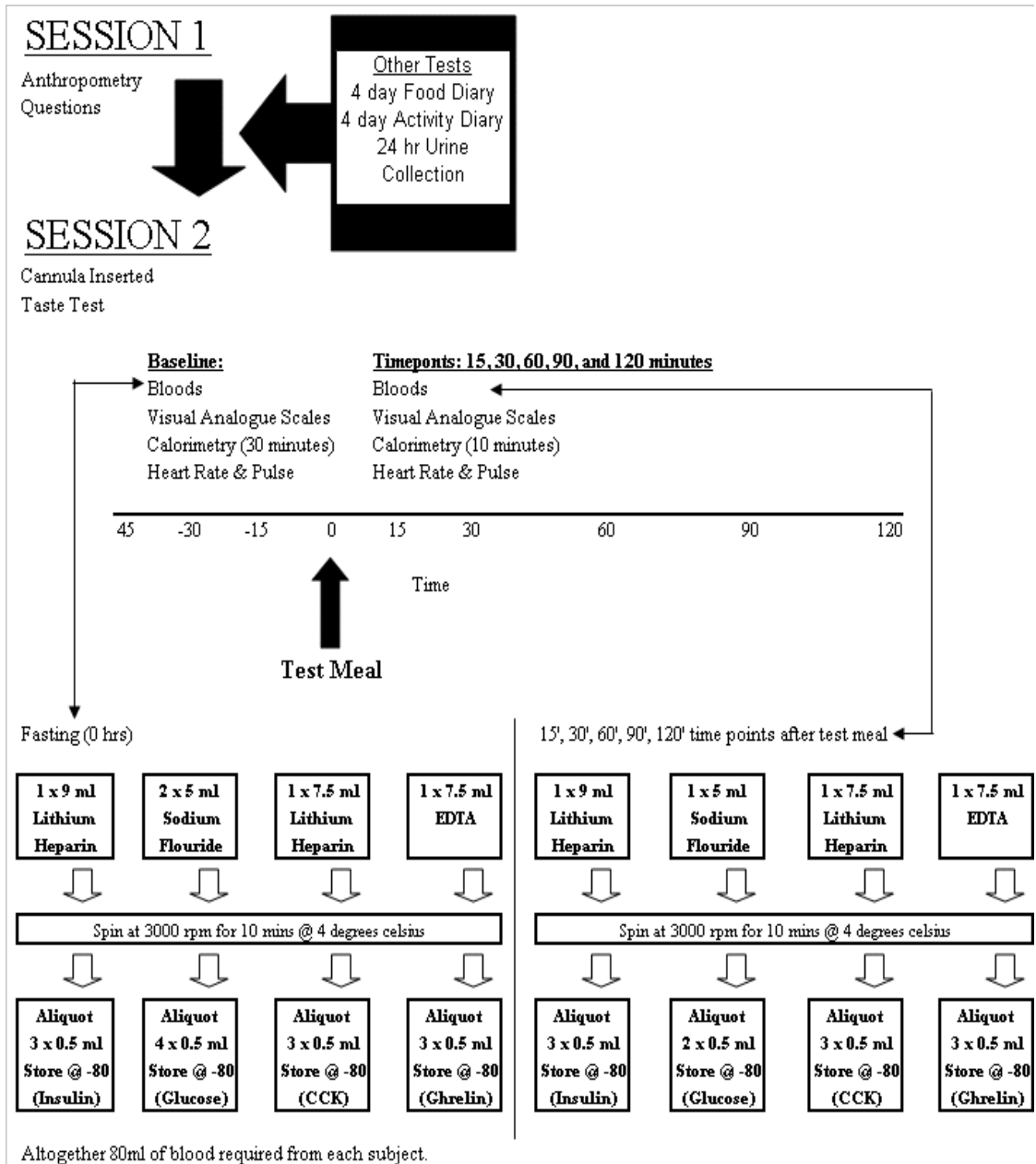
Figure 2.1 Schematic Diagram of the Study Protocol

The study was approved by the Wellcome Trust Clinical Research Facility (WTCRF) following a proposal and application for clinical assistance. A feasibility review was undertaken by them to determine costs and approval was granted for 1 year, subject to ethical, and research and development (R&D) management approval from both Lothian Primary Care Trust (LPCT) and Lothian University Hospital Trust (LUHT). R&D management approval was subsequently given by both trusts (LUHT and LPCT) and an 'honorary contract' and a 'Disclosure Scotland' check was obtained before research could commence. All participants attended the Wellcome Trust Clinical Research Facility at the Western General Hospital, Edinburgh on two occasions to complete the study.

2.1.2.1 Participant – Session 1

On the first occasion participants were familiarised with the facility, the study protocol was explained and patients were shown all equipment to be used and then written consent was obtained. Anthropometric tests were completed i.e. height, weight, BMI, waist and hip measurements. Participants were instructed on the successful completion of the 4-day diet and activity diaries and were given verbal and written instructions (appendix 4) on the successful completion of a 24-hour urine collection, all of which had to be completed for attendance at their second session.

2.1.2.2 Participant – Session 2

On admission into the unit for session two, general practitioner, medical history and current medications were recorded for each participant. Initial blood pressure (BP) was recorded. To facilitate blood taking a cannula was placed in the brachial vein along with a minimal infusion of normal saline to ensure the cannulas' patency and then baseline blood samples were recorded. At this stage, guidance was given on successful completion of the Visual Analogue Scales (VAS) and all terms were explained e.g. hunger, satiety, fullness and prospective consumption. Baseline VAS measurements were recorded and time was given for the patient to relax into the environment. Each participant then underwent an initial period of indirect calorimetry for 30 minutes, with the first 10 minutes of results being discarded to allow the participant to acclimatise to the calorimeter. Subjects were asked to lie at rest in a supine position, with their arms away from their body and palms facing upwards, with their legs slightly apart, and to remain awake during the recording period. Immediately after this, participants were given a breakfast equal in calorific value including: 1 x 30g box of Kellogg's cornflakes, 200g of semi-skimmed milk, 1 piece of white toast with a portion of butter and a 200ml carton of freshly squeezed orange juice. The time taken to eat the breakfast was recorded. At specific time points throughout the morning, BP and pulse, VAS, bloods and 10 minutes indirect calorimetry were recorded. At 120 minutes the timer was stopped and the cannula was removed.

2.2   BODY COMPOSITION MEASUREMENTS

2.2.1   Height and Weight

Subjects' height and weight were measured. Subjects were asked to remove their shoes, heavy outer garments and jewellery, loose change, keys and mobile phones. Height was measured using a stadiometer (wall fixed height measure, CMS Weighing Equipment Ltd, London, UK) to the nearest 0.1 cm. Weight was measured to the nearest 0.1 kg (Seca 761, Birmingham, UK). Body Mass Index (BMI) was calculated by the division of weight (kg), by height (m²).

2.2.2   Waist and Hip Measurements

Waist is defined as the point midway between the iliac crest (superior border of hip bone) and the costal margin (lower rib). Hip is defined as the widest circumference of the buttocks and below the iliac crest. Subjects were asked to remove all outer layers of clothing, shoes, belts and any tight garments intended to alter the shape of the body. Waist and hip were measured twice to the nearest 0.1 cm using a tape measure.

2.2.3   Waist: Hip Ratio

Waist: hip ratio (WHR is defined at the waist circumference divided by the hip circumference). It is a measure of the distribution of abdominal fat (central obesity) Raised WHR has been shown to be associated with certain health risks and may be a stronger predictor than BMI of the risk of diabetes or insulin resistance. A high WHR

has been taken to be 0.95 or more in men and 0.85 or more in women (Department of Health 1995).

2.3   PHYSIOLOGICAL PARAMETERS

Blood pressure and pulse rate were also measured throughout the study period at the six determined timepoints. These were recorded using a Dinamap Compact Vital Signs Monitor 9000EM04 (Johnson & Johnson Medical Ltd, Gwent, UK) on the arm which was not cannulated for blood letting.

2.4   TASTE TEST

A taste test was used to examine how individual sensitivities to bitter-tasting compounds affects food intake by determination of the detection threshold for the taste of 6-n-propylthiouracil (PROP). PROP taste thresholds were determined using a series of 15 PROP solutions that ranged in concentration from 1.0×10^{-6} mmol/l to 3.2×10^{-3} mmol/l PROP. This range of concentrations increases in quarter log steps as per Drewnowski (Drewnowski et al. 1991; Drewnowski et al. 1997a and 1997b) as shown below:

Log (1.0×10^{-6}) = -6 to Log (3.2×10^{-3}) = -2.5 mol/l PROP

i.e. (-6.0, -5.75, -5.5, -5.25, -5.0, -4.75, -4.5, -4.25, -4.0, -3.75, -3.0, -3.25, -3.0, -2.75, -2.5).

170g PROP =   1L   = 1mmol/l

170mg PROP = 1ml 1mmol/l =   170 x 3.2g = 0.0032 =   544mg

170 x 1.8g = 0.0018 =   306mg

The highest concentration, solution no. 15 contained 0.5446 g/l; the next concentration, solution no. 14 contained 0.3064 g/l and so on. The 4 stock solutions (15, 14, 13 & 12) were diluted by 10 times to make the less concentrated solutions. The PROP chemical was purchased from Pfaltz & Baver, Waterbury, CT, USA as per Drewnowski (1997). The solutions were prepared and numbered at least one day before testing with tap water and stored at $4^{o}C$.

2.4.1   Testing procedures

Tests were always performed in the morning of the study, either in session 1 or 2. Subjects were requested to abstain from eating, drinking and brushing their teeth for 1 hour prior to the test. Each subject was given verbal instructions (Keller et al. 2002) on the correct completion of the taste test prior to starting the test and was standing alone with the experimenter in a hospital room. The sip-swirl-and-spit method was used and a rinse with tap water separated the sampling of the pairs of stimuli.

2.4.2   Detection Threshold Determination

Subjects were first presented with the reference solution (tap water) and then with two identical glasses, one containing a solution of the tastant (about 10ml of solution) at an intermediate concentration and the other containing tap water. Solutions were at room temperature. The participant was asked "Do you taste anything?" and if the response was "no" the participant was presented with a higher concentration and the result was recorded (see appendix 5). If they answered "yes", participants were required to indicate which of the two samples was identical to the reference. Wrong

answers led to the presentation of a higher concentration again paired with tap water, and correct answers to a lower concentration. Detection thresholds were measured using an up-and-down procedure with a forced choice pair presentation method (Dixon and Massey 1960). PROP tasters were defined as having thresholds of <1.0 x 10-4mmol/l (equivalent to solution 9) and non-tasters as having thresholds in excess of 2.0 x 10-4mmol/l (equivalent to solution 10). This separation criteria was similar to that adopted by Fischer et al (1963) who regarded the concentration of 1.88 x 10-4mmol/l as the threshold, and to that of Bartoshuk (1993) who used threshold values of 2.0 x 10-4mmol/l to separate tasters from non-tasters.

2.4.3 Suprathreshold Scaling

For each tastant to be scaled, subjects were asked to taste and rate PROP solution 12. The subjects were required to rate the intensity of bitterness using the labelled magnitude scale (LMS) with 6 categories (appendix 5) from 'barely detectable to 'strongest imaginable'. Verbal instructions were given on the use of the LMS (Appendix 6). Green's strategy was to include only as many descriptors as subjects could comfortably use, as too many may confuse subjects and too few may leave gaps in the scale and thus reduce its usefulness (Newstead and Griggs 1984). The LMS is a quasi-logarithmic scale with semantic descriptors that is equivalent to magnitude estimation scaling (Green et al. 1993). The placement of semantic descriptors along the length of the scale makes this scale easy for subjects to understand and use. The scale (see figure 2.2) is 165 mm in length and is anchored at the bottom with the phrase 'barely detectable' and at the top with 'strongest imaginable'. The top of the scale, 'strongest imaginable', on the LMS refers to the strongest oral sensation an

individual has been exposed to in everyday life (Tepper and Ullrich 2002). This general description of descriptors has revealed no discernable differences in the ratings for PROP than when an explicit definition was used (Sposato et al. 2001) i.e. 'strongest imaginable oral sensation' as opposed to 'strongest imaginable sensation of any kind'. The scale is particularly useful for collecting intensity responses to strong stimuli such as PROP because it permits an individual to rate a stimulus based on the full range of his/her everyday experiences rather than truncating the individual's response to a standard descriptor (i.e. very strong). Since the intensity of PROP to supertasters can exceed the limits of a standard rating scale, the LMS avoids ceiling effects often associated with the use of standard, scaling techniques (Prutkin et al. 2000).

Participants rinsed their mouth with tap water before they began and between each sample. They were required to place the whole sample in the mouth (10ml), expectorate it and rate its intensity by making a single mark on the scale. Subjects could then rinse their mouth.

Subjects were independently classified by the one-solution test. For non-tasters, the upper limit of the confidence interval reached a value of 15.5, approximately 'moderate' on the LMS (Tepper 2001). The lower limit for supertasters occurred at a value of 51, corresponding to 'very strong' on the scale. Medium tasters fell within the intermediate range, >15,5 and <51. Some subjects gave borderline ratings for PROP. When this occurred, the individual's detection threshold determination results were considered.

Single Solution Test

```
┌─  Strongest imaginable




├─  Very Strong



├─  Strong



├─  Moderate


├─  Weak
└─  Barely Detectable
```
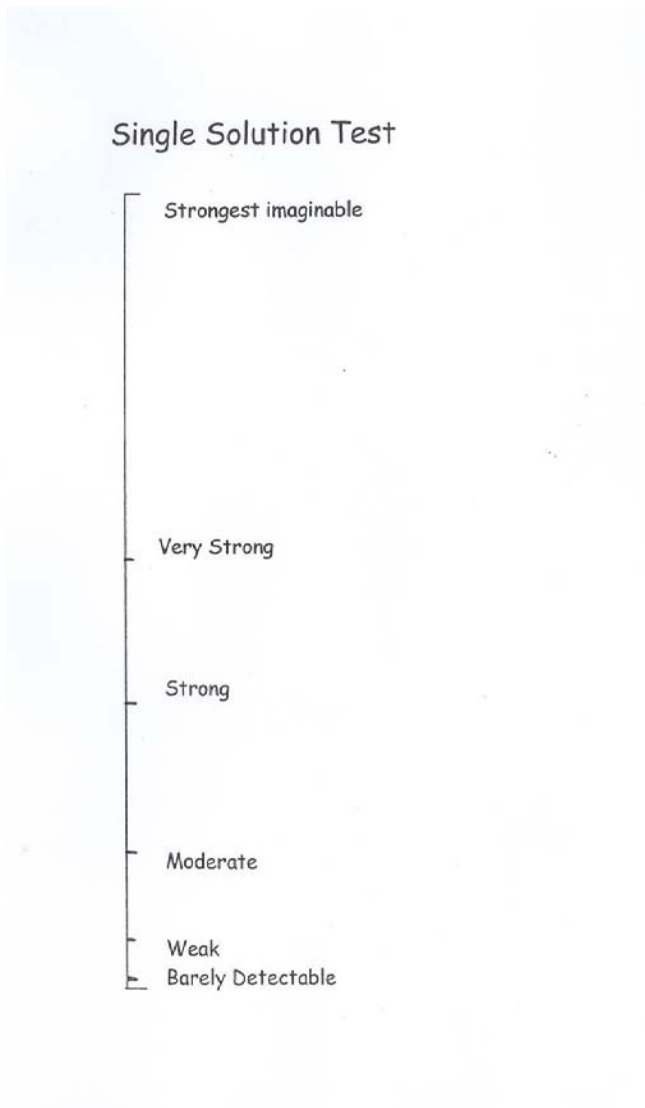
Figure 2.2 The Labelled Magnitude Scale used for the PROP Taste Testing for a Single Solution Test devised by Green et al (1993). The version used by the experimenter includes a numerical scale (Appendix 5)

2.5   FOOD INTAKE AND ENERGY EXPENDITURE MEASUREMENTS

2.5.1   Diet Diary

Subjects completed a 4-day food diary (Bingham et al. 1994) so that food intake could be measured in kilocalories per day. Subjects were given verbal and written instructions on successful completion of the diary and were also given a telephone number to contact with queries regarding the diary (appendix 7). Subjects were asked to complete the diary within the course of one week and to record three week days and 1 weekend day. This would take into account the day of the week variations. It has often been stated that there is no gold standard that can be used to validate prospective methods of dietary assessment carried out in free living subjects however, a validation protocol (Bingham and Day 1997) specified a 4 day weighed food record was developed to minimise the burden on volunteers asked to weigh and record their food for prolonged periods. Subjects also received a portion sizes photograph book (Nelson et al. 2002) to help estimate weights while out with the home. To assess the validity of the weighed-food records, average dietary nitrogen intake from the 4-d weighed food record was compared with average urinary nitrogen output from the 24-hr urine collection. All diet diaries were analysed by the author using the dietary analysis computer programme, Comp-eat 5 (Carlson Bengston Consultants Ltd, London, UK). The reported intake was analysed and percentage energy provided from fat, carbohydrate and protein was assessed.

2.5.2 Activity Diary

Subjects were asked to complete a 4-day activity diary (appendix 8) so that energy expenditure including activity could be estimated in kilocalories per day. Again, subjects were given verbal and written instructions on successful completion of the diaries and were also given a telephone number to contact with queries regarding the diaries. Subjects were required to keep a detailed record of all activities carried out during the day, including, showering, eating, walking, sitting at work/in front of the television, doing housework and also of any leisure or sporting activity. The time of day, the amount of time spent carrying out the activity and the intensity of the activity were noted in the diary. Physical activity levels (PAL) (WHO 1985) were calculated by giving 'weights' to activities depending on their intensity, e.g. sleep/rest is given a weight of 1.0 whereas heavy activity is 7.0. Energy expenditure was then calculated for each activity within the 24 hours by multiplying the total time and intensity for each activity by the calculated hourly basal metabolic rate (BMR). BMR was calculated using the equations of Schofield et al (1985) which utilise height and weight as an indicator of BMR. Total daily metabolic expenditure was then calculated by adding the sum of the energy expended for each activity (Gerrior et al. 2006).

2.6 TEST MEAL

On study days participants were booked in to arrive at their preferred time between 8-9am therefore breakfast was used as a test meal in this study. This time was booked in with the WTCRF so that they could arrange parking (if necessary) and greet the

participant on their arrival. An identical, isocaloric meal was used for all subjects so that the difference between the groups could be identified. Each test meal was made immediately prior to consumption by the researcher. The subjects were instructed to consume the test meal as quickly as was comfortably possible and the importance of finishing the meal was stressed. Time taken to finish the breakfast was recorded. The composition of the test meal is provided below:

Table 2.2 Composition of the Test Meal and Energy Value of each Component

Food Item	Quantity	Energy value from CHO (kcals)	Energy Value from Fat (kcals)	Energy Value from Protein (kcals)	Total Energy Value kcals
Kellogs Cornflakes	30g	100.8	2.43	9.48	112.8
Semi-skimmed Milk	200 ml	35.25	30.6	27.2	93.1
Brown bread toasted	25g	46.125	6.25	11.2	63.75
Butter	15g	0.34	110.97	0.36	111.69
Fresh Orange	250ml	75.94	trace	0.36	82.5
Totals (kcals)		258.45	150.25	48.6	463.84
Macronutrient (%)		(56%)	(32.5%)	(10.5%)	

2.7 MEASURES OF APPETITE BY VISUAL ANALOGUE SCALES

Visual analogue scales (VAS) measures a characteristic or attitude that ranges across a continuum of values and is a 100mm in length anchored by word descriptors at each

end. VAS were used to measure subjective sensations of hunger, satiety, fullness and prospective food consumption (see appendix 9). Each subject was given verbal instructions on the correct completion of the VAS and the four subjective measures of appetite were explained. Hunger was classified as the subjective desire to consume food, satiety as the subjective feeling of 'satisfaction' from food and was classed as a positive feeling. Subjects were asked to differentiate between the fullness sensation, which was described as a 'gastric' or 'stomach bloated ness' feeling and could be classed as a negative feeling. Prospective consumption was described as the ability to consume more food, which subjects had to quantify using the scale. Subjects were asked to mark the scale at the point which best described the extent of their feelings for each category. The visual analogue scales were quick and easily scored, measuring the distance along the 100 mm line to the mark the subject made. VAS rating scales were used as they are more sensitive and less restrictive than category scales, which may produce bias. VAS have previously been shown to encompass some ability to predict aspects of feeding behaviour and act as a useful adjunct to measures of food, energy and nutrient intake. VAS have also been shown to be sensitive to experimental manipulations, provided those manipulations exceed or disrupt the effects of the habituated motivation to eat (e.g. hunger at meal-times), and they have been shown to have good reproducibility (test-retest reliability) under controlled conditions provided that they are used in within-subject designs (Stubbs et al. 2000). Limitations of the VAS were that the subjects were still restricted by the end dimensions of the scale and that assessing the validity was difficult because they could not be compared with an objective physical measure (Maxwell 1978). The latter can be overcome if examining the VAS for the hunger measurement before and after a meal as it was in this study. The scales were used to analyse fasting (baseline)

levels and at 5 further points throughout the study period (see figure 2.1). At each timepoint a fresh VAS was used so that comparisons could not be made with previous results.

2.8   INDIRECT CALORIMETRY

Indirect calorimetry was used to determine energy expenditure and substrate oxidation in the study. Following the study protocol, indirect calorimetry was used on each subject at regular intervals of the 3½ hour study period using an indirect calorimeter (Datex Deltatrac Metabolic Monitor, Hatfield, Hertfordshire, UK). A known rate of airflow through the hood (at steady state conditions air flow is set to maintain a concentration of carbon dioxide (CO_2) in the chamber of approximately 0.5%) enables the gas analysers to interpret oxygen consumption and carbon dioxide production. Deltatrac analysers measure the volume of oxygen consumed, using a paramagnetic sensor, as oxygen has a large paramagnetic susceptibility. Carbon dioxide produced was measured using an infrared sensor.

2.8.1   Operation of the Calorimeter

Before initiation of the current study the calorimeter was serviced by a Datex engineer. Regular calibration of the flow rate and respiratory quotient (RQ) of the Deltatrac calorimeter was performed using an alcohol burn kit in accordance with the manufacturer's instructions. Flow was checked by burning a known amount of pure ethanol in a Deltatrac designed alcohol burner. This check of the flow rate enabled

adjustments to be made to the calorimeter and also checked accuracy of the RQ (Ethanol RQ = 0.67) for any problems with the linearity of the gas sensors.

The calorimeter was switched on, in the morning of the study, at least 30 minutes prior to the commencement of the study. Before usage, the gas sensors were calibrated using a gas mixture of 5% carbon dioxide and 95% oxygen (Quick CAL, calibration gas, Datex, Helsinki, Finland). Calibration for atmospheric pressure was also performed using a barometer as this may contribute to drifts in the gas sensors. Prior to the initial calorimetry measurement, subjects were informed of the basic procedure for using the calorimeter and the duration of each measurement. To ensure the subjects were in a stable state, a 10 minute acclimatisation period was included at the start of each test day. Subjects were measured in a quiet, private room and were given no stimulus. To ensure accurate readings, subjects remained awake throughout measurement periods and the researcher or research nurse stayed with the subject at all times throughout the measurement periods. Indirect calorimetry measurements were taken in a fasted state for 30 minutes, and then at 15, 30, 60, 90 and 120 minutes following the test meal, with the measurement lasting for 10 minutes on each occasion over the study period.

2.8.2   Limitations of Calorimetry

Limitations of indirect calorimetry are that the data gives no information about the substrates being oxidized in individual organs and tissues. Furthermore, indirect calorimetry assumes a single value for the volumes of O_2 (oxygen) consumed and CO_2 (carbon dioxide) produced during the complete oxidation of one gram of each of

the substrates.   However, these values depend very much upon the type of substrate being oxidised (Table 2). For example, oxygen consumption depends on whether the sugar is a mono, di, or polysaccharide. The carbohydrate energy source of the body in the postabsorptive state is glycogen, which will consume 829 ml of O_2 per gram of glycogen oxidised. If however, one is studying a subject or patient who is receiving glucose, carbohydrate oxidation expressed as glucose will be underestimated by 11% if the starch (glycogen) value is used in the calculations. Protein oxidation holds similar limitations in that the volume of oxygen consumed per gram of protein will change depending on whether the protein is primarily a meat, milk or cereal. Unlike protein and carbohydrates, the amount of oxygen consumed per gram of fat oxidised varies little depending on its source.

Table 2.3 Oxygen Consumption for Nutrient Oxidation (Jequier et al. 1987)

	Oxygen consumed per gram of nutrient Oxidised (litres)
Protein	0.966
Beef muscle	1.008
Casein	1.008
Zein (Maize Protein)	1.052
Carbohydrates	
Starch	0.829
Sucrose	0.786
Lactose	0.786
Glucose	0.746
Fat	2.019
Corn Oil	2.015
Pig fat	2.037

Also, hyperventilation during indirect calorimetry can influence the measured O_2 consumption, CO_2 production, and the respiratory quotient. During hyperventilation CO_2 is eliminated in excess of that produced by oxidative metabolism and there is a decrease in the bicarbonate pool. CO_2 elimination increases rapidly and exceeds concomitant O_2 consumption which results in a respiratory quotient greater than 1.0.

Hyperventilation is normally followed by a compensatory period of hypoventilation during which metabolically produced CO_2 is stored to re-equilibrate the bicarbonate pool (Jequier et al. 1987). To overcome this limitation it was ensured that no participants were claustrophobic and that all participants were given time to relax before the procedure commenced. Jequier et al (1987) stated that the mean values for carbohydrate and fat oxidation will be correct provided that the respiratory exchange measurements encompass these transient changes.

Lastly, the calculation of energy expenditure in indirect calorimetry cannot take into account any of the oxygen and carbon of amino acids that remain combined with nitrogen, and excreted as nitrogenous products in the urine. To overcome this limitation, urine was collected to measure urinary nitrogen and estimate the contribution of protein oxidation to the O_2 and CO_2 measurements.

2.9 RESPIRATORY QUOTIENT

Respiratory quotient (RQ) was calculated through the measurement of oxygen consumption and carbon dioxide production. RQ is the ratio of moles of carbon dioxide produced to the moles of oxygen consumed on oxidation of a given amount of each nutrient. These values are close to 0.7 for fat, 0.8 for protein, 1.0 for carbohydrate and 0.8 for a mixed macronutrient meal. Energy expenditure per litre of oxygen consumed is very similar for all three major nutrients, however RQ can be used to determine substrate oxidation (when urinary nitrogen is also calculated).

2.9.1 Non-protein Respiratory Quotient

With the knowledge of RQ, non-protein respiratory quotient (NPRQ) and substrate oxidation rates could be determined. NPRQ was estimated by subtracting the volume of oxygen consumed and carbon dioxide produced per gram of protein oxidised, from the total volume of oxygen consumed and carbon dioxide produced as shown in the equation below:

$$NPRQ = \frac{VCO_2 - PVCO_2}{VO_2 - PVO_2}$$

Whereby $\quad PVCO_2 = N \times 6.25 \times 0.774$

$\qquad\qquad\quad PVO_2 = N \times 6.25 \times 0.966$

And $\qquad\qquad N = $ Urinary nitrogen (g/day)

2.10 <u>URINARY NITROGEN</u>

Participants were asked to bring a 24 hour urine collection along on the morning of the second session at the WGH which was collected in the 24 hours prior to commencement of session 2. Urine was collected for a period of 24 hours to determine nitrogen excretion and permit calculation of the NPRQ (non protein respiratory quotient). Total urine was measured and 2 aliquot samples were frozen (-40°C freezer) for later analysis.

Urinary nitrogen was also used as an indicator of the validity of dietary intake (Bingham & Cummings, 1985) and as such was built into the methodology of the current study to objectively verify the diet diaries because it is independent of the techniques used to test dietary intake. Even with the daily variation of dietary intake the average nitrogen output was reported to be a constant fraction of nitrogen input (Bingham & Cummings, 1985).

2.10.1 Procedure for determination of Urinary Nitrogen

Urine was analysed using a Leco-FP328 protein/nitrogen analyser. The Leco was calibrated following the manufacturers instructions on a daily basis. Initially power-up checks were performed by the Leco after it was switched on. Blanks were inserted to assess the percentage of nitrogen and argon impurities in the oxygen gas used and finally EDTA was used for calibration as the nitrogen content of this is known precisely at 9.59%.

A 200 µl specimen was pipetted and weighed into a tin foil sample cup to be analysed. The Leco then performed its 3 phase analyse cycle. Phase 1 purged the sample of atmospheric gases, phase 2 burned the sample in an $850^{o}C$ furnace flushed with oxygen to ensure rapid combustion. The combusted products (mainly CO_2, H_2O, NO_x and N_2) were then passed through a thermoelectric cooler to remove most of the water. In phase 3 a piston was forced down on the gas products and a 10 cc aliquot of the sample mixture was collected through a valve. This was then swept through hot copper to remove oxygen and change NO_x to N_2 then, through lecosorb and anhydrone to remove CO_2 and water respectively. The product remaining was

nitrogen contained in a helium carrier and this was measured by a thermal conductivity cell. The result is displayed as a percentage of nitrogen. Total urinary nitrogen can then be calculated by multiplication of this figure by the subjects' total urine volume over 24 hours.

2.11   SUBSTRATE OXIDATION RATES

Indirect calorimetry measures the volume of CO_2 produced and O_2 consumed by oxidative processes, and for each litre of oxygen consumed there is a known amount of heat released. This is not constant but depends on the type of nutrients oxidised. By measuring oxygen consumption, carbon dioxide production, and urinary nitrogen, the proportion of different oxidative fuels can be calculated. The rate of substrate oxidation for each study period was calculated by the following formulas based on oxygen consumption (VO_2), carbon dioxide production (VCO_2), and urinary nitrogen excretion (Nu) and was calculated using the equations shown below (Suen et al. 1998):

Lipid oxididation (g/d) = 1.67 x (VO_2 –VCO_2) – 1.92 x Nu

Glucose oxidation (g/d) = 4.09 x (VCO_2 – 2.88) x (VO_2 – 2.59) x Nu

Protein oxidation (g/d) = 6.25 x Nu

2.12   BLOOD ANALYSIS

An indwelling cannula was placed in the brachial vein of each subjects arm at least 15 minutes prior to the beginning of the study. The cannula was kept patent with a 5ml

infusion of normal saline over the length of the protocol. Blood samples were withdrawn at various timepoints throughout the study period. To avoid any dilution effect, the first 2 millilitres (ml) of blood from each withdrawal were discarded. At each of the 6 timepoints for blood sampling 4 samples were withdrawn. These samples were withdrawn into blood tubes containing heparin, ethylenediaminetetraacetic acid (EDTA) and fluoride. These were inverted and placed into ice.

All blood samples were then centrifuged at 2500 rpm for 10 minutes (4°C), providing a relative centrifugal force of 1328. Plasma was then stored in a -80°C freezer at the Wellcome Trust research facility for subsequent batch analysis. All samples were centrifuged and stored within 30 minutes of blood letting. Analysis of Glucose, Insulin and Ghrelin concentrations were performed at the laboratory in QMUC.

2.12.1 Glucose

Glucose was analysed using the Glucose Liquid Reagent Hexokinase Method (Randox, United Kingdom). This method is totally enzymatic utilising both hexokinase and glucose-6-phosphate dehydrogenase enzymes. This is the principle reaction:

Glucose + ATP $\longrightarrow$ G-6-P + ADP

G-6-P + NAD^{+} $\longrightarrow$ gluconate-6-P + NADH + H^{+}
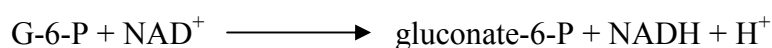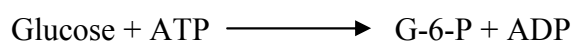
In this procedure 10μl of sample or standard was added to 1000μl of buffer solution and mixed and the absorbance (A_1) was measured at 340 nm wavelength as per the

method instructions. The enzyme reagent was then added (10µl) and the tubes were mixed again and incubated for 10 minutes at +20 - +25°C. The absorbance (A$_2$) was then measured within 30 minutes. The result of the following calculation was then calculated: A$_2$-A$_1$ = ΔA sample/standard. This test is linear up to a glucose concentration of 38.9 mmol/l. All tests were completed in duplicate and the average of the sample/standard was used in the following final calculation:

Glucose concentration (mmol/l) = ΔA sample/standard x 16.2

2.12.2 Insulin

Insulin was analysed using the Insulin Ultrasensitive ELISA kit EIA-2337 (DRG Diagnostics, Germany). This is a solid phase two-site enzyme immunoassay and it is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. In this procedure the microtiter wells, previously coated with a mouse monoclonal anti-insulin antibody, had 25µl of calibrator/samples and 100µl of enzyme conjugate added, and was incubated on a plate shaker for 1 hour at room temperature. During incubation insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing procedure then removes any unbound enzyme labelled antibody. Then 200µl of substrate 3,3',5,5',-tetramethylbenzidine (TMB) is added and the samples are incubated for a further 30 minutes at room temperature. The bound conjugate is detected by the reaction with TMB. The reaction is stopped by adding acid (0.5M H$_2$SO$_4$) to give a colorimetric endpoint and then shaken to ensure mixing. A microplate reader MRX (Dynex Technologies (UK) Ltd, Sussex, UK) with a

reference filter of 450nm was used to determine the absorbance as per the Elisa kit instruction guidelines. A standard curve was drawn for each kit and used the corresponding regression equation to determine results. All samples were measured in duplicate. The detection limit is <0.07 mU/l calculated as two standard deviations above the Calibrator 0.

2.12.3 Ghrelin

Ghrelin was analysed using the Human acylated Ghrelin enzyme immunoassay kit A05106 (Bertin Technologies, France). This EIA (enzyme immunometric assay) is based on a double-antibody sandwich technique. In this procedure the microtiter wells, previously coated with anti-ghrelin mouse monoclonal antibody (specific to the C-terminal part of ghrelin), had 100µl of standard/quality control/1:5 diluted samples and 100µl of anti-acylated ghrelin-AChE (acetylcholinesterase) tracer added (which recognises the N-terminal part of acylated ghrelin) and was incubated for 20 hours at $+4^O$C. This long immunological incubation allows the increase of assay sensitivity: 0.3 pg/ml versus 0.8 pg/ml for rapid (3 hrs at room temp) immunological incubation. This incubation period allows the two antibodies to form a sandwich by binding on different parts of the human acylated ghrelin. The sandwich is immobilised on the plate so a simple washing procedure allows the excess reagents to be washed away. The concentration of the human acylated ghrelin is then determined by measuring the enzymatic activity of the immobilised AChE. 200µl of Ellman's Reagent is added, which acts with AChE to form a yellow compound, and incubated in darkness at room tempterature for 30 minutes. A microplate reader MRX (Dynex Technologies (UK) Ltd, sussex, UK) with a reference filter was used to determine the absorbance at

405nm as per the kit A05106's instruction guidelines. A standard curve was drawn for each kit and used the corresponding regression equation to determine results. The limit of detection in the samples is 1.5 pg/ml due to the minimal plasma dilution.

2.13   ETHICAL APPROVAL

Ethical approval for the recruitment of control participants at QMU was approved on the 26th of June 2003. The study was approved by the Lothian Health Ethics Committee, Orthopaedic Surgery/Surgery Research Ethics Sub-Committee, Deaconess House, Edinburgh on 4th December 2003 and a subsequent amendment was approved on the 19th of January 2005 which approved an amendment in the criteria to the inclusion age changing it initially from 18-60 years to 18-70 years. Subsequent LPCT research and development committee approved the study on the 4th of November 2003 and LUHT research and development committee approval was gained on 24th November 2003. The honorary contract was gained on the 12th of December 2003 and from here Dr. John McKnight who is the consultant at the diabetic centre within the metabolic unit at the WGH and Dr. Casey Stewart, the consultant of OPD2 at the Royal Infirmary in Edinburgh were both contacted and consent was given to access patient records within both hospitals. Dr. Ewan Crawford who was at that time clinical director of north west Edinburgh LHCC at Corstorphine hospital became the study's independent advisor for the IGT and type 2 diabetic patient groups and he gave instruction as to which GP practices to contact regarding the study.

2.14   DATA ANALYSIS

2.14.1 Area Under the Curve (AUC)

AUC was used as a summary measure of some of the data such as, appetite parameters, EE and blood responses. AUC can be interpreted in these circumstances as the cumulative response from a series of measurements on each individual. In this study parameters were recorded at fixed time points and were calculated as per Altman (1991). The data are joined by straight lines to get a 'curve'. The AUC is usually calculated by adding the areas under the curve between each pair of consecutive observations. In the current study it was desirable to identify a single measure of primary interest to be able the comparison between the components of energy intake. AUC as a single measure enabled this to be done and was used to observe the total sensitivity or cumulative response of particular energy intake components. Interpretation is simplified by having one 'unit of measurement' per subject as opposed to many different time points.

2.14.2 Timepoints

Timepoints were used to demonstrate intensity at a certain time i.e. blood concentrations, RQ, EE and substrate oxidation. Timepoints give a record of how the intensity changes over time. In comparison to an AUC response with one value, timepoints will give information about where the changes in intensity lie.

2.15   STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, Version 15.0). To determine whether the study data was normally distributed the Shapiro-Wilks' test was used. Results with a value of $p<0.05$ were considered significant and in these cases data was analysed using the non parametric, Kruskal Wallis test and results with a value of $p>0.05$ were considered non-significant and in these cases data was analysed using parametric tests. Descriptive data has been expressed as mean ± standard error of the mean (SEM). Where data was found to be normally distributed the three groups of subjects were compared using analysis of variance (ANOVA) to reveal any statistically significant differences between groups. Subsequent Bonferroni adjustment tests were made to assess where the differences between groups occur. Repeated measures ANOVA were used to determine whether there was any statistical difference within groups over the study period.

Spearman's correlation was used to determine the strength and direction of the linear relationship between energy intake components and Pearson's correlation was used when the data was considered non parametric.

CHAPTER 3 - RESULTS

3.1   PARTICIPANTS

Forty-six participants were recruited for the research study; 11 impaired glucose tolerant participants from the diabetic clinic at the Western General Hospital and from GP practices, 17 type 2 diabetics from the Western General Hospital and 18 control participants from Queen Margaret University (QMU).   The mean age of IGT participants was 51 years, type 2 diabetic participants were 53 years, and control participants were 39 years. Age (yrs) was measured for comparison between groups and showed a significant difference between control and IGT participants (p=0.014) and control and type 2 diabetics (p<0.001). Gender profiles within the groups in the study included 5 males and 6 females within the impaired glucose tolerant participants, 8 males and 9 females in the type 2 diabetic group and 4 males and 13 females in the group.

3.1.1   Intergroup Comparisons of Anthropometric Characteristics

Anthropometric data collected included height (m), weight (kg), body mass index (BMI) (wt/ht²), waist and hip measurements (cm) and waist to hip ratio.   Intergroup comparisons of anthropometric characteristics were analysed using ANOVA with Bonferroni post hoc tests (Table 3.1).   Height (cm) was measured for comparison between groups and as expected indicated no differences.   Weight was significantly lower in control participants (65.2kg) compared with IGT participants (92.5kg),

p=0.002 and type 2 diabetic participants (91.6kg), p=0.005. BMI was significantly lower in the control group when compared with IGT participants (p=0.002) and type 2 diabetics (p = 0.000); see figure 1. Similarly, there were no differences between IGT and type 2 diabetic groups for weight or BMI. There were significantly higher waist and hip measurements in both IGT participants (waist, p=0.002 and hip, p=0.002) and type 2 diabetic participants (waist, p<0.0001 and hip, p<0.0001) compared to controls. IGT and type 2 diabetic participants did have higher waist-to-hip ratios (WHR) compared to control participants but there was no significant difference between groups. IGT and type 2 diabetics have been implicated in having impaired taste sensitivity because of the association with obesity and these two diseases (Mattes et al. 1990). However, it is not sufficiently known if the disease states of IGT and type 2 diabetes affects the sensitivity of taste to a greater degree as to alter the ratios of nontasters:tasters:supertasters.

Table 3.1 Anthropometric Measurements of IGT, Type 2 Diabetic and Control Participants

Characteristic	IGT n=11	Type 2 Diabetic n=17	Control n=18
Height (m)	1.68 (0.22)	1.62 (0.26)	1.67 (0.29)
Weight (kg)	92.5 (7.10)	91.6 (6.13)	65.2 (3.64)*
Body Mass Index (wt/ht²)	32.6 (2.23)	34.7 (2.02)	23.3 (0.80)*
Waist (cm)	108.1 (5.77)	110.8 (4.95)	83.6 (2.83)*
Hip (cm)	112.4 (4.94)	114 (4.62)	89.75 (2.41)*
Waist to Hip Ratio	0.96 (0.17)	0.97 (0.15)	0.93 (0.15)

Values are means ± SEM
* p < 0.01 compared to the other groups Bonferroni Pairwise comparison
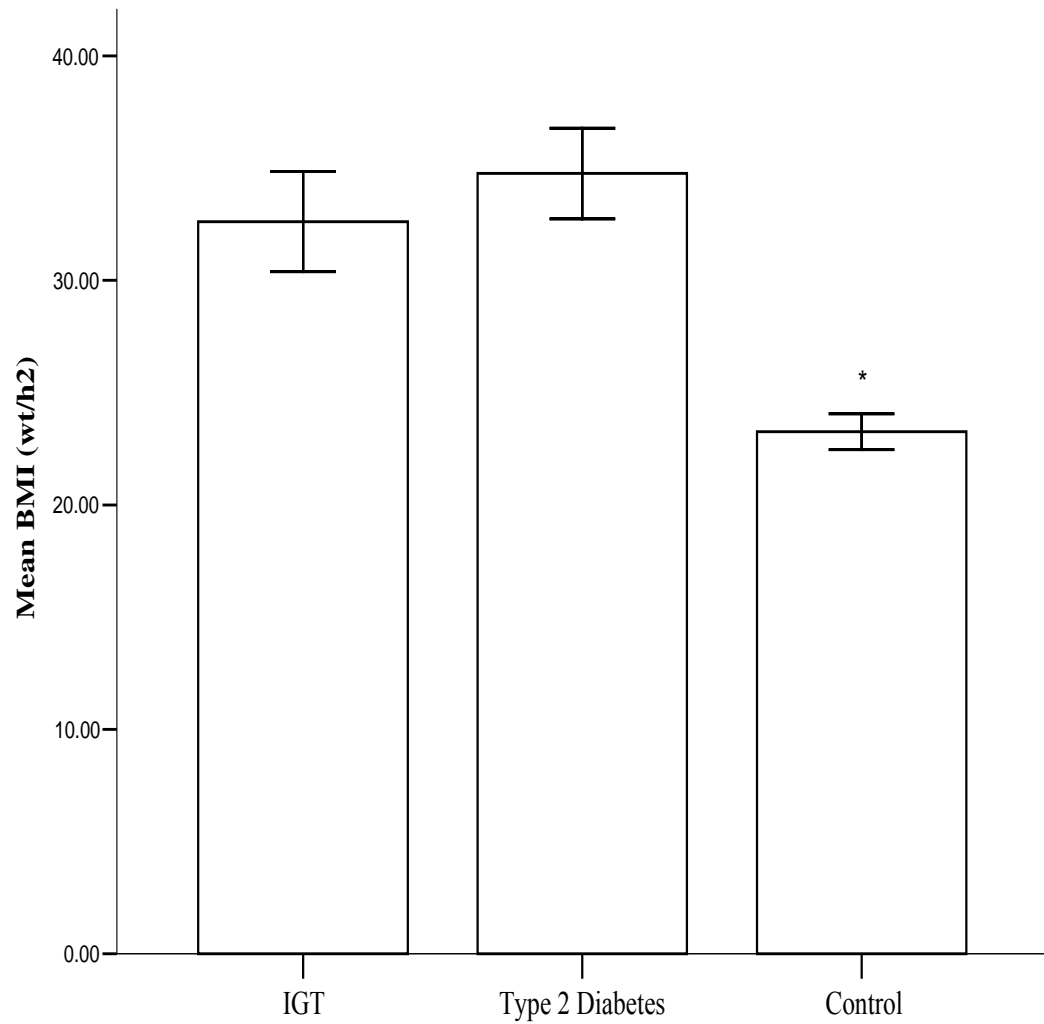
Figure 3.1 Mean Body Mass Index (BMI) of IGT, Type 2 Diabetic and Controls
* p<0.01 Control group significantly different from other 2 groups

3.2   TASTE

The protocol for the taste test is described in the methods chapter, but for clarity is described in brief below.   The values used for this analysis are from the rating of PROP solution 12 (5.75×10^{-4} mmol/l). Each person rated this solution on a 9-point category scale ranging from 1 = barely detectable to 9 = strongest imaginable taste. The mean scale tasted was analysed using Kruskal-Wallis tests to determine intergroup comparisons and Mann-Whitney tests were performed between nontasters, tasters and supertasters to determine where the differences lay between the taste groups. Furthermore, Mann-Whitney tests were also performed between IGT, type 2 diabetic and controls to determine where the differences lay between the study groups.

3.2.1 Intergroup Comparisons between Nontasters, Tasters, and Supertaster Groups

As expected non tasters rated the bitter PROP solution the lowest on the taste scale (barely detectable to strongest imaginable taste) and supertasters rated it the highest on the taste scale, close to the strongest imaginable taste. There was a significant difference between supertasters and both tasters ($p < 0.0001$) and nontasters ($p < 0.0001$), see figure 3.2. There was also a significant difference between tasters and nontasters, $p = 0.013$.

3.2.2 Intergroup Comparisons between IGT, Type 2 Diabetic and Control Groups

The Kruskal-Wallis test showed a p value close to significance, p=0.064 when using participants as a whole group and to detect an interaction with taste sensitivity rated on the taste scale. Therefore Mann-Whitney Tests were completed between the study groups. The subjective ratings of bitterness on the taste scale show that IGT participants rated the bitter taste the lowest (figure 3.3) and this was significantly different from type 2 diabetic participants and control participants ($p<0.05$).

Additionally, the taste detection test, which determined what concentration the bitter compound was first tasted, demonstrated that IGT participants first tasted the bitter solution PROP at the highest concentration when compared to the control group. The control participants were the most sensitive to PROP and first tasted the bitter solution at the lowest concentration (figure 3.4). To detect the interaction between the participants as a whole group and PROP taste detection a Kruskal-Wallis test was performed and showed significance at $p<0.01$. Therefore subsequent Mann-Whitney tests were performed. Results show that IGT participants' detection of the PROP solution was at a significantly higher concentration than control participants ($p<0.01$). There was no significant difference between type 2 diabetics and either IGT or control participants.
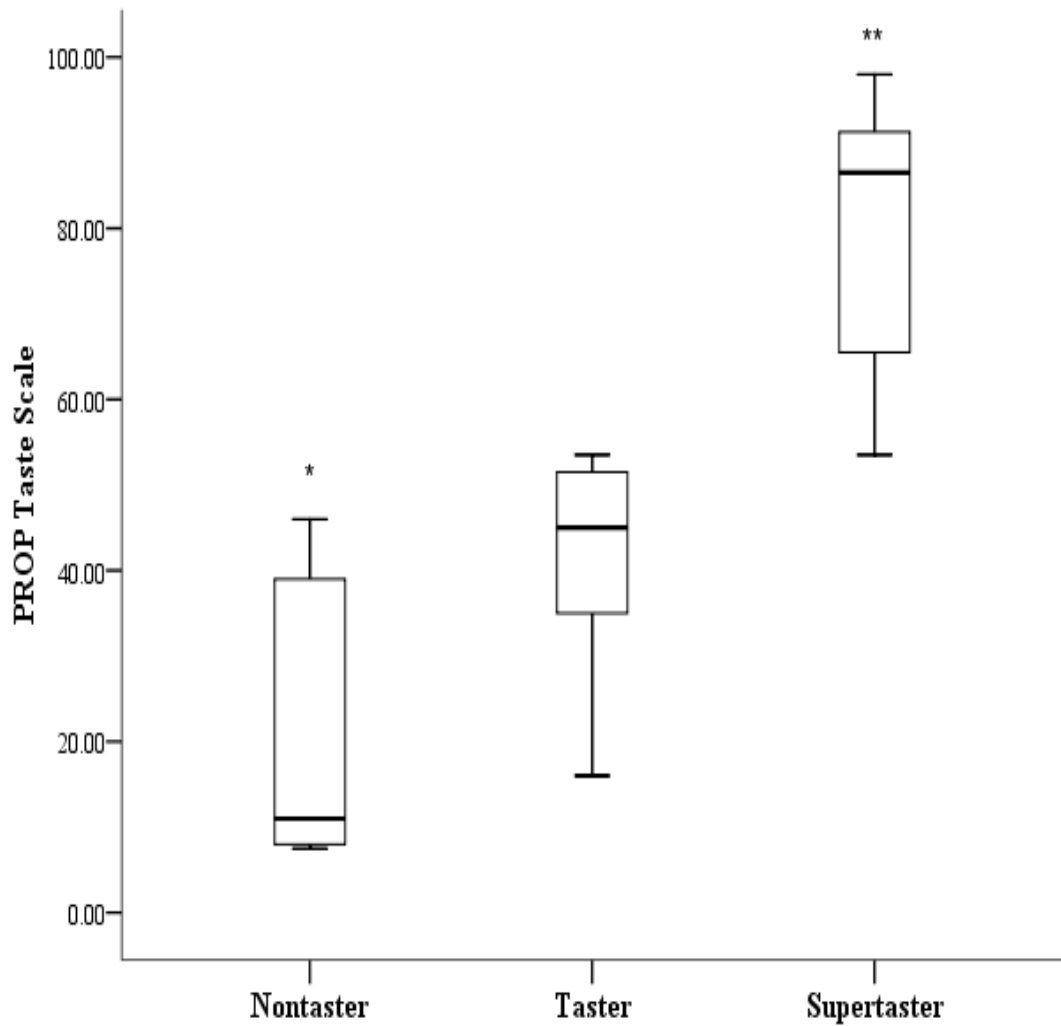
Figure 3.2 Taste Scale for bitter compound PROP for Nontasters, Tasters and Supertasters
Values are Median and Upper and Lower Quaritiles
*p<0.05 when compared to the tasters
**p<0.0001 when compared to nontasters and tasters
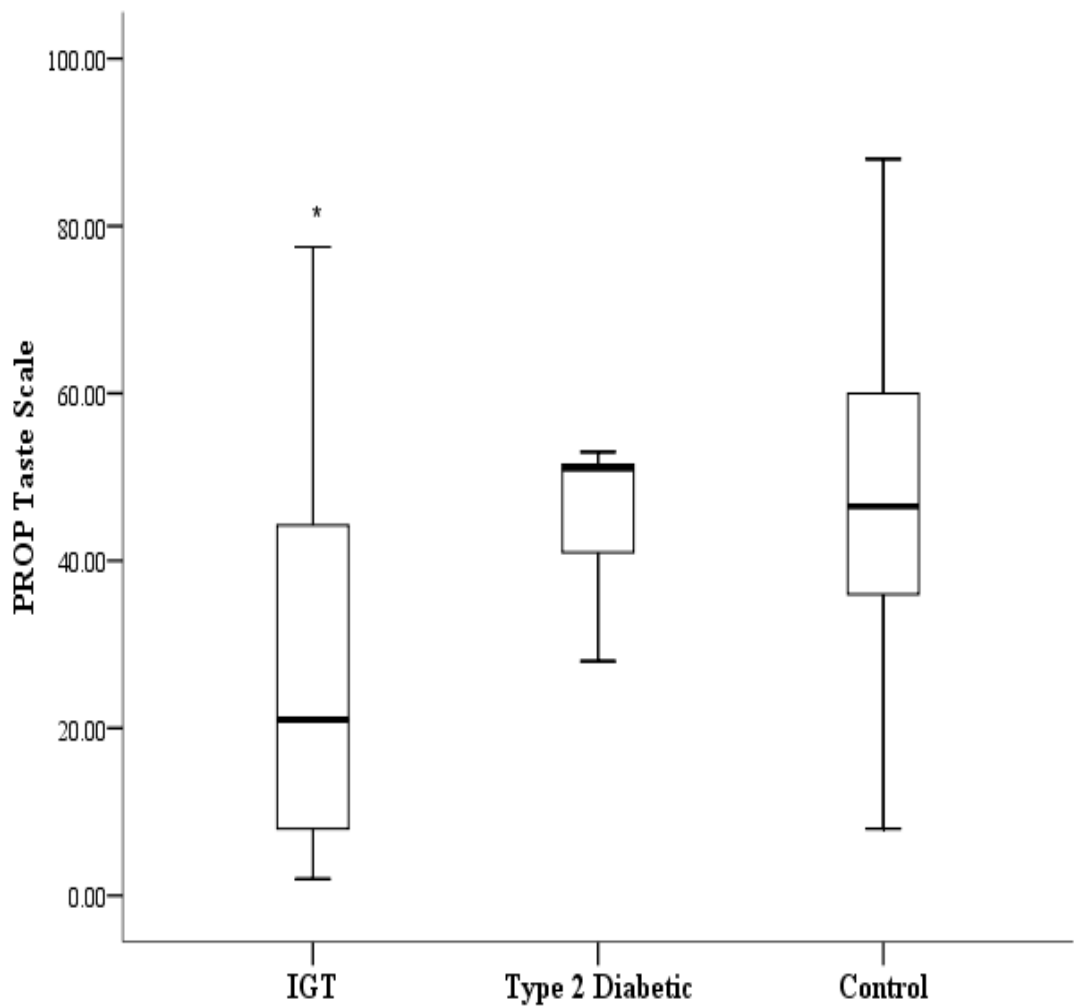
Figure 3.3 Mean values on PROP Taste Scale for IGT, Type 2 Diabetic and Control
Participants
Values are Median and Upper and Lower Quartiles
*p<0.05 when compared to type 2 diabetics and controls
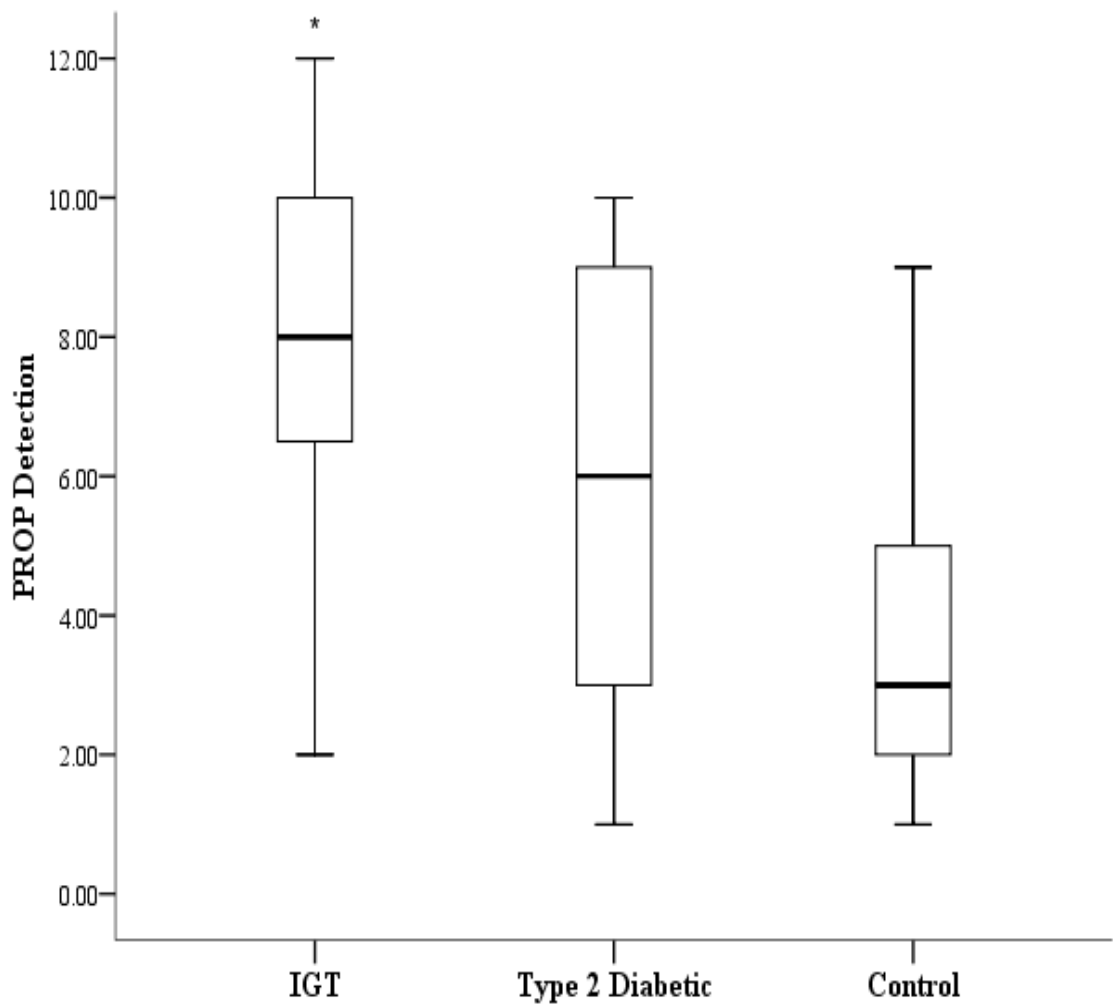
Figure 3.4 Concentration that PROP was detected by IGT, Type 2 Diabetic and Control Participants
Values are Median and Upper and Lower Quartiles
*p<0.01 when compared to control participants

3.3   HABITUAL FOOD INTAKE RECORDED BY DIET DIARIES

Diet diaries were recorded for 4 days in the week between the participant's introduction session and the test day. Results for each day of the diet diary recording were averaged to represent a single day's intake and are shown in table 3.2. The results are presented as basic macronutrient groups (protein, fat and carbohydrate (CHO)) and energy intake and expenditure. To facilitate the evaluation of the type of macronutrients consumed the fat content of the diet was fractioned into polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) and the CHO content was fractionated into starch and sugar. The results indicate averages over a 24-hour period. Due to underreporting one control subject was withdrawn from the results. Guidelines for cut off values to identify suspected underreporters has been developed using EI:BMR ratio in a series of publications (Black et al. 1991; Goldberg et al. 1991). EI:BMR is based on the assumption the total energy expenditure (TEE) is equal to EI and where TEE=BMRxPAL (physical activity level). Therefore, underreporters in the current study were identified by multiplying an individuals BMR, calculated using equations by Schofield et al (1985) by 1.2 which is the minimum EI:BMR ratio for survival and not compatible with long term health (WHO 1985).

The results in table 3.2 show that there was no significant difference in the average energy intake (k/cal), total fat (g/day), protein (g/day) or CHO (g/day) intake between groups, although the type 2 diabetic group tended to have the lowest intake of fat, CHO (g/day) and total energy (k/cals). Total fat (g/day) intakes, including MUFA, PUFA and SFA, were lowest in the type 2 diabetic participants compared to the other

participant groups, but this was not statistically significant. The type 2 diabetic participants were shown to have significantly lower intakes of sugar (g/day) compared with the control (p=0.013) and IGT groups (p=0.026) respectively. Starch intake was highest in the IGT group compared with the type 2 diabetic group and control groups respectively, but this was not statistically significant. Non-Starch Polysaccharide (NSP) intakes were lowest in the type 2 diabetics (g/day) and highest in the IGT participants, however there were no significant differences between groups. Conversely, alcohol intake (g/day) was highest in the type 2 diabetic group but no differences were found between groups.

Table 3.2 Nutrient Intakes in IGT, Type 2 Diabetic and Control Participants

Macronutrient Group	IGT n=11	Type 2 Diabetic n=17	Control n=17
Energy Intake (kcal/day)	2068 (176.12)	1729 (166.8)	1980 (179.5)
Energy Expenditure (kcal/d)	2666 (204.02)	2683 (131.15)	2210 (119.14)
Protein (g/day)	87.77 (7.0)	77.0 (6.46)	70.28 (5.98)
Fat (g/day):	81.55 (10.64)	65.32 (7.68)	81.61 (10.35)
PUFA (g/day)	15.31 (3.0)	11.07 (1.21)	17.21 (3.35)
MUFA (g/day)	26.28 (3.54)	21.96 (2.61)	25.86 (3.79)
SFA (g/day)	29.53 (3.71)	23.67 (3.51)	27.94 (4.44)
CHO (g/day):	243.55 (19.81)	196.78 (13.57)	237.03 (22.15)
Sugar (g/day)	111.26 (10.03)	71.74 (5.46)*	110.05 (11.91)
Starch (g/day)	126.05 (17.81)	120.99 (10.38)	118.16 (14.35)
NSP (g/day)	13.6 (0.83)	12.46 (0.77)	14.06 (1.55)
Alcohol (g/day)	9.41 (4.21)	13.53 (7.12)	10.64 (2.85)

Values are means ± SEM
* $p < 0.05$ compared to the other groups Bonferroni Pairwise comparison

Table 3.3 Percentage of Energy Intake from each Macronutrient

Macronutrient Group	IGT	Type 2 Diabetic	Control
% Energy Protein	17.24 (1.03)	18.55 (1.24)	14.43 (0.76)*
% Energy Fat:	34.77 (1.78)	33.31 (1.51)	35.68 (1.73)
% Energy PUFA	6.44 (0.82)	5.86 (0.53)	7.15 (1.01)
% Energy MUFA	11.16 (0.62)	11.31 (0.69)	11.04 (0.89)
% Energy SFA	12.72 (1.06)	11.89 (0.87)	12.10 (0.99)
% Energy CHO:	44.58 (1.64)	44.40 (1.96)	45.50 (2.03)
% Energy Sugar	21.04 (2.17)	16.66 (1.25)	21.55 (1.48)
% Energy Starch	22.35 (1.28)	27.0 (1.53)	22.21 (1.88)
% Energy Alcohol	3.32 (1.41)	3.70 (1.61)	4.32 (1.19)

Values are Mean ± SEM
*$p < 0.05$ control group different from type 2 diabetic group

Macronutrients were also presented as percentages of total energy intake, see table 3.3. Macronutrients were divided into protein, fat (PUFA, MUFA, and SFA), CHO (sugar and starch) and alcohol. Again, the results shown indicate averages over a 24 hour period. The control group had a significantly lower percentage of energy from protein compared to type 2 diabetic participants ($p = 0.016$). The percentage intake of CHO, fat, and alcohol were similar in all 3 groups. The type 2 diabetic participants had the lowest percentage intake of sugar and the highest starch intake but this was not significantly different from the other groups.

Figure 3.5 shows the percentage intake of each of the main macronutrients. A noted difference was in the protein intake where IGT participants had 17% intake, type 2 diabetics had a 19% intake and control participants had a 14% protein intake. Type 2 diabetics, IGT and then control participants respectively showed an increasing percentage of fat intake and there was a significant difference between control and type 2 diabetic participants ($p<0.05$)..

Diet diaries gave an indication of habitual intake but the experimental protocol required expression of short term intakes to investigate how the episodic ingestion of food contributes to overall habitual intakes. Therefore the short term effects of a test meal were investigated in the current study.

IGT Participants
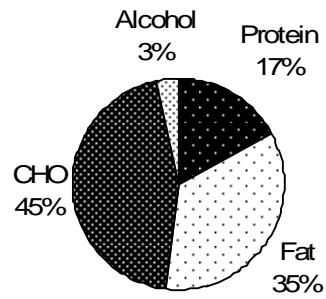
Type 2 Diabetic Participants
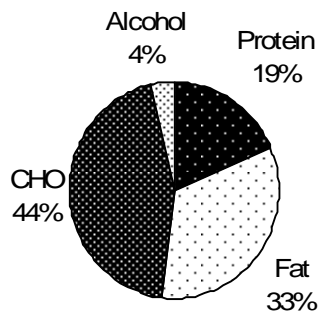
Control Participants
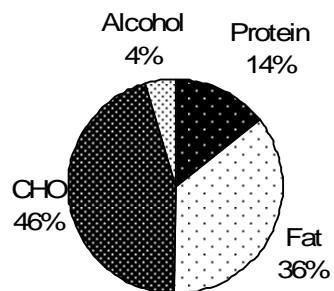
Figure 3.5 Main Macronutrients which make up 100% of Energy Intake in IGT, Type 2 Diabetic and Control Participants

3.3.1 Meal Consumption

The test meal consisted of 56% CHO, 32.5% fat and 10.5% protein and was of low calorific value to ensure a complete postprandial response within the 120 minute test period. The meal was presented when participants were 12 hours fasted. It consisted of cereal with semi skimmed milk, toast with full fat butter and freshly squeezed orange juice. Subjects were given as much time as they required to consume the meal. The length of time taken to consume the meal, see figure 3.6, was covertly recorded and participants were requested to consume all the contents of the meal. This was to ensure that all participants were consuming an equal quantity of food. Results indicate the length of time taken to consume the meal (mins) for each group. The average time to consume the test meal did not differ between groups. The average time to eat the meal was 10.63 minutes for IGT and control participants and 11.07 minutes for type 2 diabetics.
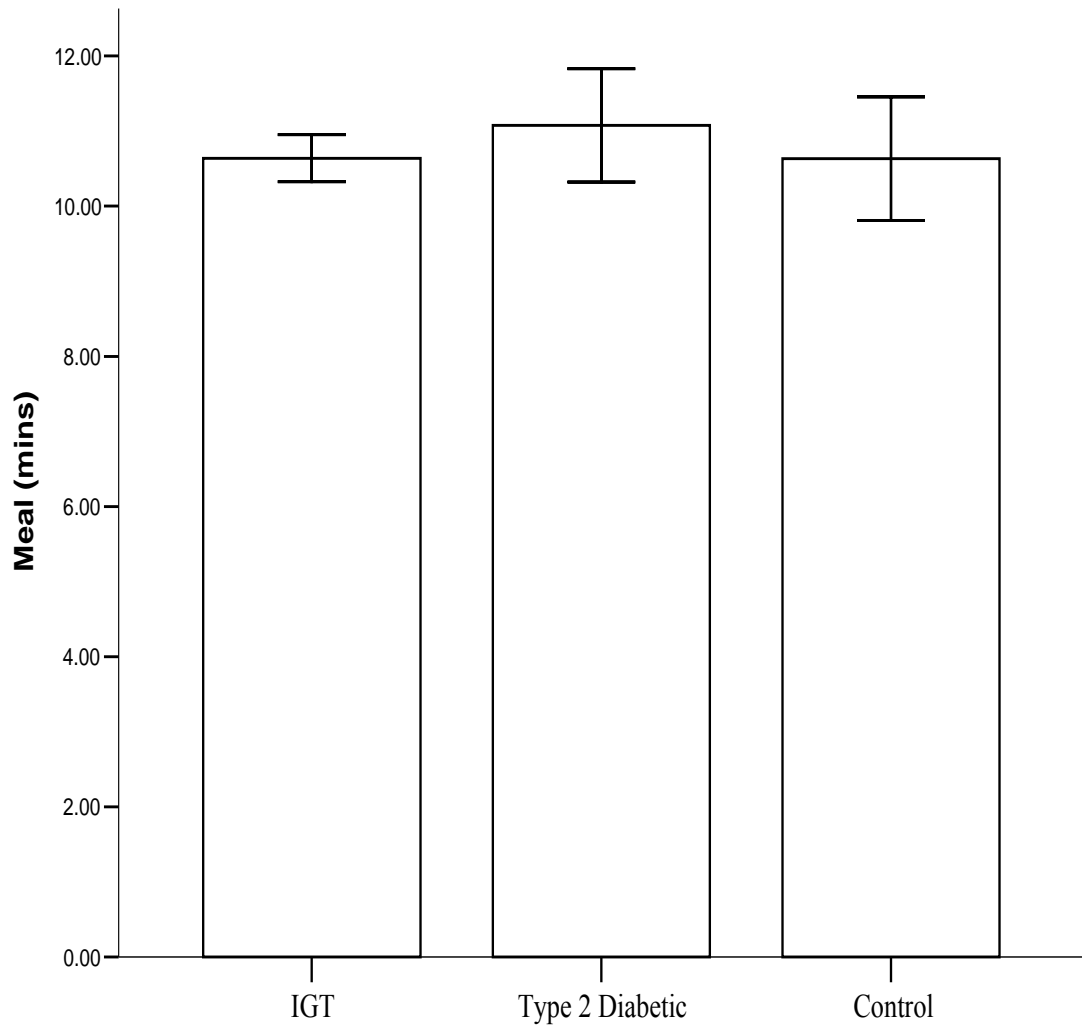
Figure 3.6 Length of time for IGT, Type 2 Diabetics and Control Participants to Consume the Test Meal
Values are Mean ± SEM

3.4 SUBJECTIVE MEASUREMENTS OF APPETITE

Visual analogue scales (VAS) were used to assess subjective measures of appetite using four parameters, hunger, satiety, fullness, and prospective consumption (PC). Each of these parameters were measured at regular intervals throughout the test periods as shown in figure 2.1. In order to analyse differences within groups (intragroup comparisons) repeated measures ANOVA with post hoc test comparisons were used. This measured differences over time. Subsequent pairwise comparisons were done where differences were identified, using independent t-tests. ANOVA with Bonferroni post hoc tests were used to analyse results between groups (intergroup comparisons).

3.4.1 Hunger

3.4.1.1 Intragroup Comparisons

For all groups the levels of hunger changed significantly throughout the study day. Following the meal there was a drop in hunger ratings in all groups. By 120 minutes after the meal was consumed levels of hunger had increased again. Repeated measures ANOVA analysed hunger across time (from fasting until 120 minutes after the test meal). There was a significant difference within-groups in all three groups ($p < 0.0001$) across time. The result of this test also demonstrated that there were differences between the control group and both IGT and type 2 diabetic participants, ($p < 0.01$) therefore ANOVA with Bonferroni post hoc tests were performed to determine at which timepoints the differences lay between groups.

Figure 3.7 Hunger ratings for IGT, Type 2 Diabetic and Control Participants over the Study Period

Values are Means and SEM

* p<0.05 when compared to the other 2 groups

** p<0.05 when compared to type 2 diabetics

*** p<0.01 when compared to type 2 diabetics

+ p<0.05 when compared to IGT group
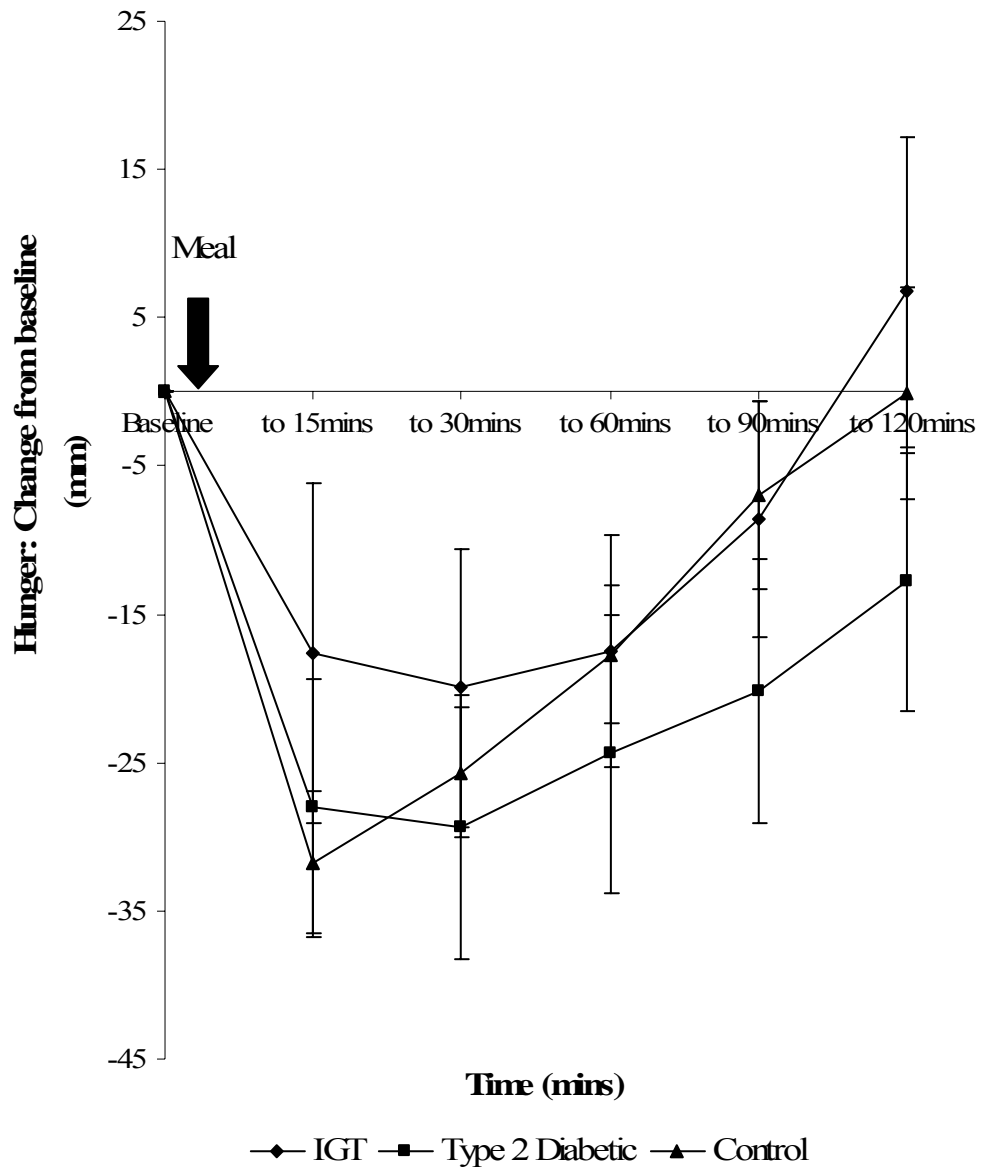
Figure 3.8 Hunger ratings for IGT, Type 2 Diabetic and Control Participants represented as the Amount of Change from Baseline Levels
Values are Means and SEM

3.4.1.2 Intergroup Comparisons

The hunger ratings were significantly different at baseline (fasting) levels between control participants ($p < 0.05$) and the other 2 groups (figure 3.7). Due to the difference in baseline values hunger ratings were also represented as change from baseline (figure 3.8). It was important to observe the change over time because the starting point (fasting level) for hunger was different between groups. After the test meal subjective ratings of hunger decreased sharply in all groups but decreased the most in the control group and resulted in no significant differences between subjective ratings of hunger at 15 minutes post-meal. At 30 minutes post-meal IGT participants hunger ratings remained steady, type 2 diabetic participants reported a further reduction in subjective ratings whereas control participants reported an increase in hunger ratings resulting in a significant difference between control participants and type 2 diabetic participants, $p = 0.017$. All 3 groups then reported increasing levels of hunger at 60, 90 and 120 minutes following the test meal. There was significant differences between control and type 2 diabetics at 60 mins ($p < 0.05$), 90 mins ($p < 0.01$) and 120 mins ($p < 0.05$) and there was significant differences between control and IGT participants at 60 mins ($p < 0.05$) and 90 mins ($p < 0.05$). At 120 minutes IGT participants reported a sharp increase in subjective levels of hunger, to a level higher than their fasting measurement, therefore there was no significant difference between IGT and control participants at this timepoint. Control and type 2 diabetic participants' hunger levels at 120 minutes did not reach as high as fasting levels.

3.4.1.3 Hunger Area Under the Curve

The AUC value was utilised to observe the whole hunger response, recorded by a single value. It is a useful tool to analyse the difference between groups for a whole response. The mean AUC response for subjective ratings of hunger was significantly different between IGT and controls ($p<0.05$) and type 2 diabetics and control participants ($p<0.01$), see figure 3.9. Control participants had the overall greatest hunger response compared to both IGT and type 2 diabetics. There was no difference between the 2 latter groups.
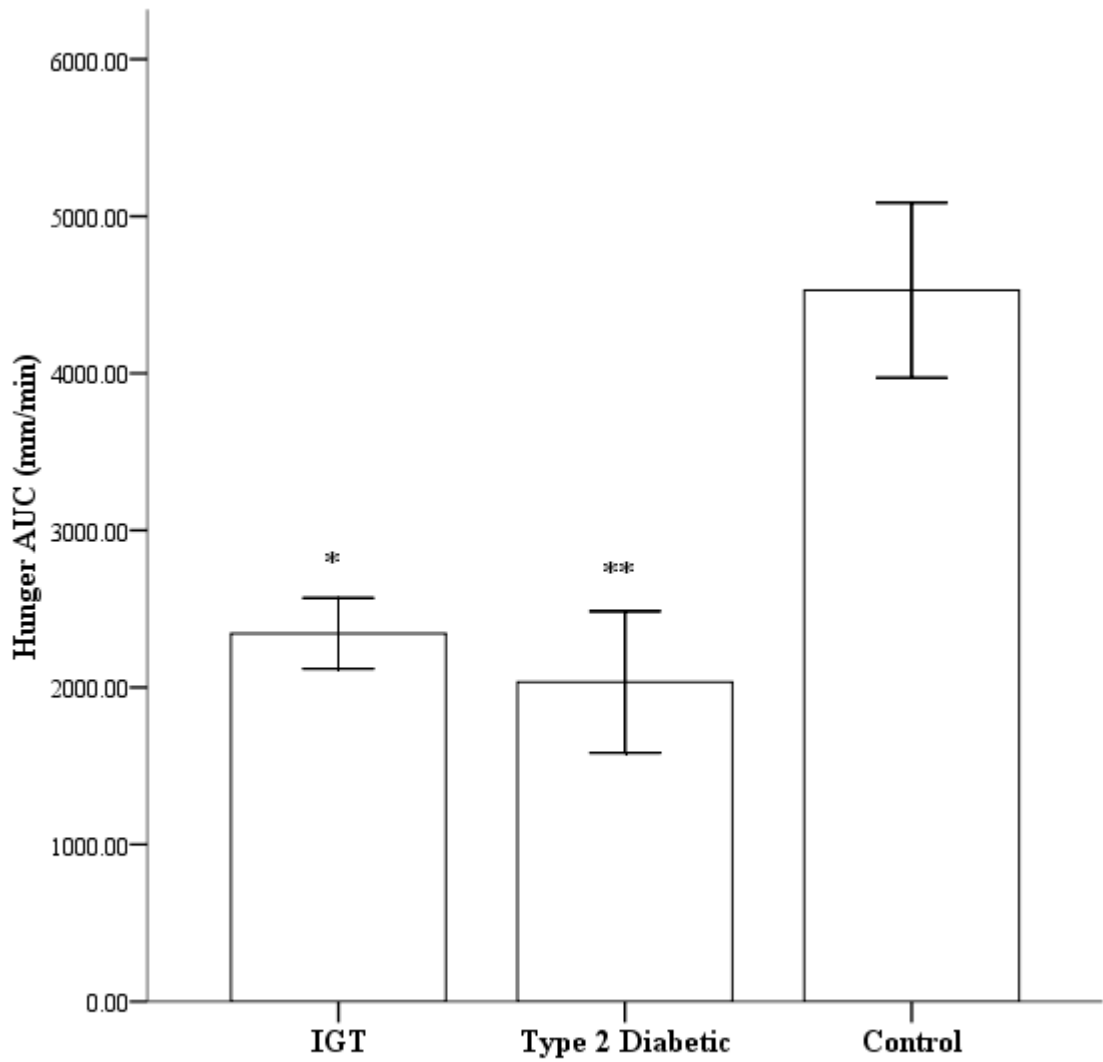
Figure 3.9 Visual Analogue Scales: AUC for Hunger
Values are Means and SEM
* p<0.05 when compared to controls
** p<0.01 when compared to controls

3.4.2 Satiety

3.4.2.1 Intragroup Comparisons

Repeated measures ANOVA demonstrated that for all 3 groups there was a significant difference in the subjective ratings of satiety over time ($p < 0.0001$). All groups followed a similar pattern as fasting subjective levels of satiety were midrange then 15 minutes following the meal there was an increase in satiety ratings for all groups and then a gradual decrease in levels until 120 minutes for all groups. The satiety ratings at 120 minutes did not fall to the same level as fasting levels for any of the three groups. The IGT participants recorded the highest level of satiety, followed by type 2 diabetics and then control participants. The result of this test also shows that there were differences between the control group and both IGT and type 2 diabetic participants therefore ANOVA with Bonferroni post hoc tests were completed to establish at what timepoints the differences were and this can be observed on figure 3.10.
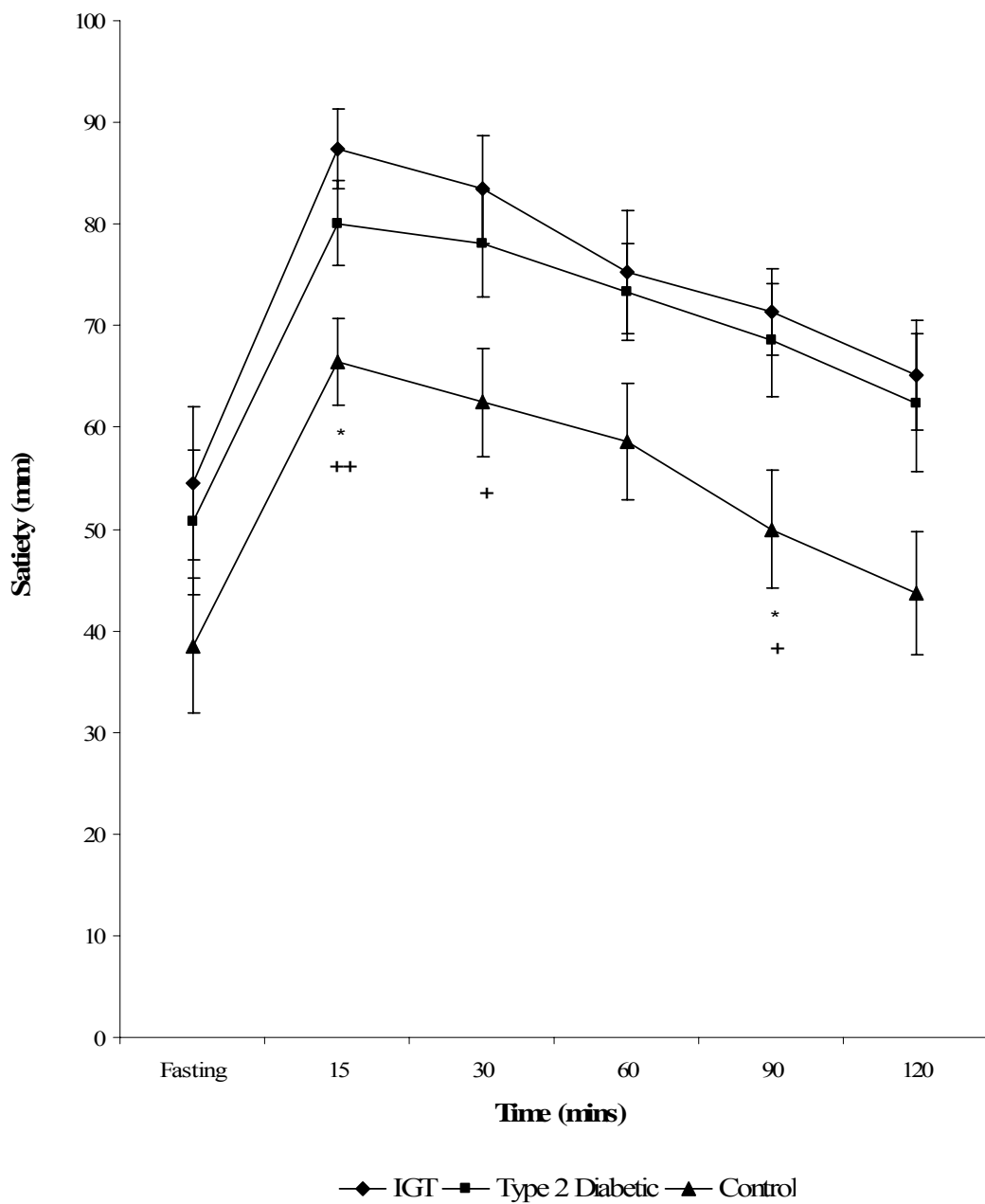
Figure 3.10 Satiety Ratings for IGT, Type 2 Diabetic and Control Participants over the Study Period

Values are Means and SEM

* p<0.05 when compared to type 2 diabetics

+ p<0.05 when compared to IGT group

++p<0.01 when compared to IGT group

3.4.2.2 Intergroup Comparisons

Fasting satiety ratings were lower in control participants but this was not significantly different, see figure 3.10. At 15 minutes post meal there was an increase in subjective levels of satiety resulting in a significant difference between IGT and control participants, $p<0.01$ and control and type 2 diabetic participants, $p<0.05$. In all groups there was a gradual decline in subjective levels of satiety at 30 minutes, with control participants recording significantly lower levels of satiety at this timepoint compared to IGT participants, $p<0.05$. At 60 minutes there was no significant differences between satiety levels and at 90 minutes there was a significant difference again between control and both IGT and type 2 diabetic participants, $p<0.05$ with control participants again still recording the lowest levels of satiety. There were no significant differences between participants at the final, 120 minute timepoint.

3.4.2.3 Satiety Area Under the Curve

The mean area under the curve (AUC) for subjective ratings of satiety was significantly different between control participants and both IGT participants ($p=0.016$) and type 2 diabetics ($p=0.032$), see figure 3.11. The control participants reported the lowest feelings of satiety and therefore had the lowest overall satiety response. There was no difference in satiety ratings between IGT and type 2 diabetics.
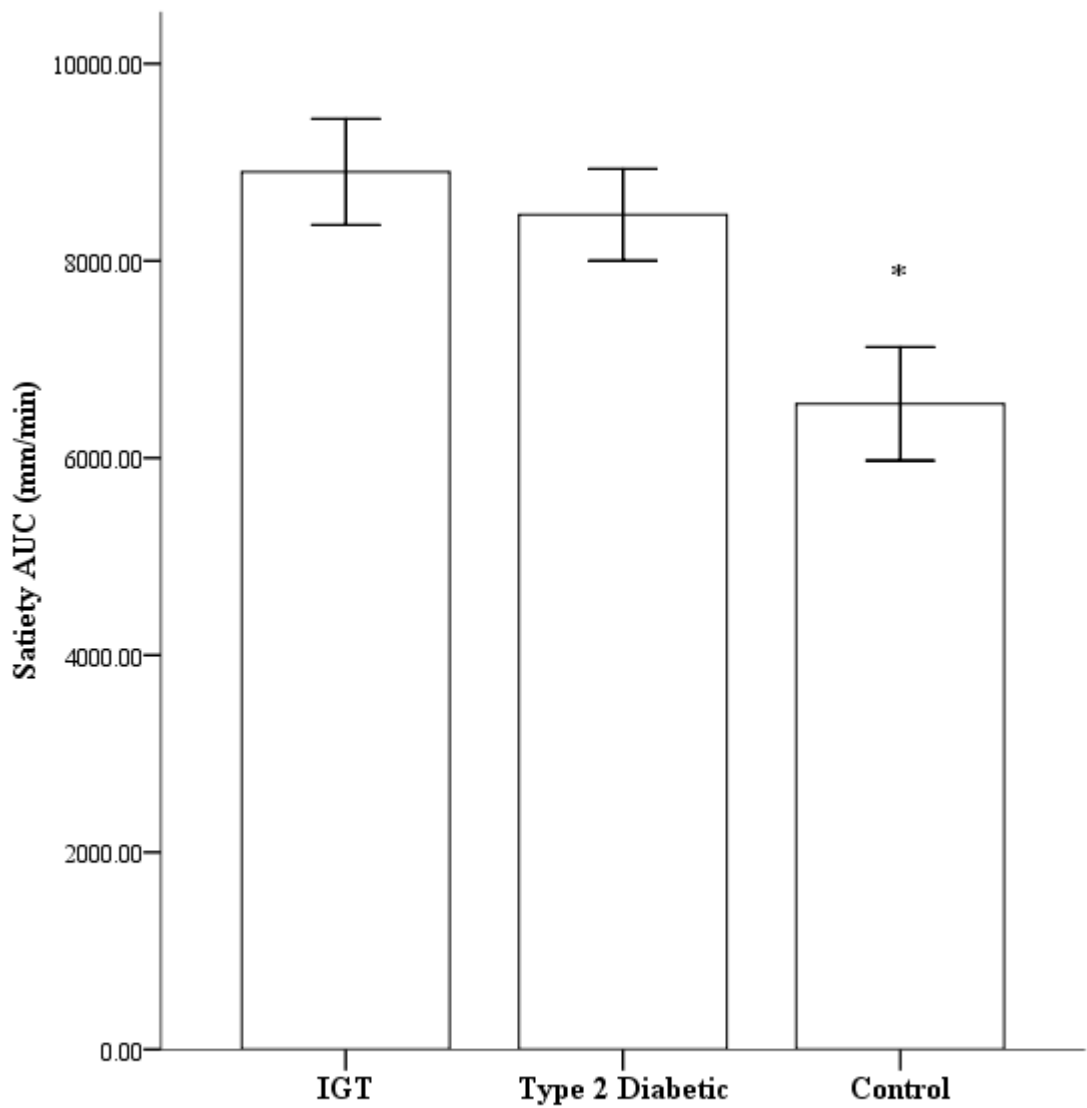
Figure 3.11 Visual Analogue Scales Intergroup Comparisons: AUC for Satiety
Values are Means and SEM
* $p < 0.05$ when compared to other 2 groups

3.4.3 Fullness

3.4.3.1 Intragroup Comparisons

For all 3 groups fasting subjective levels of fullness were low. At 15 minutes following the meal there was a sharp increase in fullness ratings. The 2 patient groups (IGT and type 2 diabetes) fullness levels increased slightly at 30 minutes, whereas the control group decreased slightly. Then there was a gradual decrease in subjective levels of fullness until 120 minutes in all groups. There was a significant difference over time within all 3 groups, $p<0.0001$. On the whole IGT participants reported the highest levels of fullness until 60 minutes when a large drop meant that their levels were slightly below the type 2 diabetic participants. Control participants recorded the lowest levels of fullness throughout the study period.   The repeated measures ANOVA indicated that there were differences between groups, therefore ANOVA with bonferroni posthoc tests was performed to determine where the differences lay, see figure 3.12.
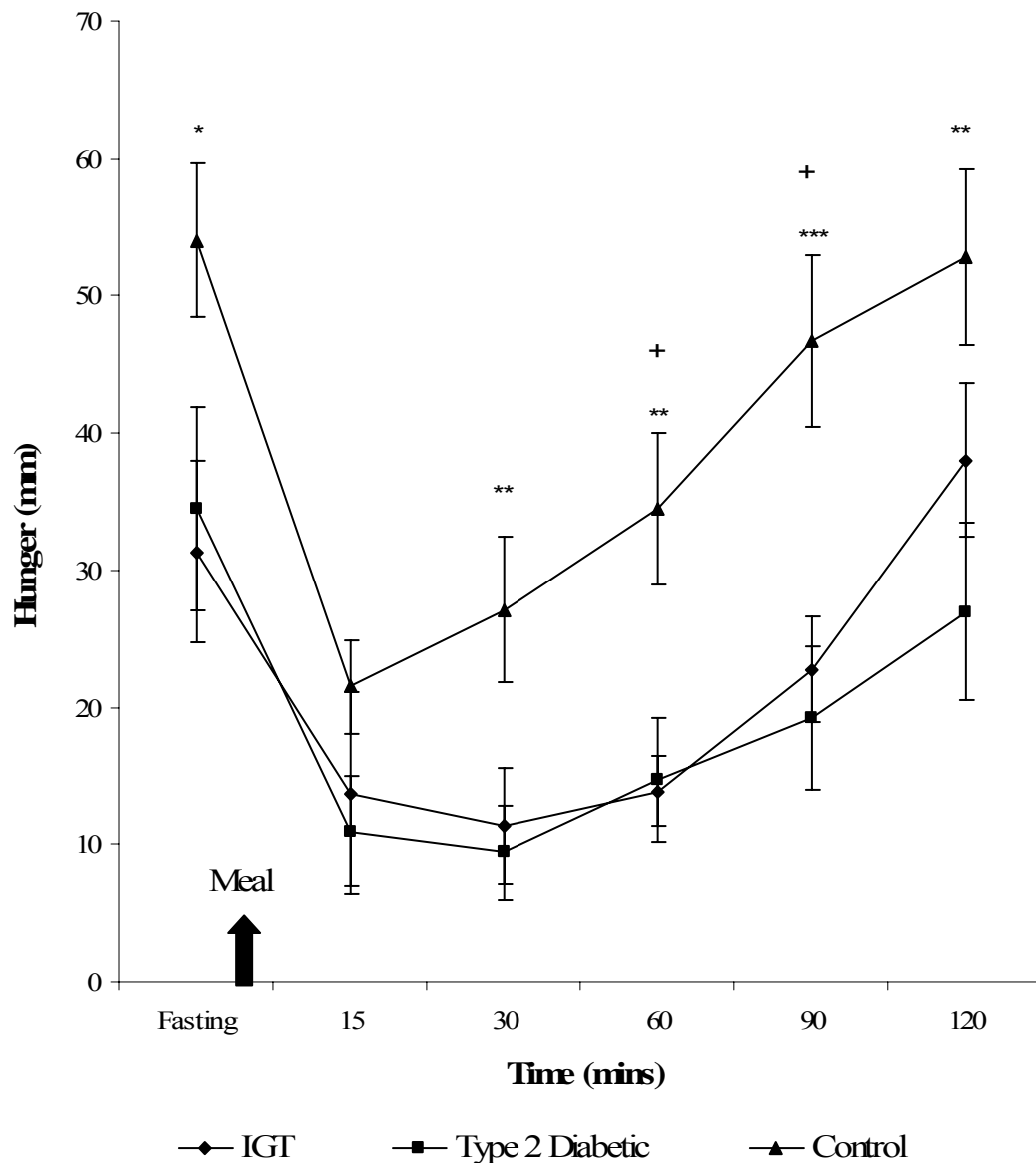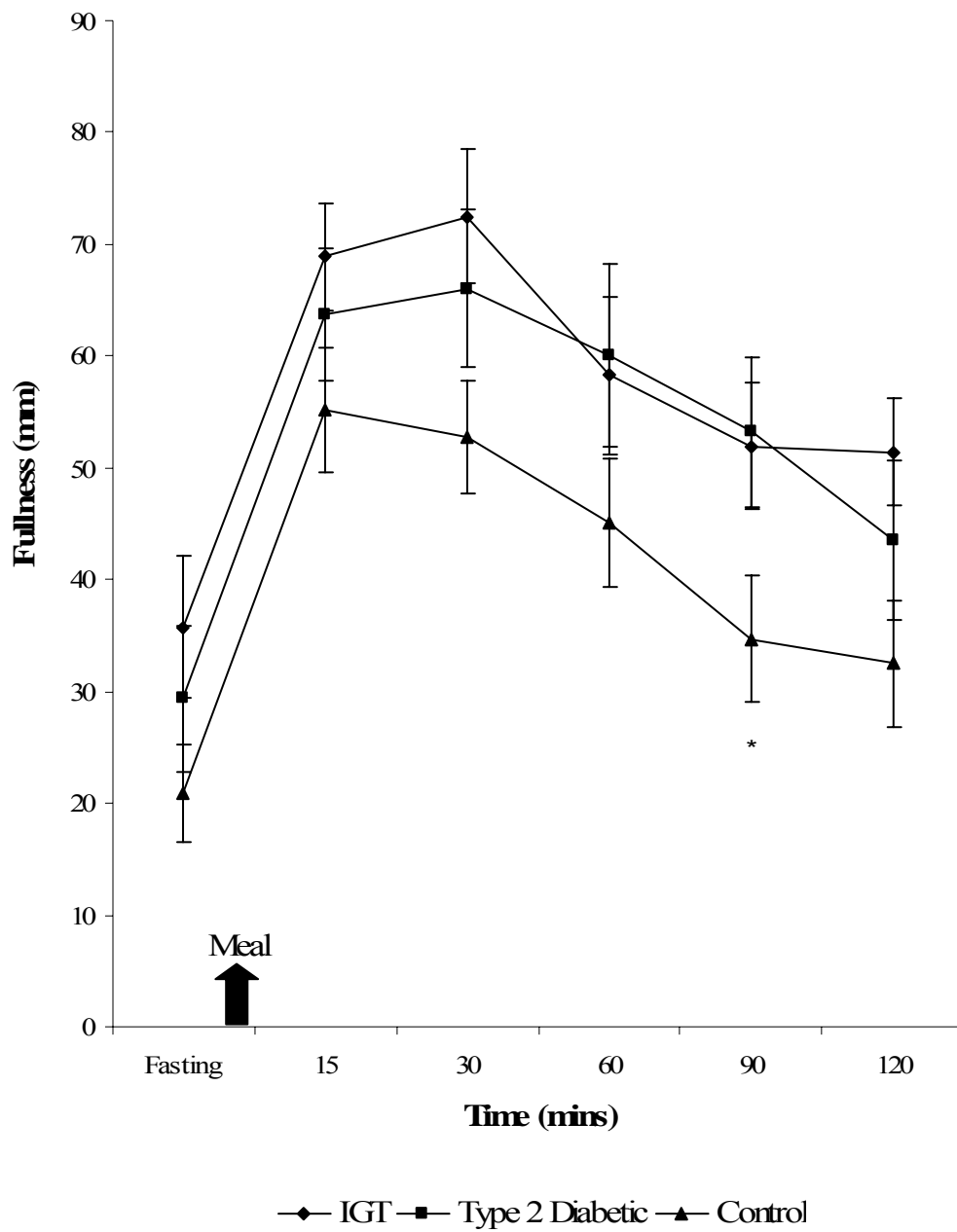
Figure 3.12 Fullness Ratings for IGT, Type 2 Diabetic and Control Participants over the Study Period
Values are Means and SEM
* p<0.05 when compared to type 2 diabetics

3.4.3.2 Intergroup Comparisons

At baseline the fullness ratings were lower in control participants but this was not significantly different. At 15 minutes post meal there was an increase in subjective levels of fullness in all groups, again there was no significant differences between IGT, type 2 diabetic and control groups. In all groups there was a gradual decline in subjective levels of fullness at 30 minutes and 60 minutes, although IGT participants demonstrated the largest decline in fullness ratings at 60 minutes, and all groups levelled off at 120 minutes. At 90 minutes control participants recorded significantly lower levels of fullness when compared to type 2 diabetics, $p=0.048$.

3.4.3.3 Fullness Area Under the Curve

Control participants tended to report the lowest rating for fullness, see figure 3.13. This resulted in the AUC response in control participants to be the smallest, however this was not significantly different. Therefore control participants felt the least full throughout the study period whereas IGT participants felt the most full.
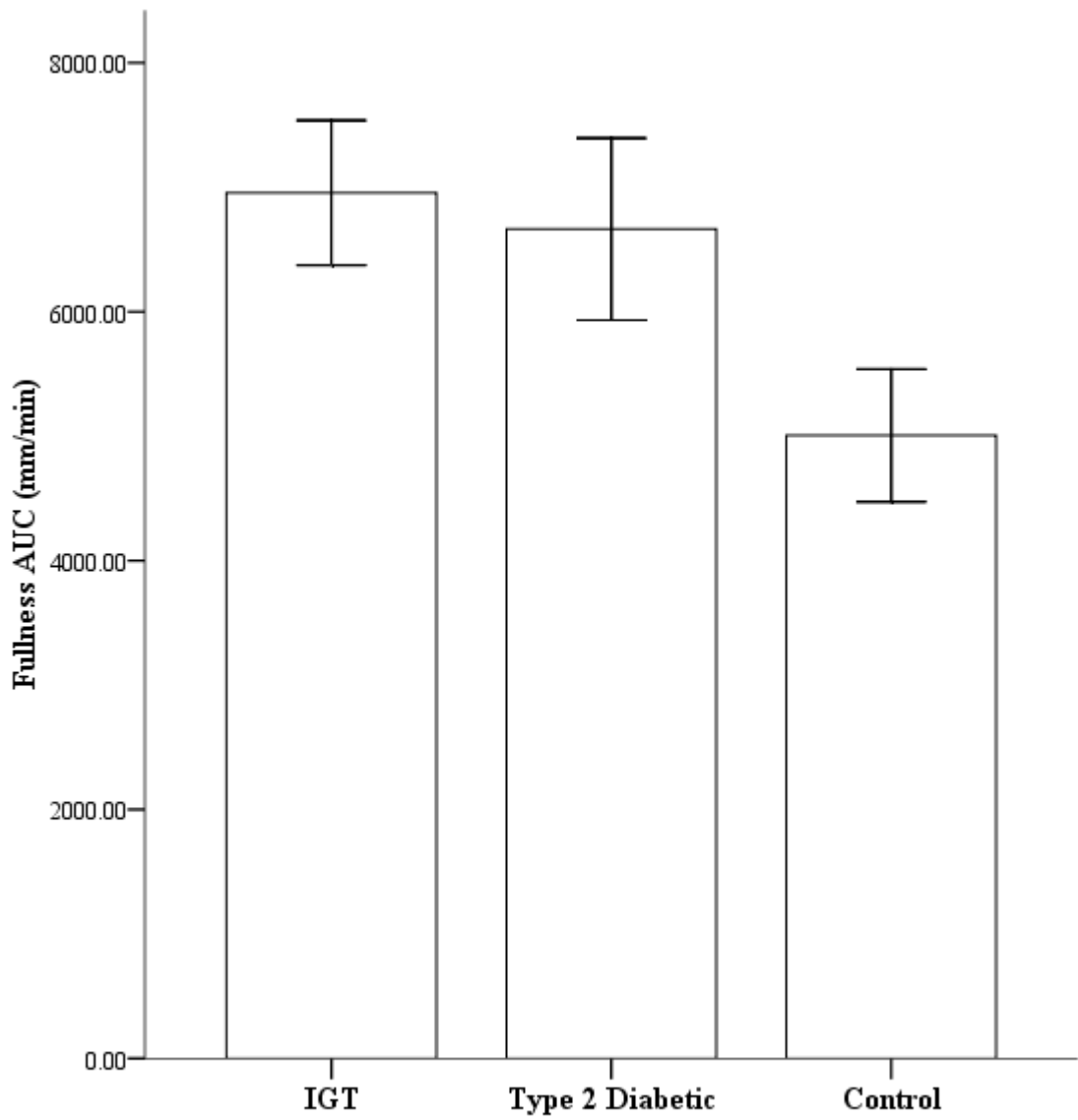
Figure 3.13 Visual Analogue Scales Intergroup Comparisons: AUC for Fullness
Values are Mean and SEM

3.4.4 Prospective Consumption (PC)

3.4.4.1 <u>Intragroup Comparisons</u>

For all 3 groups the fasting (baseline) subjective levels of prospective consumption were high. Levels started highest in the IGT participants and were lowest in the type 2 diabetic participants. Again, the recorded levels of prospective consumption followed a similar pattern in all three groups. At 15 minutes following the meal there was a sharp decrease in prospective consumption ratings, as expected. This reduction in levels was most pronounced in the IGT participants whose levels then fell below that of the control participants and remained intermediate for the rest of the study period. At 30 minutes IGT and type 2 diabetic participants reported a further reduction whereas control participants reported an increase in prospective consumption. From there, for the rest of the timepoints, all groups reported a gradual increase in prospective consumption until 120 minutes post meal. Type 2 diabetic participants recorded the lowest levels at all timepoints. The control participants came closest to reaching fasting values of prospective consumption. Across time there was a significant difference between type 2 diabetic participants and control participants ($p<0.05$). The repeated measures ANOVA indicated that there were differences between groups therefore ANOVA with Bonferroni post hoc tests was performed to determine where the differences lay.
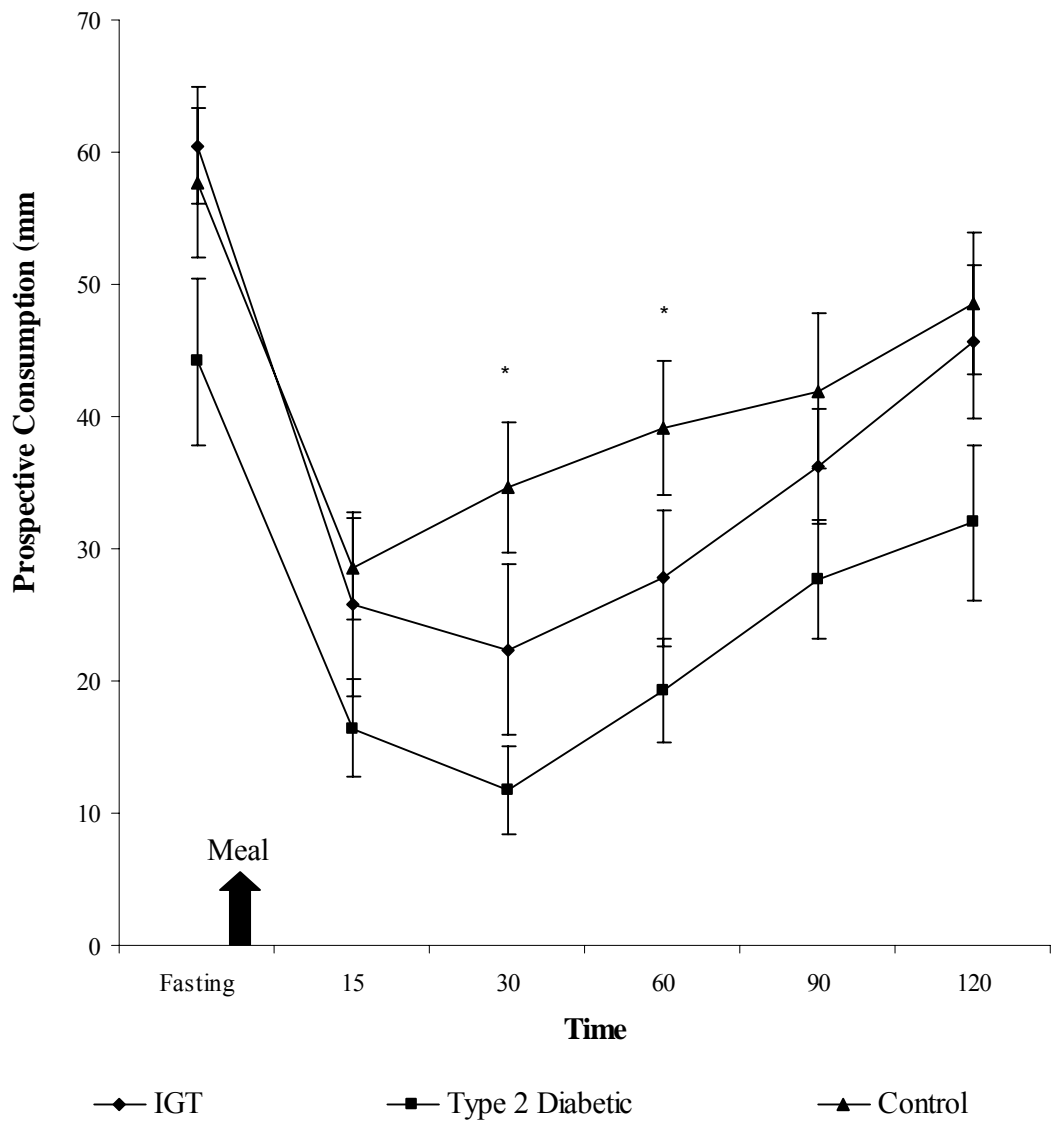
Figure 3.14 Prospective Consumption Ratings for IGT, Type 2 Diabetic and Control Participants over the Study Period
* p<0.05 when compared to type 2 diabetic participants

3.4.4.2 <u>Intergroup Comparisons</u>

At baseline there was no significant difference between subjective levels of prospective consumption (figure 3.14). Fifteen minutes post meal there was a sharp decrease in subjective levels of prospective consumption in all groups and there remained no significant difference between IGT, type 2 diabetic and control groups. In IGT and type 2 diabetic groups there was a further decrease in levels of prospective consumption but in control participants there was a small increase in subjective levels of PC at 30 minutes, resulting in a significant difference ($p < 0.01$) between type 2 diabetics and control participants. All 3 groups then recorded a gradual increase in levels of prospective consumption at 60 minutes until 120 minutes. There was a significant difference between type 2 diabetics and controls at 60 minutes ($p < 0.01$). There was no significant difference at the 90 minute timepoint. There was no significant difference between IGT participants and either type 2 diabetic or control participants.

3.4.4.3 <u>Prospective Consumption Area Under the Curve</u>

The mean AUC for the subjective rating of prospective consumption was significantly lower in type 2 diabetic participants when compared to control participants ($p = 0.016$) meaning they had the lowest desire to eat throughout the study period, see figure 3.15. There was no difference in PC between IGT and control or type 2 diabetic participants.

It is the change in appetite following a meal which allows increased energy intake. Therefore, it was an important part of this protocol that habitual energy expenditure was also recorded to determine if participants were in energy balance.
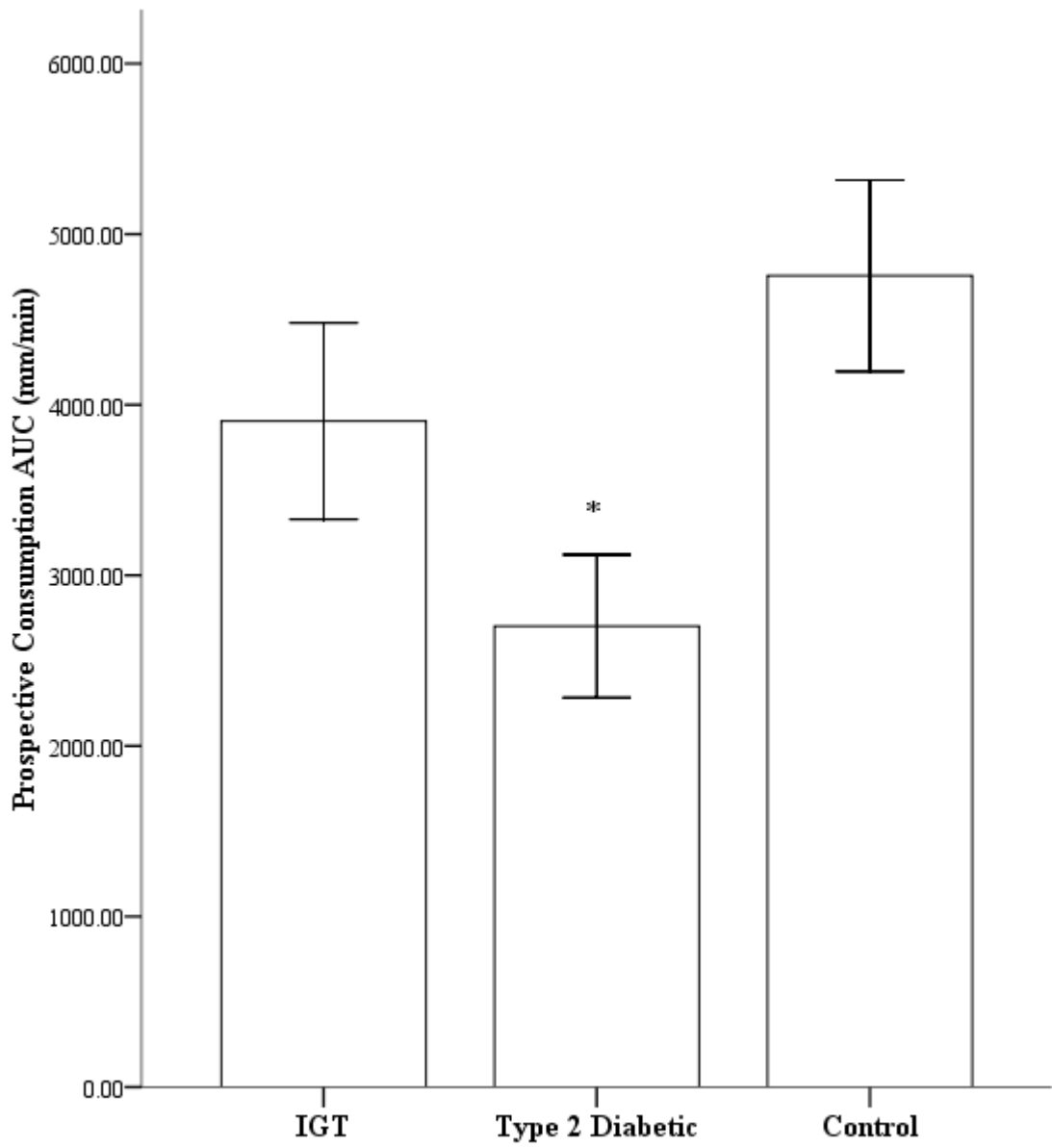
Figure 3.15 Visual Analogue Scales Intergroup Comparisons: AUC for Prospective Consumption
Values are Mean and SEM
* $p < 0.05$ when compared to control participants

3.5   ENERGY EXPENDITURE

3.5.1   Activity Diaries

Energy expenditure was measured by activity diaries over 4 days. All subjects were instructed to keep the diaries. Results of the activity diaries were averaged and are shown in figure 3.16. Results are also normalised for body weight by dividing energy expenditure (kcal per 24 hrs) values with body weight for each subject and are graphically illustrated (figure 3.17). This was done to determine differences in daily EE that were independent of weight.

Average mean energy expenditure from the activity diaries shows that IGT and Type 2 Diabetic participants have a similar EE and both have a higher mean EE than control participants (figure 3.16). The result is not statistically significant. However, once these results were normalised for body weight all 3 groups had a similar mean EE (figure 3.17). Table 3.4 shows the average time (hrs) each day spent doing specific activities to determine if there was a difference in the type and amount of activity undertaken throughout the day. One control participant failed to record their activity and therefore is discounted from this result only. The time spent doing non-physically active activities like sleeping, lying awake, sitting, sitting activities and standing were all very similar between groups. The time spent in light activity, for example standing activities, was higher in the IGT participants than in the control and type 2 diabetic participants. However, the type 2 diabetic participants spent the most time doing moderate activity such as walking and partaking in personal needs (showering, bathing, dressing etc). IGT and type 2 diabetic participants did

considerably more housework than control participants but when examining the strenuous activity (gym work, aerobic activity, cycling, martial arts etc) the control participants did twice as much as the IGT participants and a little more than the type 2 diabetic participants. Overall there were no significant differences between groups for any of the activities although it is noteworthy that the IGT participants spent more of their time doing light to moderate activity and the control participants spent more of their time doing light and strenuous activity whereas the type 2 diabetic participants spent an even amount of time doing light, moderate and strenuous activities.

The measurement of energy expenditure including activity in this type of protocol was important to assess the habitual activity of participants. However, resting energy expenditure was also measured using indirect calorimetry. This was important to assess the relationship between direct and indirect measurements of EE and also to compare resting EE between IGT, type 2 diabetic and control participants to determine differences in EE resultant of the disease status.
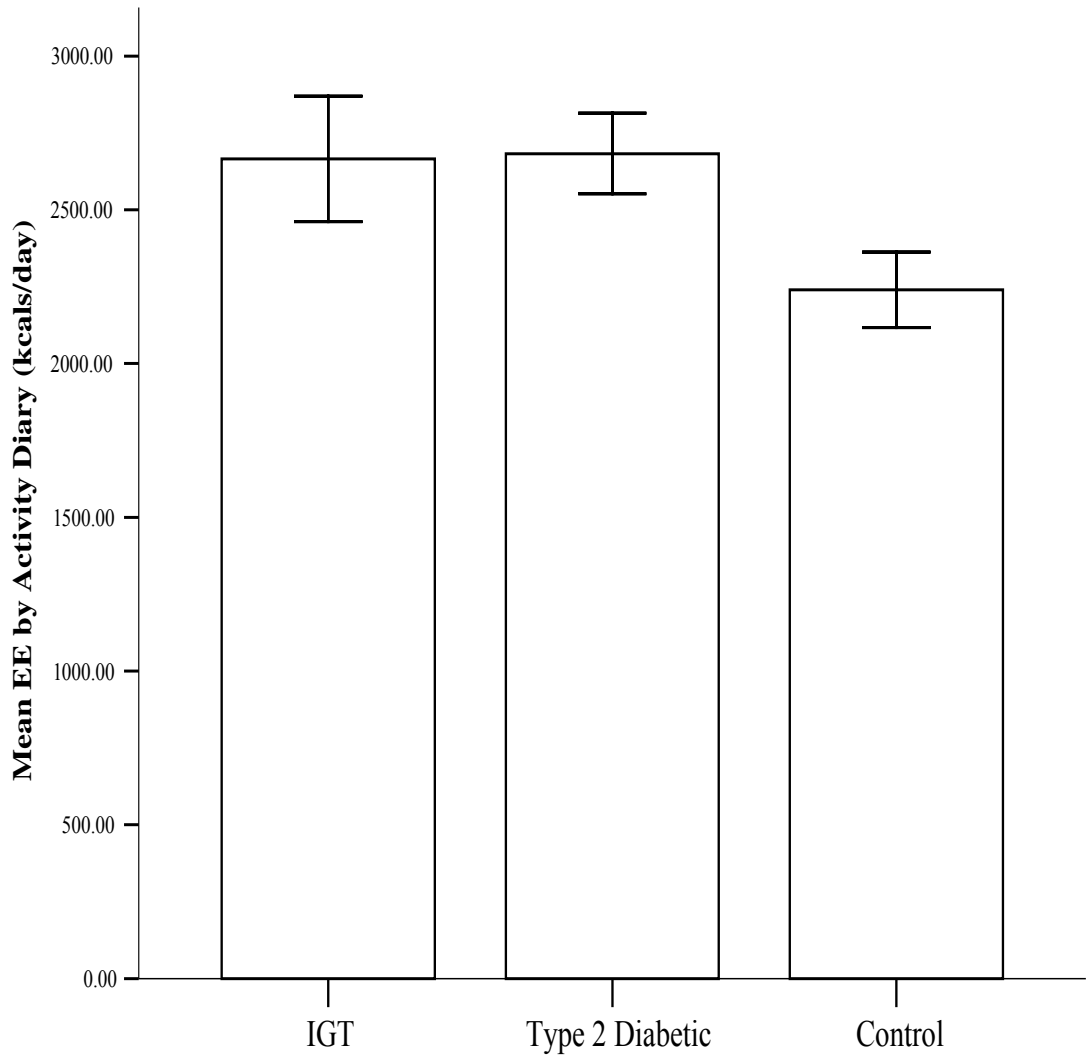
Figure 3.16 Mean Energy Expenditure recorded by Activity Diaries (kcals/day)
Values are Mean and SEM
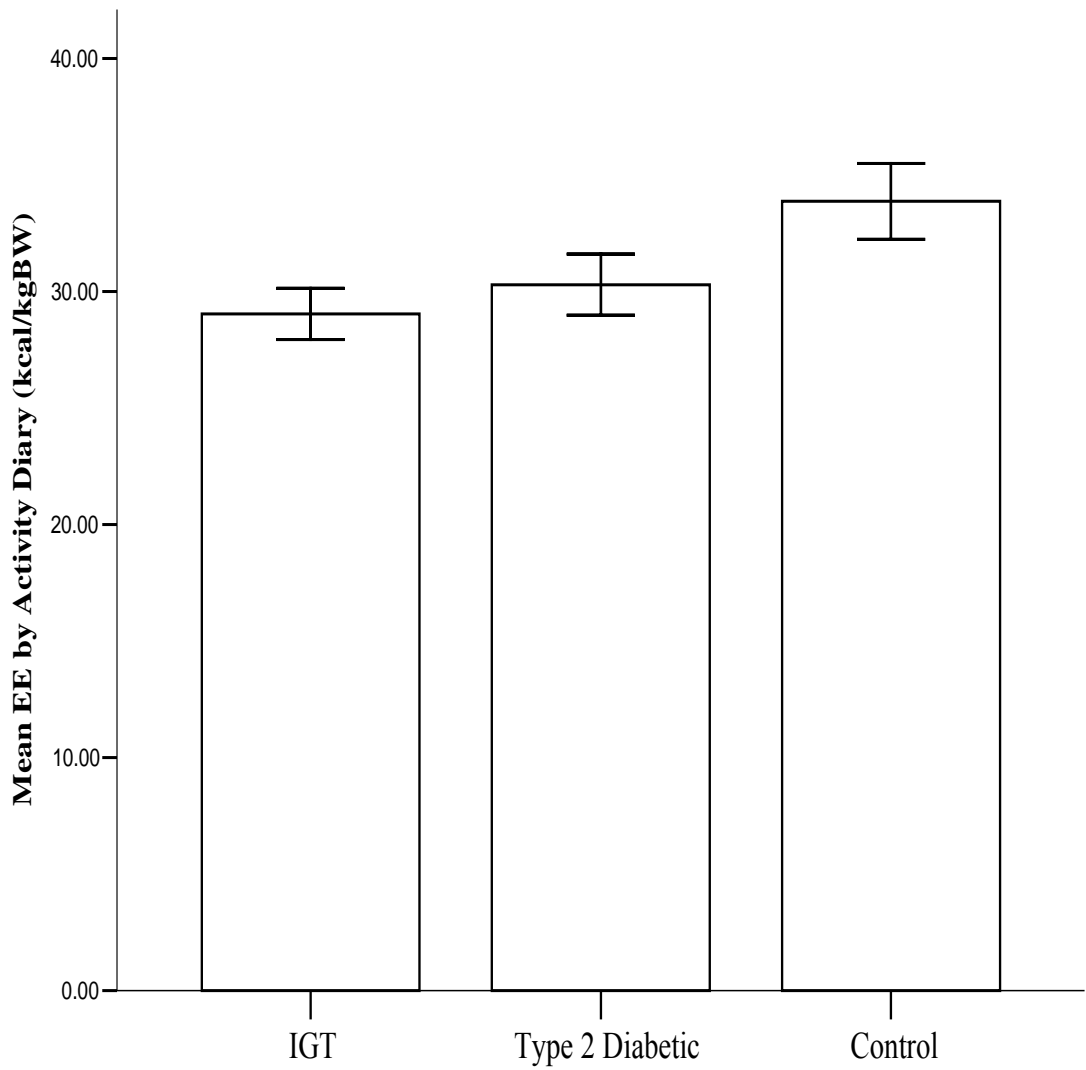
Figure 3.17 Mean Energy Expenditure (kcals) per Kilogram Body Weight

Table 3.4 Activity Diaries: Amount of time (hours) recorded doing common activities in IGT, type 2 diabetic and control participants.

Group	Sleep	Lying Awake	Sitting	Sitting Activity	Standing	Standing Activity	Personal Needs	Walking	Housework	Strenuous Activity
IGT	7.59 (1.54)	1.05 (0.93)	3.95 (3.22)	5.06 (3.53)	0.21 (0.35)	2.16 (2.72)	0.75 (0.28)	1.51 (1.29)	1.20 (1.73)	0.52 (0.62)
Type 2 Diabetic	7.76 (1.55)	0.89 (0.89)	4.04 (2.20)	4.67 (2.27)	0.66 (0.67)	1.50 (1.41)	1.07 (0.64)	1.56 (0.99)	0.94 (0.87)	0.93 (0.85)
Control	8.15 (1.42)	0.73 (0.59)	3.36 (2.85)	6.01 (1.32)	0.40 (0.46)	1.67 (0.85)	0.73 (0.37)	1.38 (0.69)	0.48 (0.51)	1.09 (0.89)

Values are Means and SEM

3.5.2   Indirect Calorimetry

Resting energy expenditure was measured 6 times throughout the test period using an indirect calorimeter. Intergroup comparisons were assessed for all subjects. Results shown are normalised for body weight by dividing energy expenditure (kcal per 24 hrs) values by body weight (kg) for each subject and expressed as kilocalories per kilogram bodyweight (kcals/kgBW), see table 3.5.   Energy expenditure is also illustrated at individual timepoints throughout the test period. Results are shown as mean resting energy expenditure and later as percentage change from resting energy expenditure, to indicate the diet induced thermogenesis (DIT) of the specific meal, see figure 3.19.   Results are also graphically illustrated as area under the curve, figure 3.18, so that the magnitude of the response across time could be observed. Results were analysed using analysis of variance (ANOVA) with Bonferroni post hoc test.

Table 3.5 Energy Expenditure Normalised for Body Weight (kcals/kgBW)

Energy Expenditure	IGT (n-11)	Type 2 Diabetic (n-17)	Control (n=16)
Fasting	18.72 (0.67) *	18.73 (0.64) **	21.8 (0.76)
15 Minutes post meal	20.62 (0.47) **	21.80 (0.85) *	25.13 (0.87)
30 Minutes post meal	20.43 (0.42) **	21.17 (0.74) **	24.78 (0.86)
60 Minutes post meal	20.45 (0.51) **	20.94 (0.74) **	24.88 (0.95)
90 Minutes post meal	20.05 (0.74) **	20.28 (0.73) **	24.12 (0.87)
120 Minutes post meal	20.06 (0.73) *	20.54 (0.81) *	23.42 (0.80)

Values are means ± SEM
* p <0.05 significantly different from control participants
** p <0.01 significantly different from control participants
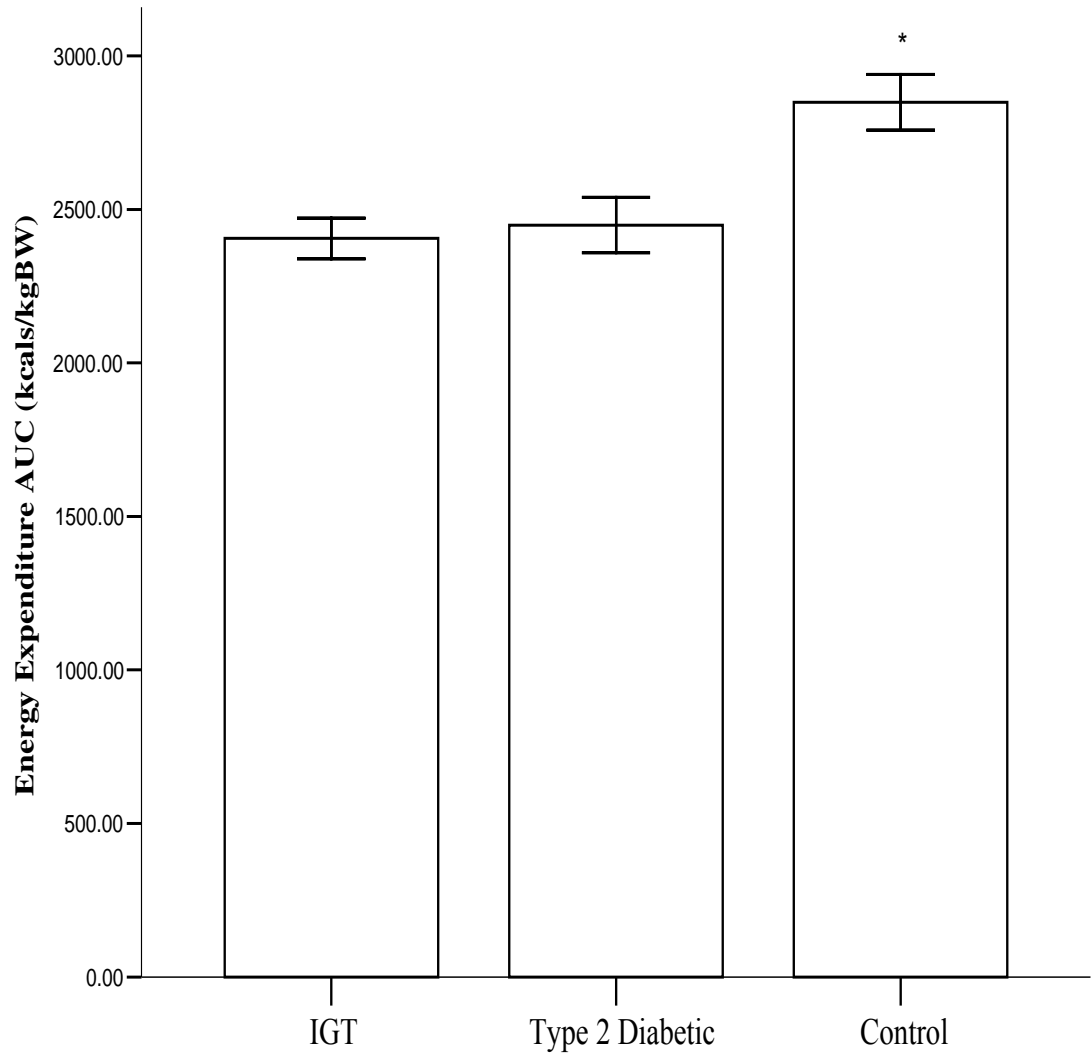
Figure 3.18 Area Under the Curve for Resting Energy Expenditure (kcals)
Values are Mean and SEM
* p< 0.01 significantly different from the other 2 groups

Resting energy expenditure data was collected and averaged to show differences between groups (table 3.5). There were significant differences between control participants and both IGT and type 2 diabetic participants at all timepoints after normalising for body weight. There was no significant difference between IGT and type 2 diabetic participants at any of the timepoints. The AUC response shows that control participants had a significantly higher EE response to the meal than IGT and type 2 diabetic participants. The AUC results are shown per kilogram body weight to achieve one value for each group. Both IGT and type 2 diabetic participants have a lower AUC value than control participants. This was statistically significant, $p<0.01$ (figure 3.18).

Percentage change from fasting was measured for energy expenditure to determine the DIT of the meal. On average the biggest change in energy expenditure (a 14.58% increase) for all groups was 15 minutes post meal. Intergroup comparisons showed no significant difference between groups in the change from fasting levels. Energy expenditure values for IGT and type 2 diabetic participants' then gradually declined throughout the other 4 timepoints (30, 60 90 and 120 mins) to 7.23% (IGT) and 9.62% (type 2 diabetics) above fasting levels. Control participants' energy expenditure remained at a higher level for 30 and 60 minutes post meal and then reduced at 90 and 120 minutes progressively to a level 7.68% above fasting levels. None or the three groups reached fasting levels of resting EE.
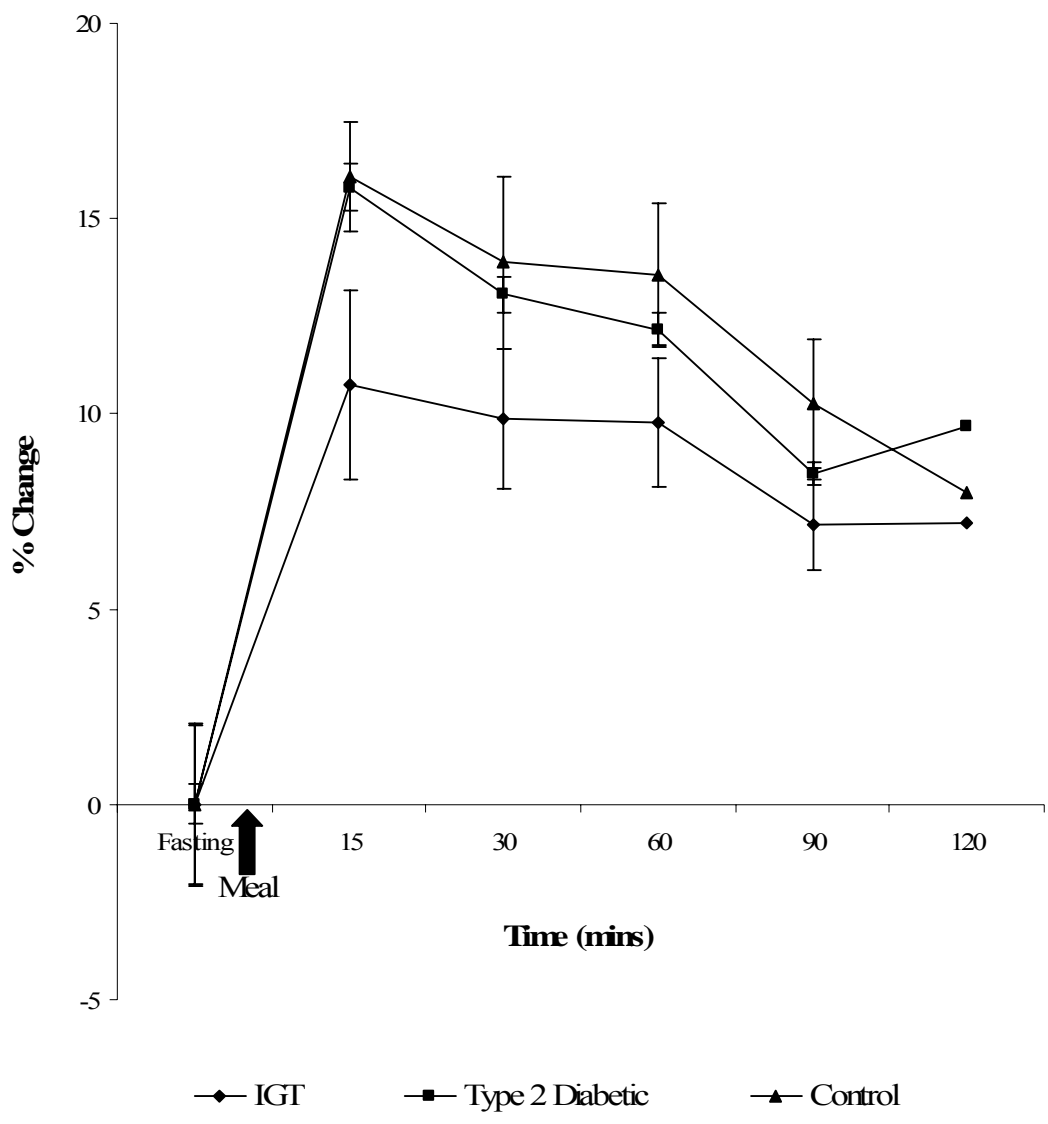
Figure 3.19 Percentage Change from Resting Energy Expenditure
Values are Mean and SEM

Energy expenditure results were integrated using correlative statistical analysis to indicate associations between the results. The integrated results assessed trends between predicted fasting EE and fasting EE measured by indirect calorimetry (figure 3.20), which is shown in the form of a Bland Altman plot, which is a statistical measure to compare two statistical methods (Bland and Altman 1986). In this method the differences between the two techniques are plotted against the averages for each technique. Horizontal lines are drawn at the mean difference between the techniques and the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences (Bland and Altman 1986). The integrated results also investigated correlations between predicted fasting EE using Schofield equations and measured total daily EE by diet diaries (figure 3.21), fasting EE measured by indirect calorimetry measured and total daily EE by diet diaries (figure 3.22), and finally the relationship between BMI and EE (figure 3.23). As all the results were considered normally distributed Pearson's correlation was used for analysis.

Graph of Predicted EE versus EE by Calorimetry
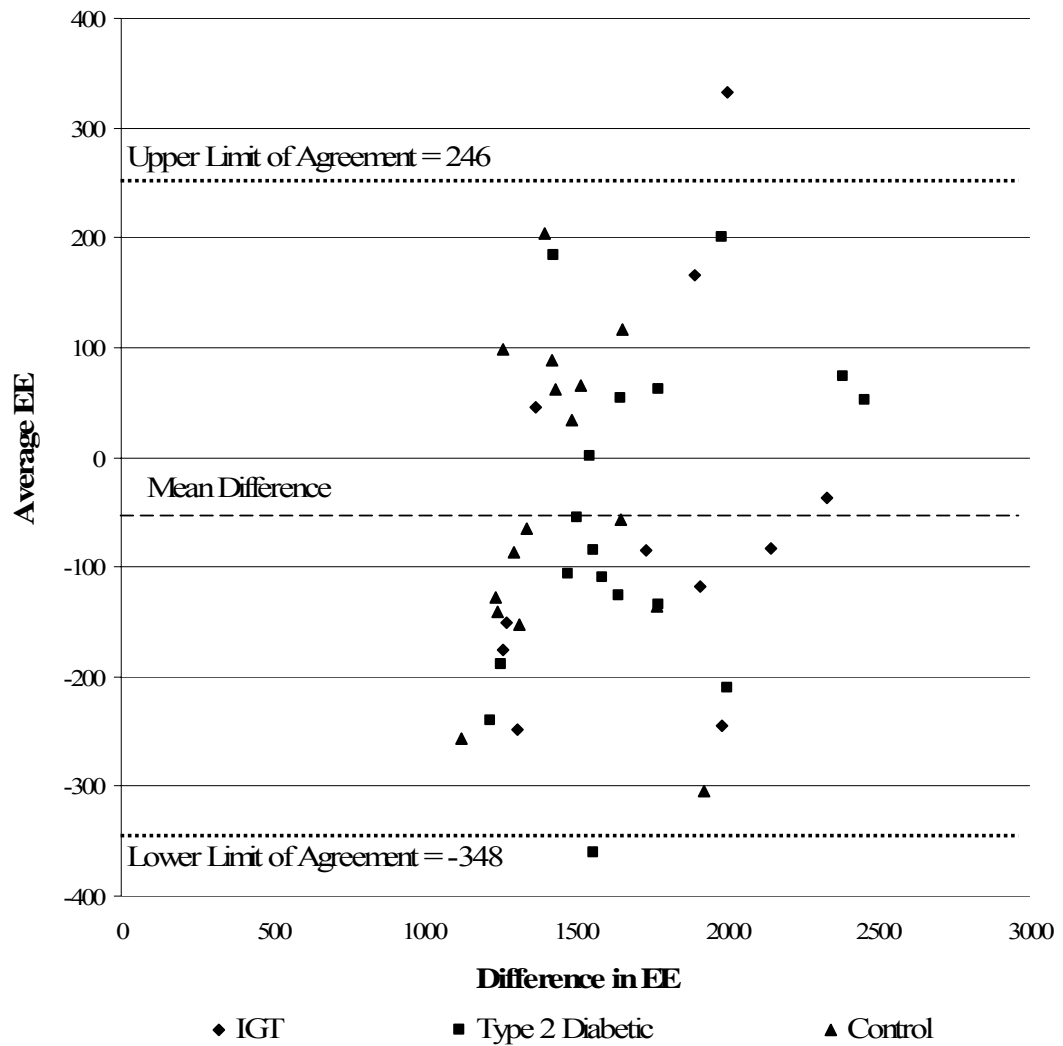
Figure 3.20 Bland Altman Plot of Predicted EE measured by the Schofield Equations and EE Measured by Indirect Calorimetry for IGT, type 2 diabetics and control participants. Lines include; ▬ ▬ the mean difference between the two methods; ·········· and both the upper and lower limits of agreement.
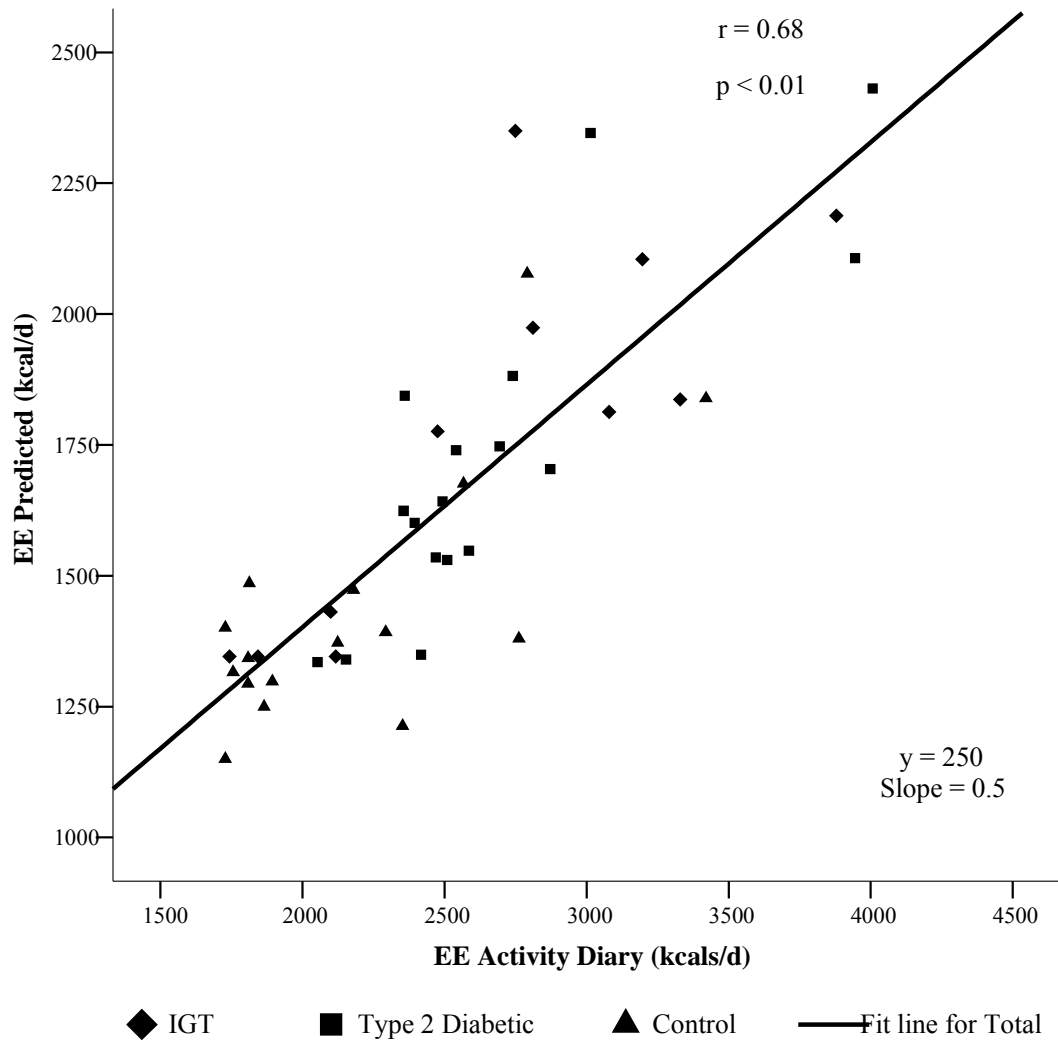
Figure 3.21 Pearsons Correlation between Predicted EE measured by the Schofield equations and EE recorded from Diet Diaries in IGT, type 2 diabetic and control participants. The line represents the linear trend. The intercept and slope of line are given on the graph.

r = 0.556

p < 0.001

y = 416.67
Slope = 66.67

◆ IGT   ▲ Control
■ Type 2 Diabetes   ──── Fit line for Total
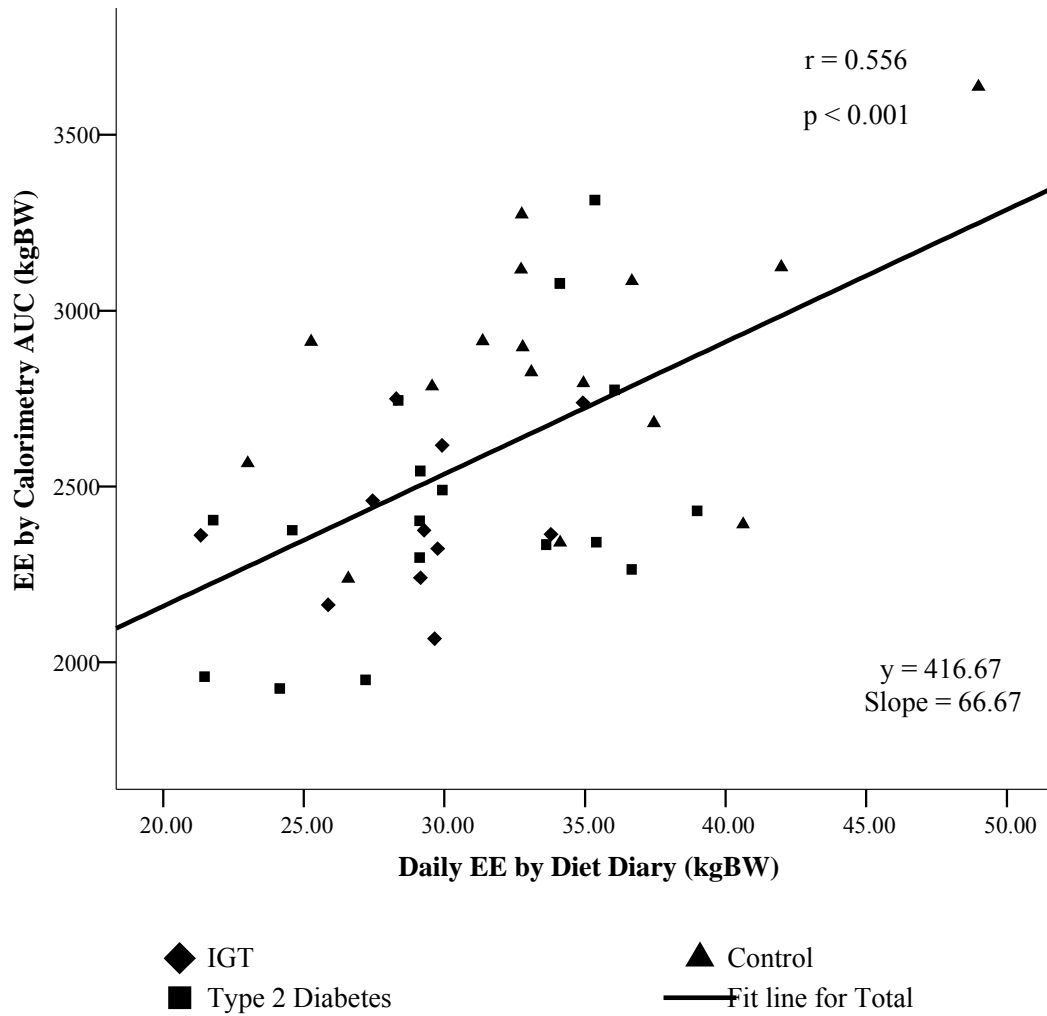
Figure 3.22 Pearsons Correlation between EE measured by Indirect Calorimetry (kgBW) and EE recorded from Diet Diaries (kgBW) for IGT, type 2 diabetics and control participants. The line represents the linear trend. The intercept and slope of line are given on the graph.
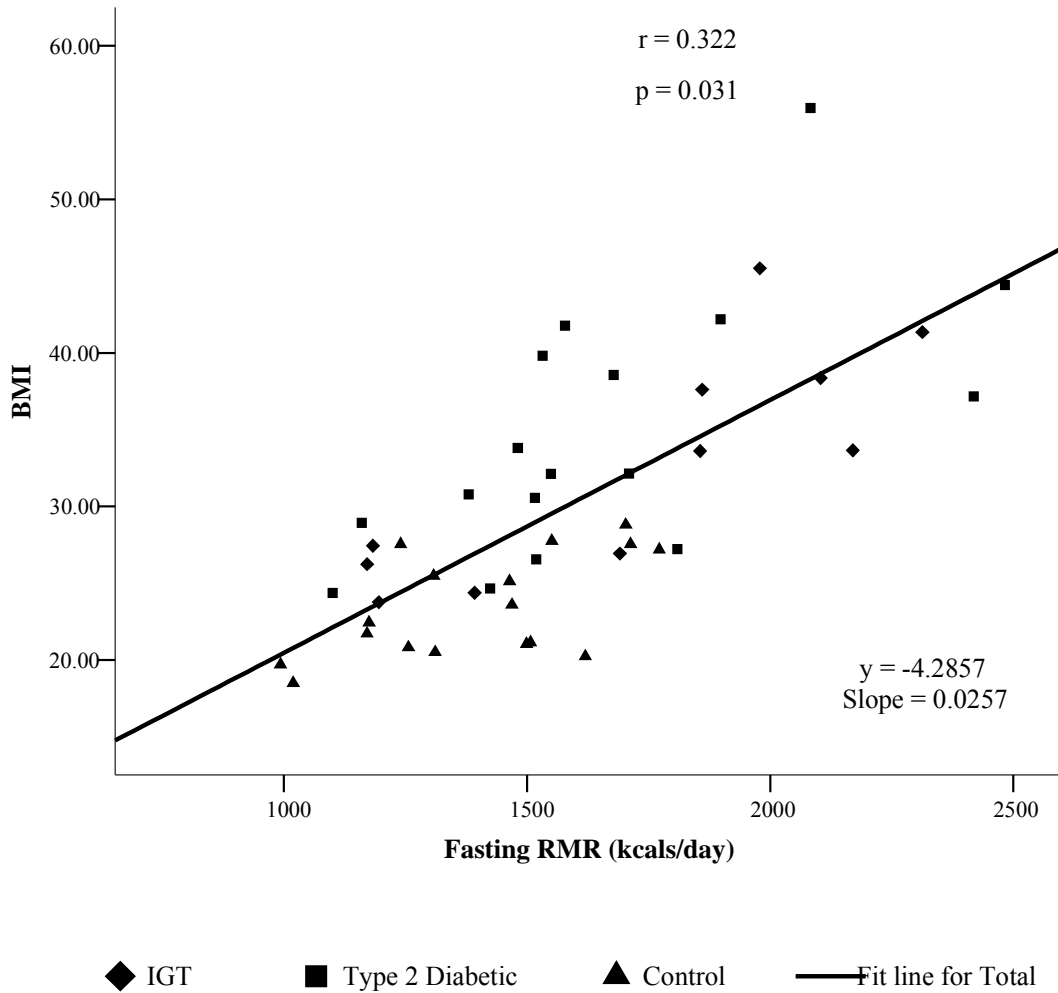
Figure 3.23 Pearsons Correlation of BMI and Fasting Resting Metabolic Rate measured by Indirect Calorimetry for IGT, type 2 diabetics and control participants. The line represents the the linear trend. The intercept and slope of line are given on the graph.

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Results show a strong positive correlation between EE predicted by the Schofield equations and calorimetry measured EE (kcals/day) as expected, $p<0.01$ (fig. 3.20). Therefore as predicted EE increases so does EE measured by calorimetry. The Bland Altman plot shows that most participants fall within the limits of agreement. There are 2 outliers, one IGT participant above the upper limit of agreement and one type 2 diabetic participant below the lower limit of agreement. Furthermore, as predicted EE increases so too does total EE measured by diet diaries, $p<0.01$ (fig 3.21). A correlation between EE measured by activity diaries and EE measured by calorimetry was done (figure 3.22). The rational for this test was to assess the association between subjective measures and indirect measures of EE which has not, to my knowledge, been done before. For the purpose of this measurement, the AUC measurement for calorimetry gave a single value to allow comparison between measurements. This association was assessed independently of body weight to determine if there was a correlation between direct and indirect measurements of EE when evaluating individuals with a metabolic disease such as IGT or type 2 diabetes. A significant positive correlation, $p<0.001$ was found between the two EE measurements therefore as EE recorded by diet diaries increased there was an evidenced increase in EE recorded by calorimetry also, independent of weight. In general, when observing the control participants in figure 3.22, they tended to be located further up the scale and therefore appeared to have a higher EE whereas IGT's and type 2 diabetics were more towards the left of the figure, indicating a lower EE. Lastly, a positive correlation was observed between resting RMR measured by calorimetry and BMI, $p<0.05$. The control participants tended to be towards the left of the figure 3.23 whereas type 2 diabetics and particularly IGT

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participants tend to be at the higher end of the graph indicating a higher BMI and resulting in a higher fasting RMR.

3.6   RESPIRATORY QUOTIENT

RQ was measured at each timepoint by calorimetry to determine fasting and postmeal values, so that an assessment could be made about the fuel mix being oxidised. The RQ values were analysed using repeated measures ANOVA with Bonferroni posthoc tests. Intragroup comparisons demonstrated that there was significant differences within groups across time for all three groups ($p < 0.0001$) as expected. The results show that the RQ of all groups rose from fasting and did not revert to fasting levels before the end of the test period. The greatest rise from fasting levels was observed in the control group between fasting and 30 minutes and their RQ remained higher than the other two groups until the end of the test period. At 30 minutes the spread of the control group was large, indicating that there were many data points far from the mean. A possible result of this has meant there is no significant difference between groups at this timepoint. The Bonferroni post hoc tests suggested there were no significant intergroup differences.
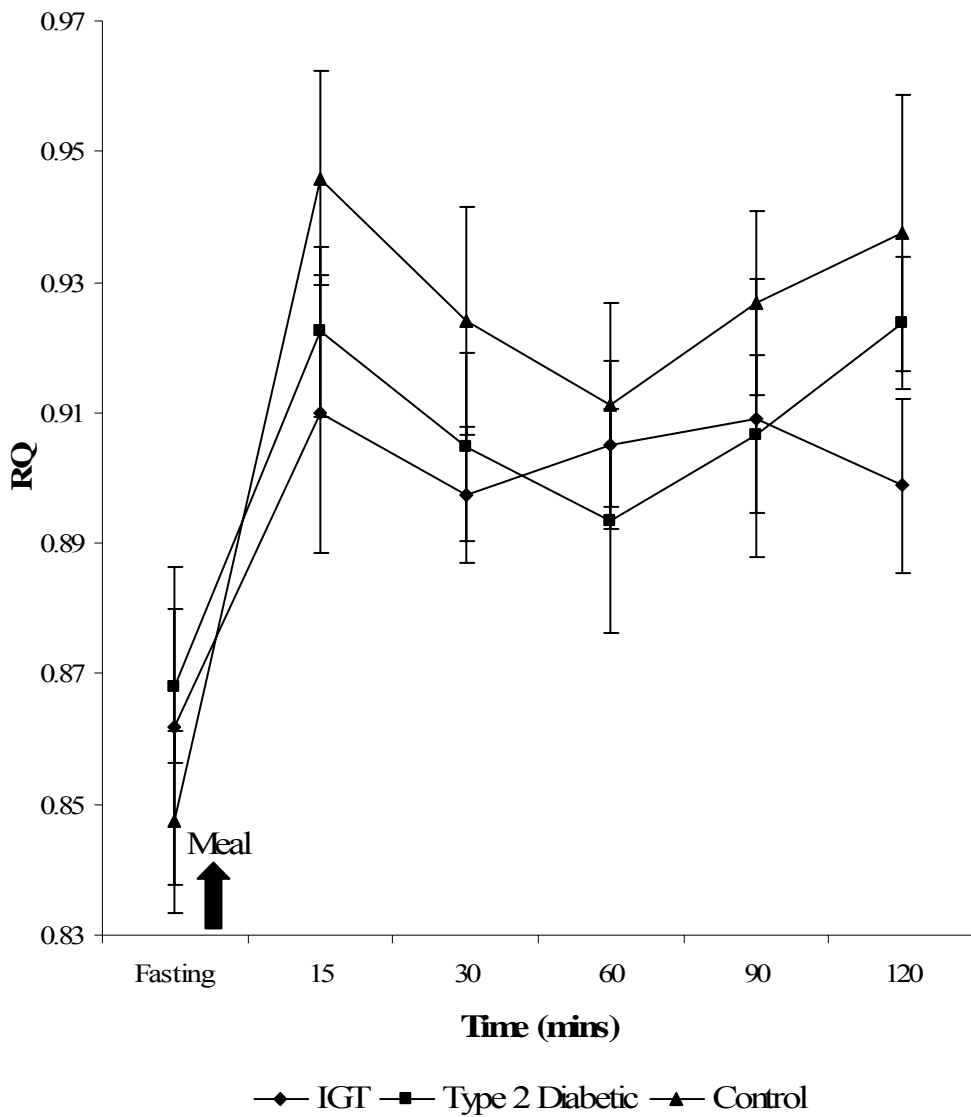
Figure 3.24 Intragroup and Intergroup Comparisons in RQ over the Study Period
Values are Mean and SEM

3.7   SUBSTRATE OXIDATION/NPRQ

Respiratory quotient was measured at each timepoint by indirect calorimetry and was combined with nitrogen analysis, to indicate the non-protein respiratory quotient (NPRQ). NPRQ was calculated using equations from Jequier, 1987. To determine an association between protein intake from individuals' habitual diet and nitrogen excreted in the urine, a Pearson's correlation was completed (figure 3.25). This would permit assumptions to be made about the accuracy of the completed diet diaries.

This would permit assumptions to be made about the accuracy of the completed diet diaries. A positive correlation was found between energy intake (r=0.337), derived from the measurement of food intake from diet diaries and urinary nitrogen. The result allows the assumption to be made that the diet diaries were recorded accurately. However, the r value shows that the degree of correlation, although significant was low.   As discussed earlier, the accuracy of diet diaries is always in question. Therefore, an investigation as to the accuracy of energy intake recorded by diet diaries was undertaken. Urinary nitrogen is a well known and established biomarker for protein intake (Tasevska et al. 2006). Therefore, total urinary nitrogen (TUN) can be used to calculate protein intake, perhaps with more certainty than diet diaries. Urinary nitrogen was used to calculate protein intake on the basis that:

protein intake (g/day) = total urinary nitrogen (g/day) x 6.25 (because 1gN – 6.25g protein) + 10 (g) (insensible losses such as fecaes, skin, fluids etc).

This calculation allowed assumptions to be made as to the level of accuracy of protein and also to make generally assumptions as to the accuracy of other nutrients. Figure 3.25.1 gives a clear indication of any outliers within each group. The control group are the only group to have a significant p value (p=0.01) indicating that they are the only group to have a significant correlation between the two measurements of protein intake. The IGT group appear to have number of participants below the correlation line indicating an under-reporting of protein intake by diet diaries and one outlier over-estimated their protein intake by 2 times. The type 2 diabetic group appears to be sporadically arranged and have the lowest correlation value, with no significance. Two of the diabetic group in particular under reported their protein intake by 50%, however some also grossly over estimated their protein intake.

Figure 3.25.2 shows that most participants fall within the limits of agreement, thus suggesting that urinary nitrogen is a plausible method for estimating energy intake. There are 3 outliers; 1 IGT participant and 2 type 2 diabetic participants. This may indicate that the methods of estimating energy intake are not as reliable for these groups as the control group. This figure may even show that energy intake, estimated by urinary nitrogen may be more useful than diet diaries because it eliminates the process of under reporting food stuffs. However, it must be used with caution, particularly in obese participants because the variety of fat intake ranges

considerably, the current study ranges between 21% and 42% fat intake for the two overweight groups.

Substrate oxidation rates were measured in the morning by indirect calorimetry after an overnight fast, with the patient awake, at room temperature. One control patient did not manage to complete the 120 timepoint in this study and is therefore eliminated from these results. At this time of the day and under these conditions, energy expenditure best represents resting metabolic rate (RMR) (Haugen et al. 2003). Results are shown with a correction for body weight so the observed aberrations were as a result of the disease status. See figures 3.26 and 3.26.1 for CHO and fat oxidation rates respectively.
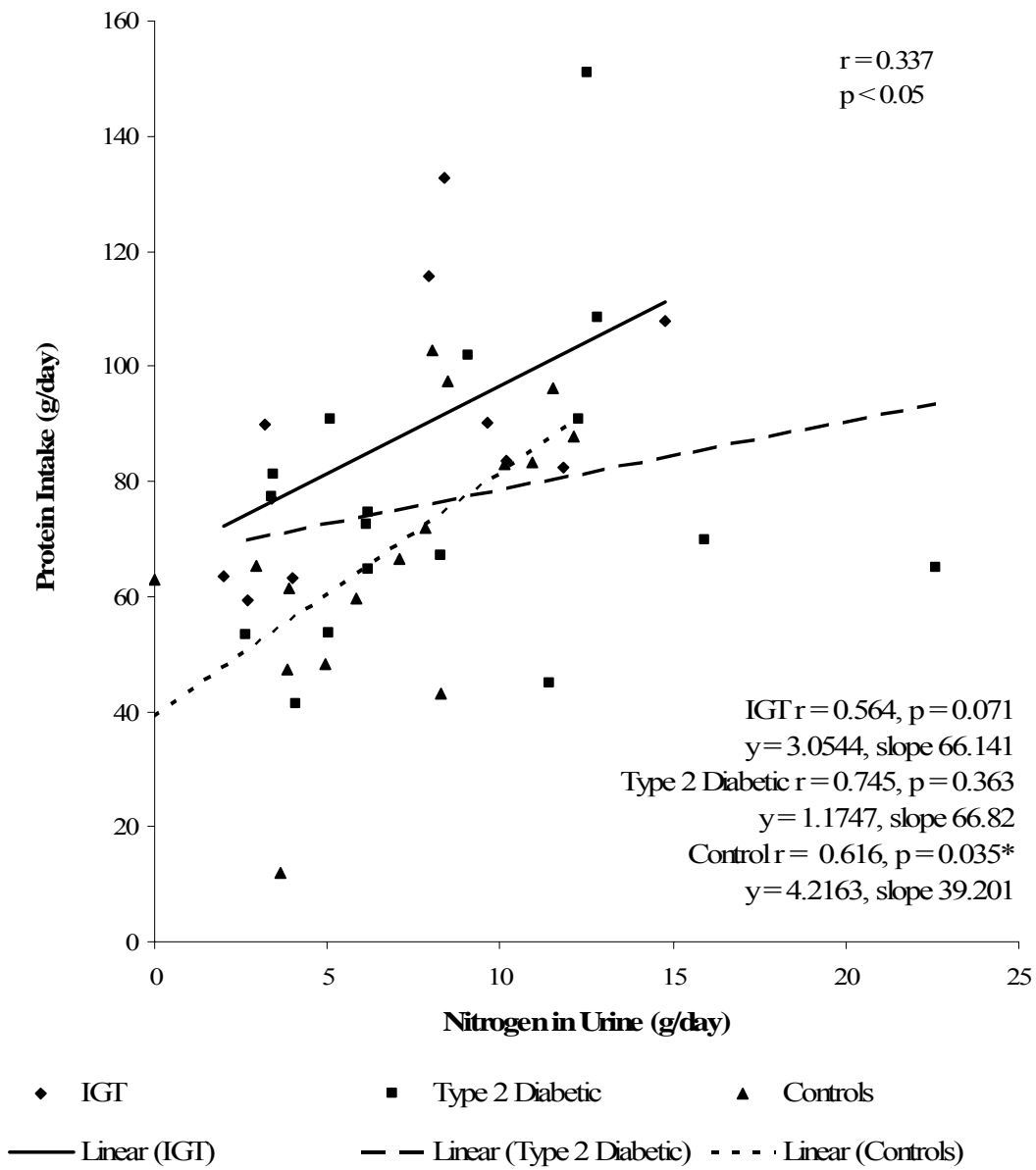
Figure 3.25 Pearson's Correlation of Nitrogen Intake recorded by Diet Diaries and Nitrogen Excreted in Urine, both in grams/day for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the slope and intercept is recorded for trend line.
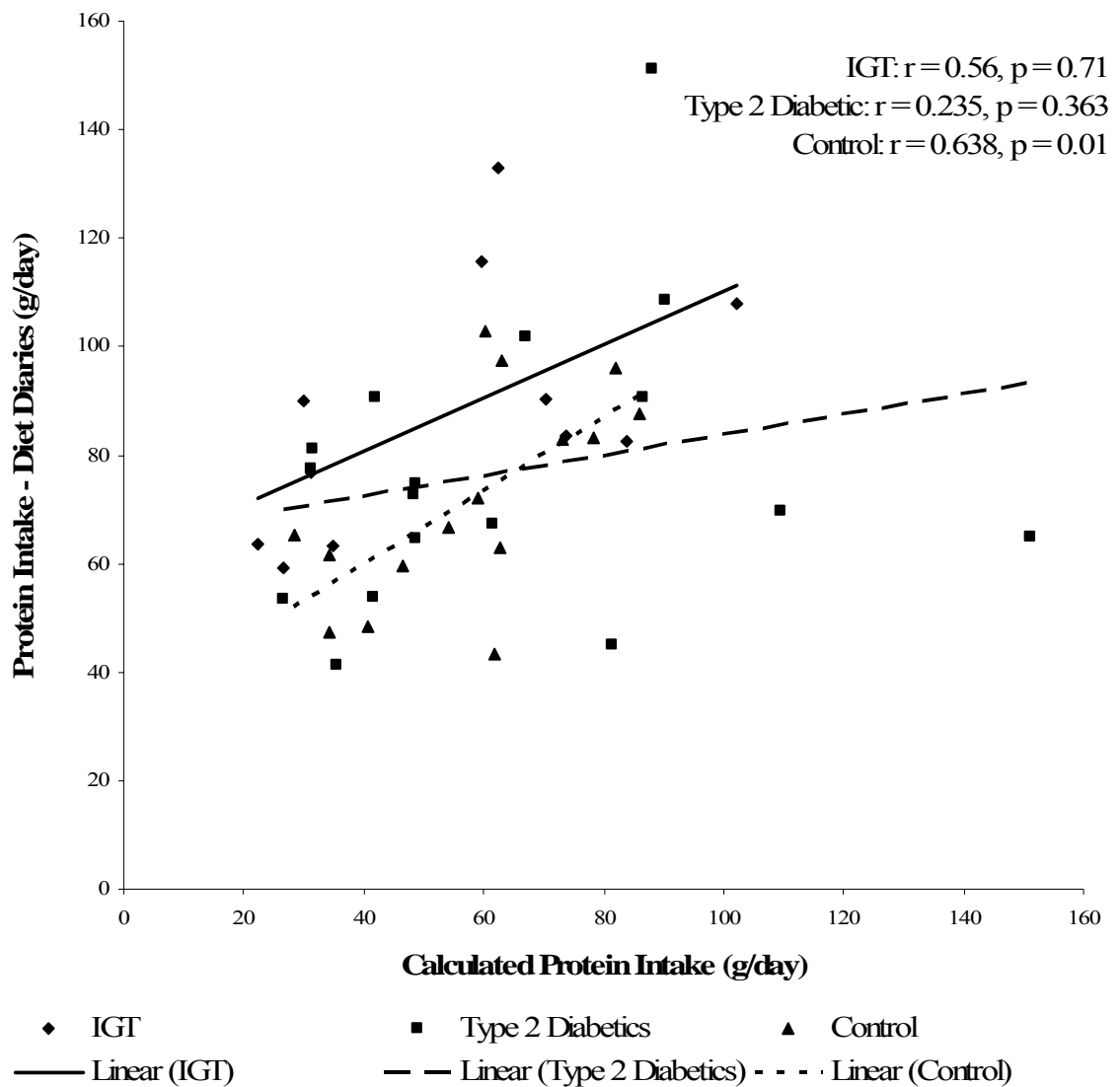
Figure 3.25.1 Pearson's Correlation of Protein intake (g/day) estimated from Diet Diaries and Calculated Protein Intake using Urinary Nitrogen for IGT, type 2 diabetic and control participants. The lines represent the linear trend for each group.
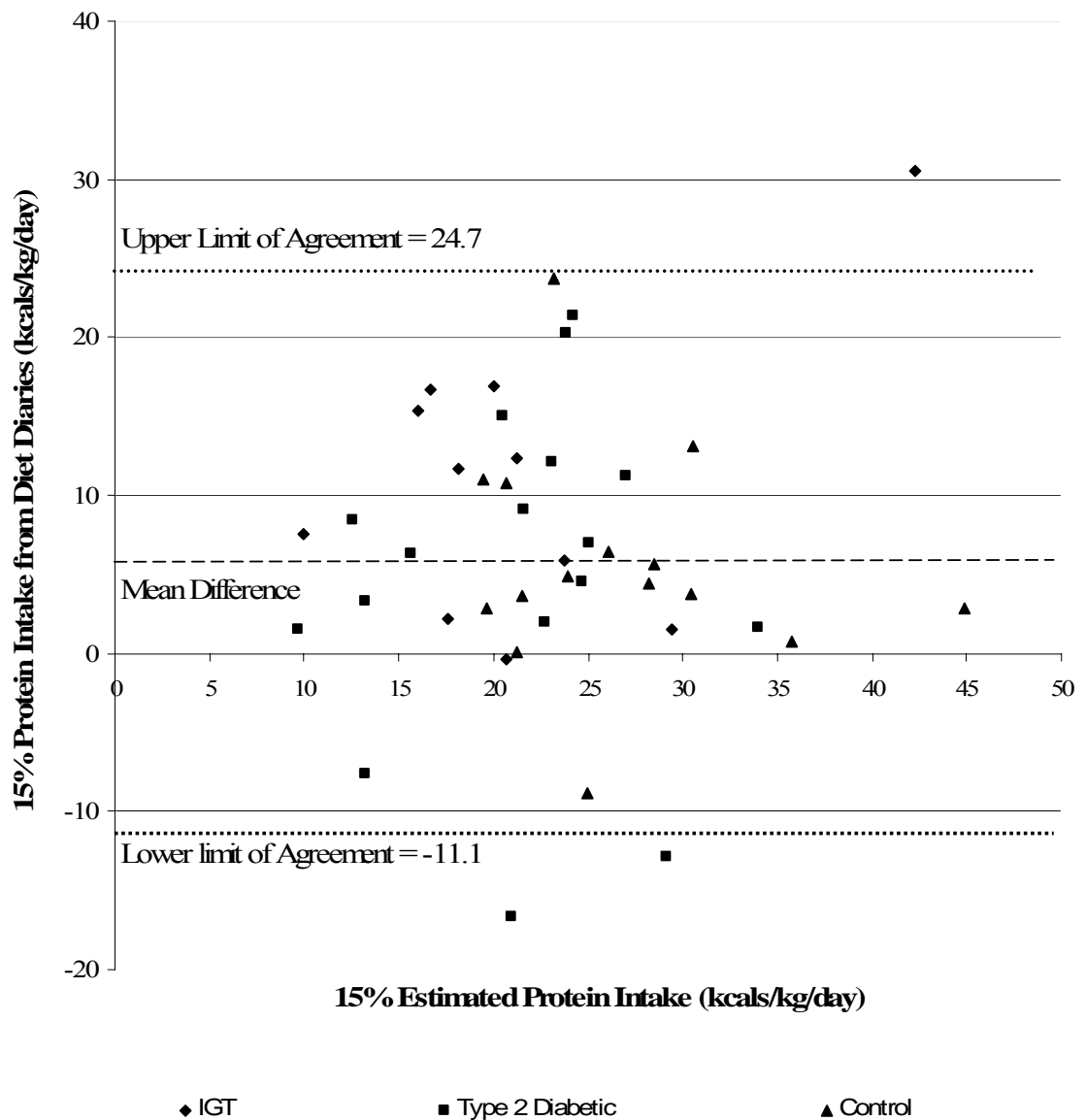
Figure 3.25.2 Bland Altman Plot of two methods of estimating Protein Intake; 1) Protein intake measured from diet diaries and 2) Protein intake measured from Urinary Nitrogen. A value of 15% protein intake was used as an average percentage intake of the 3 groups and was used to reveal the relationship between the differences and the averages and to identify possible outliers. The lines represent; ▬ ▬ the mean difference between the two methods; ········ and both the upper and lower limits of agreement.
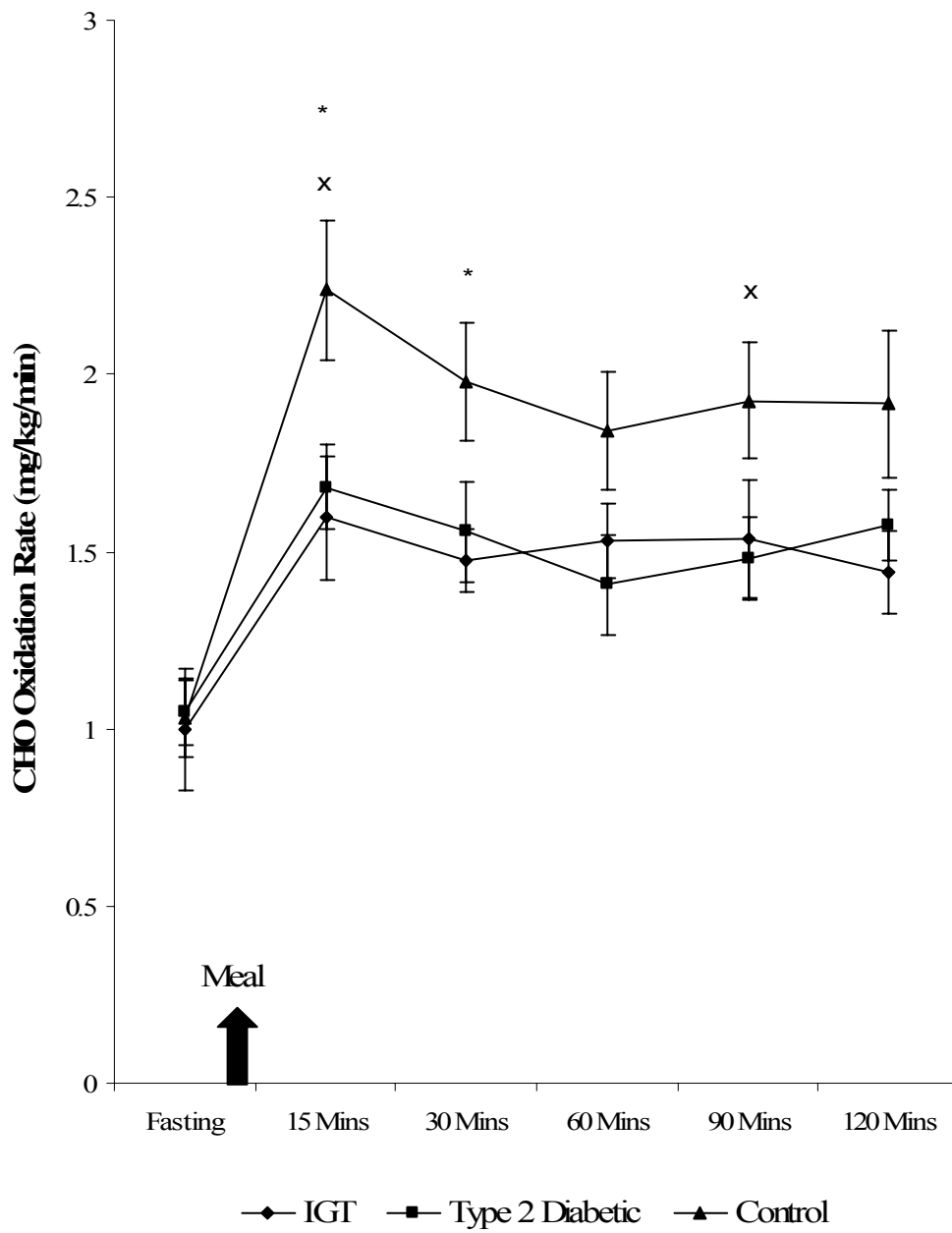
Figure 3.26 CHO Oxidation Rates in IGT, Type 2 Diabetic and Control Groups
Values are Mean and SEM
* = p<0.05 IGT group is significantly different from control group
x = p<0.05 Type 2 Diabetic group is significantly different from the control group
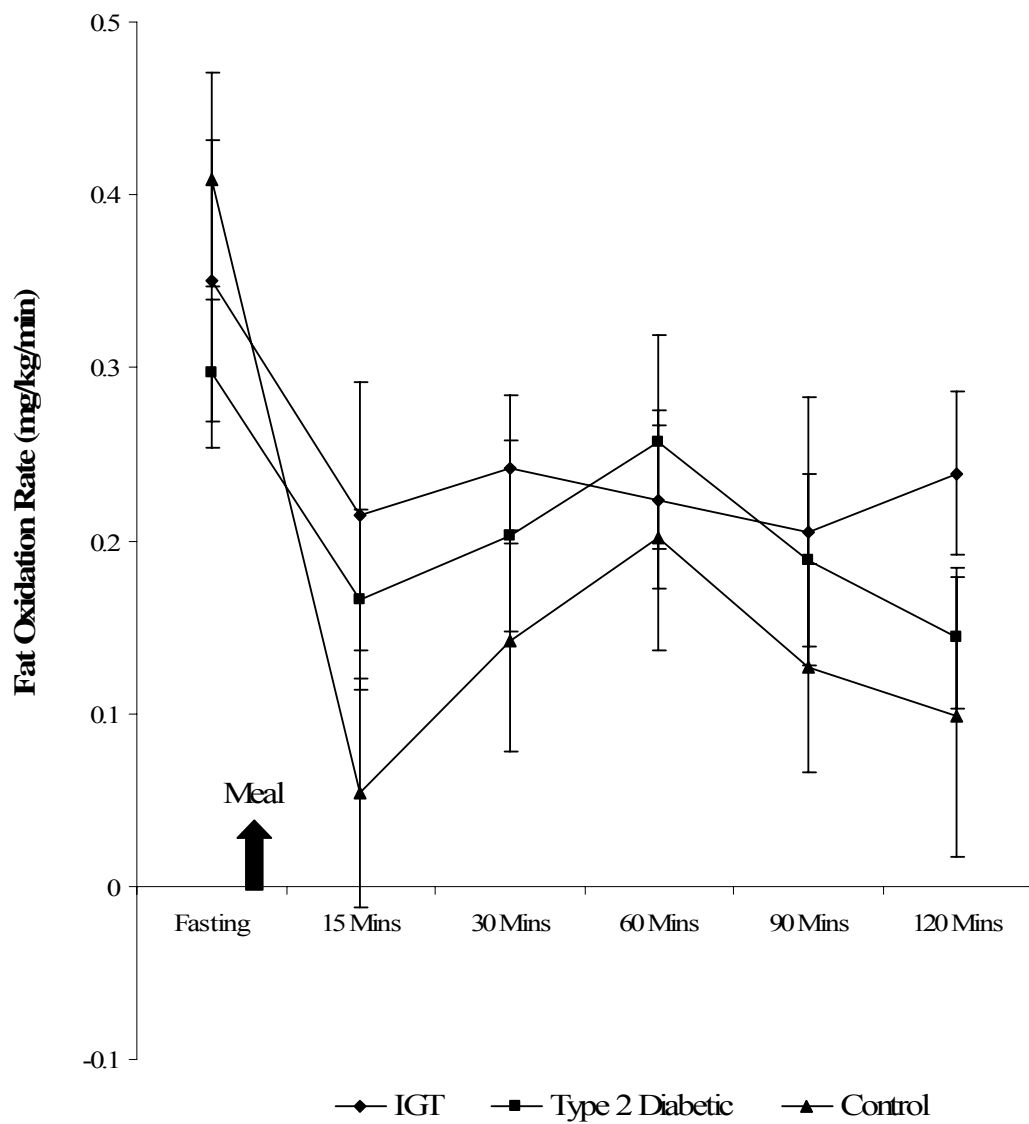
Figure 3.26.1 Fat Oxidation Rates in IGT, Type 2 Diabetic and Control Groups
Values are Mean and SEM

Figures 3.26 and 3.26.1 indicate the metabolic response to the test meal in all three groups. In each group initial substrate oxidation rates indicated comparable fat and CHO oxidation rates as may be expected in individuals after an overnight fast. Figure 3.26 shows at 15 minutes post meal CHO oxidation increased in all groups however to a significantly higher level in control participants ($p<0.05$). At 30 minutes post meal CHO rates began to decrease in all groups but to a lesser degree in control participants resulting in significantly higher CHO rates when compared to IGT participants ($p<0.05$), who had the lowest levels of CHO oxidation at that time. At 60 minutes this decrease continued in type 2 diabetic and control participants but increased slightly in IGT participants however there was no significant difference. There was a significant difference between type 2 diabetic and control participants at 90 minutes post meal when type 2 diabetic and control participants' CHO oxidation rates increased slightly as IGT participant levels decreased. At 120 minutes there was a further increase in oxidation rates in type 2 diabetic and control participants' CHO oxidation rates and a further decrease in IGT participants although this was not statistically significant.

Figure 3.26.1 illustrates large decreases in fat oxidation at 15 minutes post meal and then rates began to increase at 30 minutes for all groups. At 60 minutes post meal type 2 diabetic and control participants increased further however the IGT participants fat oxidation rates reduced slightly. At 90 minutes post meal all 3 groups' fat oxidation rates reduced and type 2 diabetic and control participant levels continued to reduce until 120 minutes whereas IGT participant levels increased

slightly at 120 minutes. There were no significant differences between groups at any timepoint throughout the study period for fat oxidation.

CHO (figure 3.26.2A) and fat (figure 3.26.2B) substrate oxidation rates were also expressed as the amount of change (g/day) at each timepoint following the test meal. Control participants appeared to show the greatest reduction in fat oxidation rates following the meal, followed by IGT and type 2 diabetic participants however this result was not significant.   Control participants also demonstrated the greatest increase in CHO oxidation rates postmeal.

Protein Oxidation rates are also illustrated independent of body weight. Results are shown as milligram of protein oxidised per kilogram body weight per minute, see figure 3.26.3. IGT participants demonstrated the lowest levels of protein oxidation followed by type 2 diabetic participants with control participants exhibiting the highest protein oxidation rates. No significant differences were observed between IGT, type 2 diabetic and control groups.

Differences in oxidation rates signify dysfunctional processes in the control of food intake. It has been hypothesised that insulin and glucose levels affect oxidation rates following the ingestion of food. Therefore, the protocol of this study incorporated the measurement of blood parameters such as ghrelin, glucose and insulin.

A
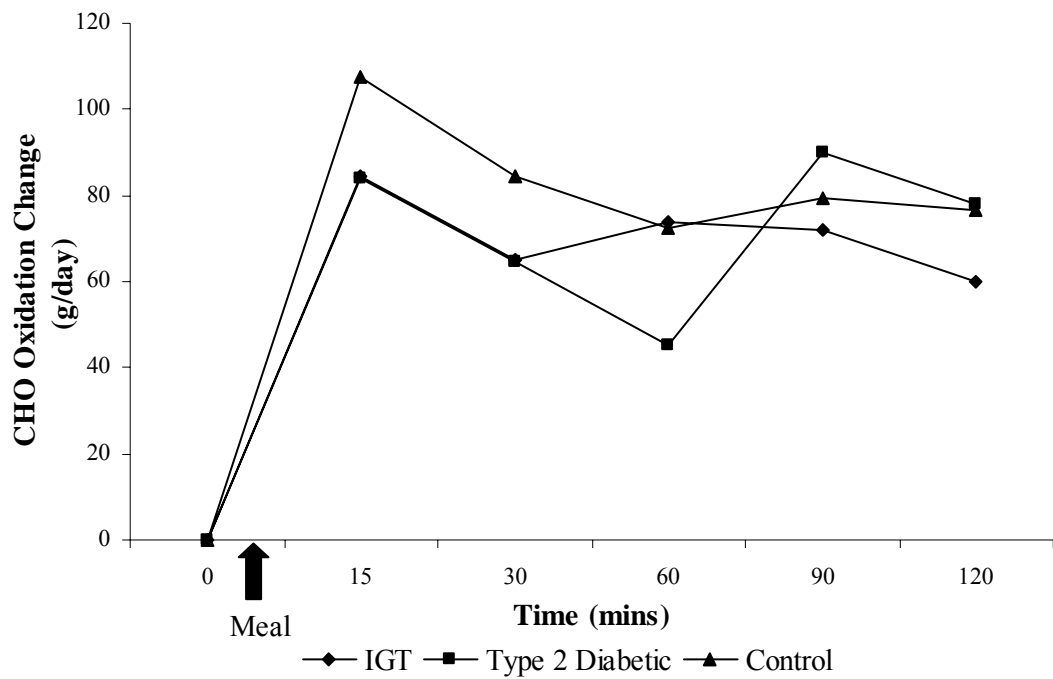
B
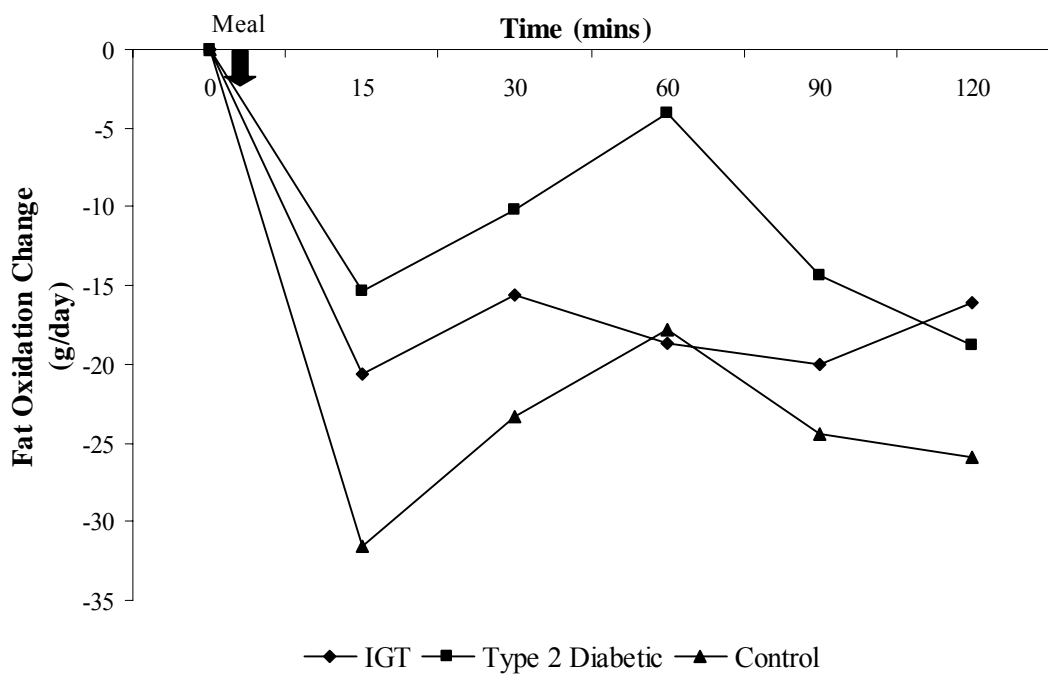
Figure 3.26.2 Change (g/day) in Substrate Oxidation in IGT, Type 2 Diabetic and
Control Participants, (A) CHO Oxidation and (B) Fat Oxidation
Values are Mean and SEM
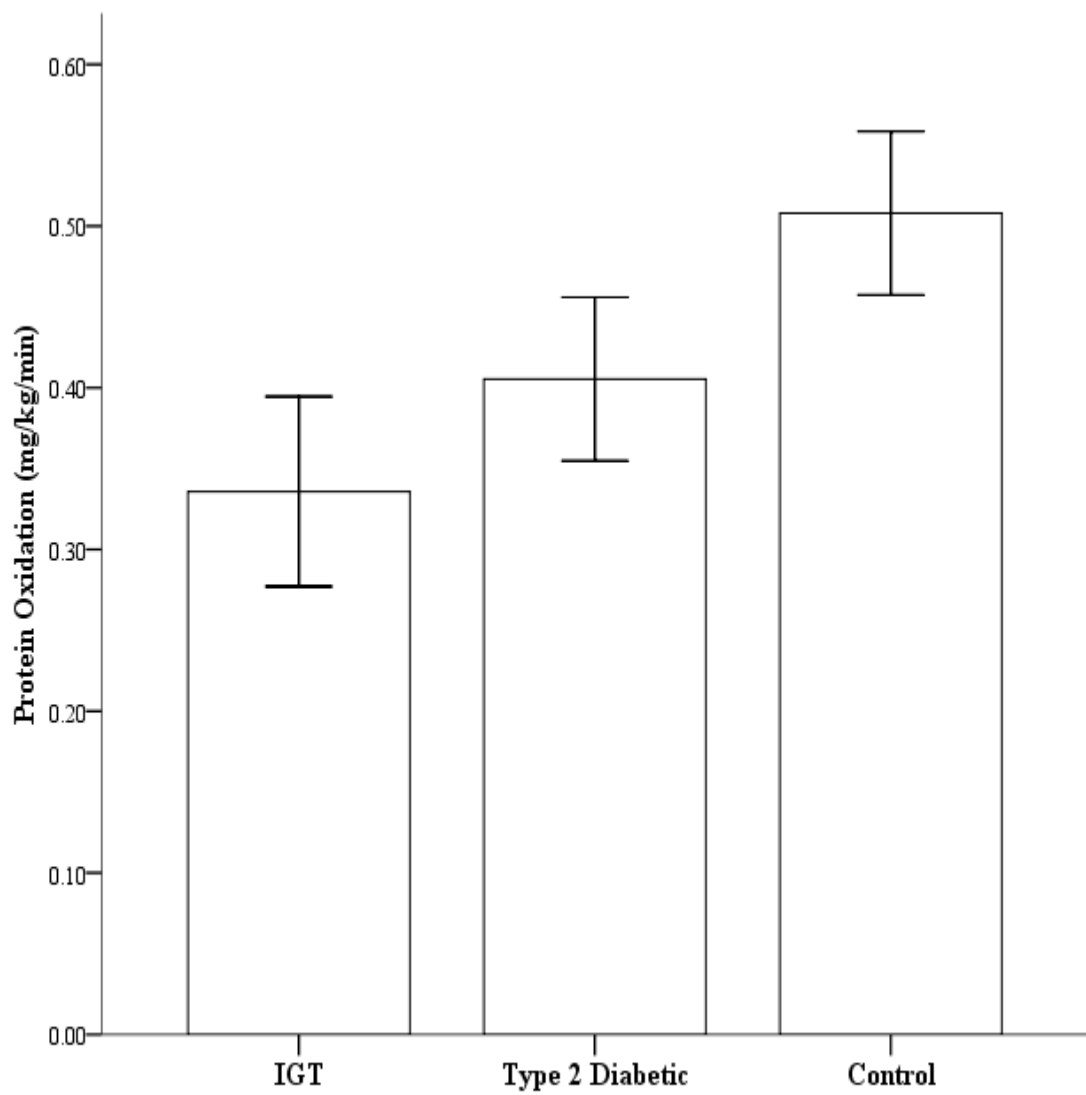
Figure 3.26.3   Protein Oxidation Rates in IGT, Type 2 Diabetic and Control Participants

3.8   BLOOD ANALYSIS

Blood sample aliquots were taken on 6 separate occasions throughout the study day. Blood was taken and analysed for glucose, insulin and ghrelin. These samples were assessed using intergroup comparisons. Results are expressed in standard international units and will be indicated on the individual graphs. Results were analysed as described in the methods chapter.

3.8.1   Glucose

Fasting blood glucose results indicated that the control group had significantly lower blood glucose than IGT (p=0.0001) and type 2 diabetic (p=0.0001) participant groups (figure 3.27). All 3 groups' plasma glucose levels increased 30 minutes postmeal and while control participants glucose levels reduced 60 minutes postmeal, IGT and type 2 diabetic participants' plasma glucose levels continued to rise. The results show that control participants had statistically lower glucose levels at 30 and 60 minutes between than both IGT and type 2 diabetic groups.

Glucose parameters were also shown as AUC (figure 3.28) and confirmed the difference in glucose response between groups as again control participants had a significantly lower blood glucose response when compared to IGT (p=0.0001) and type 2 diabetic (p=0.0001) participants.
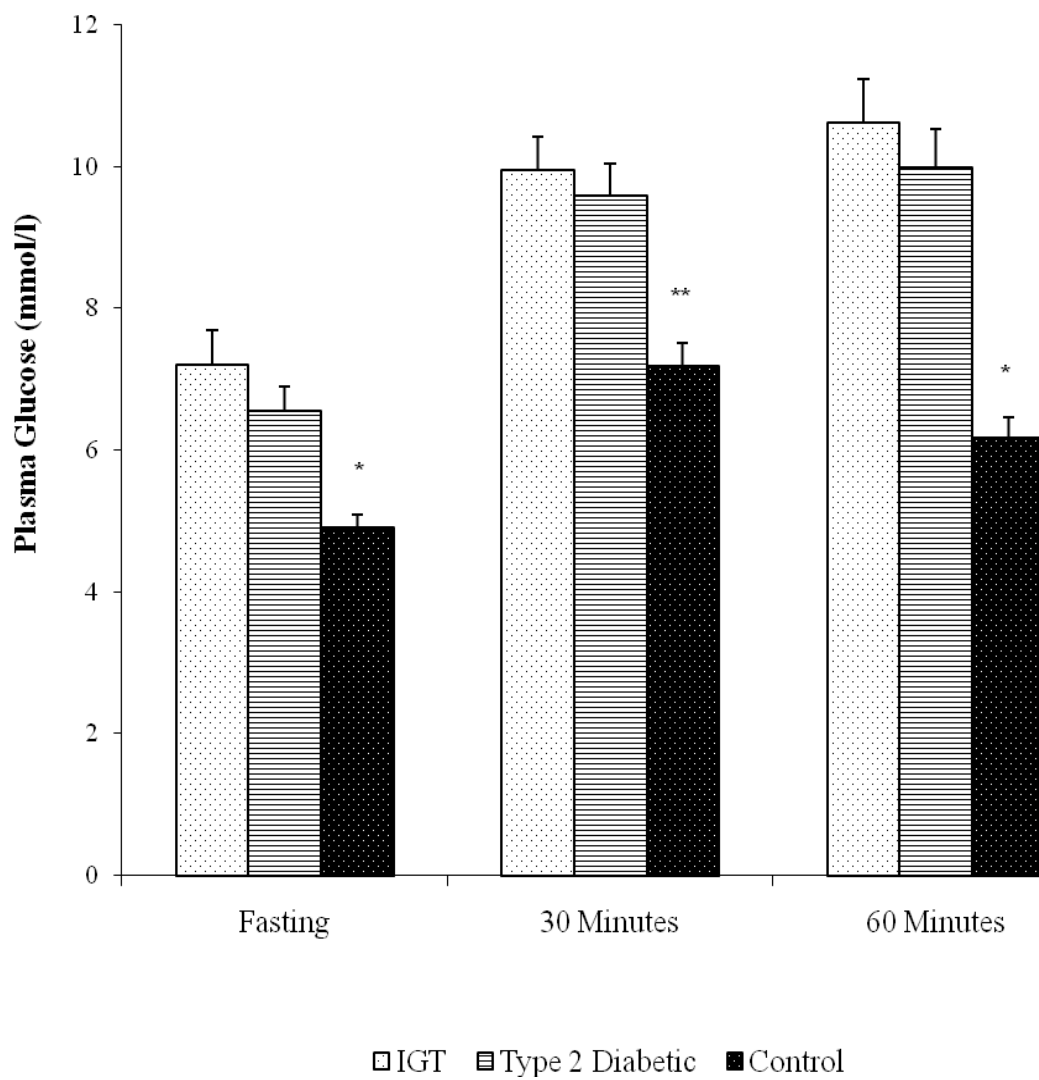
Figure 3.27 Plasma Glucose Concentrations in IGT, Type 2 Diabetic and Control
Participants
Values are Mean and SEM
* p<0.0001 Control group significantly different from other 2 groups
**p<0.001 Control group significantly different from other 2 groups
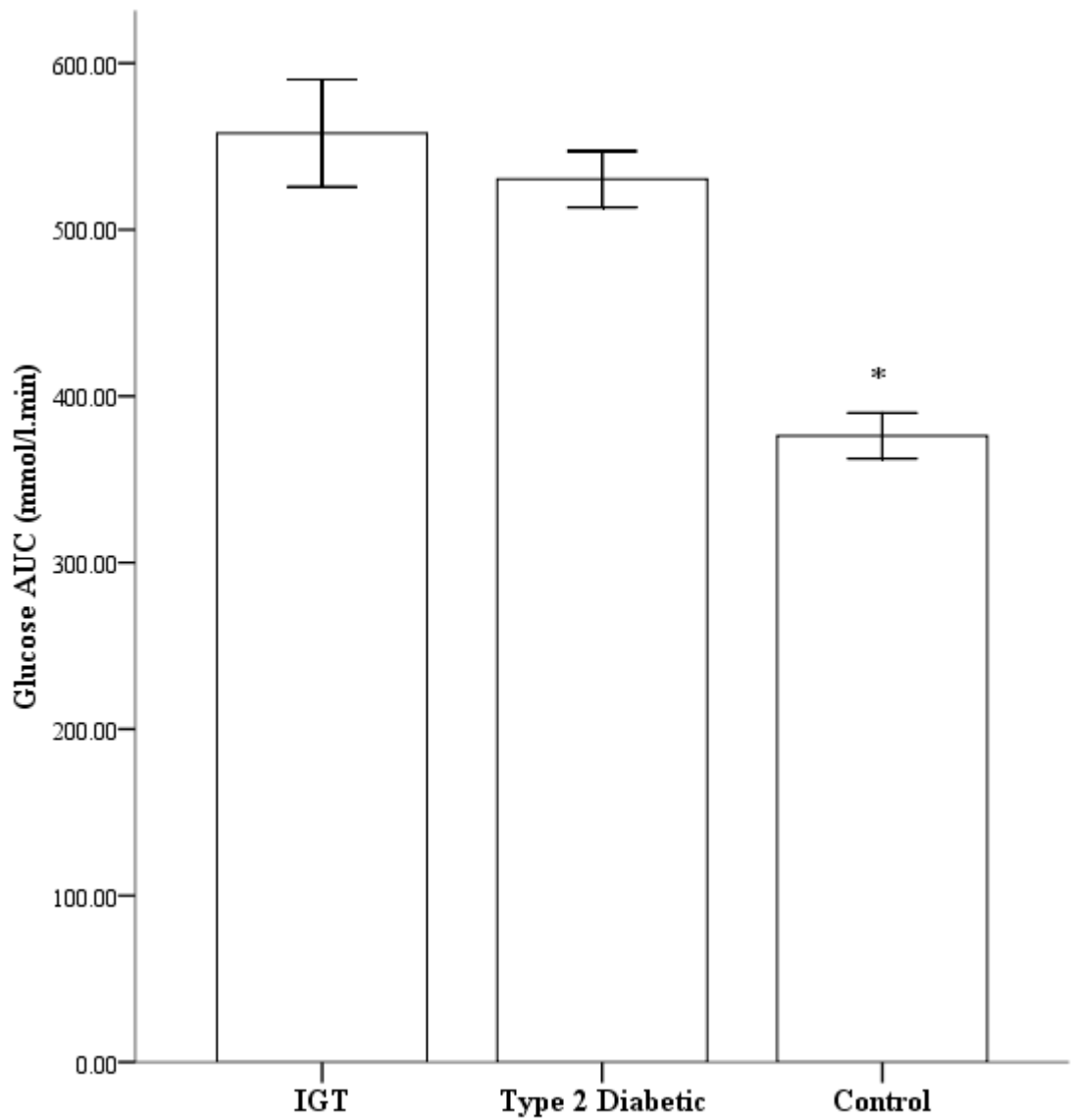
Figure 3.28 Glucose AUC Response in IGT, Type 2 Diabetics and Control Participants

Values are Mean and SEM

* p<0.0001 Control group significantly different from other 2 groups

3.8.2   Insulin

Fasting insulin levels indicated that control participants had a significantly lower insulin concentration than IGT ($p < 0.05$) and type 2 diabetics ($p < 0.01$), see figure 3.29. Thirty minutes postmeal showed an increase in insulin levels in all groups but to the greatest degree in the control participants. Control participants had significantly higher concentrations when compared to IGT participants at this stage ($p < 0.01$) but there was no significant difference between control and type 2 diabetics. As insulin levels continued to rise at 60 minutes post meal control participants again had significantly higher levels than both IGT and type 2 diabetic groups ($p < 0.05$).

Insulin parameters were also shown as AUC (figure 3.30) and the values show that the overall insulin response was significantly greater in control participants when compared to IGT ($p < 0.05$) participants and the insulin response of type 2 diabetics was very similar to that of control participants. There was no significant difference between IGT and type 2 diabetic participants.
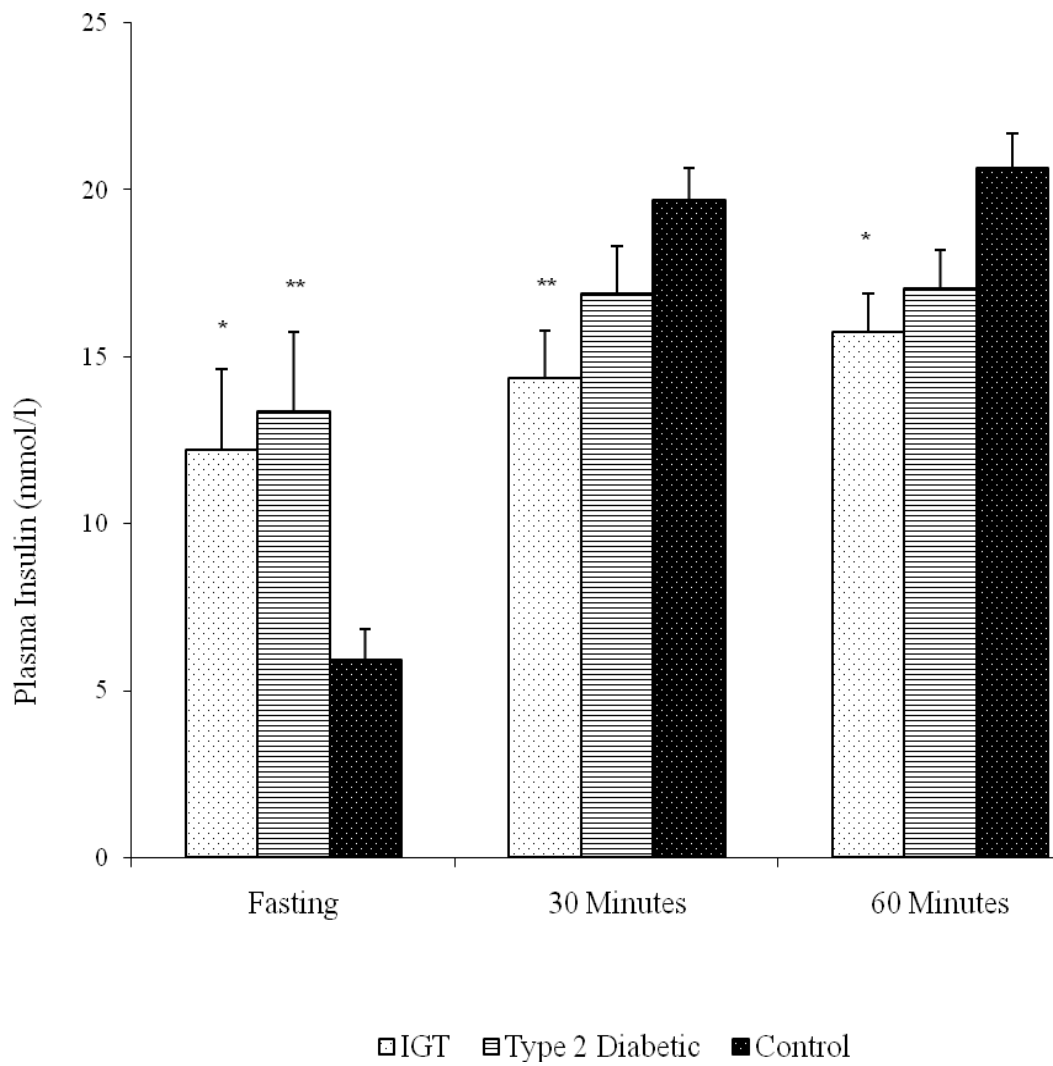
Figure 3.29 Plasma Insulin Concentrations in IGT, Type 2 Diabetic and Control
Participants
Values are Mean and SEM
* $p < 0.05$ significantly different from control group
** $p < 0.01$ significantly different from control group
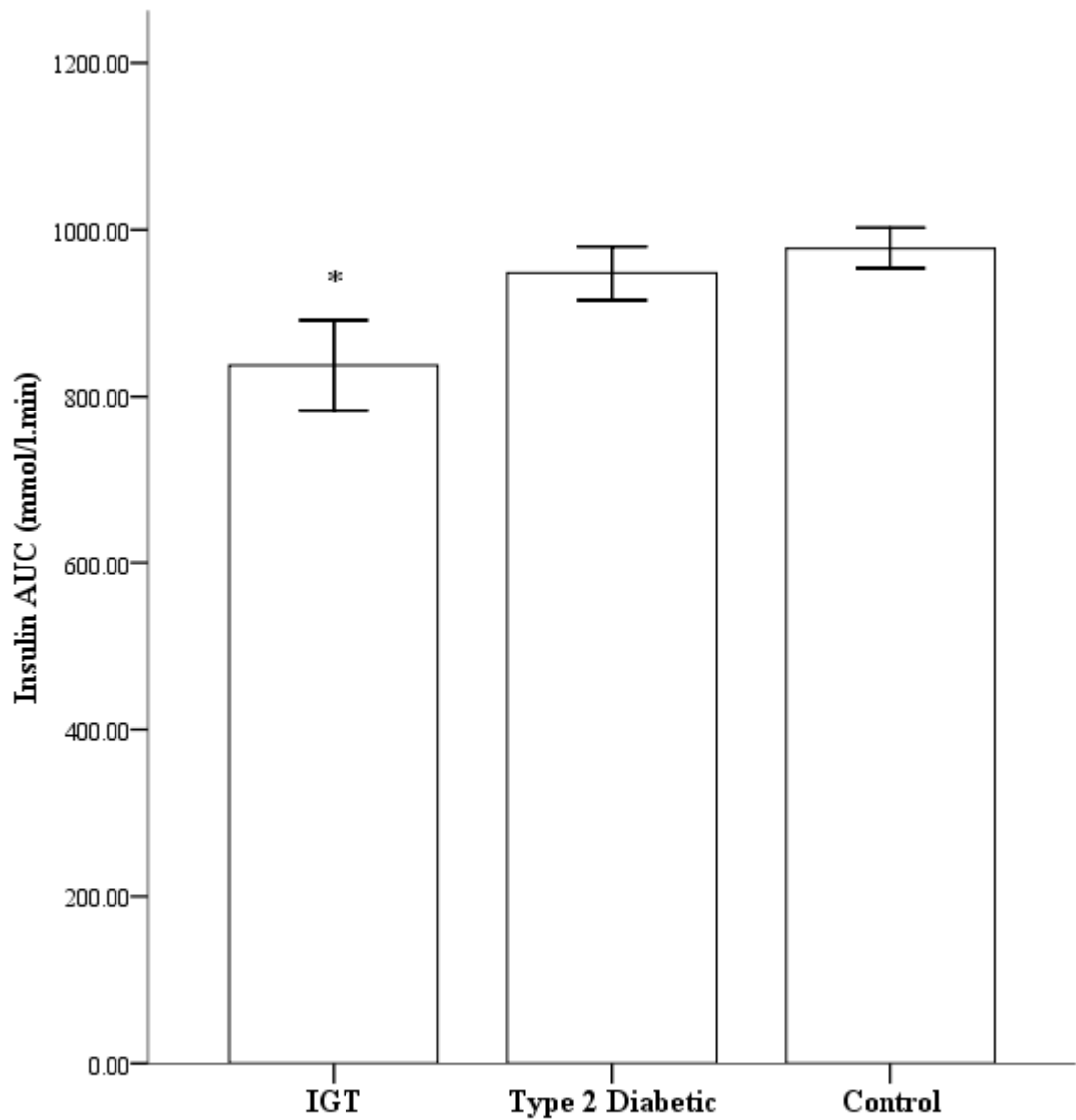
Figure 3.30 Insulin AUC Response in IGT, Type 2 Diabetic and Control Participants
Values are Mean and SEM
* p<0.05 IGT group significantly different from control group

3.8.3   Ghrelin

Table 3.6 demonstrates that fasting ghrelin results indicate no statistical differences between groups throughout the test period. IGT participants showed a reduction in ghrelin levels between fasting and 30 minutes and then a further reduction from 30 to 60 minutes (4.32%). The type 2 diabetic participants' ghrelin concentrations reduced from fasting to 30 minutes and then levels rose between 30 and 60 minutes post meal by 4.77%. Finally control participants demonstrated an increase in plasma ghrelin levels 30 minutes post meal and then ghrelin concentrations reduced at 60 minutes following the test meal.

Table 3.6 Mean Percentage Change from Fasting Ghrelin Levels

% change	IGT	Type 2 Diabetic	Control
Fast to 30 mins	-3.87% (0.05%)	-1.10% (0.06%)	4.69% (0.07%)
Fast to 60 mins	-8.19% (0.07%)	3.67% (0.06%)	1.98% (0.07%)

Values are Mean Percentage Change ± SEM

3.9   INTEGRATED ANALYSIS

This protocol involved undertaking integrated analysis between the mechanisms contributing to the control of food intake. The integrated responses were extremely important to investigate because they depict what is actually happening in the body after the consumption of food. They give an indication about what mechanisms are strongly linked and what mechanisms may only be weakly linked.

3.9.1   Taste and Habitual Diet

To establish whether there was an association between taste status and habitual food intake a correlation between subjective ratings of bitterness on the taste scale and sugar intake measured by diet diaries was performed using Spearman's correlation (figure 3.31). A significant correlation was found demonstrating that as the sensitivity to the bitter compound PROP increases, i.e increasing sensitivity from nontaster to taster to supertaster, the corresponding sugar intake decreases.
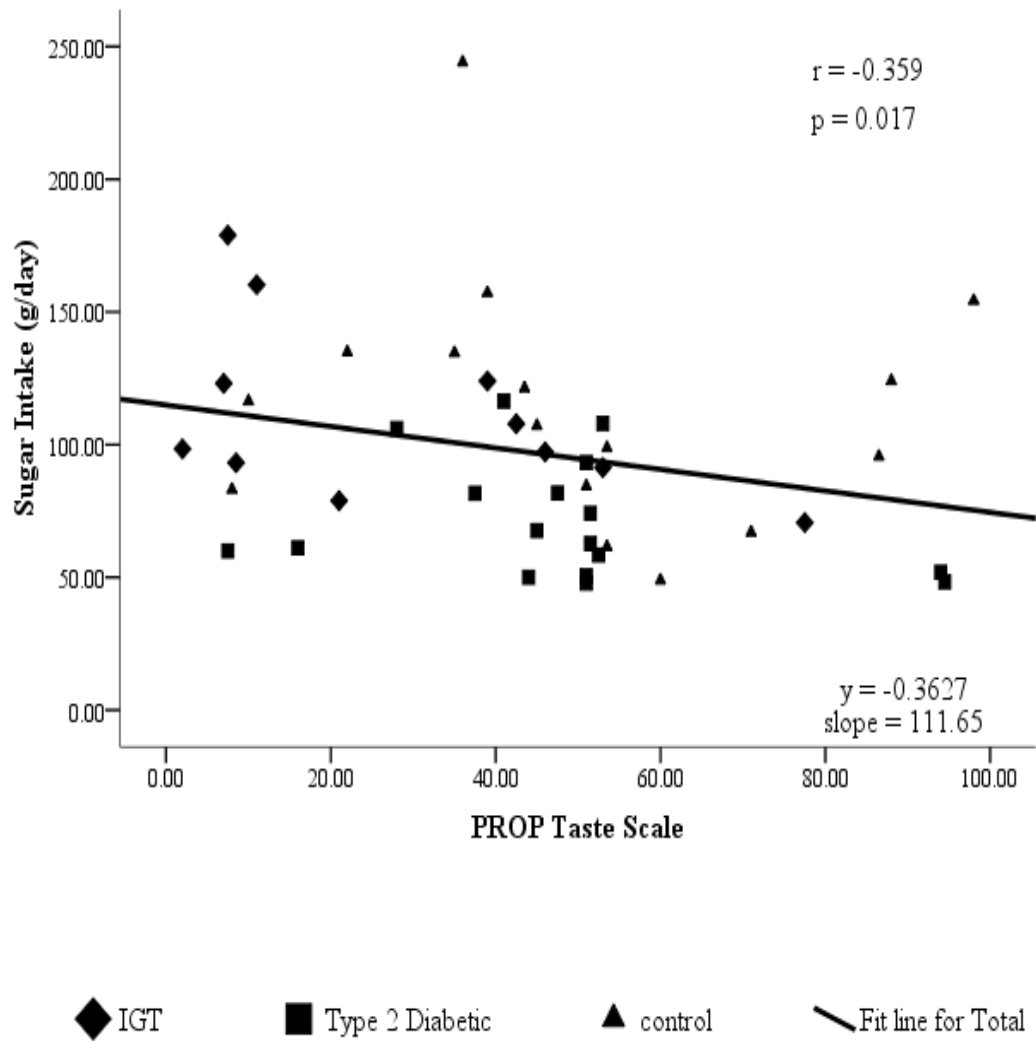
Figure 3.31 Spearman's Correlation Demonstrating the Association between Sugar Intake and Bitter (PROP) Taste Sensations Recorded on the Taste Scale

3.9.2 Appetite and Taste Analysis

Correlations were completed where earlier results showed significant differences between groups. Results were integrated using correlative statistical analysis to indicate associations between results. Pearson's correlations were used where the data was parametric and Spearman's correlations were utilised in situations where the data was considered non-parametric.

3.9.2.1 Correlation of Appetite Parameters versus Bitter PROP Taste Scale

A correlation between subjective ratings of appetite the PROP taste scale was done using Spearman's correlations (figures 3.32 to 3.32.3). There were no significant correlations between appetite parameters and the tasting of the bitter compound PROP. As the subjective rating of PROP increased, hunger (r=0.187) and PC (r=0.035) both increased indicating that the more sensitive an individual is to PROP the more hungry they will be and the tendancy to consume food earlier increases. This relationship was not anticipated even though it is not a significant result. Figures 3.32.1 and 3.32.2 show the relationship ratings of PROP on the taste scale and satiety and fullness. Both appetite parameters (satiety and fullness) indicate a reduction as taste sensitivity increase, although the relationship's are not significant. Therefore, as the sensitivity to PROP increases (i.e. non tasters are the least sensitive and supertasters are the most sensitive), levels of satiety and fullness decrease. Again, this deviates from the anticipated results, although the results are not significant.
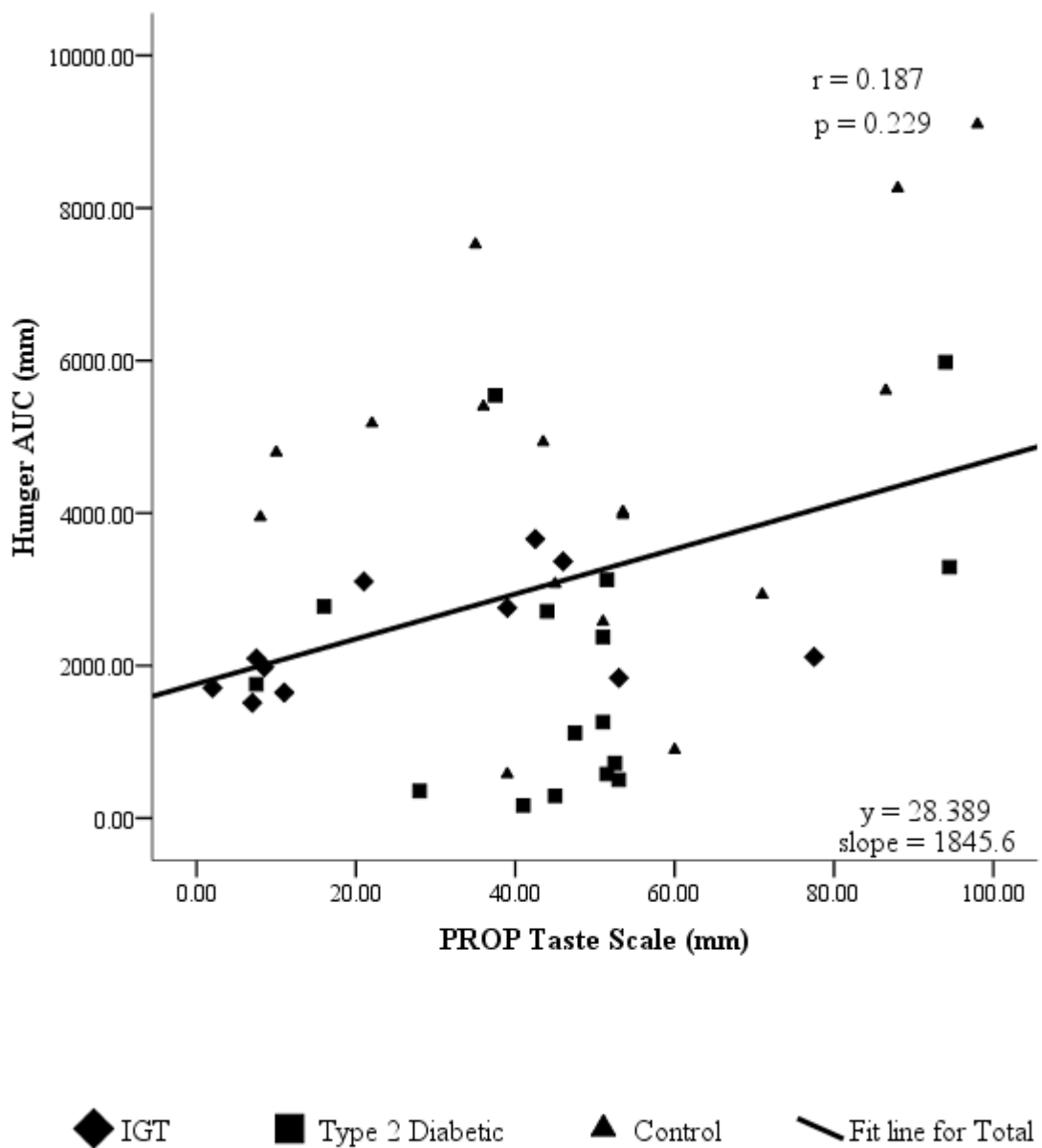
Figure 3.32 Spearman's Correlation between the AUC for Hunger and Bitter (PROP) Taste Sensations Recorded on the Taste Scale for IGT, type 2 diabetics and control participants. The line represents the linear trend and the slope and intercept are recorded on the graph.
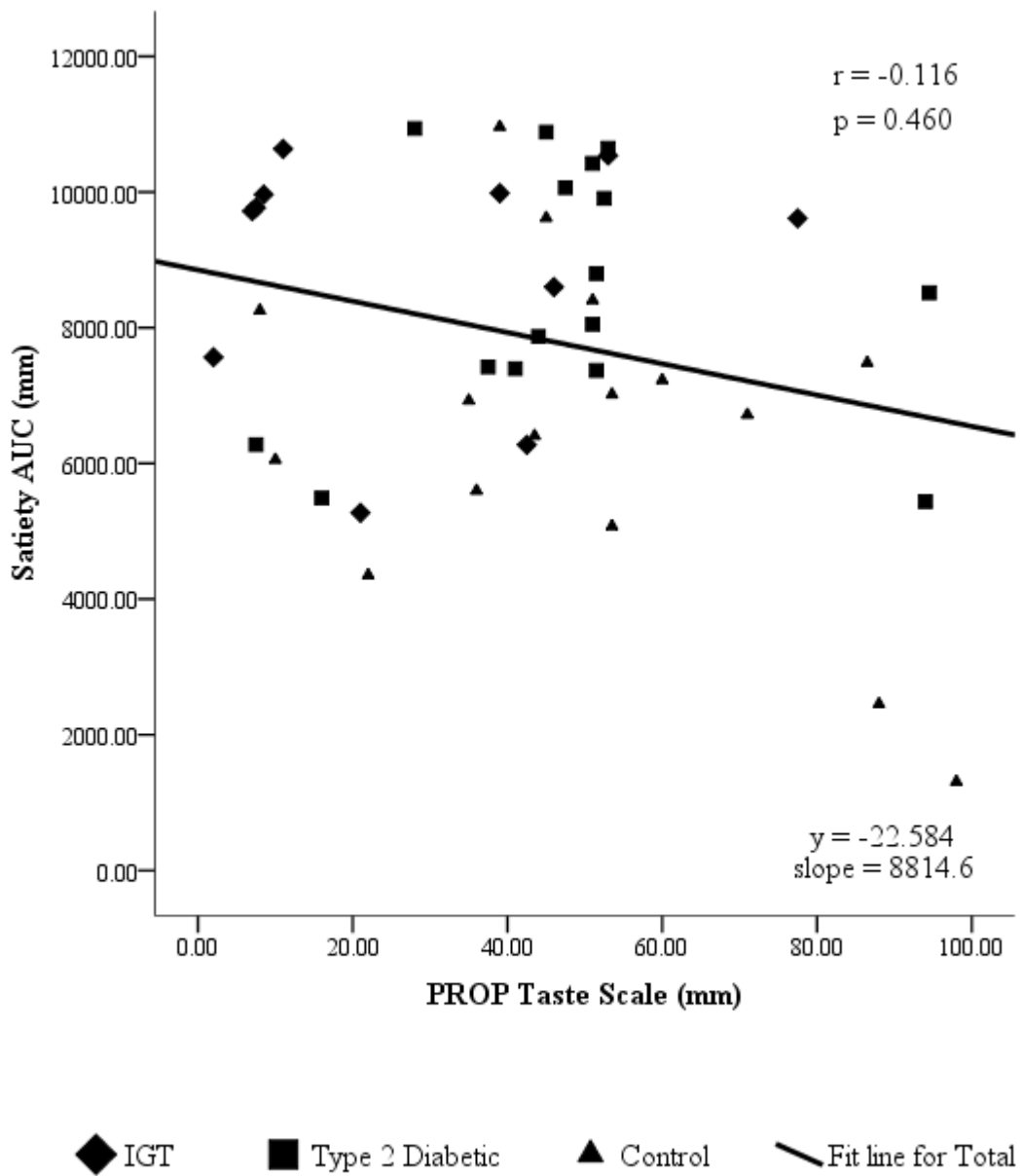
Figure 3.32.1 Spearman's Correlation between the AUC for Satiety and Bitter (PROP) Taste Sensitivity Recorded on the Taste Scale for IGT, type 2 diabetics and control participants. The line represents the linear trend and the slope and intercept are recorded on the graph.
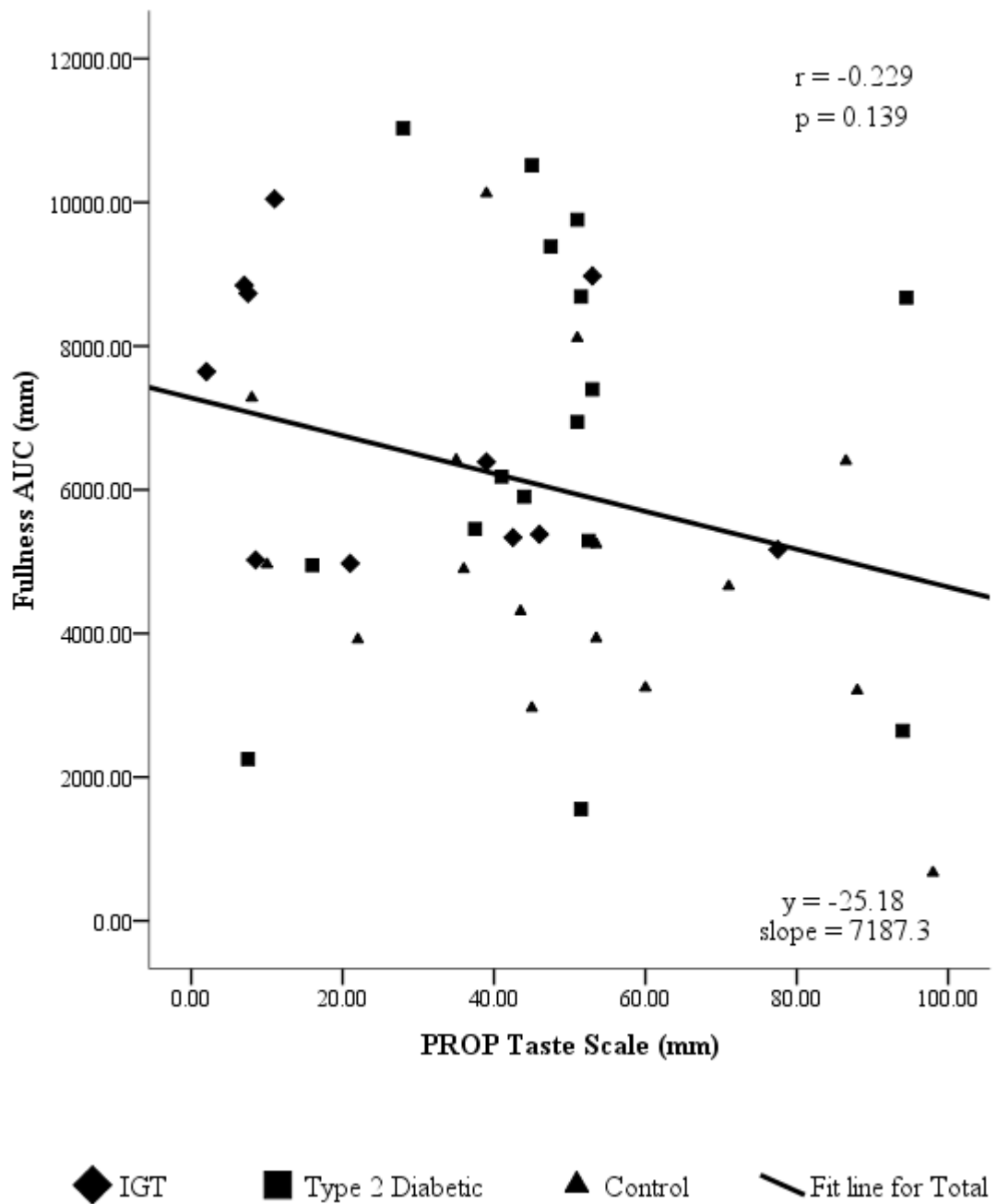
Figure 3.32.2 Spearman's Correlation between AUC for Fullness and Bitter (PROP) Taste Sensations Recorded on the Taste Scale for IGT, type 2 diabetics and control participants. The line represents the linear trend and the slope and intercept are recorded on the graph.
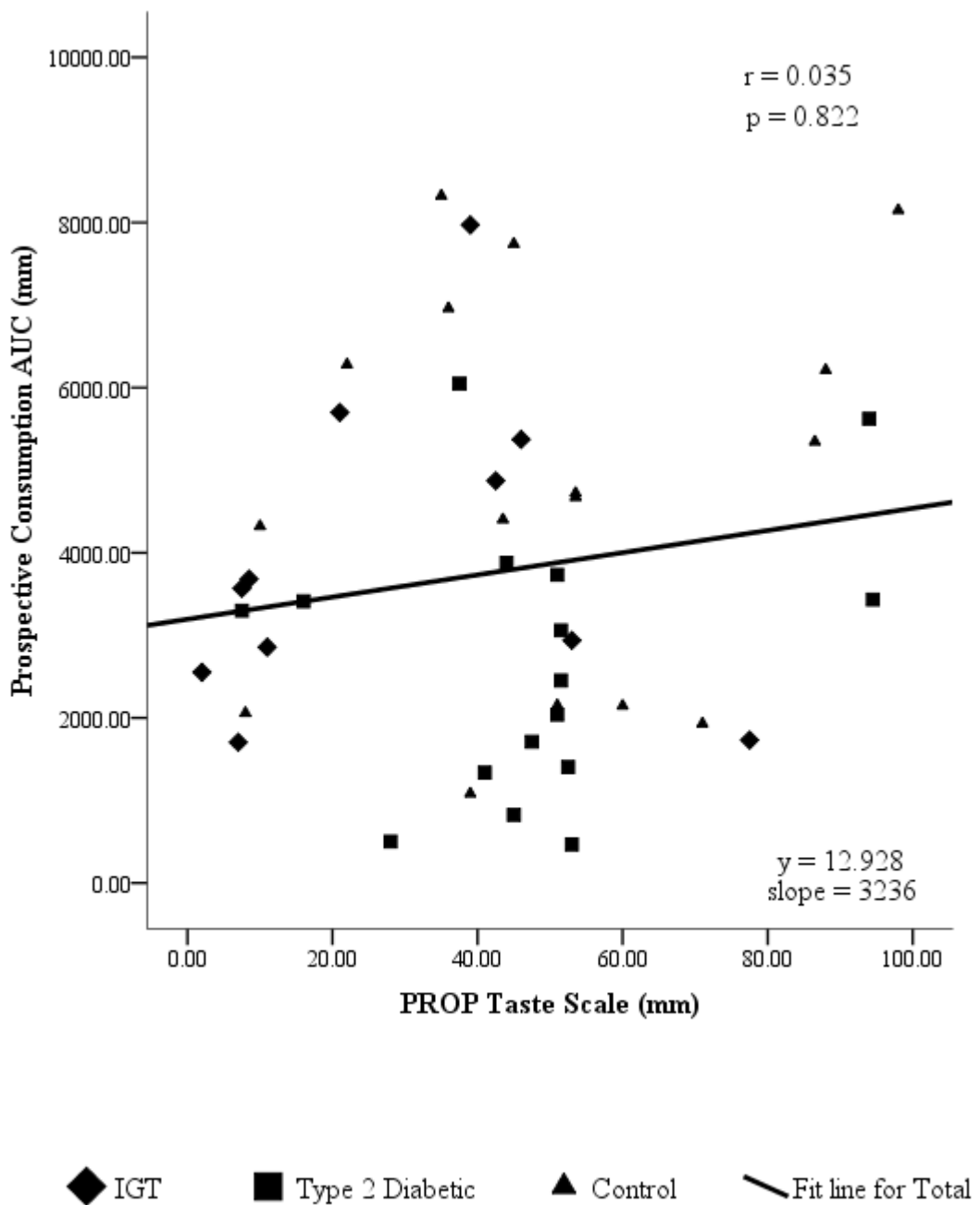
Figure 3.32.3 Spearman's Correlation between AUC for Prospective Consumption and Bitter Taste Sensations recorded on the Taste Scale for IGT, type 2 diabetics and control participants. The line represents the linear trend and the slope and intercept are recorded on the graph.

3.9.3 Appetite and Oxidation Analysis

The present study investigated the relationship between appetite sensations and oxidation rates to determine if there is a relationship between the two because the effects of this relationship may stimulate important signals which are necessary to evoke the satiety cascade and would result in the cessation of feeding. Without this relationship feeding may continue for a longer duration and this may be what results in the maintenance of obesity. The results of CHO oxidation were integrated as AUC from baseline to the end of the study period. Appetite parameters assessed were hunger, satiety, fullness and prospective consumption, and were correlated with CHO and fat oxidation.

3.9.3.1 <u>Intergroup Comparisons</u>

Comparisons were done between appetite parameters and CHO oxidation. CHO oxidation AUC was corrected for body weight to ensure that any differences observed were as a result of the disease state and not the differences in body weight.

The results show that IGT participants consistently differed from type 2 diabetic and control participants. As CHO oxidation increased, hunger (see figure 3.33) and prospective consumption (see figure 3.33.3) also increased for type 2 diabetics (hunger, r=0.217, p=0.0419; PC, r=0.302, p=0.255) and control (hunger, r=0.112, p=0.641; PC, r=0.134, p=0.609) participants whereas the IGT participants demonstrated a negative association meaning that as CHO oxidation increased,

hunger (r=-0.330, p=0.332) and prospective consumption (r=0.170, p=0.616) decreased. The results were not significantly different. As expected, opposing results were found for CHO oxidation and satiety (see figure 3.33.1) and fullness (see figure 3.33.2) however the type 2 diabetic (satiety, r=-0.476, p=0.063; fullness, r=-0.215, p=0.425) and control (satiety, r=-0.223, p=-0.390; fullness, r=-0.362, p=0.153) groups still differed from the IGT participants. As satiety and fullness increased, CHO oxidation decreased showing a negative relationship, whereas in IGT participants as satiety (r=0.237, p=0.483) and fullness (r=0.621, p<0.05) increased so did CHO oxidation. The positive relationship between fullness and CHO oxidation in IGT participants was significant (see figure 3.33.2).
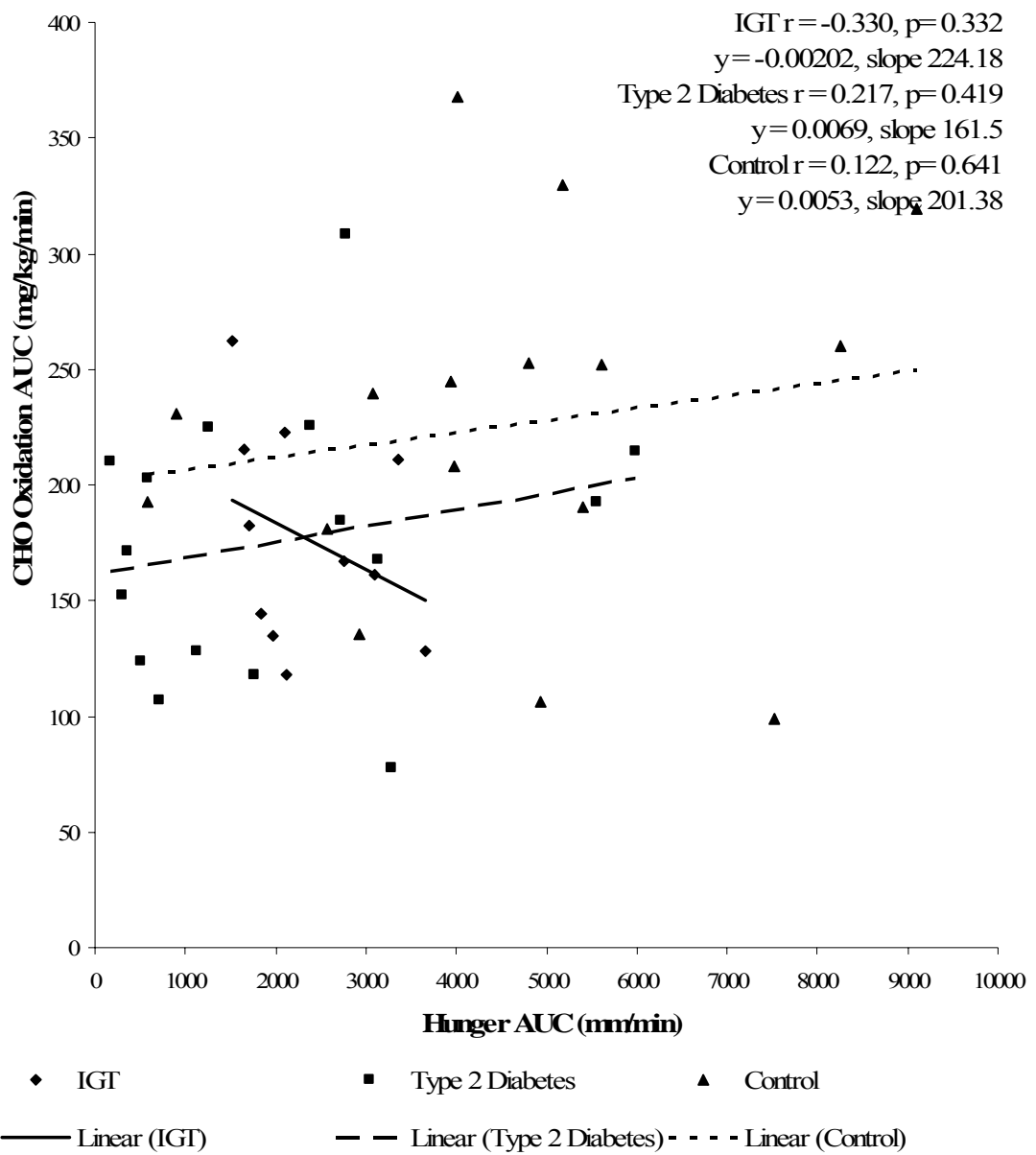
Figure 3.33 Pearsons Correlation of CHO Oxidation and Subjective feelings of Hunger for IGT, type 2 diabetic and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.
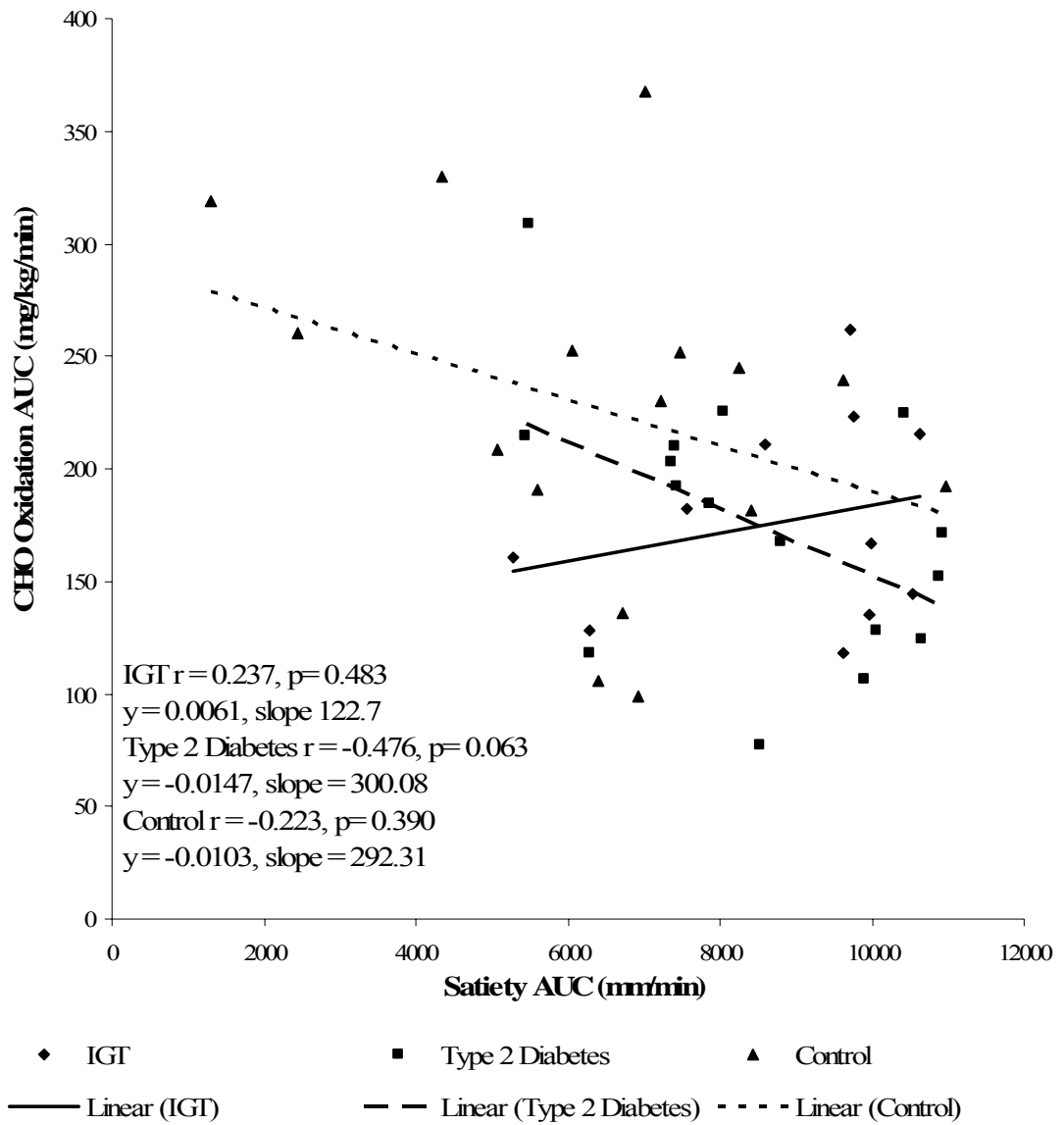
Figure 3.33.1 Correlation of CHO Oxidation and Subjective Feelings of Satiety for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.
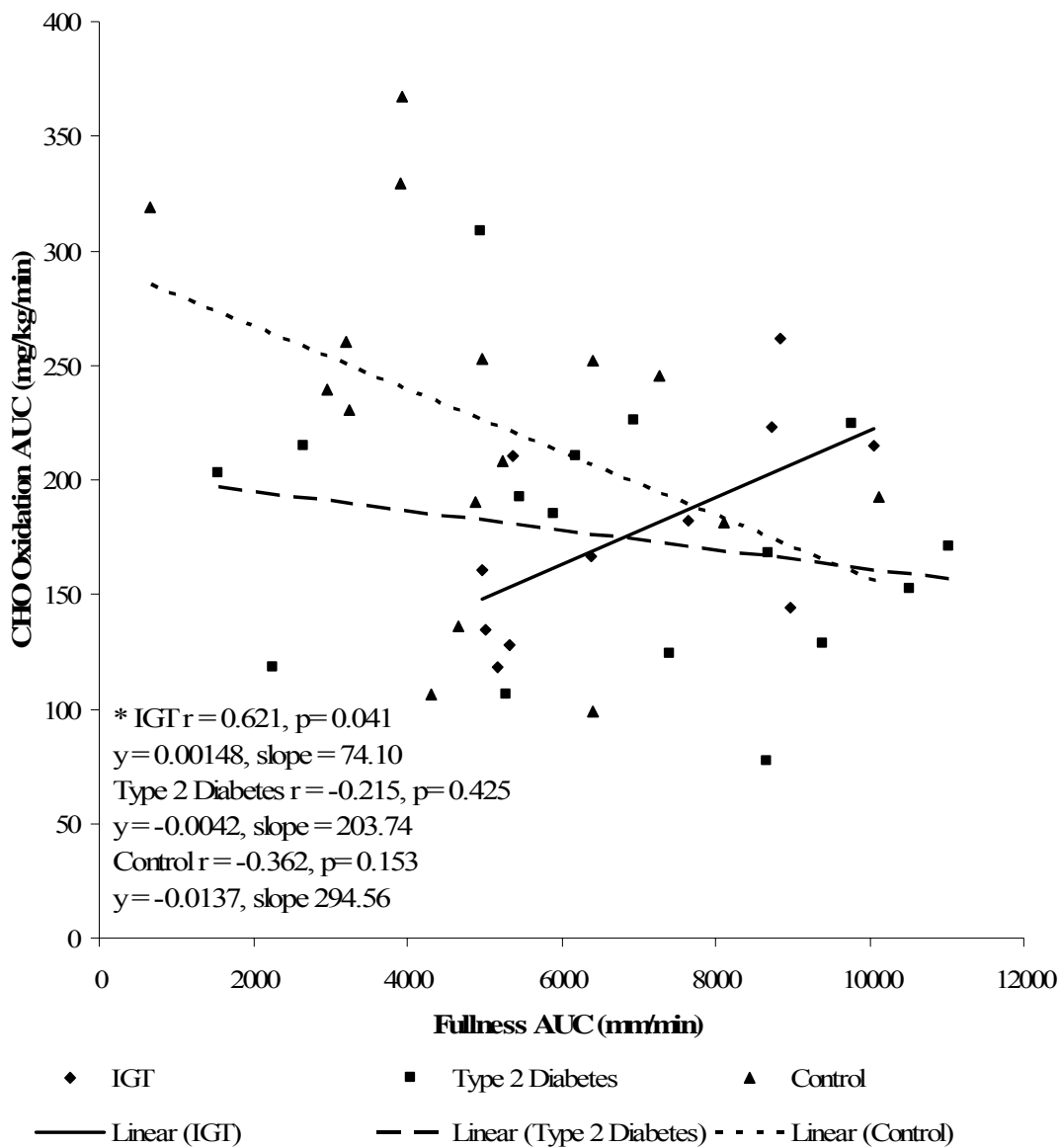
Figure 3.33.2 Correlation of CHO Oxidation and Subjective Feelings of Fullness for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.
* significance association at the level p<0.05
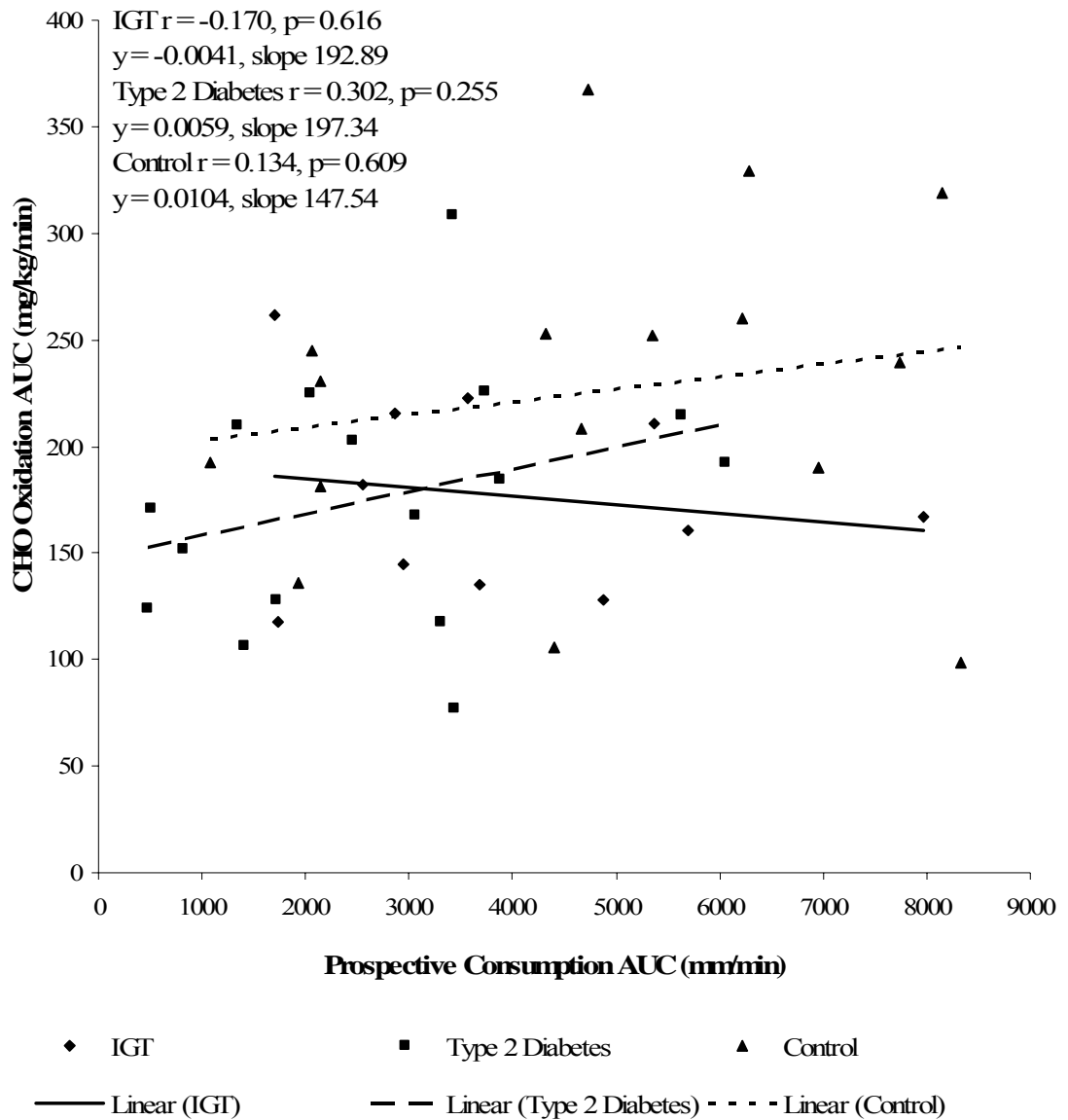
Figure 3.33.3 Correlation of CHO Oxidation and Subjective Feelings of Prospective Consumption for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.

Similar correlation tests were completed for fat oxidation and the same four appetite parameters. The trend observed between fat oxidation and hunger in type 2 diabetic (r=-0.445, p=0.084) and control (r=-0.105, p=0.689) participants was negative, therefore as fat oxidation increased, levels of hunger decreased, whereas in IGT participants (r=0.106, p=0.756) as fat oxidation increased, levels of hunger increased (figures 3.34). The p values show that the associations were not significant. As fat oxidation increased so did satiety (figure 3.34.1) in type 2 diabetics (r=0.207, p=0.443) and control (r=0.183, p=0.481) participants. In IGT (r=-0.003, p=0.992) participants as fast oxidation increased levels of satiety reduced. Fullness levels followed a similar pattern to that of satiety where as fat oxidation increases, fullness increased in type 2 diabetic (r=0.102, p=0.708) and control (0.210, p=0.419) participants (see figure 3..34.2). The association in IGT participants again differed to the other two groups. As fat oxidation increased, fullness decreased (r=-0.492, p=0.124) in IGT participants. The results of the association between PC and fat oxidation deviated from the pattern observed in the other relationships between oxidation rates and appetite parameters. As fat oxidation increased, PC increased in IGT participants (r=0.014, p=0.968) and control participants (r=0.005, p=0.985) whereas in type 2 diabetic participants as fat oxidation increased the levels of PC decreased (r=-0.452, p=0.079), see figure 3.34.3. The associations between fat oxidation and appetite parameters were not significant.
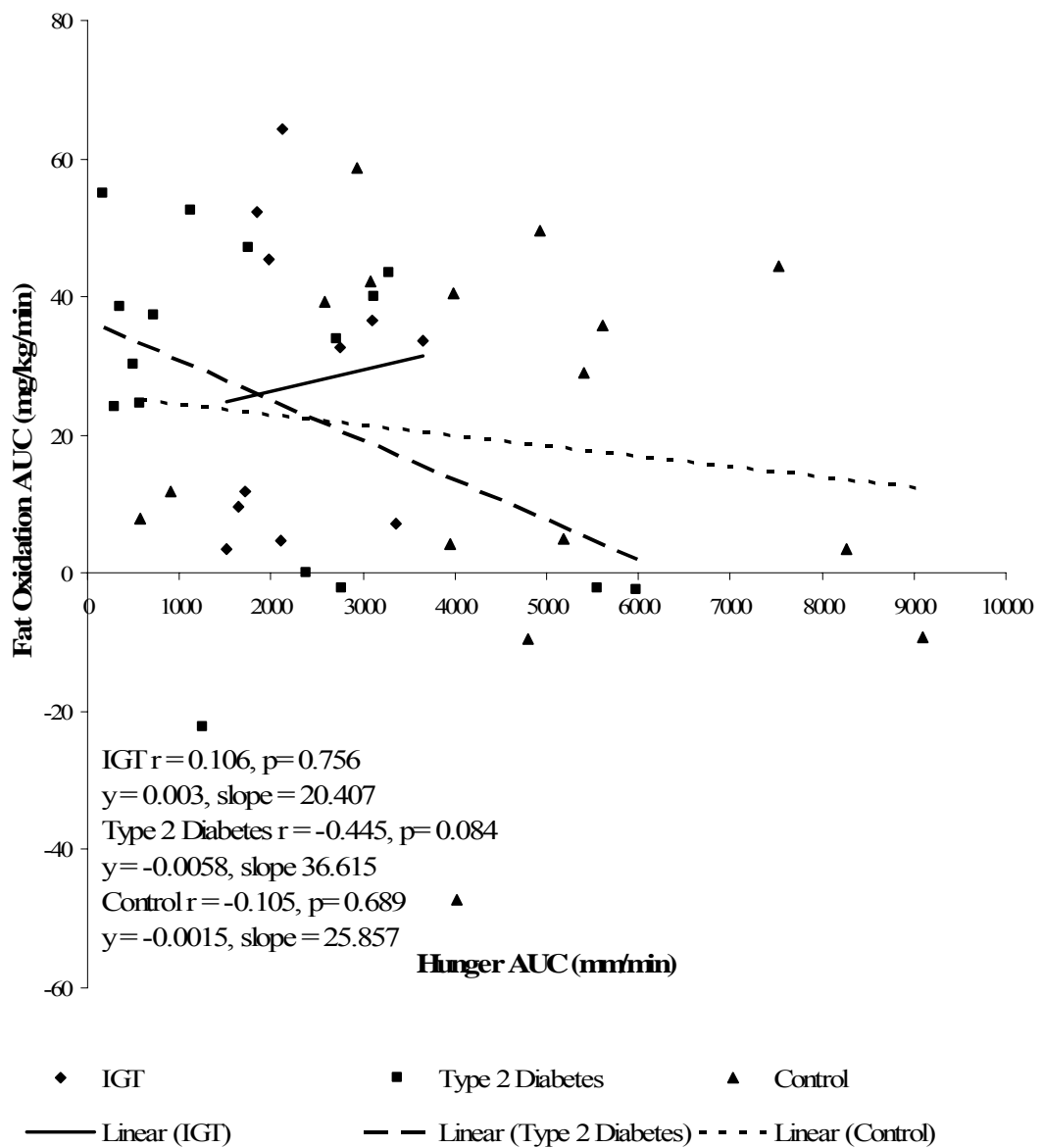
Figure 3.34 Correlation of Fat Oxidation and Subjective Feelings of Hunger for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.
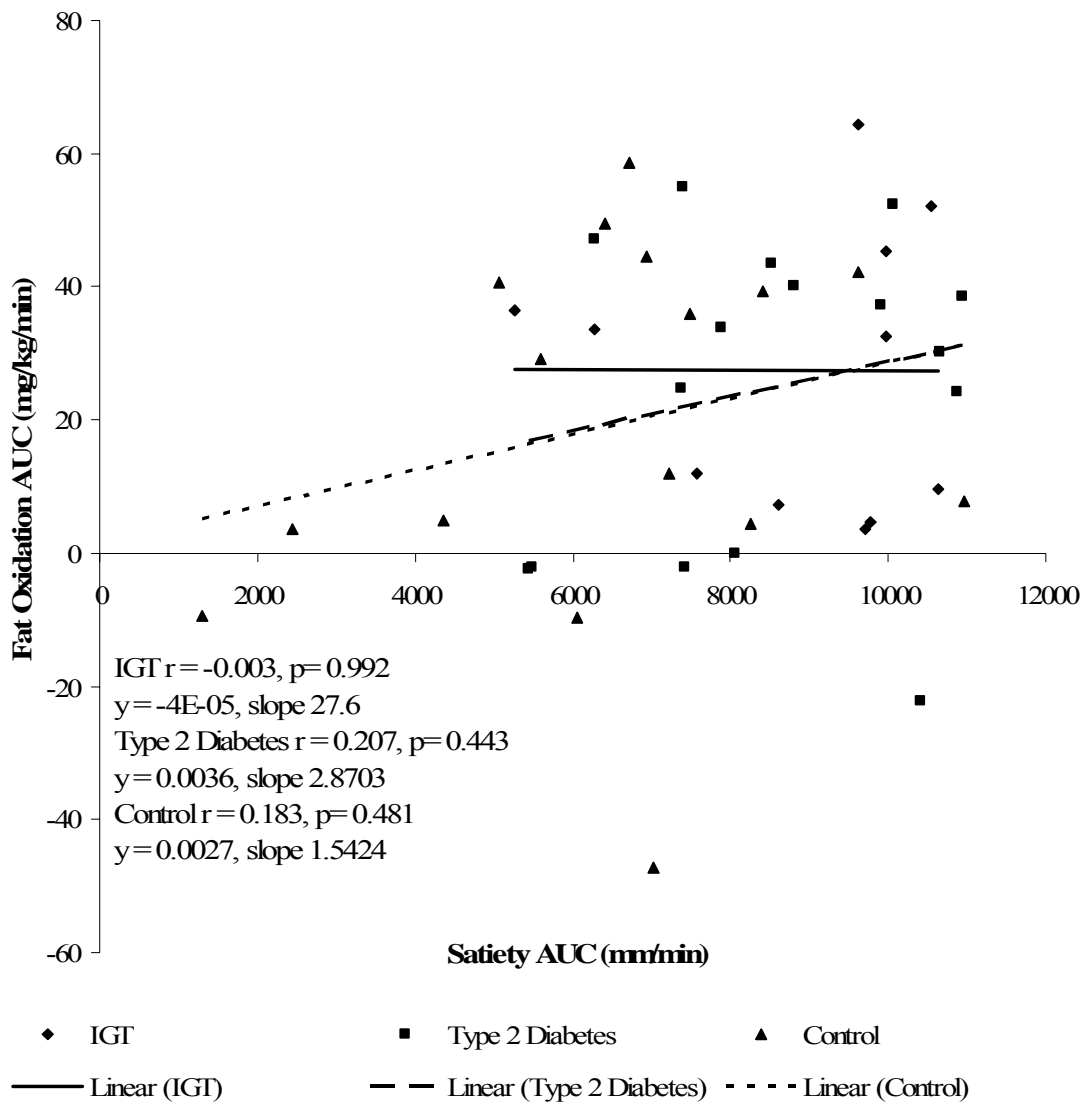
Figure 3.34.1 Correlation of Fat Oxidation and Subjective Feelings of Satiety for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.
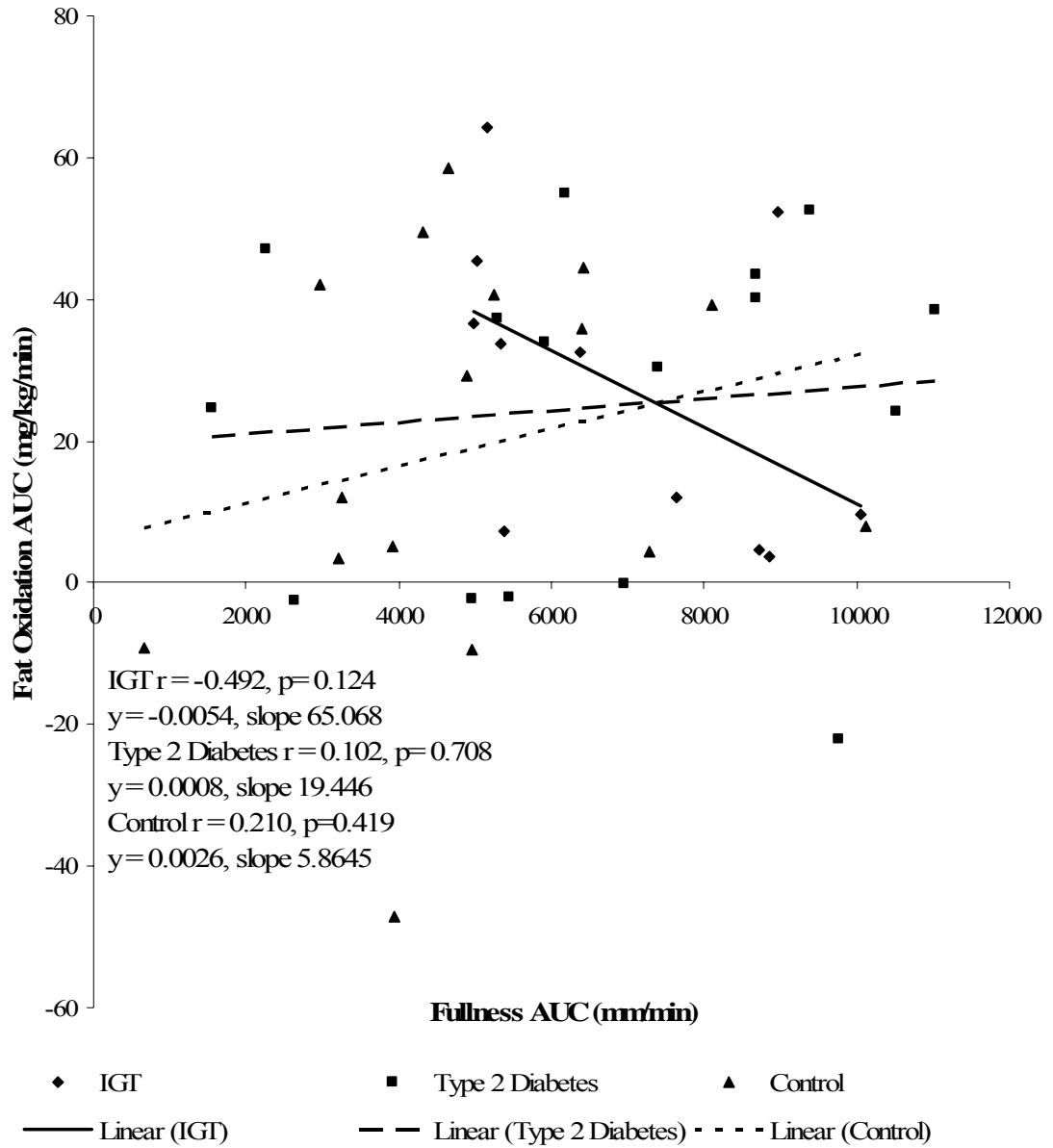
Figure 3.34.2 Correlation of Fat Oxidation and Subjective Feelings of Fullness for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.

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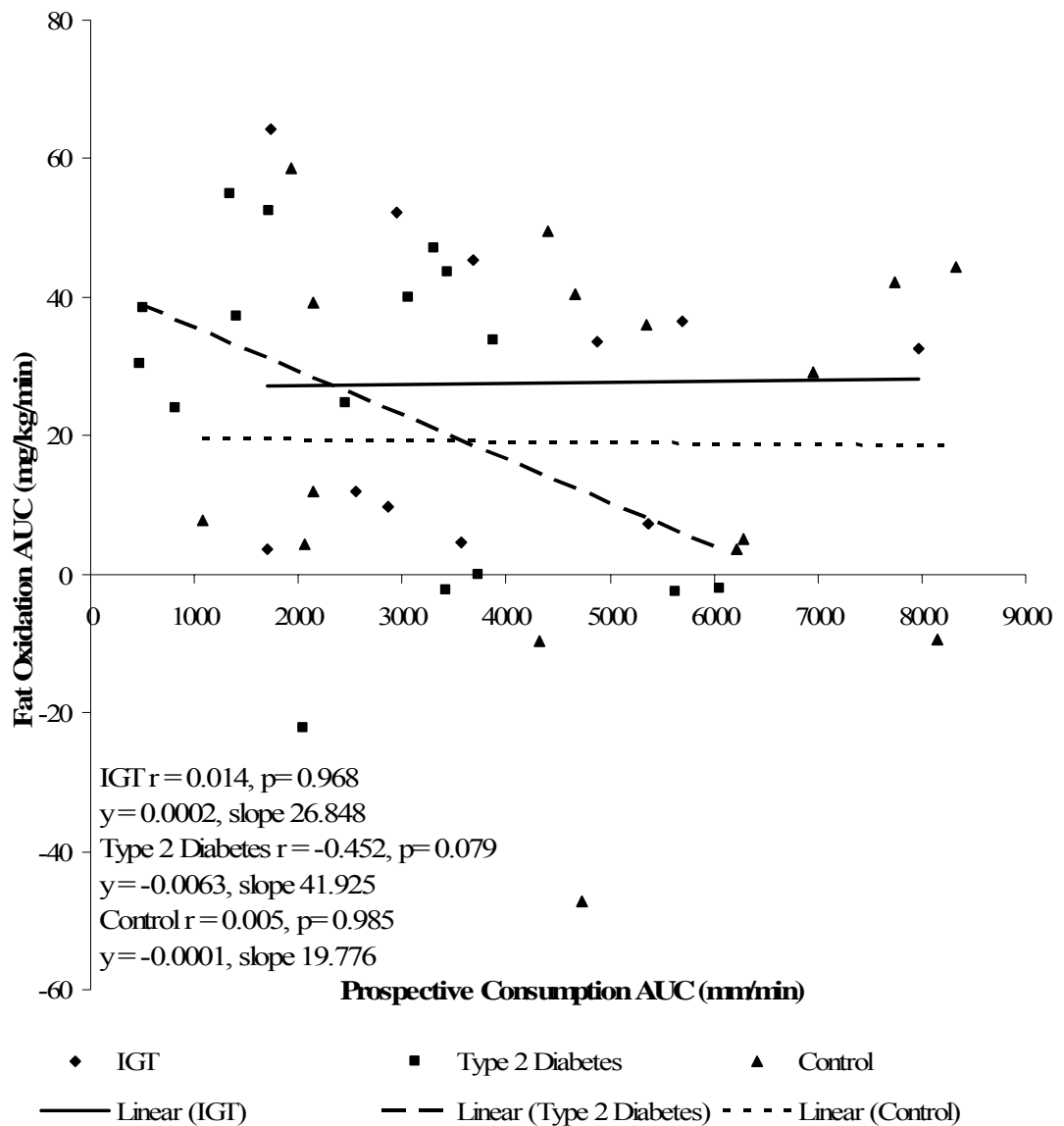
Figure 3.34.3 Correlation of Fat Oxidation and Subjective Feelings of Prospective Consumption for IGT, type 2 diabetics and control participants. The lines represent the linear trend for each group and the intercept and slope is recorded for each line.

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3.9.4 Appetite and Blood Parameters (Glucose and Insulin)

A correlation of appetite parameters and blood plasma measurements was done to determine if there was an association between subjective measures of appetite and the corresponding biological measures following a meal. The glucose and insulin results were separated by group into IGT (table 3.7), type 2 diabetic (table 3.8) and control participants (table 3.9). Both the glucose and insulin parameters were correlated using AUC values and were corrected for differences in body weight to ensure that any differences observed were as a result of the disease status.

Table 3.7   IGT Participants: Correlation between Glucose and Insulin and Subjective ratings of Hunger, Satiety, Fullness and Prospective Consumption.

	Glucose AUC		Insulin AUC	
	R-value	p-value	R-value	p-value
Hunger	-0.210	0.536	-0.122	0.721
Satiety	-0.486	0.129	0.141	0.680
Fullness	0.221	0.514	-0.137	0.689
Prospective Consumption	-0.196	0.564	0.179	0.599

There were no significant differences between glucose or insulin and any of the appetite parameters in IGT participants (table 32.7) suggesting that the differences observed in the AUC response for glucose and insulin between groups had no resultant effect on subjective appetite parameters.

Table 3.8   Type 2 Diabetic Participants: Correlation between Glucose and Insulin and Subjective ratings of Hunger, Satiety, Fullness and Prospective Consumption.

	Glucose AUC		Insulin AUC	
	R-value	p-value	R-value	p-value
Hunger	0.143	0.583	-0.523	0.031*
Satiety	-0.436	0.080	0.421	0.092
Fullness	-0.335	0.189	0.146	0.577
Prospective Consumption	0.280	0.276	0.516	0.034*

* significance at the level of p<0.05

In type 2 diabetic participants there was a significant positive correlation between hunger and insulin levels (r = -0.523, p < 0.05) so as insulin levels increased, hunger reduced. Alternatively, a greater overall insulin response is associated with reduced feelings of hunger. There was also a positive relationship between insulin and prospective consumption which suggests that as the complete insulin response increases so does the desire to consume more food. This is contradictory to the relationship between insulin and hunger and will be discussed further in the discussion chapter.

Table 3.9 Control Participants: Correlation between Glucose and Insulin and Subjective ratings of Hunger, Satiety, Fullness and Prospective Consumption.

	Glucose AUC		Insulin AUC	
	R-value	p-value	R-value	p-value
Hunger	0.193	0.474	-0.220	0.413
Satiety	-0.508	0.045*	0.147	0.587
Fullness	-0.424	0.102	-0.389	0.136
Prospective Consumption	0.132	0.626	0.033	0.902

* significance at the level of p<0.05

The control participants demonstrated a negative relationship between the overall glucose response and satiety (p = -0.508, p < 0.05). The results denote that as the glucose response increases, satiety levels decrease. Therefore, it is necessary for blood glucose to be increased to maintain a satiety response, which is what would be expected.

CHAPTER 4 - DISCUSSION

This study investigated the integrated responses to a meal and the differences found between IGT, type 2 diabetic and control participants. It is accepted that an imbalance in the integrated response of food intake can result in a positive energy balance and over time this may lead to obesity and associated chronic disease. The results have shown differences between control participants and both IGT and type 2 diabetes and differences have also been established between IGT and type 2 diabetic participants. This is important as it will confer an insight as to how the progression of disease affects the already dysfunctional mechanisms involved in the process of food intake. Definate differences observed may assist to identify individuals who are likely to develop IGT and type 2 diabetes, using a specific set of physiological, psychological and biological markers. Once a diagnosis of IGT and type 2 diabetes is made, counteracting the mechanisms which contribute to a dysfunctional satiety cascade and which affect feeding behaviour is essential and may prevent further progression of the disease.

4.1 SUBJECT CHARACTERISTICS

4.1.1 Age Differences between Groups

In the current study subjects were recruited between the ages of 20 and 70 years. In this study the IGT groups had ages ranging from 28 to 65 years, the type 2 diabetic participants age ranged from 36 to 66 years. There was no significant differences

between the mean age of these two groups. However, the control participants, who were recruited from the University population, were aged from 20 to 57 years (being the oldest participant) and this group were younger in age to the two patient groups. In the current study the control participants were not age matched. The primary reason is linked with the timescale of the study data collection period. The type 2 diabetic and IGT participants were recruited over the course of a year. Recruitment was based upon recruiting 1 patient per diabetic clinic. Therefore it would not have been feasible to recruit all the patients before advertising for all control participants as this would have taken the data collection period past the planned time period.

Studies comparing the results of type 2 diabetes and IGT with control participants often encounter similar age-related differences (Wolever and Mehling 2002). The age differences may be explained by: 1) the nature of the disease state because type 2 diabetes was commonly known as late-onset type 2 diabetes (Hansen et al. 2000) and so was initially identified as being an age-related illness, therefore older participants were expected and 2) the older range of the population had less time constraints i.e. less work or family commitments and therefore found it easier to dedicate time to be involved in the study. Some investigators have reported significant changes or differences in body composition with advancing age (Steen 1988) however studies primarily focus on subjects over the age of 70 years (Newman et al. 2005 & Ding et al. 2007) which discounts the participants in the current study as the maximum inclusion age was 66 years. Hughes et al (2008) investigated body composition changes in participants up to the age of approximately 60 years, with a 10 year follow up, and noted an increase in adipose tissue resulting in changes in

regional body composition such as increased waist and hip measurements. This age related body composition change is symptomatic of the diseases that are being studied. Therefore differences would be expected between the groups facilitating the requirement for the measurement of WHR as it is an indicator of body composition which is independent of body weight.

There are known changes in nutrient handling and metabolism in the elderly; namely changes in fuel utilisation, reduced responsiveness to energy imbalances, changes in energy expenditure, reduced dietary variety, impairments in hormonal mediators and reduced hunger and increased satiation (Roberts and Rosenburg 2006). In longitudinal studies, Keys et al (1973) recorded that basal metabolic rate (BMR) reduced 1-2% per decade over the ages of 20 to 75 yrs. However, what is not clear is whether body composition changes are reflected in metabolic responses and if so when the metabolic changes start to manifest. The World Health Organisation (WHO 1995) consider that 'elderly' is 65 years and above and this distinction is accepted in most developed countries. Delacourte et al (2004), reported older subjects in an experimental underfeeding study experienced significantly less frequent symptoms of hunger (assessed using visual analogue scales) than young subjects, despite the fact that the older subjects lost significantly more weight and would therefore be expected to experience more frequent hunger. The altered hunger versus satiety regulation seen in the study by Delacourte et al (2004) may be due to altered glucose homeostasis in old age i.e. blood glucose is regulated over a broader range (MacIntosh et al. 2001). Melanson et al (1997) reported persistently elevated postprandial glucose and insulin in older women following consumption of 2,092-

and 4,184-kJ test meals compared with younger women. Provided that central mechanisms for converting a signal of energy status into a sensation of hunger are intact in older individuals, as one study (Brand et al. 1982) but not some others (Marker et al. 1999; Meneilly et al. 1994) indicated. The elevated postprandial blood observed by Melanson et al (1999) could potentially lead to an attenuated return of hunger in the postprandial period. Melanson (1997) also reported that in old age there is a reduced insulin sensitivity resulting in elevated circulating insulin which accompanies the high postprandial glucose levels in the body and the result of this may contribute to the delayed return of hunger. The mechanism is thought to involve a central satiety effect of high insulin levels or by altering central sensitivity to other components in the cascade of mechanisms that regulate food intake such as cholecystokinin (CCK) and neuropeptide Y (NPY). Although, as aforementioned, the subjects, except one, in the current study were below that which were considered 'elderly' (WHO 2002), the age-related changes will be considered along with the continuing discussion of the current study.

4.1.2   Gender Differences between Groups

Significant differences were observed between males and females for a number of anthropometric measurements. Males were significantly taller and heavier than females and there was no difference in BMI or age between the sexes. These results were all expected and are consistent with other European studies (de Jong et al. 2001). Male participants had significantly larger waist measurements and waist to hip ratios when compared to female participants, however this could be attributed to

the gender split within groups i.e. the low number of male control participants. The two patient groups had a similar number of male and female participants but the control group was skewed towards females (76%). These findings do provide evidence for the need of combined sex and age-specific reference data. The severity of the problems concurrent with type 2 diabetes and IGT identifies the need for specific data relating to the diseases and is therefore important for future research. For example, it has been recorded (WHO 1995) that a larger proportion of females reach older, old age (+85yrs) than males and so it is important to understand the metabolic consequences surrounding this age range and the differences between males and females. Acquiring this data can provide important information for further research and may result in more specialised care and treatment of the diseases at pertinent points of the ageing process and allow an understanding of the metabolic changes that occur over time as type 2 diabetes and IGT progress.

4.2 ANTHROPOMETRIC CHARACTERISTICS

Control participants had significantly lower weight, BMI, waist, and hip measurements compared to the other two participant groups. The age-related differences discussed earlier, such as age-related weight loss can almost be discounted because the weight in the type 2 diabetic group, which was the eldest group, was significantly higher than the control group. The differences in BMI, waist and hip measurements were expected due to the significantly higher mean weight of the IGT and type 2 diabetic participants compared to the controls. There was no significant difference found in either height or waist to hip (WHR) ratio

between groups. The use of WHR as an anthropometric indicator to assess adipose tissue distribution was validated by in-vivo methods (Ferland et al. 1989). Results have varied between studies; Daniel et al (1999) found no differences in WHR between normal and clinically obese individuals and concluded at higher BMIs, overall adiposity was a better indicator of poor glycaemic status than abdominal obesity. On the other hand, Snijder et al (2003) concluded opposing results and suggested that WHR is a strong predictor of type 2 diabetes. The current study indicates that WHR was not an important indicator of IGT and type 2 diabetes but that abdominal obesity, measured by waist and hip measurements certainly did indicate poor glycaemic status.

4.3   TASTE

Previous research has indicated that taste has a genetic origin (Blakeslee and Salmon 1931). This was originally tested with the bitter compound PTC and went on to be tested on a second bitter compound PROP. It was established that humans can be separated into three taste status groups; nontasters, tasters and supertasters (Bartoshuk 1991). Tasters and supertasters were found to dislike the bitter PROP compound and correspondingly they were reported to avoid foods containing this compound in their everyday food choices. Tepper and Nurse (1998) studied the effect of fat perception with PROP taster status and found that tasters and supertasters could discriminate differences in the fat content between salad dressings (10% fat and 40% fat) whereas the nontasters could not. Tasters and supertasters showed no preference for either dressing however the nontasters preferred the high

fat dressing. The importance of the ability to discriminate food elements in the process of food intake is imperative. A preference for high fat foods, or the lack of distinction between low and high fat foods, may result in a positive energy balance on a daily basis. A consistent positive energy balance is known to result in obesity which can lead to IGT and overt type 2 diabetes.

In the current study it was to be determined if IGT and type 2 diabetic participants follow the same taste status trends as previous results which have, in general, been undertaken on normal healthy subjects (Tepper & Ullrich 2002; Stein et al. 2003; Duffy 2004). If previous research has been precise, non-tasters would be the heaviest participants because they cannot detect bitter content and corresponding fat content, differences in foods. The resultant food preferences (Tepper and Nurse 1997) would be expected to consist of both a higher energy density and fat content, so overall a greater average daily energy intake would be expected to be consumed in nontasters, than tasters and supertasters. Conversely, the supertasters of PROP should have a dislike for bitter foods and high fat foods and therefore the aversion would cause a lower energy density diet, lower fat intake and overall lower daily energy intake. In reality, the current study demonstrated that half (5 out of 10) of the nontasters were IGT participants and all except one of the nontasters were either IGT or type 2 diabetic. This was expected and did conform to previous research to demonstrate that obese participants may be nontasters of bitter/fat content. The result of the current study exposes that IGT participants are more likely to be nontasters of PROP. This suggests that IGT and type 2 diabetics participants, due to their lack of sensitivity to bitterness and fat have a propensity to avoid bitter foods

and consume fatty foods readily which, if continuous may consequentially result in positive energy balance and weight gain. The results also suggest that nontasters are pre-disposed to gain weight. Taste status may be a used as a prediction tool, along with physiological markers, to screen healthy or perhaps overweight individuals (BMI>25) to determine a pre-disposition for obesity. Furthermore, all except one of the supertasters were control participants. The results demonstrate significant intergroup comparisons between taste status and subjective ratings on the taste scale. Overall, 20% of the participants were nontasters, 64% were tasters and 16% were supertasters. A study by Bartoshuk (2000) stated that the frequency of non-tasters, tasters and supertasters was 25%, 50% and 25% respectively but that the figures must be adjusted depending on the dominancy of males or females in the sample, because more females are supertasters than are males (Bartoshuk et al. 1994). There is no research which states how the ratios should change with gender however considering both the small sample of this study and that the patients were procured with a known metabolic disorder, it would be acceptable to have a lower percentage of supertasters. Given the research, there would not be a high expectation of supertasters within the IGT and type 2 diabetic groups because of the relationship between PROP taster status and BMI (Tepper and Nurse 1998).

Differences were found between IGT, type 2 diabetics and control participants and taste sensitivity. The differences in the comparisons between groups and the taste scale for PROP were significant. In reviewing the literature there were very few studies which investigate the influence of PROP on IGT and diabetic patients, therefore it is novel to observe that a group with metabolic disease do fit in to the

profile of the research which has been undertaken on healthy control subjects. In the current study the IGT participants were significantly less sensitive to PROP on the taste scale and also identified PROP at a significantly higher concentration than control participants. The type 2 diabetic patients were intermediary in the detection of PROP and were therefore not significantly different from either control or IGT participants. It is noteworthy to mention that IGT is known to be a lesser condition than type 2 diabetes and yet IGT patients were found to be more likely to be less sensitive to the bitterness of PROP. With greater study numbers this result may have been observed more explicitly in type 2 diabetics. An explanation for why the differences were found between IGT and type 2 diabetic participants may be that the IGT participants in this study were recruited after first diagnosis of their condition whereas the type 2 diabetics may have been diagnosed some time ago. Therefore, it appeared that the type 2 diabetics had adapted to the presence of their condition and secure in their ability to control their diabetes, more so than the IGT participants. Consequently, the type 2 diabetics may have had greater habitual control over their diet and therefore their blood glucose. It may be possible that once a level of control is established over blood glucose and insulin levels that taste sensitivity improves, analogous to the improvement in diabetic symptoms with good glycaemic control. Poor insulin sensitivity found in type 2 diabetic patients has been attributed to diminished taste for PROP bitterness (Moran et al, 2007; Williams & Cummings, 2005). Furthermore, reduced postprandial ghrelin suppression has also been associated with diminished taste for PROP bitterness (Moran et al, 2007; Williams & Cummings, 2005). The blood results do show that the IGT participants appeared to have a diminished level of plasma ghrelin postprandially, whereas the type 2 diabetic

participant levels did not decrease to such a large degree. Although the results in ghrelin concentrations were not significant between IGT and type 2 diabetics this may attribute to the difference seen in the taste results as it may denote a lesser glycaemic control in IGT participants. Therefore, the results of this study show that a better glycaemic control may lead to improved ghrelin levels. As shown in the type 2 diabetics and a concomitant improvement in taste sensitivity. This hypothesis would warrant further investigation with both poorly and well managed type 2 diabetics to determine a definitive answer as to how taste can vary with different states of glycaemic control. In summary, the current study has demonstrated that IGT participants are more likely to be non-tasters and are unlikely to be supertasters. Also, taste status has the potential to be utilised as a prediction tool for obesity and may help the prevention of IGT and type 2 diabetes if weight gain can be avoided.

The intake of fat and sugar have been implicated as being influenced by the taste sensitivity to PROP due to sharing similar pathways in the body that sense bitterness (Drewnowski 1997a). In the current study, fat intake was not significantly different between IGT, type 2 diabetics and controls or between taster groups (nontasters, tasters and supertasters). However, there was a decreasing trend observed between the taster groups in fat intake with increasing taste sensitivity (nontasters are least sensitive to taste, whereas supertasters are the most sensitive). The average fat intake was 80g/day for non-tasters and 76g/day for both taster and supertaster groups. Furthermore, there was a similar trend in the intake of SFA. Nontasters had a 28.8g/day intake, tasters had a 27g/day intake and supertasters had a 25.5g per day intake. The differences were not large enough for them to be significant differences

between taster groups and this may be an indicator of the compliance shown in the control of the habitual diets by IGT and type 2 diabetic participants in this study.

Total fat intake in IGT participants was comparable with control participants but IGT participants had the highest amount of SFA in their diet whereas, type 2 diabetic participants had a lower fat intake and a correspondingly lower SFA intake. The intake of fat was not significantly different between groups, however, again these small aberrations observed in IGT participants in the current study may have synergistic effects in the control of food intake and energy balance. The occurrence of differing control over metabolic systems between IGT and type 2 diabetic participants is emerging. IGT participants had the highest SFA intake and highest sugar intakes which habitually indicates less well controlled blood glucose levels. Thus, proposing the hypothesis that sensitivity to taste may be affected to a greater degree in an individual with poor glycaemic control than in an individual where glycaemic control is well managed. This demonstrates how important a healthy habitual diet is, particularly in IGT and type 2 diabetics.

4.4   HABITUAL FOOD INTAKE AND ENERGY EXPENDITURE

4.4.1   Nutrient Intakes Assessed by Diet Diary

The habitual dietary intake of the groups in the current study were examined to determine differences between groups. Diet diaries were returned for all participants which was significant as it demonstrated the participants' commitment to the study.

Differences included a significantly lower sugar intake (g/day) in type 2 diabetic participants when compared to IGT and control participants. Also, control participants showed a lower percentage intake of protein (14%) when compared to IGT (17%) and type 2 diabetic (18.5%) participants. It was important to consider how habitual diet would influence long term energy intake in the normal settings of these groups. Food intake was assessed in this study by the use of four day estimated diet diaries. Although seven day weighed diaries were considered for use it was decided that this may be too onerous a task in addition to the other components of the study. Diet diaries were used to determine any causative factors that may lead to positive energy balance and weight gain commonly seen in the IGT and diabetic patient groups (Klein et al. 2004).

The type 2 diabetic participants had the lowest intakes (in grams and also percentage intake) of total fat and also saturated fatty acid (SFA). Although this result was not significant, it is important because diabetes is associated with a high dietary fat intake (Rolls et al. 1994) and high fat intake may be key in the aetiology and maintenance of obesity. The fat intake is important because it reflects the fact that the diabetics in this study were in contact with a dietician and were gaining dietary advice. The IGT participants in the current study were not yet in contact with a dietitian as they were newly diagnosed. The study investigated saturated fatty acid intake because it is known to be detrimental to body insulin sensitivity (Marin et al. 2005) which is vital to the good (or bad) management of IGT and type 2 diabetes. When the digestion products of fat, FFA are elevated for prolonged periods it is known to lead to the progression of insulin resistance because elevated FFA affects

cellular glucose uptake and oxidation. Elevated FFA cause a lowering of the RQ and the propensity to store rather than utilise fuel. Therefore, the hypothesis can be made that the high total fat and SFA observed in the IGT participants may result in lower RQ values and a different ratio of CHO to fat oxidation.

It has not been elucidated if the reduction in SFA desirable in diabetic diets should be substituted by CHO or MUFA and dietetic advice does not appear to favour one or the other macronutrients. In the current study it appears that the fat intake may have been substituted for protein in IGT and type 2 diabetics. Type 2 diabetic participants had a significantly higher percentage intake of protein (18.55%) compared to control participants (14.33%). Gannon et al (2003) investigated the effect of a high protein diet (15%) and found a significantly reduced 24-hr integrated glucose response in type 2 diabetic patients and an increase in insulin response and therefore concluded that a high protein diet may improve blood glucose control in type 2 diabetic patients in the short-term. Long term studies are required to determine the potential long term effects of such a diet. The dietetic contact in the current study may explain the replacement of total fat for protein, particularly in the type 2 diabetic participants. It is difficult to determine if the protein content has changed only when the participants' diets have been under scrutiny or if this is truly a reflection of the habitual diet consumed. However, it certainly appears that the habitual diets of IGT and particularly type 2 diabetics were well managed.

The significance of an increased intake of protein in the habitual diet of the type 2 diabetic participants is in its recorded influence on satiety. Protein has consistently

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been reported to be the greatest promoter of satiety (Stubbs 1998; DeCastro 1987). The protein effects of satiety are only short term, i.e. meal to meal. Although successful in promoting weight loss (Brown et al. 1983), the effects of a long-term high protein diet are still not clear. It has not been elucidated what the classification of a high protein diet is or what harm the effects of prolonged ketosis produces as this may be a problem for susceptible individuals. The significance of a high protein diet is in its association with microvascular disease. AGEs (advanced glycation endproducts) induce significant increases in inflammation and endothelial dysfunction, which is thought to be a major risk factor for atherosclerosis (Negrean 2007). Dietary factors such as high protein diets can increase AGE production, when blood glucose is elevated, and this is even more important in type 2 diabetics for this reason because CVD is already the main cause of mortality. Therefore, a continuous high protein diet may not be beneficial in IGT and type 2 diabetic participants. Furthermore, it should be noted that protein intakes are markedly constant when comparing one study population with another (Stubbs 1998) and it takes a lot of effort to maintain a high protein diet. This highlights the potential problems associated with the misreporting of dietary intakes and it is also possible that misreporting may be specific to certain nutrients (Stubbs 1998). Nevertheless, in investigating the self-reported results of the EE with energy intake, it would appear that half of the IGT and more than three-quarters of the type 2 diabetics were in weight loss because the average daily EE exceeded the intakes. Whether this is a result of increased satiation, due to the increased protein intake, is difficult to say but there is certainly an argument to strengthen the hypothesis that protein is the most satiating macronutrient.

Accurate estimates of the dietary intake of free-living individuals are essential for nutritional research. The degree of underreporting is positively correlated with body mass index (Schoeller 1990). However, there are two explanations of underreporting; underrecording is the failure to record everything that is consumed, without a change in body mass; and undereating is the consumption of less than usual because of the requirement to record food intake, with a reduction in body mass (Goris et al. 2000). Indicators of underreporting are selective underreporting of snacks (Livingstone et al. 1990) and fatty foods (Goris et al. 2000). Poppitt et al (1998) studied nonobese women in a metabolic facility. Subjects had ad libitum food intake, which was covertly measured and the next day were asked to report everything they ate and drank during the previous 24 hours. It was found that snack foods were underreported, which are mostly carbohydrate rich. This was another reason why the current study implemented the use of recorded food diaries which were to be completed at the time of food consumption. Of course, all dietary assessment techniques rely on information supplied by the participants themselves and therefore often, their validity is questioned. No method has been shown to be free of systematic error, for example, systematic underestimation of intake using the 24 hour recall has been reported when compared with records of food consumed (Acheson et al. 1980). Therefore, an independent verification method of dietary assessment was developed initially by Isaksson in 1980. Twenty four hour urinary nitrogen showed that a food record and a diet history gave valid estimates of the average protein intakes (Isaksson 1980). Consequently, protein intake in the current study was validated by the significant relationship found between protein intake

(g/day) in the diet and the amount of nitrogen excreted in the urine collection. This gives validity to the accurate completion of the diet diaries. Only one participant was discounted from the study due to over/underreporting (i.e. intakes below 1.2 x BMR). This individual was a control participant. There were other participants who fell into the bracket of under/over reporting however it was decided that intakes matched their activity levels or their body weight status and they remained included in the study.

The type 2 diabetic participants had the lowest intake (g/day) of sugar of the three groups. Research has consistently shown that high CHO/low fat diets influence energy balance (probably by reducing food intake by greater satiety effects, reducing energy density and displacing fat from the diet) and thus help in body weight control (WHO 2002). Improved body weight control has been shown in high CHO diets, even those containing a higher than normal amount of sugar. Sugar intake has been investigated as prevention against weight gain (Saris et al. 2000). Generally the amount of 'added sugar' is restricted to <10% of total energy intake (WHO 2002) and the consensus is that the intake of sugars does not appear to have a deleterious affect on the primary care of diabetes (Nadeau et al. 2001; Janket et al. 2003). Indicating that the IGT participants in the current study, who had the highest sugar intake, would not be negatively affected by the level of sugar intake. However, an increase in sugar intake is almost always associated with an increased fat intake as well (WHO 2002). This appears to be verified by the IGT participants in the current study because they had the highest intakes of fat and sugar. As suggested earlier, the high sugar intake may be associated with the lack of taste sensitivity found in IGT

participants and may signify dysfunction in that mechanism of the metabolic control of food intake. The reason that this may not have been as obvious in the type 2 diabetics is because their recorded dietary intake seemed to be well controlled, as the sugar intake was significantly lower.

While the type 2 diabetic patients had the highest intake of alcohol (g/day) the differences were not significant. Shai et al (2007) studied the glycaemic effects of wine intake in diabetic patients who had previously abstained from alcohol intake. The results indicated reduced fasting plasma glucose levels but not postprandial (2 hour) glucose levels. Lapidus et al (2005) concurred with these results and also concluded that alcohol had a significant inverse association with the incidence of diabetes.

The results of the percentage intake of the main macronutrients demonstrate that none of the groups were achieving the national guidelines set out by the British Nutrition Foundation of 50% CHO, 36% fat and 14% protein. Control participants were the closest to meeting the CHO guidelines as their CHO intake was 46% and in fact met the fat 36% and protein intake 14% guidelines. The IGT participants' CHO intake was lower than the guidelines at 45%, their fat intake was 35% and the protein intake was higher than the guidelines at 17% and the type 2 diabetics had the lowest CHO intake at 44%, a 33% fat intake and the highest protein intake at 19% of the total intake. As discussed earlier, this may be due to the dietary intervention that the type 2 diabetic patients received and although no diet is specifically discussed, the reduction of fat ultimately leads to an increase in the alternative macronutrient(s).

The potentially positive effect of a high protein diet is to increase satiety and consequently lower calorific intake. A high protein intake helps to augment postprandial insulin secretion, promoting glucose transport and utilisation and thereby promoting improved glycaemic control in type 2 diabetics (Promintzer and Kreb 2006). It has been found that a high protein diet (30%) (Gannon et al. 2003) improved 24-hr integrated glucose response by 40% but again the long-term effects of such a diet have not yet been identified therefore optimal macronutrient protein composition for the treatment and prevention of obesity and related diseases such as IGT and type 2 diabetes is not known.

Habitual diet is only one of the considerations in balancing energy intake with expenditure. It has been demonstrated that sedentary living may be related to obesity and metabolic syndrome (Hardman 1996).

4.4.2   Differences in Habitual Activity

Activity diaries were used to record the participants habitual activity and total daily energy expenditure (kcals/day). There were no significant differences between any of the groups' daily energy expenditure. The control group had the lowest overall daily energy expenditure (2210 ± 119.14) when compared to the IGT (2666 ± 204.02) and type 2 diabetic participants (2683 ± 131.15). This may be attributed to the higher energy costs of sedentary and light activity in the obese which has similarly been recorded in previous studies (Blair and Buskirk 1987). After normalising for body weight the three groups had very similar energy expenditure results, as expected.

When investigating the types of activities undertaken by each group there were no significant differences between groups although an observation was noted between the control participants and the IGT groups. It was observed that the amount of strenuous/physical exercise completed by the IGT participants was less than control participants. Daily physical activity guidelines state that adults should accumulate 30 minutes of moderate intensity physical activity on most days of the week (WHO 2002). The similarity in EE between the groups may be explained by dietetic intervention as 'lifestyle' advice is given concurrently with dietary advice. Another possible reason for the similarity in EE per kilogram bodyweight may be due to the average age of the type 2 diabetic participants. Many of the participants were retired, giving them more time for moderately active activities such as walking, gardening etc, compared with the control group, all of which worked or were full time students. The results may suggest that the type of activity is important in the development of obesity and that strenuous physical activity may play a role in preventing weight gain. Further investigation would need to be undertaken to understand the benefits of strenuous physical activity compared to moderate physical activity in IGT and type 2 diabetic participants.

4.4.3 Biases and Limitations of Energy Intake and Energy Expenditure Methods

4.4.3.1 Energy Intake

Information on the usual macronutrient intakes of individuals is frequently a central component of nutrition studies. Such information is used, for example, as the basis

for defining relations between macronutrient intakes and morbidity and mortality and for determining the role of dietary energy in the development and treatment of obesity (Acheson et al. 1980). Inaccurate information on dietary nutrient intakes will therefore lead to spurious conclusions regarding the importance of diet in the maintenance of long-term good health. It is widely recognised that dietary assessment methodologies used currently to determine the usual nutrient intakes of individuals are open to several general criticisms (Bingham 1985). In particular, subjects may bias the information obtained by not consuming typical amounts of food during the period when food intake is being recorded, and in addition, they may not accurately report the food that is consumed. A special concern is that different groups of subjects may not exhibit the same degree of over or underreporting relative to their usual diet. Thus, in addition to the widely recognised problem that energy intakes determined by a weighed food record tend to underestimate usual dietary nutrient intakes of individual subjects (Schoeller 1990), it is also possible that differences in group means for reported nutrient intakes may in part simply reflect group differences in methodological bias rather than being caused exclusively by differences in food consumption.

The lack of detailed information on the bias inherent in different food intake assessment methods is due primarily to the absence, until recently, of an accurate reference method against which different methods could be compared. The successful validation of the doubly labeled water method for measurement of total energy expenditure (TEE) has now provided such a reference method (Schoeller 1988). Sawaya and colleagues (Sawaya et al. 1996) studied weighed food records

and 3 other widely used methods of food intake (24-hour recall (Gibson 1990), Willett food frequency questionnaire (Willett et al. 1985) and GHCRC/Block food frequency questionnaire (Block et al. 1990)) and compared them with doubly labeled water measurements of TEE in young adults and children. Results of this study showed that no method gave accurate information on the usual energy intake of individual subjects. In addition, there was an indication that old age may cause different relative biases in the four methods that were evaluated, although further studies are needed to confirm this suggestion. In young women, 24-h recall data gave mean energy intakes that were closest to measures of TEE, and food-frequency questionnaires provided the only data that correlated with individual values for TEE. In older women, the Willett food-frequency questionnaire gave values for energy intake that were closest to measures of TEE, but there was no method that gave values for energy intake that correlated significantly with TEE. Seven-day weighed dietary intakes, although by far the most difficult and time-consuming measurements, were neither more accurate nor more precise than the other, simpler methods, although they did provide the most accurate estimate of the mean difference in energy intake between the two groups. The results indicate that EI derived by weighed intakes and food frequency questionnaires were in excellent agreement with mean EE measured by DLW therefore in the current study weighed records were the method of choice due to the low cost in comparison to the doubly labeled water method. Furthermore, as already discussed, there was a positive correlation between protein intake and urinary nitrogen which again validates the data obtained in the diet diaries in the current study. However, the correlation was a low correlation and this may be due to that some participants in the IGT and type 2

diabetic groups may have been in weight loss, which was reflected in their overall energy intake values (kcal/day). Weight loss was not measured within the study and to incorporate a dietary restraint questionnaire to eliminate potential restrainers was too costly for the current study. Therefore, it was difficult to eliminate under-reporters in the current study because it was unclear if the low energy intake was due to under reporting or a true reduction in food intake due to dieting. Consequently, only 1 individual was discounted from the study due to under reporting and this was a control participant, as it would have been expected that they were in energy balance and therefore, within the upper and lower limits of over and under reporting.

4.4.3.2 Energy Expenditure

The validity of the activity diary method is open to doubt. It relies on the accurate description and timing of activities and their exact reproduction for measurement of their cost. In addition the cost of some activities, the most important being sleep, is difficult or impossible to measure in the field, and assumptions have to be made for them.

Inherent sources of error in the activity-diary method are incorporated into the process of recording activities and the conversion of these activities to energy expended. Additionally, it is unlikely that a normal activity pattern can be maintained when activities are being recorded every minute. Influencing activity seriously undermines the validity of any method for measuring habitual energy expenditure. Some investigators have tried to overcome this problem by having observers record activities. This may be feasible and socially acceptable in some

situations but not in others. In the current study this would have been too time consuming to consider. The component of error associated with converting recorded activities into their energy equivalent is as important as the precision in recording activities. In many situations it is not possible to measure the energy costs of activities and consequently values found in the literature are used. Even when the energy costs of activities are measured there is no guarantee that intensity and effort during measurement will accurately reflect those in a free-living situation. Because both sources of energy costs are subject to error it is important to know whether energy expenditure calculated with measured values is more accurate than that calculated with the most widely used literature values. Most of the published values used in this study were from studies of women of comparable body weight (Durnin 1967) which suggests that overweight individuals may have an even higher error rate.

4.4.4   Meal Consumption

The test meal chosen was a low calorific meal designed to show a rapid and complete postprandial response. There were no significant differences in the time taken to eat the test meal. This is important because it has been hypothesised that the time taken to eat a meal and greater exposure time to food stimulates additional food intake and so also a greater total energy intake (Hetherington et al. 2006). This was an important finding because it is one of multiple possible mechanisms that leads to a positive energy balance, which may consequently lead to obesity. Varma et al (1999) found that energy intake was influenced by the time taken to consume a meal,

causing an increase in meal size. In the current study a conclusion can be made that the feeding behaviour of IGT, type 2 diabetic and control participants investigated, in relation to meal consumption, was normal and the time taken to consume the meal was not an important factor in influencing food intake. However, an argument could be made that the meal design in this study was not conducive to the discovery of time differences as the meal was not large enough to illustrate this effect. Furthermore, it has also been hypothesised that future meals are affected by either high energy density (HED) or low energy density (LED) preloads. For example, Mazlan et al (2006) discovered that only partial compensation (~40%) occurs after a HED pre-load and compensation only occurs at the next meal so any subsequent meals are not affected and normal food intake occurs resulting in a short-term positive energy balance. If this positive energy balance is not balanced out over time then it will result in slow weight gain. Again, this is crucial to understand for people with IGT and type 2 diabetics because if their habitual diet incorporates sporadic inclusion of HED and/or high fat meals, as is common (Schulze et al. 2004), then weight gain is implicated over time. High fat content is a feature of high energy dense foods and both are associated with poor glycaemic control (Ello-Martin et al. 2007). The concept of the glycaemic index of food was introduced to distinguish and quantify the variability of the glycaemic responses to the CHO in different foods (Jenkins et al, 1981). High glycaemic index foods result in increased blood glucose concentrations and an increased insulin demand which Schulze (2004) understood to lead to an exhausted pancreas resulting in glucose intolerance and then a consequential insulin resistance. Poor glycaemic control has been associated with the risk of developing type 2 diabetes which is particularly important for the IGT

participants in this current study. The discussion of whether IGT is an intermediary in the progression to type 2 diabetes is still under investigation. Various studies (Marshall et al. 1994; Saldana et al. 1994) associate the fat intake with the progression of IGT to type 2 diabetes. The current study shows that daily fat intake was within guidelines and therefore there should not be high progression rates from IGT to type 2 diabetes. Saldana (2004) stated that a diet containing 41% fat would increase the risk of diabetes. Therefore fat intake, energy density and glycaemic control are all important in the influence and control of food intake for these patient groups. Importantly, it is necessary to understand how the effects of glycaemic control affects appetite and the ability to cease eating. The type of macronutrients consumed may have a role in influencing subjective feelings of appetite, i.e. satiety and prospective consumption.

4.5   VISUAL ANALOGUE SCALES

4.5.1 Hunger and Prospective Consumption

In this study subjective ratings of hunger, satiety, fullness and prospective consumption (PC) were measured from fasting levels and then following a test meal, of the same content for all three participant groups, to determine whether appetite ratings differed between groups and to discover what impact this had in the integrated process of food intake. Prospective consumption is becoming increasingly important to record when studying IGT and type 2 diabetic patient groups because as previously mentioned obese groups tend to feed on highly palatable foods even when

sensations of hunger are low and satiety is high. The foods tend to be high in energy density and so have a high fat content resulting in a positive energy intake, which may lead to further weight gain if not controlled over time. This could contribute to the progression of their chronic disease.

The overall hunger response was significantly higher in control participants demonstrating that they felt hungrier throughout the study. Control participants also had lower fullness ratings which was only significant at the 90 minute timepoint and higher PC ratings when compared to the other two groups. This may be indicative of an overall increase in appetite sensitivity which may be associated with taste sensitivity. Supertasters, which were primarily control participants, demonstrated the greatest sensitivity to taste and therefore because the supertaster range of sensitivity is greater in taste it may also be the case in measures of appetite. Furthermore, supertasters should consume an overall lower intake of food based on the hypothesis that BMI is associated with taste status and this may also contribute to the higher feelings of hunger observed in the controls compared to IGT and type 2 diabetic participants. The current study shows that the greater feelings of hunger are not associated with a greater food intake, compared by the energy intakes from the diet diaries, but are associated with greater sensitivity to appetite.

Hunger ratings were inversely correlated with satiety and fullness in the current study whereas prospective consumption positively correlated with hunger which validates the parameters of appetite ratings. Looking at the overall response, type 2 diabetic participants showed high fullness ratings, low hunger ratings and also rated

prospective consumption the lowest. IGT participants rated high fullness ratings and hunger levels as being low. This was similar to type 2 diabetics however, IGT participants recorded higher levels of prospective consumption than type 2 diabetic participants although this was not significant. The difference in PC ratings between IGT and type 2 diabetic participants may be put down to the subjectivity of the test. On the other hand, it is possible that IGT participants are displaying a greater desire to consume food when they do not feel particularly hungry which may of course be the factor responsible for the positive energy balance which leads to obesity, i.e. want versus need.   The appetite results of the current study differed to a similar study by Chapman et al (1999) who recorded appetite ratings in obese and non-obese subjects. Chapman's (1999) study recorded higher hunger ratings in obese patients before food intake and then recorded no differences in hunger during feeding. In studying the diet diaries of IGT, type 2 diabetic and control groups, it was noted that many participants of both the patient groups missed breakfast out of their daily food routine whereas all control participants included breakfast in their habitual diet. Missing breakfast may contribute to the lower ratings of hunger observed in the IGT and type 2 diabetics on the study morning. Because it was not 'normal' for these two groups of participants to eat this early in the day it may have resulted in the overall lower hunger sensations. Meal placement may be a key factor when investigating IGT and type 2 diabetics because when food is not consumed in the morning this may result in increased hunger intensity and increased ratings of prospective consumption later in the day. It may be that hunger intensities grow throughout the day and the amount of food consumed in subsequent meals and snacks may be more important than the first meal of the day. Therefore, further research would be

required to determine the effects of meal skipping on the intensity of appetite parameters. Many overweight dieters adopt this strategy of missing breakfast in an attempt to reduce their overall daily food intake (Rogers 1997). However, it is unclear how this influences the next meal, food intake over the course of the day and if and how the body compensates over coming days. This is an area that requires investigation, in particular in clinical groups such as IGT and type 2 diabetic populations.

4.5.2   Satiety and Fullness

Satiety may be defined as the state of inhibition over further consumption and is the interval between differing episodes of food consumption (Rogers and Blundell 1991). Satiety can be assessed as a behaviour of a physiological response and a reduction in satiety creates a stimulus for eating. Studies have indicated that satiety ratings of sweet and fatty foods differ between obese and normal weight individuals (Salbe et al. 2004). Therefore it was essential to determine how ratings differed between patient groups following a mixed meal where the content was the same for all three groups. In the current study satiety ratings were highest in the IGT group and control participants rated satiety the lowest, which corresponds with the hunger ratings. However, the expected result would have been to observe the highest satiety and fullness ratings in the control group because previously it has been shown that the satiety response in obesity is attenuated. The current study indicates that the satiety response in type 2 diabetics and IGT participants in this study is normal

following the consumption of a mixed meal and is not associated with a reduction in satiating effects.

When the satiating effects are diminished in obese individuals then the effect is mediated elsewhere in the complex pathways that control food intake. Alternatively, the satiety response may be normal to a meal but the satiety effects may be overridden as a consequence of behavioural or sensory factors, such as emotions or palatability respectively. Short-term intake of foods (i.e. snacking) due to palatability is associated with high fat, high energy dense foods which are consumed out-with mealtimes resulting in satiety responses being overridden (Yeomans et al. 2005). In this instance cessation is strongly affected by sensory-specific satiety, the intake of a single food relative to the intake of other foods. Therefore it is also important to distinguish between satiety and fullness. Satiety is a neural response as opposed to fullness which is a physiological response to stretch recepters. The IGT participants had the highest fullness ratings, followed by the type 2 diabetics and then the control participants. There was a significant difference at the 90 minute timepoint only between control and type 2 diabetic participants where the type 2 diabetics rated feelings of fullness greater than IGT participants. The trends throughout the study period for IGT participants, as well as type 2 diabetics, showed high satiety and fullness and low hunger and prospective consumption, as shown by the area under the curve results.

In summary, the appetite parameters were not what was expected however they were consistent. The current study may indicate that control participants display increased

appetite sensitivity analogous to increasing taste sensitivity which may lead to a greater range of sensitivity i.e. higher when hungry and lower when full, but does not appear to result in altered food intake. Obesity has been associated with dysfunctional satiety mechanisms thought to be due to the large storage capacity for fat (Flatt et al. 1995). Obesity occurs as a result of a high intake of energy from the overeating of high fat foods, consumption of a high energy density diet and the high palatability and weak satiation of high fat foods (Snoek et al. 2004). This was not observed in the current study, however, as aforementioned a second meal may be more suited to elicit the attenuated satiety response that has been reported in other studies. The possible reason for this is due to the irregularity of consuming breakfast at all, let alone so early in the day for the IGT and type 2 diabetic participants. Consuming food prior to the time a first meal would normally be consumed in the day may have resulted in raised satiety levels due to initially feeling less hungry, which was the case in this study. This may have resulted in altered responses for all parameters of appetite in the two patient groups.

The measurement of appetite parameters in this type of protocol is important to assess the dynamic changes which occur following a meal. It is the change in appetite sensitivity which allows increased energy intake, as well as behavioural factors and decreased activity.

4.6   CALORIMETRY

4.6.1   Respiratory Quotient and Energy Expenditure

Most individuals maintain a steady body weight for long periods throughout their lifetime indicating a good control of energy intake and expenditure. A steady state is likely to be maintained if the fuel mix oxidised in the body is equal in composition to the nutrient flux of the diet consumed thus balancing, protein, CHO and fat in the body. The composition of the fuel mix oxidised and therefore respiratory quotient (RQ) are influenced by circulating substrate and hormone concentrations, which reflect the degree of replenishment of the body's fuel reserve. The composition of the fuel mix oxidised to drive oxidative phosphorylation changes considerably throughout the day. However, nitrogen content undergoes minimal change and CHO content is maintained within a tight range (McDevitt et al. 2000). Reasons for this are due to the important functional roles of proteins and the essential role of the body's systems to sufficiently supply glucose to the brain. Therefore, changes in the fuel mix oxidised alters an individuals overall energy balance primarily with changes in fat balance, which can easily accommodate gains or losses due to the large storage capacity for fat.

When insulin is released postprandially, this initiates a rise in RQ and promotes the storage of nutrients. Whereas, the release of hormones between meals activates mobilisation of the body's glycogen and fat stores and ensures an adequate supply of ATP production and circulating glucose. The composition of the fuel mix between

meals is also influenced by the body's protein pools, the degree of repletion of its glycogen reserves and the size of the adipose tissue mass. This is a consequence of the fact that rates of mobilisation, which are regulated by hormone concentrations, have to be multiplied by the size of the adipose tissue mass to which the hormone signals are conveyed. For most individuals this is remarkably effective but the body composition for which this adjustment is achieved varies greatly between individuals and depends on genetic interactions and circumstantial factors. Following a CHO-containing meal RQ increases because CHO is favourably oxidised and after a protein-containing meal, amino acid oxidation increases. However, fat oxidation is reduced after food consumption even when the meal contains fat, which suggests that control over fat balance is not effective (Flatt 1987). In the current study RQ increased in all three groups postmeal as expected, as the body preferentially oxidises CHO and emulates the results of similar studies (Flatt 1995a & b). Although there was no significant difference in RQ between groups, at 15 minutes postmeal the largest increase was in the control group. It is known that when fat is consumed together with CHO (as in a mixed meal) it is followed by an increase in CHO oxidation and a reduction in fat oxidation, which is resultant of insulin release following the ingestion of CHO (Flatt 1995). A study by Acheson et al (1987) in lean and obese subjects found that RQ rose similarly postmeal in both groups but dropped earlier and more rapidly in obese individuals. The current study did not show the same results as Acheson et al (1987) as there was an increase in RQ, in response to CHO ingestion and oxidation, over all groups but to a greater extent in the control group. The control group then had the larger decrease back to normal levels whereas the IGT participant group levelled off and decreased more gradually.

The type 2 diabetics also had a gradual reduction following the initial increase in RQ, but then increased further towards the end of the study period. RQ levels did not reach fasting levels within the study period. This study shows that RQ levels in IGT and type 2 diabetics did not rise as much as control participants suggesting that fat was being oxidised and CHO was being spared. It was expected that the RQ would have remained higher for a longer period in the control group when compared with the two obese groups and that IGT and type 2 diabetic RQs would have reduced quicker towards fat oxidation. A study by Schutz et al (1992) found obese subjects to have increased fat oxidation postprandially, compared to non-obese subjects, as the body attempted to regulate energy and fat balance in the long-term. Therefore, it appears be that fuel oxidation in obese individuals is controlled firstly by their fuel stores and what fuel is currently circulating and then secondly by the type of fuel consumed by the most recent meal. This insinuates that the body's need to regulate long-term energy balance may be more important than the short-term regulation of food and consequently will also impinge upon the release of neuro-endocrine control mechanisms and may alter fuel oxidation. Further results in the study of Acheson et al (1987) recorded a reduced diet induced thermogenesis (DIT) response to food in obese subjects. The current study showed a similar lower DIT response for the IGT participants whereas type 2 diabetics had a very similar DIT response to the control participants. The IGT and type 2 diabetic participants in the current study had a BMI greater than 30 and therefore a blunted DIT was expected as it has previously been shown that obesity results in a reduced DIT response and that there is an inverse relationship between percentage body fat and DIT (Schutz et al. 1984). Differences seen between this study and previous studies may be due to the smaller size of the

test meal in this study. The differences observed between IGT and type 2 diabetics may again be due to the fact that the type 2 diabetics controlled their habitual diet well. Inadvertently, this may have resulted in some weight loss and consequently a reduction in fat mass and a reversal in the symptoms of type 2 diabetes.

Obesity is a multifactorial and complex disorder that is characterised by a long-term energy intake that is above energy expenditure (EE). Many studies have examined the regulation of both these components to provide an understanding of energy balance in obesity. In the current study daily EE was measured by activity diaries and there were no significant differences in EE between IGT, type 2 diabetics and control participants. However, both IGT and type 2 diabetics had higher EE values throughout the study day when EE was measured by calorimetry. This higher EE was expected and attributable to their greater weight, which results in high energy costs for innocuous activities. Other studies have also shown EE to be higher in obese groups (Marques-Lopes et al. 2001). To be able to discuss the changes in EE independently of weight, EE was normalised for body weight. EE was significantly higher in control participants both in a fasting state and postprandially throughout the study period when normalised for body weight. The overall changes in EE in response to the test meal are shown by the AUC. The control participants' response was significantly higher when compared to IGT and type 2 diabetic participants. This indicates a lower DIT in IGT and type 2 diabetics and therefore suggests, according to Stock (1997), that they are metabolically more efficient. This means that a far greater proportion of ingested energy is deposited as fat, and much less is dissipated as heat, which is thought to be due to a reduced action of the sympathetic

nervous system (Stock 1997).   Together the low EE measurements, with possible low DIT response, and lower RQ values lead to the conclusion the metabolic handling of food results in a propensity to store fat to a greater level than control participants.

When evaluating the differences between the resting metabolic rate (RMR) assessed by calorimetry and the total EE assessed by activity diaries in the current study, there was a positive correlation which validates both the use and the results of the activity diaries. The differences observed in the 3 groups in RMR over the study are not seen in total daily EE recorded by activity diaries which suggests that the blunted EE response observed following food intake in the IGT and type 2 diabetic participants may contribute to a reduced daily EE which in turn may perpetuate weight gain. On the other hand, the observed total daily EE by activity diaries would have been expected to have been significantly higher in the control group, particularly given the differences in the RMR, however no significant differences were observed. The reasons for observing no differences in the total daily EE between groups may be due to the interventions initiated by IGT and type 2 diabetics post diagnosis to counteract their condition. The interventions may have resulted in an increase in the amount of daily physical activity in the type 2 diabetic participants. The activity diaries highlighted that some of the IGT and type 2 diabetic participants were reasonably active compared to the normal sedentary lifestyle expected to be observed in these patient groups: one was an avid cyclist, two participants were in professions which involved manual labour and another had recently started brisk walking.

4.6.2 Thermic Effect of Food

Energy expenditure or thermogenesis may play a roll in body fatness (Miller and Payne 1962) therefore interest in DIT has emerged over time. Energy balance control has always been associated with the regulation of energy intake however, animal experiments have shown that at excess energy intake, the regulation of thermogenesis is important for the control of body fatness (Thurlby and Trayhurn 1979). Numerous studies have been undertaken investigating energy expenditure or another component of daily energy expenditure i.e. RMR and DIT, in obese subjects with non-obese counterparts. With respect to DIT, the most significant outcome of all these studies was that the DIT might be blunted in obese subjects and therefore may be significant in the pathogenesis of obesity (Bessard et al. 1983). The evidence is conflicting (Samueloff et al. 1982) and therefore has had a negative effect on the elucidation of the etiology of problems of energy balance. The disagreement in results in this field of study, particularly in postprandial metabolism, may be related to the constant reappraisal of the methodology for assessing RMR and DIT in humans. The EE results suggested that there may have been reduced DIT in IGT and type 2 diabetic participants. In the current study RMR and DIT were obtained with a ventilated-hood system. However, no significant differences were found in DIT between groups. This result was unexpected due to the reduced EE and RQ values in IGT and type 2 diabetics compared to control participants. The trend was that the IGT participants had a blunted DIT response but the reduction was not significant. Type 2 diabetic participants had a DIT response very similar to the control group. Previous studies have shown that the differences in DIT between

control and obese subjects could result from a lower stimulation of the sympathetic nervous system in obese subjects due to a low ratio of diurnal/nocturnal norepinephrine (NE) excretion (Schutz et al. 1984). Schutz et al (1984) specifically demonstrated that urinary excretion of NE during the day and night is increased however the relative increment in diurnal NE excretion over sleeping values was less, i.e. a lower ratio, in obese than control subjects. This is important because NE is a hormone and neurotransmitter, secreted by the adrenal medulla and the nerve endings of the sympathetic nervous system to cause vasoconstriction and increases in heart rate, blood pressure, and the sugar level of the blood. Welle (1995) indicated that postprandial NE concentrations are caused by an increased spillover from sympathetic nerve endings in muscle brought about by glucose induced hyperinsulinemia. Any messages conveyed via the sympathetic nervous system to the NTS in the medulla and are then relayed to a variety of other brainstem and hypothalamic areas. A disturbance in the concentrations of NE will result in reduced sympathetic nervous system stimulation postprandially (in this case) which results in reduced EE. Another hypothesis is that there may be a resistance to the thermogenic effects of NE in obese subjects. Therefore these studies only go a little way to understanding the role of NE and the sympathetic nervous system in the etiology of obesity by reduced EE. One major problem is that comparisons of lean and obese subjects cannot tell us whether a difference in sympathetic nervous system activity is the cause of or the result of obesity or its side effects, such as hypertension or hyperinsulinemia.

4.7   SUBSTRATE OXIDATION

4.7.1   Fat Oxidation

Studies have reported that fat oxidation rates reduce in obese individuals postprandially (Flatt 1995; Marques-Lopes et al. 2001). Obesity occurs frequently together with both IGT and type 2 diabetes. They are all characterised by insulin resistance, disturbances in intermediary CHO and fat metabolism, and most often by an increased adipose tissue mass, increased FFA, as well as increased triacylglycerol (TAG) storage within skeletal muscle. Increased TAG storage is thought to be observed in the body by a blunted fat metabolism following catecholamine release postprandially (Blaak 2003). These disturbances perpetuate the progression of the said diseases (obesity, IGT and type 2 diabetes) because they play a role in the development and maintenance of adipose tissue stores, accumulation of lipid intermediates (strongly linked with skeletal muscle insulin resistance (Blaak 2003)) and TAGs in skeletal muscle, all risk factors for the development of whole body insulin resistance and type 2 diabetes. In the current study there was no significant difference in oxidation rates between groups for protein, fat or carbohydrate when in a fasted state. At fifteen minutes postmeal fat oxidation had reduced in all groups but to the greatest degree in the control group and then there was an increase in fat oxidation in all groups up to 30 minutes postmeal and again the control group had the sharpest increase. Following this the IGT participants fat oxidation slightly reduced until 90 minutes and then slightly increased up until 120 minutes whereas the type 2 diabetics and control participants levels of fat oxidation increased again until 60

minutes and then reduced until the end of the study period. In the current study, after normalising for body weight, it appears that there was no tendency for obese individuals to have suppressed fat oxidation levels following a mixed meal as previous studies have shown (Owen et al. 1986; Acheson et al. 1987). The current study shows that fat oxidation is higher after the meal consumption in IGT and type 2 diabetics than in control participants. This corresponds with a study by Astrup et al (1996) where obese subjects were found to have higher fat oxidation, however this was thought to be due to their increased weight and therefore enlarged body fat stores. The current study corrected for body weight and an increase in fat oxidation was still observed. The RQ of the IGT and type 2 diabetic participants was lower than that of the control participants suggesting that they were indeed oxidising fat at an increased rate and sparing CHO. A study similar to the current study by Owen et al (1992) investigated oxidation rates for 4 hours following ingestion of a mixed meal and also found no differences between obese and lean individuals. Therefore, in the current study fat oxidation does not appear to be one of the mechanisms which contributes to a blunted satiety response and dysfunctional feeding behaviour.

4.7.2   CHO Oxidation

In the current study overall CHO oxidation rates were similar between groups, as discussed earlier, due to the tight metabolic control over blood glucose levels however the control group did have the highest RQs throughout the study period suggesting that they were oxidising more CHO than the other groups. When oxidation rates were normalised for body weight, CHO oxidation was significantly

higher at certain points throughout the study period in control participants compared to IGT and type 2 diabetic participants. It was important to look at oxidation rates when normalised for weight to indicate the difference between groups as a result of the disease status. The literature is sparse surrounding the assessment of oxidation rates when normalising for body weight. It has been reported that in IGT and type 2 diabetes there is impaired metabolic flexibility (Storlien et al. 2004), for example, an impaired switching from fatty acid to glucose oxidation in response to insulin (Phielix and Mensink 2008) which is again associated with insulin resistance and in particular skeletal muscle insulin resistance which impairs the cellular uptake of glucose causing an accumulation of lipid inside the muscle cell. This process is under positive feedback control so as FFA levels increase in the cell, glucose uptake is further inhibited and in fact it is not clear if FFA is a cause of reduced cellular uptake or if it is the effect of it. The impairment however, would be ever more apparent in IGT and type 2 diabetic patients when glycaemic control is poor and the positive feedback effect perpetuates the problem. Furthermore, the lower CHO oxidation rates in the current study in the two patient groups may be driven by the body's lack of metabolic need and drive to generate ATP for metabolic function. This may be a consequence of low levels of physical activity which is common in obesity (McGough et al. 2001). Flatt (1995c) suggested that oxidation rates are dictated by the body's need to generate ATP used in performing metabolic functions, in moving and in physical activity. Therefore, if leading a sedentary lifestyle the requirement to sustain ATP regeneration will be minimal and oxidation rates will fall. Conversely, it may be the amount of physical activity that is driving the fuel mix towards an increase in CHO oxidisation in the control participants.

In summary, the IGT and type 2 diabetic participant levels of CHO oxidation was lower than control participants once normalised for body weight indicating that this is one of the dysfunctional mechanisms involved in the complex interplay of mechanisms controlling food intake. The impairment may be due to insulin resistance which impairs the uptake of glucose from the blood. Therefore, the current study measured indicators in the blood which give information about the digestive process.

4.8 BLOOD PARAMETERS

4.8.1 Glucose

In the current study the CHO content of the testmeal was 58%. A similar study by Wolever and Miller (1995) investigated different CHO contributions in a meal (5%, 30%, 45% and 60% CHO) and found that the meals with the highest CHO content produced the lowest blood glucose response. This is significant because the dietary management of IGT and diabetes aims to optimise blood glucose control and to decrease the risk for the long-term complications associated with diabetes, i.e. retinal eye, kidney and nerve and cardiovascular disease. In the current study, as expected when fasted, the control participants had a lower blood glucose concentration than the 2 patient groups and post meal all three groups' blood glucose concentration rose. Wolever and Miller's (1995) study showed no difference in blood glucose between type 2 diabetic and control groups with the 60% CHO meal. However, in

contradiction, the blood glucose concentrations in the IGT and type 2 diabetics in the current study rose to a high level and at 60 minutes there was a significantly greater blood glucose profile in the two patient groups compared to control participants. Other studies have shown results similar to this study (Manders et al. 2005). The raised blood glucose profile suggests that the IGT and type 2 diabetic groups in the current study had disrupted glucose uptake via GLUT 4 transportation, which is known to be a cause of insulin resistance (Bilan et al. 1992) and may be the cause of the previously noted low RQ, CHO oxidation rates and EE. There was no significant difference between IGT and type 2 diabetic participants, however the type 2 diabetic participants had an intermediary blood glucose profile between the IGT and control participants. The results expected would perhaps have shown the IGT participants to have the intermediary blood glucose levels because it is known to be a less severe disease, however the results remain consistent because it is likely that the outcome is due to the well controlled diets of the type 2 diabetics and may also be associated with the lower taste sensitivity identified in the IGT participants, but not in the type 2 diabetic participants. When good glycaemic control is achieved in type 2 diabetes the short term responses change i.e. increased satiety, improved insulin profile and therefore improved glucose transport. Furthermore, the long term micro and macrovascular complications are improved as shown by the UK Prospective Diabetes Group (U.K.P.D.S) who has published literature investigating blood glucose and blood pressure in over 5000 people with type 2 diabetes. Hyperglycaemia and hypertriglyceridemia can lead to oxidative stress and endothelial dysfunction, which in itself is a known cause of cardiovascular disease (CVD) (Negrean et al. 2007). In diabetes, endothelial dysfunction is triggered by

the production of advanced glycation end products (AGEs). AGE concentrations are elevated in type 2 diabetes and come from the uncontrolled non-enzymatic reaction of sugars with proteins, particularly when blood glucose levels are high. Increased AGE concentrations have significant pro-inflammatory (cause increased cytokine levels) and pro-oxidative effects, therefore play an important role in the development of diabetic complications as discussed earlier. Insulin resistance is an effect of the inflammation associated with AGE concentrations, perhaps due to damage to specific neurons which sense plasma glucose levels and control insulin release from the pancreas. Therefore it is imperative that glycaemic control is maintained and when hyperglycaemia is prevented some of the postprandial effects can be minimised which can reduce the long-term complications of diabetes. In the current study the type 2 diabetic participants and in particular the IGT participants should look to improve blood glucose control to prevent the progression of their disease. Certainly, it appears that an aberration in blood glucose profile may contribute towards dysfunctional food intake and may be one of the triggers which causes alterations in consequent physiological parameters, such as EE, RQ and CHO oxidation, in the regulation of food intake.

Of course, the crucial component in glucose transportation into the body cells is in insulin. Therefore, it was important to record insulin levels in the study participants to determine if: a) these participants had the traditionally high levels of insulin found in obese subjects and b) if this contributed as one of the dysfunctional pathways in the control of food intake.

4.8.2   Insulin

Insulin sensitivity is central to IGT and type 2 diabetes. Studies have shown that fasting insulin levels are higher in IGT and type 2 diabetic populations than in healthy, normal weight individuals (Wolever and Mehling 2002). The current study also showed that control participants had lower fasting insulin levels. However, the postprandial insulin levels increased significantly more in the control participants in this study and were actually higher than the IGT and type 2 diabetic participants. This result was not anticipated. The insulin concentrations followed that of the blood glucose changes except at 60 minutes when the insulin levels continued to rise in the control subjects when the blood glucose concentrations had fallen. The overall glucose AUC response for control subjects was the lowest whereas their AUC for insulin was the highest. The AUC for glucose in IGT participants was the highest and their AUC response for insulin was the lowest with the type 2 diabetic participants having the intermediary result. It is assumed that in obese individuals regulation of the normally high insulin response is desirable but in this case with a high CHO mixed meal, a higher insulin response would have been expected in the patient groups. A reason for the response seen in the IGT participants is that the body's response to a high carbohydrate meal, including the insulin response, is influenced by the rate of absorption of the meal (Collier and O'Dea 1982). The AUC for insulin suggests that the insulin secretion in IGT participants in the current study was less sensitive to the meal than in control participants. It is known that the release of insulin is a satiety factor and causes inhibition of feeding (Havel 1999). If, however, the insulin response is blunted and satiation does not occur the result may

be abnormal ratings of appetite parameters i.e. reduced satiety and fullness, also increased hunger and prospective consumption. This may potentially account for an increase in food intake and weight gain. In the current study, although a reduced insulin response was seen in the IGT participants, a corresponding change in the appetite parameters was not observed and the reason for that is not clear. A possible explanation for the low hunger and high satiety ratings may be a result of the low insulin response may slow down glucose uptake because the glucose uptake mechanism is insulin-induced. This may have resulted in an overall slower processing of the breakfast. The opposite may be true of the control participants, the high hunger and low satiety ratings may be a result of the high insulin response which promoted quick glucose storage and therefore quicker ingestion of the breakfast. It is also possible that the dietary intervention which was received in the type 2 diabetic participants may have resulted in the improved insulin profiles observed when compared to the IGT participants. A study by Mann (2000) has shown similarly improved insulin profiles as a result of dietary intervention, although further long-term studies are required to elucidate the mechanisms surrounding this change.

Insulin is a known mechanism in the suppression of food intake (Blom et al. 2005) and exerts its action via the central nervous system. In contrast, ghrelin also exerts its action via the CNS however has an opposite role to that of insulin, in the stimulation of food intake.

4.8.3   Ghrelin

Ghrelin has an important role in food intake therefore it is important to understand the relationship between glucose, insulin, ghrelin and also appetite. Plasma ghrelin concentrations rise gradually before a meal and decrease rapidly (Cummings et al, 2001) following a meal in normal weight individuals. Obese individuals have been shown to have reduced ghrelin concentrations (Shiiya et al. 2002) and furthermore, plasma ghrelin levels fail to decline following food intake (English et al. 2002). The lack of suppression, following food intake, in obese individuals may contribute to increased food intake and potentiate obesity. Plasma ghrelin concentrations are known to decrease after oral and intravenous administration of glucose whereas lipid or high fat diets suppress the postprandial ghrelin concentration less effectively (Blom et al. 2005). The current study testmeal contained a high CHO content to assist the observation of plasma ghrelin changes.   Postprandially, IGT participants showed reductions in plasma ghrelin levels at both timepoints. The type 2 diabetics only showed a very small reduction in plasma ghrelin levels at 30 minutes postprandially as expected, but then ghrelin levels increased at 60 minutes suggesting that the type 2 diabetic participants would have accepted further food intake at this stage. The control participants did not show a reduction in plasma ghrelin levels. Insulin is essential for meal-induced ghrelin suppression and to acutely increase leptin in healthy persons (Saad et al. 1998). An inverse relationship has been found between leptin and ghrelin (Kalaitzakis et al. 2007) and it has been proposed that leptin is important in the suppression of basal ghrelin concentrations in normoinsulinaemic individuals.   Therefore in obese individuals ghrelin

concentrations have been found to be reduced which is thought to be due to being in a state of positive energy balance (Mingrone et al. 2006) and is caused by insulin resistance which is due to consistently high blood glucose levels. Furthermore, the lack of ghrelin suppression following food intake may contribute to abnormal appetite parameters in obese individuals such as individuals with IGT and type 2 diabetes. Changes in ghrelin levels did not correspond with other studies. In the current study the lack of suppression of ghrelin concentrations observed in the type 2 diabetic participants may affect the satiety cascade by promoting reduced satiation and suggests that ghrelin is involved in the dysfunction of the satiety cascade and therefore also in the pathophysiology of obesity. It was possible in the current study that the ghrelin assay was compromised and that these results may be distorted. Therefore it is impossible to make any genuine statements about the contribution of ghrelin in the control of food intake. There was no correlation between plasma ghrelin levels and appetite parameters as would perhaps have been expected due to the suppression of ghrelin concentrations postmeal. However as has often been observed, particularly in obese individuals, people do not always eat when they are 'hungry' and do not always refrain from eating when 'satiated' (de Graaf et al. 2004). Therefore, it is imperative to understand the integrated responses involved in the process of food intake i.e. those which combine to initiate feeding and the mechanisms which contribute to the cessation of feeding.

4.9   INTEGRATED RESPONSES

The integrated responses in the control of food intake are probably the most important to understand because there are a constellation of adverse metabolic, psychological and physiological changes that occur in chronic diseases such as IGT and type 2 diabetes. Increased knowledge about the underplay of events involved in food intake may help in the prevention and treatment of such conditions. Much of the research has focused on individual components in the process of food intake and this ongoing research is imperitive to fully understand the explicit involvement of each component i.e. how knowledge about fluctuations in ghrelin concentrations may be a feeding indicator. However, it is now vital that researchers understand how these components interact to control food intake. Every individual eats episodically therefore the 'end point', which in this case is cessation of eating, is universal. Each person has the capacity to be satiated or has the means to initiate feeding, whether this occurs for different reasons in obese individuals compared to normal, healthy individuals and to what extent is not fully known. The current study aimed to investigate the interactions between components to determine if the 'end point' is reached by similar means in IGT, type 2 diabetics and control participants.

4.9.1   Influences of Taste, Appetite and Habitual Diet

Appetite, in humans, appears to be unique to the species and to the individual person. Phylo-genetically this specificity relates particularly to the evolution of the cerebral cortex system and the complex interplay of the limbic and neocortical brain. Such

specialised nervous and psychological control is not available to humans when they are born (Hamburger 1960) and therefore is learned by associated psychological factors such as emotional conflict and personal gratification. Consequently, the nutritional aspects of appetite are sometimes distorted. Taste can influence appetite because it is clear that some individuals can consume foods even when they do not 'feel' hungry. In this scenario, investigators have shown that often the food eaten is high in fat content which is consistent with the idea that fat tends to be less satiating than protein and CHO respectively (Chapman et al. 1999). There is compelling evidence for innate preferences for sweet tastes and aversions to bitter tastes (Yeomans 2006). In this study the relationship between taste and appetite was investigated by looking at taste status to bitterness and the amount of fat consumed in the habitual diet and then by how taste is correlated with subjective feelings of hunger, satiety, fullness and prospective consumption. Looy and Weingarten (1992) were the first to suggest a link between sweet taste preferences and obesity and investigators have continued on to explore how appetite is influenced by PROP taste status. Results have shown that PROP supertasters and tasters have an aversion to bitter and sweet food items and are more sensitive to the fat content in foods leading to avoidance of these food items. Therefore supertasters and tasters tend to be normal weight whereas non-tasters have a propensity to be overweight as they are not sensitive to fat content and so do not have aversions to sweet/high fat foods. The current study exhibited no correlations between taste and appetite parameters, however does demonstrate that control subjects tasted PROP at a significantly lower concentration when compared to IGT participants. The trend also showed that control participants tasted PROP at lower concentrations than type 2 diabetic

participants, however the result was not significant. All control participants were either tasters or supertasters and there were no control participants in the non-taster status group. Conversely, there were no IGT participants in the supertaster group and only one type 2 diabetic in this group. These results provide further evidence that body weight is indeed related to taste status. Salbe et al (2004) found a positive relationship between weight gain and hedonic responses to sweet and creamy solutions in Pima Indians. Therefore, it was important to determine how taste status affects an individual's habitual diet. In the current study there was no relationship between habitual fat intake and taste status as some literature has suggested (Salbe et al. 2004). However, a significant negative relationship between sugar intake and taste scale was found; in that the stronger the taste for the PROP compound was rated, the lower the dietary sugar intake. Given that the supertasters rated the PROP compound the strongest, this result can be extrapolated to mean that supertasters had the lowest dietary sugar intake whereas non-tasters had the highest sugar intake in their diet. This high sugar intake may in fact perpetuate obesity and the related cardiovascular disease. Yudkin (1964) proposed in the early 1960s that the Western diets that were high in fat were also high in sugar and he proposed that the sugar intake may also play a role in cardiovascular epidemic. In the current study, although no significant differences were recorded in fat intake from the 4-day diet diaries, IGT participants did record the highest fat intakes. Recently, in the UK, although a low fat intake has been promoted, rates of obesity have continued to increase as has sugar intake (FSA 2008). Even in children non-milk extrinsic (NME) sugar intakes increase with age. The main contributors to NME being non-diet soft drinks along with confectionary, cakes, biscuits and pastries (FSA 2008). Earlier, it

was discussed that sugar may actually prevent weight gain however the current study shows conflicting results. This study shows that when taste sensitivity is poor this may cause an increase in the amount of sugar consumed in the diet. This may mean an increase in cakes and biscuits which can result in positive energy balance and obesity. By decreasing sugar intake a resultant reduction in the energy imbalance between intake and expenditure would be expected and overall may make a positive contribution to the prevention of obesity.

4.9.2   Influences of Appetite and Substrate Oxidation

The oxidation of nutrients will lead to either the storage or utilisation of nutrients and will ultimately lead to satiation. The fuel mix is important and will determine the release of biomarkers which affect satiety (de Graaf et al. 2004) so it was essential to investigate the relationship between oxidation and appetite. Appetite was analysed in relation to substrate oxidation in the current study, to assess if the differences in fuel usage are reflected in the appetite parameters. The relationship reflects the metabolic profile of each group. The aim of the current study was primarily to investigate the differences between IGT, type 2 diabetic and control participants. Therefore it was important to investigate the correlations between appetite and substrate oxidation between groups (IGT, type 2 diabetics and controls). Correlations were performed taking away the influence of body weight so that any differences observed were a result of the disease status. This was important because the effects of obesity are different to the effects of IGT and type 2 diabetes. This much is clear because there are obese individuals who never develop these

conditions and therefore it would be expected that the metabolic responses to food are different.

Correlations were performed on AUC calculations taken from fasting until the end of the study period. There were no significant correlations between fat oxidation and any of the appetite parameters in the current study. Some studies suggest that the ability to induce satiety is influenced by the ability of fat to be readily oxidised (Bendixen et al. 2002). In obese subjects basal fat oxidation has been shown to be reduced (Solomon et al. 2008) when compared to healthy control subjects. This reduction is thought to be due to the accumulation of free fatty acids and results in increased intracellular fatty acid metabolites and disrupts insulin-signalling pathways which lead to insulin resistance and IGT. The current study did not show differences in fat oxidation between either of the obese groups (IGT or type 2 diabetics) and the control participants, so although there were differences in the appetite parameters between groups it does not appear to be due to differences in the oxidation of fat. It seems that the ratio of CHO to fat oxidised is more important than the fat oxidation itself.

IGT participants demonstrated that hunger and prospective consumption reduced as CHO oxidation increased, whereas an increase in fullness and satiety increased. Conversely, type 2 diabetic and control participants showed an increase in hunger and prospective consumption as CHO increased and a corresponding reduction in satiety and fullness. Therefore IGT participants in the current study are showing a response similar to those which Astrup et al (1996) recorded in normal control

participants. Because of the high levels of satiety recorded in the current study it would be expected that the duration of satiety, i.e. the period between meals, in the IGT participants would be greater than in type 2 diabetics and control participants. To determine the effects of appetite and oxidation on subsequent meals further investigation would be required. In contrast to CHO oxidation increasing in direct response to CHO intake, fat oxidation is not directly linked with intake (Astrup et al. 1996) and this results in less accurately maintained fat balance. In the current study the relationship between fat oxidation and appetite varied between groups. The IGT participants again, for the most part, showed contrasting results to the type 2 diabetic and control participants. As fat oxidation increased in IGT participants so did hunger and prospective consumption, whereas satiety and fullness reduced. In type 2 diabetics as fat oxidation increased, hunger and prospective consumption reduced and satiety and fullness increased. Finally, in control participants as fat oxidation increased hunger reduced and satiety, fullness and prospective consumption all increased. The results demonstrate the lack of any definitive relationship between appetite and fat oxidation. A possible hypothesis that the increased fat oxidation postmeal observed in the IGT and type 2 diabetic groups, although not significant may be associated with the increased levels of satiety ratings recorded. Although the differences in fat oxidation were not significant there was a notable increase post meal compared with the control group. This hypothesis has been previously suggested back in the late 1990's by Himaya (1997) however it does not appear to have been avidly pursued. The current study may necessitate a re-evaluation of the satiety value of fat. It clearly indicates that research aimed at providing an insight into the physiological and biochemical phenotypic expressions of the obesity genes

and how they interact with dietary fat should be conducted with caution as the results are extremely difficult to interpret.

It is however, important to determine associations between measured processes in the control of feeding behaviour and subjective measures because it gives an idea of the influence that psychology has in food intake and the role that subjective feelings have in the control of food initiation and cessation. Hence, associations were completed with appetite and taste, oxidation rates and biological markers.

4.9.3   Influences of Appetite and Blood Parameters

4.9.3.1 Glucose and Appetite

As expected, in the current study, carbohydrate oxidation was higher in the control participants compared to the other two groups. This was also reflected in the blood glucose profile as a subsequent lower concentration of glucose was found in the control participants. Consequently it was noted that a decrease in blood glucose concentrations result in decreased hunger and satiety levels. This does not concurs with previous research in that the drive to consume food is promoted following a decrease in blood glucose (Mayer 1960; Van Itallie and Hashim 1960) which is thought to reflect a sudden decrease in the supply of immediately available glucose (Melanson et al. 1999). The correlations with hunger, fullness and prospective consumption were not significant and demonstrate conflicting results from the anticipated outcomes. The results of the current study showed that hunger and

prospective consumption increased, whereas satiety and fullness decreased as a result of an overall increased glucose response. Perhaps the meal was not large enough to elicit large changes in the subjective responses even though the physiological changes were there. The glucose response was greater in both the IGT and type 2 diabetic groups however, the correlations between appetite ratings and the blood glucose response were different. The type 2 diabetic participants correlations followed the same pattern as control participants whereas IGT participants demonstrated opposing results, as the blood glucose response increased, there was reduced hunger, satiety and prospective consumption and increased fullness, although these results were not significant. So to summarise, all three groups demonstrated a negative association between satiety and blood glucose although the result was only significant in the control group, that is as the blood glucose response increases, satiety reduces. It is thought that if blood glucose is modulated, there is a subsequent effect on satiety which is the basis for the glucostatic theory of food intake regulation (Mayer 1953). Conversely, satiety and the termination of eating will only occur following an increase in blood glucose. A study by Anderson et al (2002) demonstrated that high GI carbohydrates more effectively promote subjective feelings of satiety and reduce feelings of hunger at 60 minutes postmeal. The current study does not concur with the glucostatic theory that a meal which results in a high glucose response suppresses food intake. In fact, this study demonstrates that as the blood glucose response increases, satiety reduces, concurring with the hypothesis that foods which elicit a large glucose response, i.e. high GI foods, promote excessive energy intake. This hypothesis is based on the hypothesis that meals which produce a quick initial glucose response, i.e. high glycaemic index (GI) foods,

then produce a corresponding postprandial dip in blood glucose, which again initiates feeding (Anderson et al. 2002). The current study did not observe a corresponding correlation between the glucose response and prospective consumption which suggests that there was no corresponding dip in blood glucose in the participants. The reason for this is unclear. The type 2 diabetic and control participants did demonstrate a positive relationship between PC and blood glucose but this was not significant. The IGT participants showed a negative relationship between PC and blood glucose which again was not significant. The current study is consistent with studies which identify a strong satiety response with low GI foods. Therefore the participants in this study would benefit from a low GI meal, thus reducing blood glucose levels, reducing the glycaemic response and lowering food intake. Therefore, the habitual diet of IGT and type 2 diabetics should include foods which will help to control blood glucose levels because consistently high blood glucose levels increase the risk of metabolic syndrome and cardiovascular disease. This is also consistent with the idea that an improved, or lower, glycaemic response may improve secretion of ingestive hormones such as GLP-1 and CCK which help to promote the suppression of food intake. A weak satiety response as a result of a high GI meal may promote the early initiation of feeding and the response is likely to be different to a healthy individual who is not concerned with blood glucose levels. A healthy individual may make a different CHO choice and have a full and sustained satiety response. Granfeldt et al (1994) investigated the response of low GI foods, which had high levels of resistant starch and is digested and released slowly and so helps to control blood glucose levels, for up to 180 minutes after feeding. The results of this study showed a greater overall satiety response with the low glycaemic food.

However, this response was compared with white bread rather than a mixed meal. The comparisons between the different types of resistant starch were comparable to the study by Anderson et al (2002) in that the differences in satiety were only observed in the late phase, denoted from 95 minutes onwards and similarly demonstrated that in the short-term there is no 'satiety' benefit to consuming either a high or low GI diet. Granfeldt et al (1994) concluded that long-term GI foods may in fact promote satiety however, further research would need to be done before it could be concluded that overall daily or weekly energy intake was influenced. It is important to stress that these two studies have been carried out on normal subjects therefore the same conclusions cannot be assumed for subjects with IGT and type 2 diabetes as their responses may differ. Therefore, it is important that future research investigates the appetite and blood responses to different types of meals over both the short and long term. Arguably a long term study is more important to determine if energy balance is achieved and maintained. The answers may also explicitly lead us to conclusions about what food sources can more easily promote negative energy balance and weight loss.

4.9.3.2 Insulin and Appetite

It is well established (Lavin et al. 1998) that there is an increase in insulin secretion following a meal causing a rise in plasma insulin. In the current study the overall insulin response measured by AUC was significantly lower in the IGT participants and intermediary in the type 2 diabetic participants compared to the controls. A study by Lavin et al (1998) on normal subjects where insulin was initially injected to

simulate the conditions of type 2 diabetes investigated appetite levels following intra duodenal glucose infusions. Results showed that the appetite ratings of hunger and fullness were not affected by the simulated condition of hyperinsulinemia and that a 'normal' satiety response was seen following duodenal glucose infusions in that there was a reduction in hunger and fullness and subsequent food intake (Lavin et al. 1998). Lavin et al (1998) concluded that insulin was not a physiologic mediator of satiety. In the current study there were no significant relationships between appetite parameters and the insulin response in IGT or control participants. However, in type 2 diabetics there was a significant inverse relationship between hunger and the insulin response, that is as insulin increases, hunger consequently reduces and a positive relationship between prospective consumption and the insulin response. Thus, as the insulin response increased so did the desire to consume food in type 2 diabetics. Therefore, the current study agreed somewhat conflicted with the results of Lavin et al (1998) because there were correlations between insulin concentrations and appetite parameters. The claim that foods which promote a reduced insulinaemic response, i.e. low GI foods, have a positive effect on fuel oxidation by reducing CHO oxidation and fat storage and by increasing fat oxidation (Astrup et al. 1996). The current study showed that a high insulin response resulted in an increased desire to consume more food in type 2 diabetics therefore the participants would benefit from aiming to achieve a reduced insulinaemic response by choosing appropriate food items to elicit a low glycaemic and insulinaemic response. However, in the current study the inverse correlation between hunger and the insulin response is conflicting and suggests that as the total insulin response increases, hunger reduces. Warranted, for satiety to occur an increase in insulin concentration must occur however, as stated

earlier in the discussion of the glucose results, it has been demonstrated that a low insulinaemic response helps to maintain satiety and prevents further food consumption. Therefore, this result was unexpected and may be put down to 1) the complexities of metabolic disease or 2) the biases observed in the completion of visual analogue scales, discussed earlier. However, throughout the study period there were differences in appetite ratings between control participants and both IGT and type 2 diabetic participants. This suggests that other studies which simulate conditions of diabetes may not give the same results as studies including patients who actually contain the disease. This is because in IGT and type 2 diabetes there are known to be a number of dysfunctional mechanisms that interact and lead to the development of IGT and type 2 diabetes. The lower insulin response identified in the IGT participants therefore may have contributed to the differences observed in appetite parameters i.e. satiety and hunger. The possible mechanism for this is because of the low insulin levels, this resulted in slower glucose utilisation and storage and delayed the meal processing, leading to increase in satiety. The reason for a failure to demonstrate a correlation may be due to the small sample size. The current study has shown that insulin may not have a primary role in the differences established in subjective appetite ratings because of the erratic pattern of correlations observed.

CHAPTER 5 - FINAL DISCUSSION

Impaired glucose tolerance and type 2 diabetes are now common chronic diseases which have resulted in copious amounts of research that aim to investigate both the prevention and treatment of these conditions. The most important element in the prevention of these diseases is the ability to maintain a balance of energy intake with energy expenditure. Research has primarily focused on the side of energy intake due to the innumerable mechanisms which influence food intake including; physiological, sensory, cognitive and behavioural mechanisms. The longer term complications of IGT and type 2 diabetes can lead to morbidity, reducing the quality of life or mortality and this makes the current and ongoing research so very important. Understanding the factors which contribute to the long-term complications of IGT and type 2 diabetes may help to predict or prevent the onset of disease and lead to a reduction in the number of people with IGT and type 2 diabetes. Alternatively, an understanding will allow more effective treatment of the symptoms and reduce the associated morbidity and mortality.

Obesity is a key component in both these diseases and results in changes in the metabolic handling of food. The aberrant responses in the metabolic handling of food affects consecutive food intake and therefore may perpetuate the problem of weight gain. The IGT and type 2 participants in the current study were all classified as clinically obese and were therefore a suitable sample to compare the effects of food intake with a control group of healthy participants. The test meal given was a mixed meal and identical for all participant groups. This was important to enable the

investigation of the responses to food intake in an everyday situation in IGT and type 2 diabetic participants and to determine if the response differs to healthy controls. Previous studies have investigated single components or perhaps two components i.e. the sensory and biological components of food intake. The current study aimed to assess as many of the factors which influence the control of food intake without being overly invasive for the participant. The sensory element investigated was specifically taste, the physiological element investigated included blood hormones and oxidation rates, and the cognitive element investigated were participants' subjective feelings of appetite.

The current study demonstrated the complexities involved in the investigation of food intake. The mechanisms involved in food intake and satiety comprise of a complex interplay of events which result in either the desire to consume food or the inhibition of further food intake. The similarity of food intake in the habitual diets of IGT and type 2 diabetic participants to the control participants was unexpected given the BMI of the two patient groups. However, this may have been better assessed if patients were currently in weight loss. If weight had been recorded in both sessions in the current study this knowledge would have allowed a definitive answer as to whether patients were in weight loss or not. The two main differences between the groups' habitual diet were in percentage of protein and sugar intakes of the participants. Type 2 diabetic participants had the highest protein intakes followed by IGT and then control participants with the lowest protein intakes and type 2 diabetics had a lower sugar intake compared to IGT and control participants. These two results in themselves were significant as they conveyed a message regarding self-

mediated changes made to the participants' dietary intake following diagnosis. For type 2 diabetic participants this may have been associated with the dietary advice gained within the diabetic clinic. Furthermore, the activity recorded by diaries was also very similar in all groups showing that the two patient groups were not leading a sedentary lifestyle as was anticipated. There were differences in the intensity of activity undertaken, as the IGT participants had undertaken approximately a third less the amount of time undertaking strenuous exercise when compared to the control participants, however IGT's executed more moderate activities such as housework and walking. Therefore, including strenuous activity into their lifestyle may be an important factor to consider in IGT and type 2 diabetes as this may help to improve blood glucose profiles and oxidation rates. Although this difference was not significant it may go some way to explain the behavioural disparity between groups as it is commonly known that exercise plays an important role in the maintenance of weight loss, prevention of weight gain and (Anderson and Konz, 2001) promotion of long-term healthy living (Klein et al. 2004).

The taste results demonstrated that the IGT participants first tasted PROP at a higher concentration compared to the control participants. The IGT participants were also less sensitive to the bitterness than PROP compared to type 2 diabetic and control participants. Therefore, IGT participants were likely to be nontasters and were unlikely to be supertasters, whereas control participants were likely to be supertasters in the current study. Tepper and Nurse (1998) suggested that nontasters had a preference for fat. Therefore it was important to test that hypothesis and look at the habitual diet to determine if there were differences in fat intake between IGT, type 2

diabetics and control participants. The current study demonstrated that the sugar intake of IGT patients is influenced by a lack of taste sensitivity and this may lead to an associated increase in fat intake although this was not definitive because there was no significant difference in self-reported dietary fat intakes. The relationship may be more complex or indirect and would be worth further investigation at a more basic level. This is because the effect of taste may manifest itself to a greater degree when making individual food choices such as in-between meals and snacks. Whereas a main meal, such as lunch or dinner, may be considered 'healthier', per say, because it is a planned nutritive meal to appease a whole family. Food preferences in IGT and type 2 diabetes would also merit investigation as it is imperative to understand how the effect of taste changes with these conditions and how this has an influence on habitual food choices.

Appetite ratings showed the changes in satiety in response to the test meal. In the current study the differences in appetite parameters between IGT, type 2 diabetic and control groups were observed particularly in subjective feelings of hunger, satiety and PC. It is unclear why the differences were not observed in feelings of fullness. Significantly different results were observed in control participants compared to IGT and type 2 diabetic participants and this may be linked with the differences distinguished in taste status. Increased taste sensitivity may intimate an increased sensitivity to appetite, as observed in the control participants without a simultaneous increase in food intake. The opposite may be true of IGT participants i.e. lack of taste sensitivity may indicate a lack of appetite sensitivity. Additionally the reduced hunger and PC and increased satiety observed in IGT and type 2 diabetics may be a

consequence of reduced EE compared to control participants. The energy expended was an average of 24kcals/kgBW in control participants compared to 20 kcals/kgBW in IGT and type 2 diabetics therefore this may account for the associated differences seen in the subjective feelings of hunger. The process by which these effects may transpire may be due to afferent signalling which provides information on nutrient intakes and status. Furthermore, the results show that IGT and type 2 diabetics both had lower rates of DIT, RQ and CHO oxidation compared to control participants. These results, although not all significant are important and go some way to facilitating the understanding of the mechanisms which are influencing energy intake. It appears that a reduced DIT and CHO oxidation, which will cause a reduction in RQ (because if more fat is being oxidised then it is sensible to assume that less CHO is being oxidised) somehow inhibits the stimulation of the sympathetic nervous system, which causes reduced EE. This however does not explain the appetite ratings as the opposite results would have been expected, especially given the results of the physiological components but those differences may be related with sensitivity to taste and appetite. The current study may indicate however that subjective markers of appetite do not appear as accurate as the physiological markers. This may be due to IGT and type 2 diabetic participants conforming to what results they think the researcher wants them to feel rather than what they actually feel. Reporting biases are commonly discussed with regards to the underreporting of food intake in the obese, however it has also been noted that there is a lack of data concerning the psychometric properties of VAS to assess weight related biases in this tool (O'Connor et al. 1996).

Low fat oxidation rates have been suggested to be associated with an increased risk of weight gain. This was not demonstrated in the IGT and type 2 diabetics in the current study. In fact when observing oxidation rates as a result of the disease and independent of weight these two patient groups had an increased level of fat oxidation. This concurs with a study by Astrup et al (1994) where 24hour calorimetry demonstrated that overweight and obese subjects had a higher level of oxidative fat energy compared to control subjects. Astrup et al (1994) then concluded that this was due to overweight and obese subjects consuming a diet higher in fat content. However, in the current study the results were two-fold as this may have been true of the IGT participants and would then demonstrate why the oxidative fuel mix differed to control participants as a result of the disease. The same pattern was not observed in type 2 diabetic participants as they had the lowest fat intake, although this difference was not significant. What is clear is that the result of the aberrant changes in fat and CHO oxidation rates affected appetite levels differently in the two patient groups (IGT and type 2 Diabetics). IGT participants demonstrated greater satiety as CHO oxidation increased and less satiety as fat oxidation increased. Type 2 diabetics showed decreased satiety as CHO increased and increased satiety as fat oxidation increased. The reason for the differences observed between groups is not clear because the test meal was the same for all groups. Given that the overall levels of fat and CHO oxidation postmeal were similar, and not significantly different, the explanation for the differences in the correlations between appetite parameters and oxidation levels reflect that their body's were reacting differently to the same meal. The reasons for the differences observed may indicate differences in the ratio of CHO to fat oxidised which would

undoubtedly be controlled by the body fuel stores. Therefore, it appears that the body fuel stores, at the time of food consumption, continue to influence to the greatest degree, the macronutrient which will be oxidised, following a mixed meal. The increased fat and reduced CHO oxidation may play a role in the low RQ and EE observed postmeal in IGT and type 2 diabetics compared to control participants and this may be attributable to the increased glucose response to the meal. The raised blood glucose profile suggests disrupted uptake of glucose via GLUT-4 transport in IGT and type 2 diabetics, associated with insulin resistance and elevated FFA levels. The low RQ's also promote preferential fuel storage over oxidation and this may potentiate further weight gain, in already obese individuals. The current study demonstrates the complexity of mechanisms involved in the satiety cascade and the control of food intake.

Metabolic parameters have been implicated in the control of ingestive behaviour by their availability in the circulating pool. Metabolites associated with the ingestion of food (glucose, insulin, and ghrelin) were investigated and indicated a higher glucose response, measured in IGT and type 2 diabetic participants compared to control participants. The control group had significantly lower fasting insulin levels and significantly higher postmeal insulin levels which resulted in a significantly higher AUC response in control participants compared to IGT participants, who had the lowest insulin response. The glucose response was expected however the postmeal insulin response was surprising. It is difficult to elucidate the reasons for the insulin response observed postmeal in the current study however one conceivable theory may be related to the acute insulin response. Insulin resistance is a core component

in the development of IGT and type 2 diabetes and a compensatory hypersecretion of insulin is the normal response but β-cell dysfunction is also a pre-requisite for the development of IGT and type 2 diabetes (Kahn, 2003). Individuals who progress from normal glucose tolerance to IGT and type 2 diabetes are characterised by a lower first-phase insulin response (up to 30 minutes postmeal (Laaksonen et al. 2005)) and an additional decline with time in the acute insulin response, even though the second-phase response is initially exaggerated. In contrast, nonprogressors generally show little decline in β-cell function (Osei et al. 2001). Improvement in the acute insulin response in the absence of deleterious changes on insulin sensitivity may therefore lower the risk of worsening glucose tolerance and the development of diabetes. Thus, the lower 30 minute insulin levels in IGT and type 2 diabetics may suggest a lower acute phase insulin response due to β-cell dysfunction in these participants. The result of the reduced insulin response may have caused a reduction in insulin-induced glucose transport and an overall lower CHO oxidation response to the meal. Furthermore, the reduced insulin response may be responsible for the increased satiety and reduced hunger observed in the IGT and type 2 diabetic participants. It is important to note that the current study does not give a true reflection of the full second phase insulin (which can last up to 180 minutes postmeal (Chiu et al. 2004)) response. Assays were completed up to 60 minutes only and insulin levels were still increasing at this time. It is possible that in observing the full insulin response, subjective ratings of appetite may have been more in line with what was expected in the IGT and type 2 diabetic groups.

CHAPTER 6 – CONCLUSIONS

Many of the results have shown subtle differences in the metabolic handling of food in IGT and type 2 diabetics. The results suggest that the overall response of IGT and type 2 diabetic populations deviate from the normal and this may be a result of the said diseases. In the current study, it is clear that the dysregulation of the pathways observed, which contribute to the process of food intake (taste, DIT, EE, oxidation and blood profiles), leads the body to relay erroneous afferent messages to the brain which may lead to further metabolic changes which are to the detriment of maintaining energy balance. A definitive answer cannot be made from the current research as to which mechanism(s) of the pathway is (are) most influential as that will require further extensive and detailed studies. However, the influence of taste sensitivity in IGT and type 2 diabetics was one of the most significant findings. Taster status can be used as a tool in the treatment of IGT and type 2 diabetes as this study has shown that it may pre-dispose individuals to an increased sugar, and perhaps fat intake. Having this knowledge makes it easier to tackle dietary issues found in IGT and type 2 diabetic nontasters. Also, the current study highlights that subjective ratings do not appear to be as useful a tool as thought because the differences seen in biological markers of appetite were not conveyed in the subjective ratings. The differences observed in metabolic status between controls and the two patient groups were enough to make inferences about the dysfunctional mechanisms which occur in these diseases. The most prominent aberrations were observed in the IGT participants and were found in the pathways of taste, DIT, CHO oxidation and blood glucose levels. These abnormalities between IGT and type 2

diabetics, although not conclusive to determine how progression of the diseases results in an altered physiological and metabolic response, did give an insight as to how individuals view the severity of their disease. The results also highlight that deviations in metabolic pathways may potentially be reversed, as observed in type 2 diabetics, and with good management of IGT and type 2 diabetes the diseases can definitely be managed. This was an interesting aspect of the study and showed that the severity of the disease did appear to be associated with the level of management (dietary and activity levels) of the disease. Knowing how the body changes with progression of the disease is fundamental when looking at opportunities to reverse this advancement and is a key component in the prevention of IGT and type 2 diabetes. Furthermore, not all components of energy intake are within the human limits of control, such as taste, but understanding how the genetic implication of taste elicits changes in food choices can ameliorate the management of habitual diet which will go a large way to facilitate in the control of energy balance. This may be particularly important in the control of IGT and type 2 diabetes.

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APPENDIX 1

Dear 'patient',

I am writing to you on behalf of Jill Somerville of Queen Margaret University College who is a research nutritionist and is working on her PhD. Jill has to complete a research project for this and is looking to recruit volunteers for a study she is doing looking at food intake in people with higher than normal blood sugar levels who do not have diabetes. This letter explains what it is all about. I would emphasise that there is no commercial interest in the study and it has full ethical approval. The study does not involve you participating in anything painful, unpleasant or dangerous.

Any decision that you might make about whether you take part or not will have no impact on the treatment you receive from the practice in the future.

We would like to see patients between 20 and 70 years of age who are controlling their condition by diet.

Volunteering for the study would involve 2 visits to the Wellcome Trust Clinical Research Facility at the Western General Hospital. The first visit will take no longer than 30 minutes and the second visit will take a morning (3-4 hours). Parking arrangements will be made for you at the Research Facility on the days of your visit. The first visit to the clinic will involve completing the following tests:

- Question/answer session
- Have height, weight, waist & hip measurements taken
- Completing a urine sample
- Completing a diet diary
- Completing a taste test
- Have activity monitored.

Research suggests that by losing weight, or preventing further weight gain, you can slow down the development of your disease. The advantage to you in taking part is that you will benefit from diet advice and will receive feedback on your diet and exercise. The help given will aim to make it easier for you to balance your daily food intake against the energy you use, helping you to maintain a healthy lifestyle and good eating habits.

If you would like to help with this study, or if you have any questions, please contact Jill Sommerville on the telephone number or email address below, or complete the Patient Details Form attached and return it to Jill on the address below and she will contact you:

Jill Sommerville   Tel: 0131 317 3525
Research Nutritionist   Email: jsommerville@qmuc.ac.uk
Queen Margaret University College
Clerwood Terrace
Edinburgh, EH12 8TS

Thank you for taking the time to read and respond to this.

APPENDIX 2

Patient Details Form

Name: Date of Birth:

Address: Gender: **M / F**

                                                         Smoker/Non Smoker

Tel: Email:

Mobile:

_____

When is a good time to contact you?———————————————————————

Medication: _____

_____

_____

Questions: ——————————————————————————————————————

_____

_____

Send to:

Jill P. Sommerville
Queen Margaret University College
Department of Dietetics, Nutrition & Biological Sciences
Corstorphine Campus
Clerwood Terrace
Edinburgh, EH12 8TS
Tel: 0131 317 3525
Email: jsommerville@qmuc.ac.uk

APPENDIX 3

7 March 2005
Version 2

Integrating Components of Energy Intake in Impaired Glucose Tolerant and Non-

Insulin Dependant Type 2 Diabetic Populations

Patient Information/Consent Form

I would like to invite you to take part in a nutritional study that will examine responses to feeding. As you may know, overweight and obesity is known to be a health risk and we are keen to have a better understanding of the role of the main components involved in the feeding process so we may be able to help subjects who find it difficult to lose weight. The study will particularly explore subjects who have normal blood glucose levels and subjects with blood glucose higher than normal, which results in impaired glucose tolerance (IGT) and type 2 diabetes.

The aim of this research project is to compare subjects who have IGT and type 2 diabetes with healthy control, non IGT, subjects to determine if there are any underlying reasons for increases in weight that come about as a result of a difference in the systems operating to control food intake.

The study is designed to investigate:

- The differences in appetite following a meal, which may be predicted by certain hormones (chemicals) in the blood between IGT, type 2 diabetics and control subjects.
- The inherited control of taste and its influence on food intake.
- Information to be used in the treatment and prevention of Impaired Glucose Tolerance, type 2 diabetes and obesity.

What will be your level of participation in this study?

1. We would like to ask you to attend two sessions that are convenient to you. The first session will last approximately 1-2 hours and the second session will last approximately 3-4 hours. (Travel expenses will be reimbursed).

2. We would ask you to undergo a taste test, body weight, height and waist:hip ratio measurements and to complete an Eating Inventory.

3. Between sessions you will be asked to complete a 4 day weighed intake of foods eaten, a 4 day physical activity diary and a 24hour urine sample.

4. We would like to ask you to fast (12 hrs) the night before the second session of study and you will be required to consume a test meal.

5. A needle will be placed in your arm to allow blood to be withdrawn (which may cause bruising), just over 5 level tablespoons of blood (80mls), over a 2 hour period. This will allow the monitoring of responses to the test meal.

6. Your energy expenditure will be determined by measuring how much oxygen you use and how much carbon dioxide you expel at various time points throughout the study.

   - For this procedure we would like to ask you to lie down (without falling asleep) with your arms by your sides and would ask you to be silent throughout the procedure.

   - This will involve the wearing of a light hood, initially for 30 minutes to get a stable reading and then 5 subsequent measures lasting 10 minutes each.

7. We would like to ask you some questions about your level of hunger.

(Please can you let me know if you are allergic to latex (or have any other known allergies)).

The information obtained from this study may be used to aid weight loss or reduce weight gain therefore promoting a positive effect on blood glucose levels.

May I remind you that participation is purely voluntary and you are free to withdraw at any time without giving a reason. If you would like to enter this study you are

required to sign a consent form. Altogether the participation time will be 6 hours, not including the food and activity diaries. If you object to your GP being informed of your participation in this study please let me know.

Should you wish any further details please contact – Jill Sommerville, Queen Margaret University College, Department of Dietetics, Nutrition and Biological Sciences, Corstorphine Campus, Clerwood Terrace, Edinburgh, EH12 8TS, or telephone on 0131 317 3525.

The study's independent adviser is:

Dr Ewan Crawford
Clinical Director North West Edinburgh LHCC
Murrayfield Medical Centre
35 Saughton Crescent
Edinburgh
EH12 5SS

Email: ewan.crawford@lothian.scot.nhs.uk

Tel: 0131 334 5713

Dr. Crawford is a suitably qualified person who can advise you on the study but is not directly involved in the study and therefore will act independently, to give you sound advise, regarding the research. Please do not hesitate to contact him or myself if you have any questions or require clarification about any part of the study.

QMUC indemnity will not cover non-negligent (no fault) harm- a side effect or harm that comes about due to no-one's fault.

Thank you for taking the time to read this.

Nutritional Study/Consent Form

I _____ have had this study fully
explained to me and agree to participate.

Signed:_____ Date: _____

Witnessed by: _____ Date:_____

Reimbursement of Expenses Form

I agree that I have received the travelling expenses from my home, which were incurred whilst coming to take part in the above study.

Please try to provide receipts.

Amount of expenses_____

Signature of subject _____ Date _____

APPENDIX 4

Integrating Components of Energy Intake in Impaired Glucose Tolerant

and Non-Insulin Dependent Type 2 Diabetic Populations

15 February 2005   Version 2

COLLECTING A 24 HOUR URINE SAMPLE

- To collect a 24 hour sample of your urine you should get up in the morning at your usual time, empty your bladder and discard the urine **but take note of the time.**

- Enter the time that you passed urine on the label of the container. After this time until the same time the following morning put **all** the urine you pass into the container.

For example

- If you get up at 8.00am, empty your bladder as soon as you can after getting up. **DO NOT PUT THIS URINE INTO THE CONTAINER** - this is overnight urine and is not part of your test BUT 8.00am would be the **start** and **end** TIME ENTERED ON THE LABEL.

- Collect all the urine passed after 8.00am and put it into the container. Even if the bowels move, try to collect urine separately.

- Get up at the same time the following morning (8.00am) and empty your bladder. **COLLECT THIS URINE AND PUT IT INTO THE CONTAINER.**

That is your twenty four hour collection of urine complete! Please remember to complete the label on the container by entering the start and end date and time and your name.

Please note
If you happen to miss putting urine in the container or a mishap occurs, please can you empty the container and begin the test again. It is better to begin the test again rather than obtain inaccurate results.

APPENDIX 5

PROP taste testing   Name:

15 Solution Test

Solution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1st tasted															
Repeat															

Single Solution Test

```
          ┌─── Strongest Imaginable
     90 ─┤
          │
          │
     80 ─┤
          │
          │
     70 ─┤
          │
          │
     60 ─┤
          │
          │
     50 ─┤─── Very Strong
          │
          │
          │
     40 ─┤
          │
          │
          ├─── Strong
     30 ─┤
          │
          │
          │
     20 ─┤
          ├─── Moderate
          │
          │
     10 ─┤
          │
          ├─── Weak
          ├─── Barely Detectable
      0 ─┘
```

291
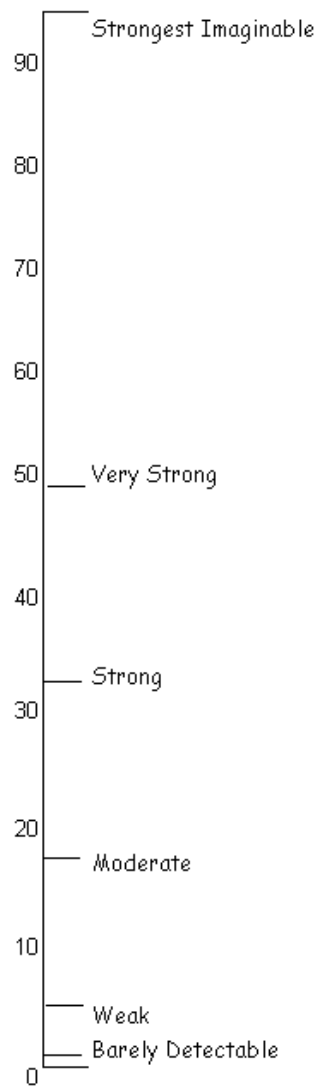
APPENDIX 6

The following verbal instruction were read to aid completion of the suprathreshold scaling test and to establish the perceptual context for the ratings of taste intensity:

In making your judgements of the taste, you should rate the stimuli relative to other tastes of all kinds that you have experienced. Thus, 'strongest imaginable' refers to the most intense sensation of taste that you can ever imagine experiencing. This includes such varied sensations as those produced by a fresh lemon, a piece of celery, or spicy mustard. Note that by 'taste' we do not mean the pain produced by a physical trauma like biting or burning your tongue. Simply rate the samples relative to tastes that you experience in daily life (Green et al. 1996).

4 DAY DIETARY RECORD

Start Date:_____

Finish Date:_____

Diet Sheet Number:_____

Guidelines for Recording Dietary Intake

All the information you need to fill in this record should be in this booklet,
but if you have any difficulties please phone for help.

0131-317-3525 (ask for Jill) or
<u>jsommerville@qmuc.ac.uk</u> **(e-mail)**

Do's

- do **fill in this record each time you eat or drink anything**
- do **take your booklet with you if you will be eating or drinking away from the home**
- do **record everything you eat and drink for the whole day from waking to sleeping**
- do **use as many pages as you like**
- do **record any vitamin / mineral supplements you take**

Dont's

- don't **write from memory at the end of the day - you may forget some foods that you have eaten**
- don't **forget to record any between-meal snacks like sweets, chocolate, crisps, nuts, ice-cream, fruit, cups of coffee etc...**
- don't **include leftovers, write only what you actually eat**
- don't **forget to write the date and day on every page**

How to describe the type and amount of foods eaten

Amounts

You can use general kitchen utensils to record your foods e.g.
- teaspoon (tsp.) - quarter, half, one, two etc.. heaped or level
- dessert spoon (dsp.) - quarter, half, one, two etc.. heaped or level
- tablespoon (tblsp.) - quarter, half, one, two etc.. heaped or level
- ladel - quarter, half, one, two etc.. heaped or level
- teacup
- mug
- wine glass
- tumbler (half pint/pint)
- soup bowl - small/medium/large
- dessert bowl – small/medium/large
- cereal bowl - small/medium/large
- dinner plate - small/medium/large
- side plate – small/medium/large

Detail

Try to give as much detail as possible e.g.:
- **Bread - was it white, brown or wholemeal? Was it thick, medium or**
  thin sliced?, large or small loaf?, pre-sliced or did you cut it yourself?
- **Milk - was it full fat, semi-skimmed or skimmed?**
- **Meat - what type of meat?, what cut of meat?, was it fatty or lean?,**
  roasted, fried or grilled?

Brand Names

Write down the brand names of makers of foods where possible e.g.:
- **Robinson's unsweetened orange juice**
- **Heinz baked beans in tomato sauce**
- **Safeway Cheese & Onion Crisps**
- **Flora Sunflower Margarine**

Cooking Methods

Give details of the cooking methods used e.g.:
- **Potatoes - were they boiled, mashed or baked or chipped?**
- **Fish - was it grilled, poached, steamed or fried?**

Commercial Foods

**These include soups, meat and fish products, ready prepared meals,
puddings, sweets etc.. Please write down:**
- **The volume or weight written on the product or**
- **the number of pieces/spoonfuls/slices/items**

Meat, Fish and Poultry

When describing meat or fish write down:
- **the type of animal or fish**
- **the cut of the meat and whether lean or fatty**
- **the number of slices/portions/pieces/rashers**
- **the part of the animal e.g. leg/drumstick/breast**
- **the cooking method**

Fruit & Vegetables

When describing fruit and vegetables write down:
- **Whether raw or cooked**
- **Whether peeled or eaten with the skin on**
- **Whether the vegetables were old, new, frozen or canned**
- **The number of spoonfuls/pieces/segments of whole fruits eaten**

Pasta & Rice

Pasta includes spaghetti, lasagne, macaroni, noodles etc..
Rice may be sweet or savoury, long or short grain, brown or white etc.
Write down:
- **The type of pasta or rice**
- **The number of spoonfuls/bowls (small, medium or large portion) when cooked**

Soups & Stews

Write down the number of ladels/spoonfuls/cups

Sauces

Please don't forget any sauces used e.g.:

Tomato ketchup	**Brown sauce**
Cheese or White sauces	**Salad Dressing**
Mustard	**Gravy**
Salt, Pepper,	**Spices, Herbs**

Breads, Rolls & Crackers

Write down:
- **The type of bread and the thickness of the slice**
- **The number of slices/rolls/crackers**
- **Is anything spread on the bread/roll/cracker? Is it thickly or thinly spread?**

Breakfast Cereals

These include Cornflakes or other 'box' cereals, porridge, muesli etc.

Write down:
- **The type or brand name of the cereal**
- **The number of spoonfuls/bowls (small, medium or large portion)**
- **Don't forget to note the type and amount of any milk used**

Milk & Milk Products

These include milk, cream, yoghurts or sauces.

Write down:
- **Milk or cream put into tea and coffee etc. (e.g. small dash or milky coffee)**
- **The amount drunk in spoonfuls, glasses, cups or fractions of a pint**

Cheeses

Write down:
- **The type of cheese (e.g. cheddar, edam, brie etc.)**
- **The size of a piece, e.g. compared to a matchbox**
- **The strength of the cheese (e.g. mature, medium, mild)**

Eggs

Write down the size, number and cooking method

Fats, Spreads, Jams, Sauces, Pickles

Write down the number of teaspoons or portion packs used

Confectionery, Savoury Snacks, Biscuits, Cakes

Write down:
- **weights from the packet**
- **small/medium/thick slice of cake or fraction of whole cake**
- **number of biscuits/sweets**

Sugar

Write down the type of sugar and the number of teaspoons, level or heaped.

Drinks

These include soft drinks e.g. tea, coffee, squash, juice, cola, etc.. as well
as alcoholic drinks (see below). Write down:
- **The type of drink**
- **The number of cups, glasses, pints, cans, bottles etc..**
- **Diet or non diet, sugarfree**

Alcohol

Write down:
- **The type of alcohol, number of measures and any mixers added**
  e.g. dash of diet coke

Made-up Dishes

There is space at the end of this booklet for you to write the recipes
of homemade dishes. Also, please write down or cut out and keep
the Nutritional Analysis **from the labels of ready prepared foods.**

Recipes and Labels

Please write below any recipes you have used while filling in this record.
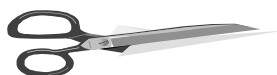
**Please cut out the Nutritional Information from the labels
of any commercial made-up foods you have eaten, not forgetting
the name
and brand of the item.**

**Please attach recipes and labels to this diary and hand in with
the
completed record.**

Thank you for all your help and co-operation.

Please could you return this record to myself at:

**JILL SOMMERVILLE
Research Nutritionist
Queen Margaret University College
Clerwood Terrace
Edinburgh
EH12 8TS**

APPENDIX 8

Queen Margaret University College

Energy Expenditure Assessment

4-Day Activity Diary

Name …………………………………………………

Start Date …………………………………………

Finish Date …………………………………………..

Hour/Min — minute columns: 60 58 56 54 52 50 48 46 44 42 40 38 36 34 32 30 28 26 24 22 20 18 16 14 12 10 8 6 4 2

Hour rows: 05:00 06:00 07:00 08:00 09:00 10:00 11:00 12:00 13:00 14:00 15:00 16:00 17:00 18:00 19:00 20:00 21:00 22:00 23:00 24:00:00 01:00 02:00 03:00 04:00

Guidelines for Recording Physical Activity

Activity Codes

SL	Sleeping	W	Walking
L	Lying, awake	HW	House work
S	Sitting still	DR	Driving
SA	Sitting activities	J	Jogging
ST	Standing	AE	Aerobics
STA	Standing activities	S	Squash
MW	Manual work	G	Gardening
PN	Personal needs (i.e. washing, getting dressed, etc)		

If you are frequently involved in some activity which is not coded for, please make your own code (i.e. Martial Arts = MA).

Guidelines for Recording Physical Activity

All the information you need to fill in this record should be in this booklet, but if you have any difficulties please phone (e-mail) for help on:

0131-317-3525 (ask for Jill) or
jsommerville@qmuc.ac.uk **(e-mail)**

1. Using the codes on the previous page, record your activities in the daily sheets provided.

2. Your activities will start from when you awake in the morning and will finish when you go to bed at night. It is important therefore to carry the activity record sheets with you at all times.

3. Record your activity each time you <u>change</u> activity, to the nearest minute using the codes on page 2.

For example- (see overleaf):
You get up to wash and dress at 8.02am, walk to work at 8.43am,

start work at 9.00am (e.g. standing in a shop), sit for lunch at

1.00pm, and re-start work at 13.30pm, walk home from work at

17.15pm and sit down to watch television at 17.35pm.

In an hour you get up to make a meal. When your meal is ready and you sit down to eat at 19.10 etc.

4. Please record your activities in as much detail as possible.

5. Please use centre pages of booklet for any notes

Name:			Date:	Day:

Hour/Min	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	30	42	44	46	48	50	52	54	56	58	60
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APPENDIX 9

Subject Number:

VISUAL ANALOGUE SCALE

How hungry are you?

| I DO NOT FEEL HUNGRY AT ALL |————————————————| I FEEL EXTREMELY HUNGRY |

How satisfied are you?

| I DO NOT FEEL SATISFIED AT ALL |————————————————| I FEEL EXTREMELY SATISFIED |

How full do you feel?

| I DO NOT FEEL FULL AT ALL |————————————————| I FEEL EXTREMELY FULL |

If you could eat now, how much could you eat?

| NOTHING AT ALL |————————————————| A VERY LARGE AMOUNT |