

Energy dysfunction in humans:
The obesity syndrome

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ABSTRACT

Hyperphagia, rather than reduced energy expenditure, is generally accepted to be the main cause of obesity. As such, the disruption of the appetite-satiety mechanism has been blamed for the relative excess in energy intake that is characteristic of the obese. Factors affecting appetite regulation, and the causes of this dysregulation in the obese remain to be established. Putative mechanisms regulating the physiology of appetite include concentrations of various hormones and macronutrient content. These, in turn, may be influenced by meal frequency, and different hormonal and appetite responses to these changes in meal frequency may be evident in the lean and obese.

In an attempt to elucidate the link between the observed incidence of obesity and elevated serum insulin concentration, I examined in a controlled setting, the effects of altered feeding frequencies, varied energy intake and changes in macronutrient composition on appetite control and insulin concentration in lean and obese males. Furthermore the correlation between serum insulin concentrations immediately before a meal and energy intake was also investigated, as was the incidence of fasting hyperinsulinaemia with concomitant euglycaemia in non-diabetic Caucasian South African adults and its relationship to BMI (Body Mass Index).

A strong correlation between fasting insulin and BMI categories was evident in both the men ($n = 34$; $r = 0.645$; $P < 0.05$) and women ($n = 45$; $r = 0.751$; $P < 0.05$) examined. There was a greater incidence of fasting

hyperinsulinaemia evident in the obese, and based on the premise that insulin plays an integral role in energy metabolism, I proposed the hypothesis that insulin action may be more involved in the control of appetite and energy intake than a mere facilitative role in energy transportation at the level of the periphery.

The effect of meal frequency on appetite control was assessed in lean and obese men. Study participants reported to the laboratory in the morning in a fasted state where they were subjected to an eating test based on the *pre-load – test meal* paradigm, using a double-blind protocol. These study participants were given three different pre-load treatments varying in energy content and macronutrient content. The three pre-loads were a) 33.3% of their average daily energy requirement, (ADER) with the proportions of the various macronutrients following a prudent diet (60% carbohydrate; 25% fat; 15% protein); b) energetic restriction of low-fat pre-load meals (20% ADER: 60% carbohydrate; 13% fat; 27% protein); and c) a high-fat, energy-dense meal that contained 55% of the ADER (35% carbohydrate; 43% fat; 22% protein). Pre-loads were administered either as a SINGLE meal, or as five smaller MULTI meals with hourly temporal spaces. 5½ hours after the first meal, an *ad libitum* test-lunch was given to determine how much energy was consumed. In the pre-load period, whole blood samples were collected hourly for the analysis of serum insulin and plasma glucose concentrations, and subjective hunger ratings (through the use of visual analogue scales (VAS)) were assessed simultaneously. These variables were also measured at 30-minute intervals for 75 minutes after the *ad libitum* meal.

This study protocol allowed me to answer three specific questions namely:

1) Does meal frequency influence appetite? 2) Do lean and obese people show differential recognition of energetically altered pre-loads? and 3) Was there a correlation between insulin concentration immediately before a meal and energy consumption at the consequent meal?

Regardless of the energetic and macronutrient content of the pre-load, when the pre-load was supplied as five smaller meals fed at hourly intervals (rather than as a SINGLE pre-load), the study participants exhibited smaller fluctuations in insulin and perceived hunger, and significantly greater appetite control at the outcome meal. Food intake in the outcome meal declined by 17-27% with increased pre-load meal frequency.

When fed a prudent pre-load, the effect of meal frequency on appetite control was assessed in eight lean men ($BMI = 23.1 \pm 2.8 \text{ kg}\cdot\text{m}^{-2}$) and seven obese men ($BMI = 40.2 \pm 10.9 \text{ kg}\cdot\text{m}^{-2}$). Under these testing conditions, both groups consumed ~27% less at the *ad libitum* meal when the pre-load was consumed more frequently. When different lean ($n = 6$, $BMI = 22.6 \pm 1.1 \text{ kg}\cdot\text{m}^{-2}$) and obese groups ($n=6$, $BMI = 39.1 \pm 11.6 \text{ kg}\cdot\text{m}^{-2}$) of males were exposed to the energy overloaded, high-fat pre-load, the increased feeding frequency reduced energy consumption by 24% and 29%, in the obese and lean groups respectively.

When these same study participants (used in the overloaded trial above) were exposed to the energy-restricted low-fat pre-loads, the obese group consumed 17% less ($t = 3.651$; $P < 0.05$) energy at the *ad libitum* test meal ($4,736 \pm 1,308 \text{ kJ}$), after more frequent smaller meals compared to that eaten after the SINGLE pre-load ($5,709 \pm 923 \text{ kJ}$). This reduction in

energy intake occurred with less fluctuation in subjective hunger ratings [$F_{6,120} = 3.7$, $P < 0.01$]. On the SINGLE treatment, the obese group consumed 24% more [$F_{1,20} = 14.39$; $P < 0.01$] energy at the test meal compared to that which the lean group consumed. Peak insulin concentrations were significantly higher [$F_{6,120} = 10.5$, $P < 0.01$] on the SINGLE treatment ($105.3 \pm 25.7 \mu\text{U}\cdot\text{ml}^{-1}$) than on the MULTI treatment ($53.5 \pm 27.6 \mu\text{U}\cdot\text{ml}^{-1}$). Furthermore, serum insulin levels remained elevated for longer on the MULTI meal treatment, resulting in no difference in the area under the insulin curves between the two feeding treatments. These findings suggest that an enhanced appetite control (with increased feeding frequency) occurred to a greater degree in the obese group compared to that observed in the lean group. Despite this difference, the obese group still consumed 16.4% more than the lean group at the test meal (following the MULTI treatment), although this was in keeping with mass specific energy requirements. This enhanced control over appetite in an obese population, with increased feeding frequency on a low-fat diet, may have positive implications for those individuals who wish to lose weight through low-fat, energetically-restricted techniques.

In an attempt to determine a possible explanation for the relative excess in energy intake in the obese, I tested the null hypothesis that varied energetic and fat intake at a SINGLE pre-load (served either as a high-fat (Overfeeding) meal or as a low-fat (Underfeeding) meal) had no subsequent effects on blood glucose, serum insulin, appetite ratings, and subsequent energy intake in (the same group of) lean and obese males. When the pre-load was served as a high-fat, energy-dense meal that

contained 55% of the ADER, only the lean group exhibited physiological recognition of the excessive pre-load, while the obese group did not compensate for the energy overload and consequently consumed 56.4% more [$F_{1,20} = 11.45$; $P < 0.01$] energy at the *ad libitum* test meal than their lean counterparts did. In contrast, such eating behaviour was absent when the pre-load meal was a low-fat, energetically-restrictive meal (20% ADER). This finding supports the notion of *passive overconsumption* with high-fat foods, with the concomitant over-riding of the intrinsic energy-regulating system, in the obese group. Insulin concentrations were greater [$F_{1,20} = 43.4$; $P < 0.01$] following a high-fat meal when compared to the low-fat meal in both lean and obese males. Accordingly, since it has been shown that obese individuals prefer energetically-loaded foods, *passive overconsumption* with energy dense foods may substantially contribute to the aetiological dysfunction of appetite leading toward the obese state.

Correlating serum insulin concentration and energy consumption in the prudent pre-load trial revealed a positive correlation ($r = 0.87$, $P < 0.05$) in the obese group on the SINGLE treatment while no such relationship was evident on the MULTI treatment. No relationship was evident in the lean group under these conditions. Furthermore, no significant relationships between serum insulin levels and energy intake were observed in either group on the high-fat trial. However, responses to the low-fat pre-load treatment were markedly different between the groups: there was a strong negative correlation between insulin and subsequent energy intake observed in the lean group on both the SINGLE ($r = -0.68$, $P < 0.05$) and the MULTI ($r = -0.73$, $P < 0.05$) treatments, while no relationship was

observed in the obese group on either treatment. These data show that when fed a low-fat pre-load, irrespective of the pattern in which the pre-load was consumed, the lean males consumed less energy with higher serum insulin concentrations. Moreover, this phenomenon was not noted in a high-fat setting, suggesting a potential disruption to the regulatory system with high-fat foods. These findings give further credence to my hypothesis that insulin dysfunction may disrupt the appetite regulating system, which in turn may exacerbate the problems of hyperphagia and passive overconsumption that is characteristic of the obese.

These findings implicate insulin in the appetite-satiety complex, and in the regulation of energy intake in humans. Under these testing conditions, increasing the frequency with which food was consumed, and thereby attenuating insulin fluctuations, enhanced appetite control and ultimately reduced short-term energy consumption. When confronted with an energy dense high-fat meal, obese males were prone to *passive overconsumption*. However, these data have shown that this phenomenon can be controlled, to a certain degree, by increasing the frequency of eating episodes. Accordingly, the therapeutic treatment of obesity may be more successful through the combination of low-fat diets and more frequent meals, which may improve appetite regulation in the obese.

To
Toni

DECLARATION

I declare that this dissertation is my own work, except where others have helped as quoted in the acknowledgements and the reference list. This dissertation is being submitted for the degree of Doctor of Philosophy in the Faculty of Medicine at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in this, or any other university.

Furthermore, I certify that the studies contained in this dissertation have the approval of the Committee for Research on Human Subjects at the University of the Witwatersrand (Protocol numbers: M 960425; M 980624).

Signed in Johannesburg on this the second day of August 2000.



David P. Speechly

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DEFINITIONS

BMI	Body mass index Calculated by dividing weight (kg) by height (m). Units given as $\text{kg}\cdot\text{m}^{-2}$
VMH	Ventromedial hypothalamus
DREQ	Dutch Restrained Eating Questionnaire
VAS	Visual Analogue Scales
ADER	Average daily energy requirement Units in $\text{kJ}\cdot\text{day}^{-1}$
LF	Low-fat: normally reflecting the macronutrient value of a dietary plan.
HF	High-fat: normally reflecting the macronutrient value of a dietary plan.
HI	Hyperinsulinaemia
IR	Insulin Resistance
VLDL	Very low density lipoprotein
DIT	Dietary Induced Thermogenesis
GIP	glucose-dependent insulinotropic polypeptide
PHDC	Pyruvate dehydrogenase complex
LDL	Lipase dehydrogenase
RIA	Radioimmuno assay

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5. Speechly DP, Buffenstein R. The association of hyperinsulinaemia in white South African adults. Submitted to the *International Journal of Obesity* (United Kingdom), February, 2000.
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Samuel Johnson: "... a man who was grown very fat, so as to be incommoded by his corpulency ..."

Boswell (Biographer): "He eats too much, Sir"

Samuel Johnson: "I don't know, Sir; you will see one man fat, who eats moderately, and another lean, who eats a great deal"

Boswell (Biographer): "Nay, Sir, whatever may be the quantity that a man eats, it is plain that if he is too fat, he has eaten more than he should have done"

Chapter One

**Energy dysfunction in humans leading to obesity:
a review of the literature.**

1.1 INTRODUCTION

Obesity is one of the most important avoidable risk factors for a number of life-threatening diseases: the statistics linking chronic pathologies such as heart disease (Wannamethee *et al.*, 1998; Himeno *et al.*, 1999; Mikhail *et al.*, 1999; Mark *et al.*, 1999), diabetes (Bjorntorp, 1984; Caro, 1991; Ferrannini *et al.*, 1991; Skelton & Skelton, 1992; Snehathatha *et al.*, 1999), and cancers bear testimony to this (Garrow, 1992). In the United States, which has the highest prevalence of obesity compared to all other countries (Kuczmarski, 1992; Popkin & Doak, 1998; Flegal *et al.*, 1998; de Wit, 2000), it has been estimated to contribute to between 8-10% of all illness costs (around \$240 billion per year) (Colditz, 1992; Allison *et al.*, 1999; Melcher & Bostwick, 1999; Pharma Marketletter, 1999). Not surprisingly, in the affluent world characterised by sustained economic growth, more people die of too much food than of too little (James & Ralph, 1999).

The incidence of obesity is increasing in all developed nations of the world (Flegal *et al.*, 1998; Popkin & Doak, 1998; James & Ralph, 1999; Investors Chronicle, 2000). When the incidence of obesity in the United Kingdom from 1980 to 1991 was considered (Prentice & Jebb, 1995), it was found that the rising prevalence (of obesity) occurred in both men and women (FIGURE 1.1, *overleaf*).

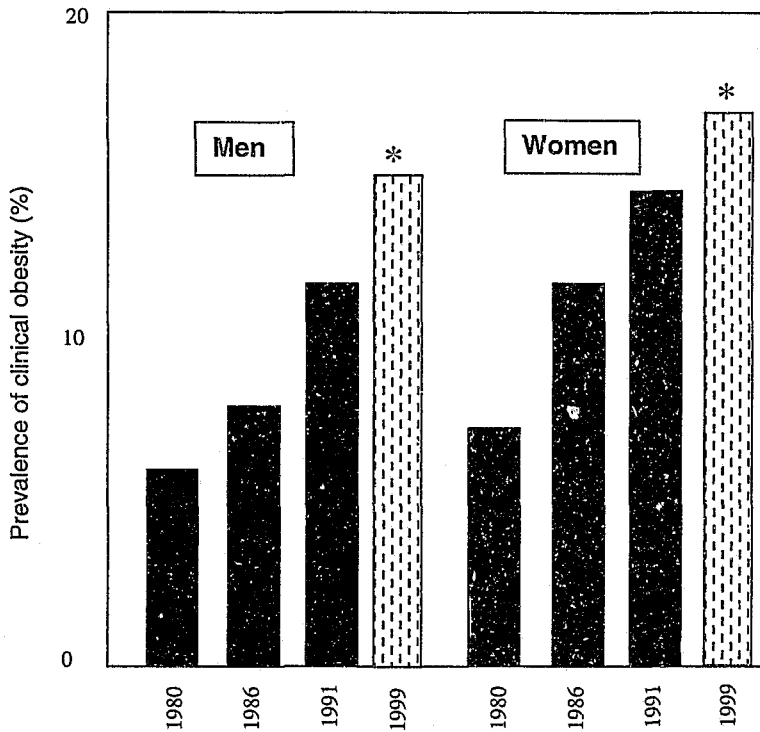


FIGURE 1-1. Increasing prevalence of clinical obesity in England.

(Data from the Office of Population Census and Surveys, 1991)
* Jebb (1999)

Despite countless efforts from the nutritional, physiological, psychological, and medical fraternities, the fundamental understanding of obesity remains unclear. Huge strides have been made toward uncovering those factors which contribute to the induction and maintenance of the condition: these include the genetic predisposition to obesity, socio-economic status, food selection and palatability of foodstuffs, and learned behaviours. Jebb (1999) has argued, however, that a number of decades of intensive research into obesity has resulted in relatively little evidence of genetic or metabolic defects that can explain the majority of cases of human obesity.

Rather, she proposed that factors which are underpinning the current epidemic of obesity, may lie in the behavioural and/or environmental fields, and be primarily involved in the regulation of energy intake (Jebb, 1999).

Obesity is a syndrome of energy imbalance that results in the storage of excess fuel as fat. With particular reference to the intake of food, a number of hypotheses have been proposed to explain the mushrooming prevalence of obesity within the parameters of appetite dysfunction. The 'carbohydrate' hypothesis states that obesity occurs through increased rates of *de novo* lipogenesis as a result of consuming excessive carbohydrate (Dunlop & Court, 1978; Hellerstein, 1999). Conversely, the 'fat' hypothesis is based on the positive correlation that exists between the amount of fat intake and the degree of adiposity (Hill & Prentice, 1995; Prentice & Jebb, 1995; Hirsch *et al.*, 1998; Prentice 1998), and that fat has a greater energy density (than carbohydrate) enabling a more efficient fat deposition. The Booth hypothesis postulates that the modern trend toward snacking between meals may be the chief culprit for the rise in obesity (Booth, 1988). This has recently been supported by epidemiological findings (de Wit, 2000).

1.1.1 Defining Normal Weight and Obesity

Any discussion pertaining to obesity requires a thorough definition of the condition and the terms associated with it. An investigation into obesity draws, by inference, on the principle issue that it is an abnormal state, but with reference to what normal state of body weight? The concept of an 'ideal body weight' was first proposed by the Metropolitan Life Insurance

Company Tables in 1959, and took no account of an individual's somatotype at all (Walker, 1998). In assessing the parameters of normality regarding body weight, the mitigating factors of potential changes in muscle mass and adipose tissue should be considered. Accordingly, given the various somatotypes, normal body weight does not have much significance without further qualification.

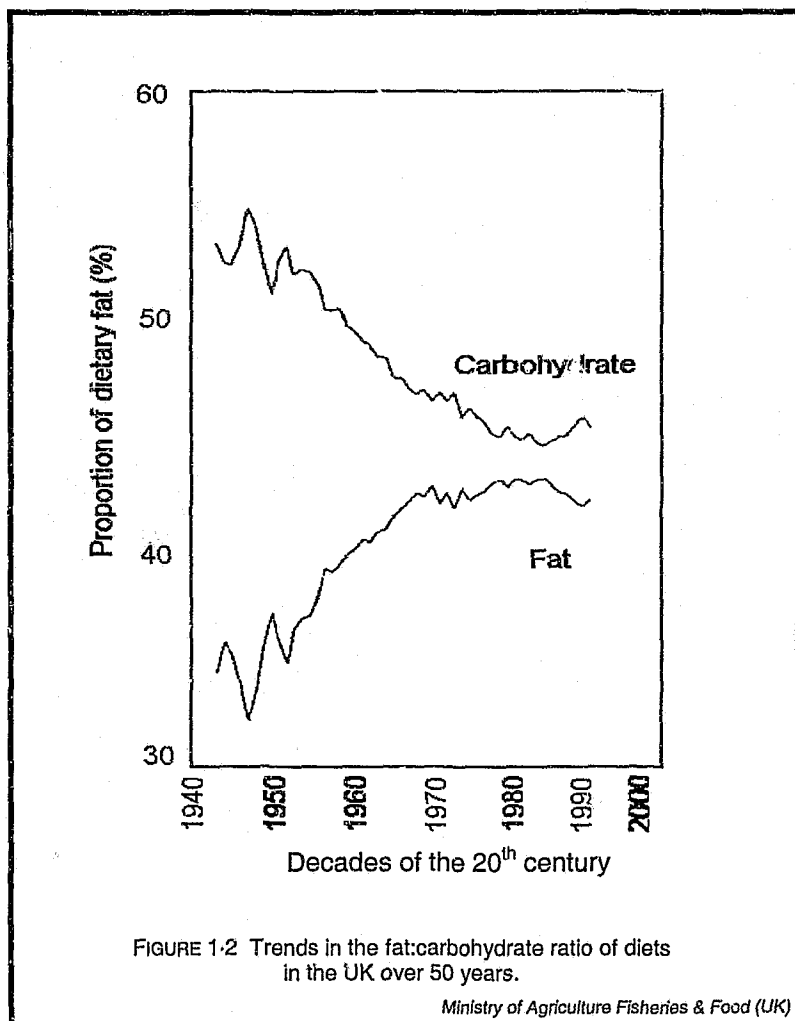
However, with the inaccuracies involved in measuring techniques for body fat and the profound differences between the genders in adipose distribution (Durnin & Womersley, 1974), the *Quetelet Index*, or Body Mass Index (BMI) became the most commonly referred to measure of a given weight relative to an individual's size.

The BMI is obtained by using the following equation:

$$\text{BMI (kg}\cdot\text{m}^{-2}\text{)} = \frac{\text{Weight (kg)}}{\text{Height}^2 \text{ (m)}}$$

Although no strict categories have been qualified for normal weight, it has been shown that people with BMI's between 21.9 – 22.2 kg·m⁻² were least likely to be affected by maladies and morbidity (Tokunaga *et al.*, 1991; Garrison & Kannel, 1993). Values of BMI > 30 kg·m⁻² are generally defined as the cut-off for obesity (Kissileff *et al.*, 1984, Bray, 1987; Caro, 1991; Bouchard, 1996; Campbell & Gerich, 1990; Cox *et al.*, 1991; Kuczmarski, 1992; McAnarney & Stevens-Simon, 1993; Albu *et al.*, 1997; Prentice 1998; Maillard *et al.*, 1999). A report issued by a Committee of the American Institute of Nutrition maintained that after reaching early adulthood, the weight gain in subsequent years should not exceed 4.5kg,

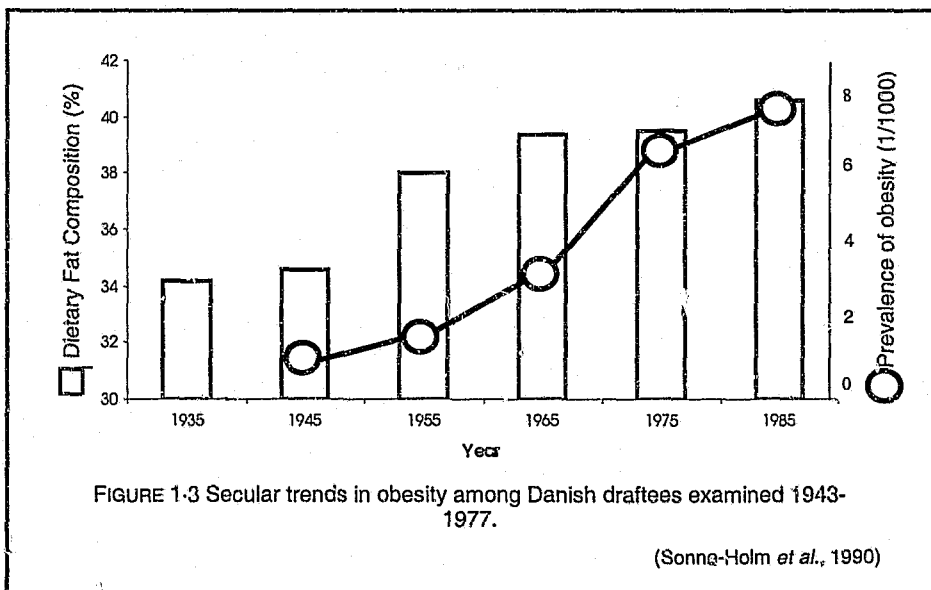
or at most 6.7kg (Committee Report, 1994). It was also considered that waistline measurement, a practical measure of fat gain, should not increase in a lifetime by more than 5 - 7.5cm. The Committee preferred the formulation of a table using a single BMI criterion. Their gradations of risk to health were given as: 18-23kg·m⁻², lowest risk; 24 - 25kg·m⁻² mild risk; 26 - 29kg·m⁻² medium risk; and ≥ 30kg·m⁻² greatest risk (Committee Report, 1994).



1.1.ii The putative causes of obesity

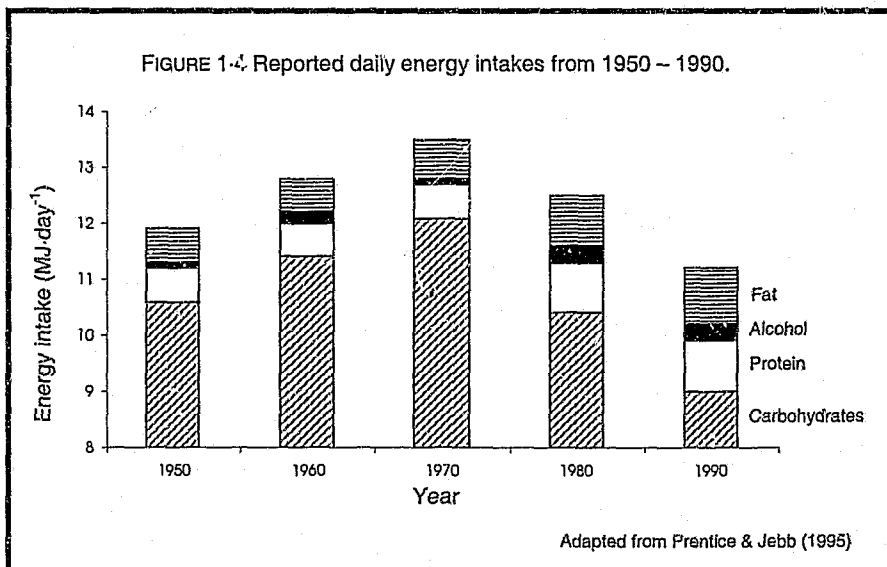
The role of dietary fat in the induction of obesity has received a great deal of attention in the past few years with the progression of trends in obesity: attention has also been drawn to the dietary fat:carbohydrate ratio as a predictor of obesity. FIGURE 1-2 depicts the changes in the composition of dietary fat and carbohydrate in the UK. The very high-fat intakes peaked in the 1970's and have since been maintained at these values.

Analyses of these changes show that the rising prevalence of obesity in the industrialised countries has not only been accompanied by an increasing proportion of energy derived from fat, but also by the associated decreasing proportion derived from carbohydrate (Danforth, 1985). Although our ancestors lived on a diet that was fairly high in fat (Garn, 1997; Harrison, 1997), diets in excess of 10% to 15% of total energy only became routine after the domestication of animals.

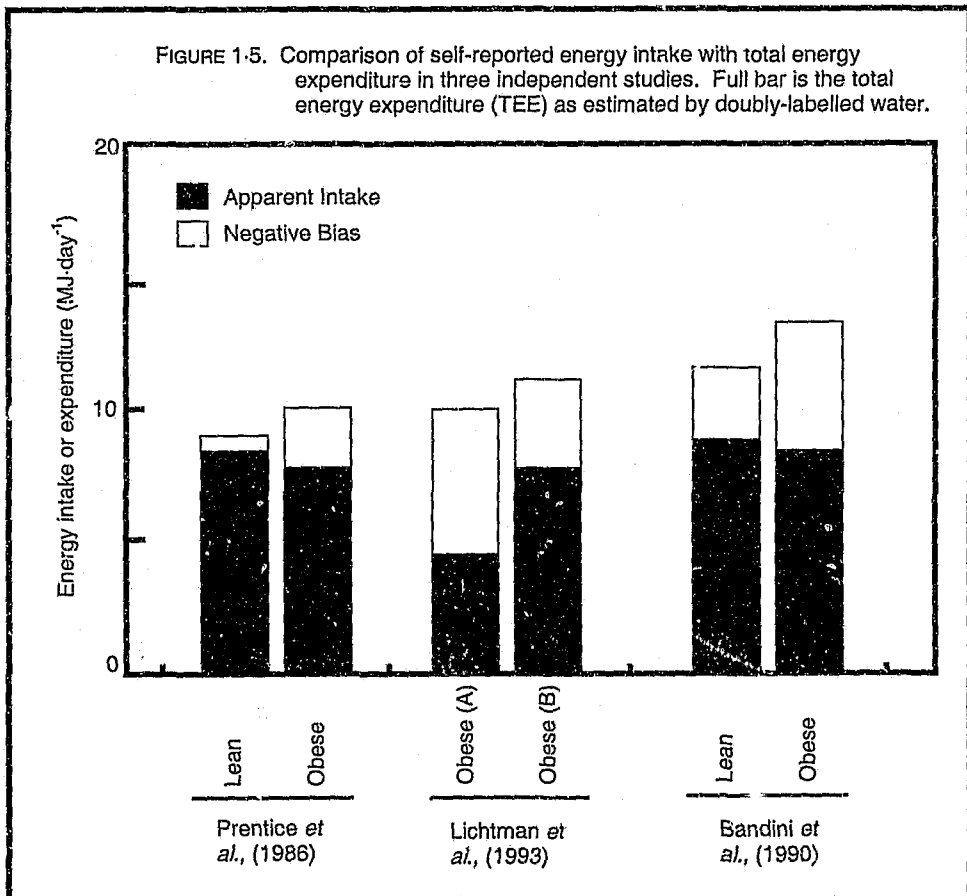


These unprecedented increases in fat intake are closely correlated with increases in rates of, and the prevalence of obesity in the western world. This is made even clearer by FIGURE 1-3, where the prevalence of obesity is plotted on the same graph as dietary fat consumption. As the fat component of the diet increased from 1945 at approximately 32% to over 40% of dietary intake in 1985, there is a close correlation as the prevalence of obesity increased from under 1 per 1000 in 1945 to reach 8-9 per 1000 (Sonne-Holm *et al.*, 1990).

Another interesting epidemiological phenomenon as reported by Prentice & Jebb (1995) is that according to feedback from surveys, people have reported consuming less energy over each decade since 1970 (FIGURE 1-4, *below*). These data, coupled with the reports of rising rates of obesity prevalence, present an obesity paradox where the apparent energy consumption is declining with a concomitant increasing prevalence of obesity.



A number of studies have investigated this paradox, and have proposed that the most plausible answer to this paradox lies in *dietary under-reporting* (Prentice & Jebb 1995; Prentice *et al.*, 1996; Bellisle *et al.*, 1997). As highlighted in FIGURE 1-5 below. Lichtman *et al.*, (1993) have found an this phenomenon to exist in obese individuals who reported only 50% of their energy intake, whilst Bandini *et al.*, (1990) have found the phenomenon to exist in both lean and obese, although the obese under-report their intake far more than lean individuals. Issues that arise from these works highlight the need for caution when considering obesity-related research, and equally important, is the need for tight control when conducting research with obese subjects who are prone to under-report energy intake.



1.1.iii Genetic factors associated with obesity

Understanding of the genetic influences on obesity has increased at a tremendous rate in recent years. By some estimates, 40 to 70 percent of the variation in obesity-related phenotypes in humans is heritable (Commuzzie & Allison, 1998; Winick & Friedman, 1999). Although several single-gene mutations have been shown to cause obesity in animal models (*fa/fa* (Zucker) rat (Sclafani, 1984)), the situation in humans appears to be considerably more complex. The most common forms of human obesity arise from the interactions of multiple genes (Winick & Friedman, 1999), environmental factors (Jéquier & Tappy, 1999) and behavior (VanItallie & Kissileff, 1985), and this complex aetiology makes the search for obesity genes especially challenging. Adoption studies in humans have reported that BMI of adopted children correlate well with BMI indices of their biological parents (Stunkard *et al.*, 1986; Price *et al.*, 1987), and these findings are further reinforced by the observation that monozygotic twin pairs resemble each other (by way of their topographic body fat characteristics more closely than do dizygotic twin pairs (Bouchard, 1985). A recent *meta-analysis* of data proposed that since genetic studies of complex traits are accelerated by the use of candidate genes, a multiplex of 12 previously cloned genes should be placed on the human physical map, which will facilitate the genetic studies of human obesity (Winick & Friedman, 1999). Additionally, the chromosomal location of three (of the twelve) of the genes, ART, NYP Y6R, and PPAR γ , have been reported for the first time, which will identify the genetic factors responsible for human obesity (Winick & Friedman, 1999). This work will expand our understanding even further on the genetic contribution toward obesity.

1.1.iv The "Set – Point" Theory related to obesity

The "*set-point*" theory is based on an intrinsic regulatory mechanism that maintains the relative constancy of body weight in adult humans and other animals (Vander *et al.*, 1997). This constancy is characterised by a spontaneous reversion to the '*normally maintained*' weight level whose weight has either been previously reduced by dietary restriction, or raised by forced-feeding (Lan & Hui, 1994; Keesey, 1986; Proietto & Thorburn, 1994). The *set-point* theory implicates a central role of the hypothalamus in the dysregulation of body weight (Proietto & Thorburn, 1994; Levin & Routh, 1996; Keesey, 1989). It is this central role which integrates those mechanisms originating in the brain (responsible for the regulation of energy) with the autonomic nervous system (and its associated thermogenesis), the hyperphagia associated with excessive energy intake in obese (Ramirez *et al.*, 1989) and the hyperinsulinaemia associated with obesity (Woods, 1997). The brain has central processing and a storage capacity for handling this afferent information, and can change both structurally and functionally in response to its internal and external milieu (Levin & Routh, 1996). Work in animal models suggests that the central system in obese individuals largely ignores signals of excess adiposity from the periphery, keeping the body weight set point at pathologically high levels (Levin & Keesey, 1998). Disordered regulation of NPY and monoamine metabolism within the ventromedial hypothalamus is a consistent finding in the brains of obesity-prone and obese rodents (Levin & Keesey, 1998). Such dysregulation may cause inappropriate neurohumoral control of metabolism and autonomic output to organs such as the pancreas, resulting in increased metabolic efficiency and persistent adiposity. It has been proposed that the high failure rates in obesity

treatment may be due to a chronic central nervous system dysfunction, which perpetuates the abnormally high set point of body weight that characterises the obese (Hirsch *et al.*, 1998).

Obesity is a function of energy dysregulation (Melcher & Bostwick, 1998; Jebb, 1999), and it would appear that control over appetite is the elusive component in the complete understanding in the aetiology of obesity. What follows is a review of the literature of the regulatory factors and processes controlling appetite, and the potential factors that may contribute to its dysfunction, leading ultimately to obesity.

1.2 PERSPECTIVES IN INGESTIVE BEHAVIOUR

Coincidental to the rising prevalence of obesity, control over human appetite and the regulation of energy intake became a highly debated issue for much of the 20th century (Cannon & Washburn, 1912; Anand & Brobeck, 1951a; Le Magnen, 1956; Kissileff, 1984; Rolls *et al.*, 1990; Blundell *et al.*, 1994; Prentice *et al.*, 1998; Poppitt *et al.*, 1998; Porrinni *et al.*, 1995). Human appetite is a highly complex process, and explaining a dysfunction on this axis as a means of explaining the aetiology of obesity has been a complicated and thorough procedure. Whilst most theories have postulated psycho-physiological factors controlling appetite, there are others that have also incorporated the necessary social and behavioural issues involved in the ingestive process.

1.2.i The development of the theories surrounding appetite research in the 20th century

The first investigations into human appetite were based on fundamental observations (using the x-ray technique of Röntgen) about the type and frequency of gastric and small intestinal motility (Cannon & Washburn, 1912). Using an inflatable gastric tube, Walter Cannon, the Professor of Physiology at Harvard Medical School, observed that pressure changes (in the inflated gastric tube) were produced by gastric contractions: and he found that hunger correlated with gastric contractions of increased frequency and magnitude. Accordingly, he called this the *gastric contraction theory of hunger* (Cannon, 1915).

The next step in the understanding of ingestive behaviour came from a more psychological perspective: behaviourism. Behaviourism was the dominant movement in American psychology in the 1920's and generally explained all human behaviour according to the environment in which the human is placed. By designing a cage that was able to record the perpetual activity of rats, a behaviourist student, Curt Richter, was able to isolate eating behaviour as a complete scientific for the first time (Richter, 1922). He found a tendency for bouts of eating – what we call meals – to occur roughly every 4 hours. There was, however, considerable variability in the interval between meals: the rats ate about 10 meals per 24 hours and ate more of them in the night than in the day. The size of the meals also varied, irrespective of the amount of food present. This brought to the fore the most interesting question of all: when food was continuously present, why did the rats eat discrete meals of various sizes about 10 times per day rather than take one large meal or nibble continuously?

In an effort to answer this fundamental question of ingestive physiology, came a paradigm based entirely on pure medical science: the neurological technique of stereotaxis as developed by Horsley & Clarke (1908). The stereotaxic machine was designed to make accurate lesions of deep structures in the brain, such as cerebella nuclei. So, in an effort to explain and understand the regulation of ingestive behaviour, lesions made to the ventro-medial hypothalamus (VMH) led to chronic hyperphagia in rats, implying that the control centre for appetite lay in the hypothalamus, and that when it was destroyed, all regulatory mechanisms for eating were destroyed with it.

Observations that lesions in the VMH of rats resulted in hyperphagia whereas lesions to the lateral hypothalamus induced anorexia in otherwise healthy rats (Anand & Brobeck, 1951b). This led to the hypothesis that the hypothalamic control of food intake was centred in the ventromedial and lateral hypothalamus. The lateral hypothalamus was subsequently proposed to be the *feeding centre* (Anand & Brobeck, 1951b), and the ventromedial hypothalamus was the *satiety centre*, and it acted by inhibiting the *feeding centre* (Anand & Brobeck, 1951a).

Thus up until the early 1950's we knew that there was a neural control of feeding, but the question as to what controlled the initiation and termination of individual meals remained unanswered. The first form of the answer was given in 1952, when Jean Mayer postulated the glucostatic theory: that decreased glucose utilisation by neurons in the brain initiated eating and that increased glucose utilisation terminated eating (Mayer, 1953; Mayer & Bates, 1952). This logical principle of autoregulation gave birth to numerous other theories based on the principle that a metabolic/hormonal variable would regulate energy intake: these include the lipostatic theory of Kennedy, (1952); the aminostatic theory (Maier & Pies, 1974; Rozin & Schulkin, 1990), insulinostatic theory as postulated by Woods (1979), appetite-suppression effect of increased cholecystikinin (CCK) proposed by Gibbs *et al.*, (1993) and Schwartz *et al.*, (1988). Leptin (also known as the OB protein) has been proposed as a potential regulator of energy in the body (Schwartz *et al.*, 1996; Montague *et al.*, 1997; Friedman, 1998).

In accordance with the set-point theory described above, the *internal milieu* is maintained constantly, despite frequent and acute changes in the external environment that challenges its integrity (Vander *et al.*, 1997).

Regulation of energy intake (the ingestion of food), however, incorporates cognitive factors over and above the physiological variables that control energy intake. Consequentially, ingestive behaviour relies on a myriad of factors – the control over which requires a symphony of regulatory mechanisms. Using the 'orchestra' metaphor, the search for the conductor in this regard is crucial to our understanding of energy regulation, and in finding this elusive piece of the puzzle, may provide the key to determine its dysfunction.

Jéquier (1993) has reported that the body has no energy sensors *per se*, but rather proposed that the body regulates its energetic requirements according to its nutrient levels. In contrast, Friedman (1997) has suggested on a number of occasions that the body does indeed have a hepatic energy-regulatory system – a concept based on the foundation of other work in this field (Ramirez *et al*, 1991; Friedman, 1995). In this light, the discussion now turns to the control of ingestive behaviour, and the theories surrounding the regulation of energy intake.

1.3 CONTROL OF INGESTIVE BEHAVIOUR

1.3.i Defining hunger, appetite, satiety, and satiation

Hunger is defined and characterised as a physiological state resulting from an alimentary deficit in the organism and varying according to the degree of this deficit (Le Magnen, 1956). This *alimentary deficit* may be subjectively expressed in humans by a diffuse complex of sensations, of which epigastric pang is one (Le Magnen, 1956). The voluntary intake of food is the amount of food that is eaten in a given period, while appetite is the drive to eat a specific nutrient (Forbes, 1988). Satiation refers to the process that brings a period of eating to an end, or, it is that process which occurs while foods are being eaten (De Graaf *et al.*, 1999). Satiety is the inhibition of hunger and the inhibition of further eating which arises as a consequence of food ingestion; it is satiety that is engendered as a consequence of consumption (Blundell & King, 1996). It is generally accepted that satiety influences appetite, and that satiation is both a consequence, and an influential factor over voluntary food intake (Blundell *et al.*, 1994).

It has been proposed that satiation influences the size of meals and snacks (De Graaf *et al.*, 1999), and satiety measures the capacity of food to control subsequent hunger and the process of eating. Different foods have been shown to exert different satiating effects (Rolls *et al.*, 1981; Rolls *et al.*, 1988; Rolls *et al.*, 1990; Rolls *et al.*, 1995; Rolls & Bell, 1999), which has been referred to as the different *satiating efficiencies* of food (Kissileff, 1984). When food choice is unrestricted, any accompanied weight-gain is

greater when dietary intake is high in fat [high food quotient (FQ)] (Tremblay *et al.*, 1989). In the current context, fat would have a low satiating efficiency, insofar as it has only a weak suppressive effect on subsequent food intake (Kissileff, 1984; Cotton *et al.*, 1994).

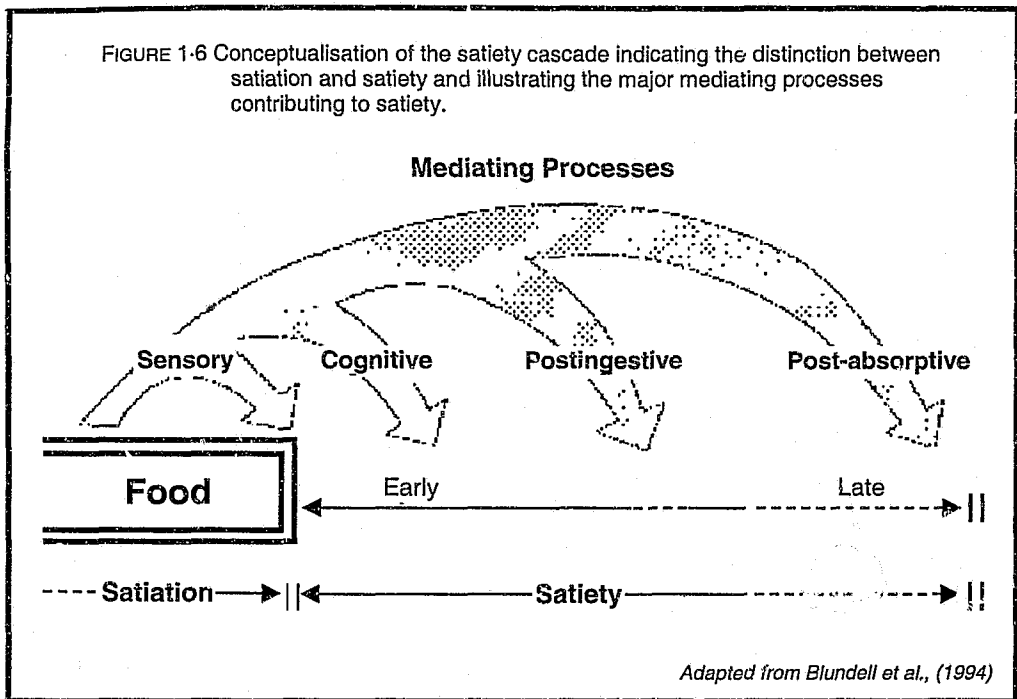
It is worth keeping in mind that hunger is a biologically useful sensation. It is a nagging, irritating feeling that prompts thoughts of food and reminds us that the body needs energy.

Blundell *et al.*, (1994)

1.3.ii The Satiety Cascade (Blundell *et al.*, 1994)

In an effort to integrate satiation and satiety, these two processes have been described as those that control events going on within meals or between meals (Burley & Blundell, 1990; Blundell *et al.*, 1994). As detailed in FIGURE 1.6 *overleaf*, food brings about satiation via various 'sensory' processes. These somatic and social influences on choice and intake depend on the type of food that is present (in terms of its taste, sweet, salty, sour, and bitter), the smell of the food, the temperature of the food, the sight of the food, and the surroundings in which the food consumption occurs (Booth, 1990).

FIGURE 1-6 Conceptualisation of the satiety cascade indicating the distinction between satiation and satiety and illustrating the major mediating processes contributing to satiety.



The satiating effects of a consumed food are generally agreed to be mediated by key features of the food itself generating physiological processes. These physiological processes arise from sensory qualities of food stimulating afferent receptors in the mouth or gastrointestinal tract, the volume and osmolality of food in the stomach, the release of gastrointestinal hormones and the post-absorptive effects of digested products being metabolised in the liver or stimulating specific chemoreceptors in the various sites in the brain or periphery (Blundell, 1991; Cotton *et al.*, 1994). When the two processes of satiation and satiety are integrated following the ingestion of a meal, they effectively create the *Satiety Cascade* (Burley & Blundell, 1990; Blundell *et al.*, 1994). Satiety incorporates the 'cognitive', 'post-ingestive', and 'post-absorptive'

processes. Food consumption usually suppresses hunger, and (increases feelings of fullness) and inhibits further eating for a given period of time. This inhibitory action is called satiety. The potential dysfunctional eating pattern that may be responsible for weight-gaining or obese individuals may be linked to a disruption in either the satiety or satiation processes, or both.

Essentially, this hypothesis proposes the way in which food (or energy) is sensed and processed by the human biological system, generates a signal(s), which may be neural and/or humeral, that controls appetite. Current understanding proposes that the neural mechanism of appetite regulation is based on the dual system of a lateral hypothalamic feeding system and a medial hypothalamic satiety system (Hoebel & Hernandez, 1993; Hoebel, 1997). The humeral system involves primarily the glucose-insulin dynamic, leptin, cholecystokinin, and glucagon. From this perspective, the most plausible explanation for an integrated control of energy intake is a two-way process between the centrally mediated neural system and the peripherally-mediated humeral system. However, the exact interaction between these two systems is as yet uncertain (Hoebel, 1997).

TABLE 1-1. Cues that may contribute toward appetite stimulation in the induction of an acute eating episode

External cues	Internal cues	
Environmental - Place of eating - Availability of food - Time of day - Ambient conditions Social - Number of people present - Occasion (celebration vs normal meal) Opportunity - Coffee break Convenience - Habit	Psychological - Cognitive - Consciousness - Learned behaviour - Conditioning - Stress levels	Physiological Metabolic - glucose - FFA Hormonal - Insulin - Leptin - Glucagon - Neuropeptide Y (NPY) - Menstrual cycle changes Mechanical - Degree of gastric fullness - Rate of gastric emptying

The control of appetite is a highly complex phenomenon which is unique from other physiological process in that it is governed by more than pure fundamental physiological regulators (TABLE 1.1). Appetite has been shown to be influenced by psychological and social factors. It follows that control over such a process would require a highly complex regulatory system. Despite countless efforts to propose a comprehensive hypothesis to explain the control over appetite, few have made significant in-roads into the phenomenon. Recent evidence that the consumption of high-fat foods may induce a disruption to the appetite satiety cascade (through an associated passive overconsumption) which leads to obesity may unlock the key to a more thorough explanation to appetite dysfunction in the obese (Blundell, 1990; Blundell & Macdiarmid, 1997a; 1997b).

1.3.iii The control of energy intake at a meal: intra-meal regulation

The amount of energy that is ingested at any one time is a function of its macronutrient content (or food type). If the quality of the food is altered in such a way that the macronutrient balance tends to have increased amounts of fat in its composition (relative to carbohydrate and protein), satiety and satiation may only occur at an increased energetic cost (Kissileff *et al.*, 1984; Booth 1990). Based on this premise, it may be hypothesised that it is the more expensive satiety and satiation that results from high-fat foods, that contribute to obesity (Blundell *et al.*, 1994).

There are often choices of what food to eat, and how much to consume. Furthermore, these choices change during the meal itself, where the cessation of the intake is specific to the food that is being eaten (Rolls *et*

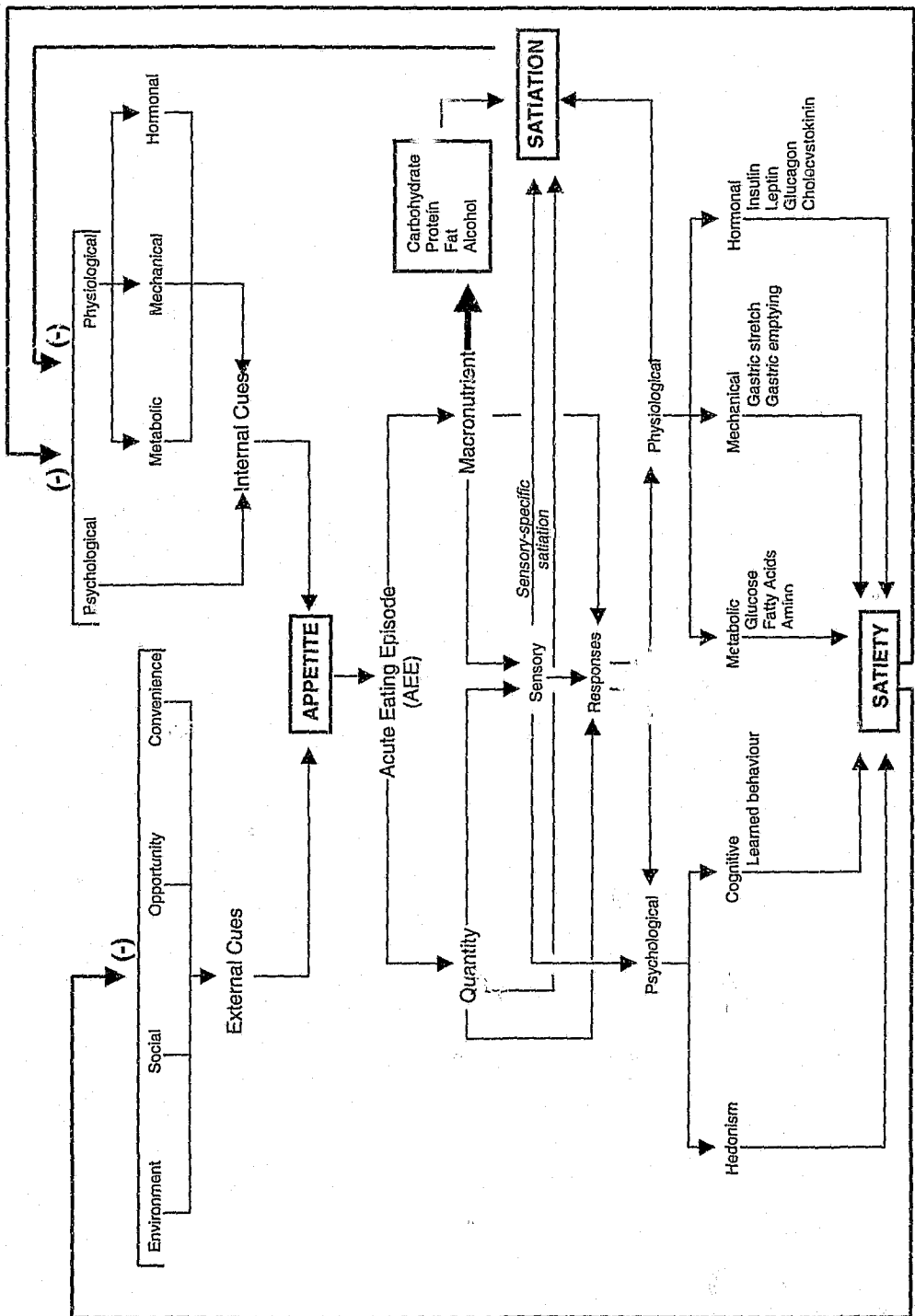
al., 1981; Kissileff, 1984; Rolls *et al.*, 1988). However, if a different food is presented, it is likely that eating will start again, which provides evidence for the stimulating effect of sensory variety and boredom in satiation (Booth, 1976). This would suggest that the eating process induces a change in relative preference between eaten and uneaten foods (Booth, 1990). Based on this premise, Rogers (1999) has proposed that the current trend in consumer-oriented marketing to overload consumers with wide varieties of (often high-fat) foods as the fundamental pre-cursor for obesity in the western world.

FIGURE 1-7 depicts the schematic representation of an appetite model which integrates both physiological and psychological variables contributing to the regulation of energy intake. The chain of events follows the hunger signals, which fire on the appetite centre, and induce the eating process. The amount of food ingested at any one time is a function of the degree of hunger that the individual is experiencing, and the type of food that is being eaten (De Castro, 1990; Rolls & Shide, 1999). The combined effects of these two factors (macronutrient value and the pattern of eating) consequently exert their effects on both the psychological and physiological profiles, which in turn lead to an eventual state of satiety. Learned changes in relative food preference from early to late in the meal also affect intake. These may be culturally based, or based on sensory factors like eating salty, savoury, or meaty foods before sweet foods (Rolls, 1988; Booth, 1990). A more thorough discussion of this figure is conducted in section 1-7 (page 46) where the concept of "inter-meal control" shall be considered.

1.3.iv The motivation to eat

Food has a lot more meaning than just sustaining life – the ritual of the hunt and the feast are very much a part of the culture of modern day life – these are principally psychological in nature (Morris, 1987), but sincerely manifest themselves in a physiological manner. These are the external cues that are intrinsically, and instinctively set, and influence our behaviour, albeit subconsciously. The social effect on eating has been well documented in a wide range of animals, including humans (De Castro, 1990; De Castro, 1997). In 1929, Bayer allowed a chicken to completely satiate, eating as much wheat as it wanted. He then introduced a hungry chicken, which began to eat. The first chicken, although just satiated, began to eat immediately (Bayer, 1929). This exact same phenomenon has been replicated in pigs (Hsia & wood-Gush, 1984), fish (Welty, 1934), rats (Hoyenga & Aeschelman, 1969), and humans (De Castro, 1988). Furthermore, John De Castro (1990) has further shown that the more people present at meal increases the amount of food consumed proportionately. These findings reinforce the hypothesis that the regulatory process controlling appetite – satiety is not a function of energy intake, and that other factors influence the eating process.

FIGURE 1-7 Schematic representation depicting those factors that contribute toward appetite build-up, and the integration of those factors responsible for the achievement of satiation and satiety.



1.3.v The Eating Episode

The physiological process of eating starts with chewing the food; the enzyme salivary amylase is secreted immediately, and starts breaking down the food (Vander *et al.*, 1997). As it enters the gastric pouch, the hormones gastrin and pepsin are released and the protein starts breaking down in the stomach. Carbohydrates are broken down into their fundamental compounds, and as the chyme starts moving into the small intestine, nutrients are absorbed from the GIT and start accumulating into the blood.

After meal ingestion, the increase in glucose and insulin plasma levels induces a stimulation of glucose uptake and oxidation in muscle and other tissues and an increase of glycogen synthesis in both liver and muscles. In contrast, insulin inhibits lipolysis, plasma free fatty acid levels decrease, and fat oxidation is inhibited (Jéquier, 1998). The intake of carbohydrate has the effect of reducing the rate of fat oxidation, which occurs in the presence of insulin (Friedman, 1995; Woods, 1997).

We know that a symphony of physiological phenomena occur prior to, during, and after the ingestion of food, but what is it that brings the process of eating to an end? Factors that have been suggested to play a causal role in the satiating effects of appetite are the volume of food consumed; the energy and macronutrient value of the food being consumed; the rate of consumption; the sensory properties of the food; and the personal beliefs about the satiety value of a food (Booth, 1976; Kissileff, 1994; Booth, 1990).

Depending on the various permutations that these factors assume, various sets of physiological responses may be elicited to result in satiety, and the consequential cessation of eating. These responses are metabolic, hormonal, and mechanical. Perturbations in blood glucose, free fatty acids and proteins in response to feeding have been discussed above, and the general understanding of hormonal intervention on mechanical function is discussed here.

Since insulin is so integrally linked to the post-absorptive phase of cellular metabolism, it is an attractive hypothesis to link its dynamics as the main contributor to the initiation, cessation and inhibition of eating (Woods, 1997). This large molecule was recently found to enter the brain and inhibit the expression of the feeding peptides neuropeptide Y (NPY) and galanin (GAL) (Schwartz *et al.*, 1992; Woods, 1997). Insulin is released in anticipation of a meal (Morrel *et al.*, 1988), and during eating (Vander *et al.*, 1997). Insulin is actively transported into the extracellular fluid of the hypothalamus where it can interact with insulin receptors and start a series of reactions that inhibit NPY and GAL, which inhibit the eating process. Leibowitz (1990) reported that it was this co-ordinated dynamics in insulin concentrations that regulate the body's short-term energy supplies, which are stored as glycogen. Similar effects have been observed on long-term energy supplies, which are stored as fat (Hoebel, 1997). In light of these phenomena, the insulin insensitivity that is characteristic of obesity may potentiate the disruption to the appetite – satiety complex which leads to an inability to regulate energy intake.

1.3.vi The effect of varied energy density on satiety and obesity

The amount of food that is consumed at any one eating episode is closely linked to its macronutrient composition. The more energetically-dense foods have higher percentages of fat as their components, and accordingly, the amount of energy that is ingested is largely dependent on the macronutrient composition value of the food.

The hypothesis that the ingestion of dietary fat may *induce passive overconsumption* is reinforced by the finding that obese individuals are more likely to consume fat (Drewnowski, 1983; Drewnowski, 1997; Seidell, 1998; de Wit, 2000). The action of dietary fat on the control of appetite has shown that subjects exposed to a high-fat diet consume more energy and gain more weight than subjects obliged to eat from a range of low-fat foods (Lissner *et al.*, 1988; Cotton *et al.*, 1994). These findings would suggest that fat has a low satiating efficiency (Kissileff *et al.*, 1984) compared to carbohydrate (Hill *et al.*, 1987; Rolls *et al.*, 1991) and protein (Rolls *et al.*, 1988). Because fat and carbohydrate are known to undergo different processes of digestion, the post-absorptive satiating effects of the nutrients are likely to have different temporal profiles (Cotton *et al.*, 1994).

1.4 MEASURING APPETITE AND SATIETY

Since measuring appetite and satiety is mostly a subjective activity, it is an extremely difficult methodology to quantify, and more importantly, to reproduce. It has been suggested that hunger (appetite) correlates well with the rate of salivation (Durrant, 1978). Accordingly, it has suggested that the use of salivary rates as a means of appetite measurement (Durrant, 1978). However, assessing this technique in obese subjects showed that alterations in energy density without changing energy intake had no effect on salivation, hunger or appetite (Durrant & Royston 1979). A follow-up study, revealed that salivary rates in obese subjects correlated well with energy intake, hunger and appetite ratings did not, (Durrant & Royston, 1980). Given the mixed reports of this technique, more research is required in this field.

An alternative to the salivary rate technique in the quantification of appetite and satiety ratings is the visual analogue scales (VAS) technique. This technique uses a line, 100mm in length, anchored on either side with a series of questions around *subjective hunger rating, amount an individual feels they can eat?; and strength of the urge to eat?* These scales have been used previously in many studies and are known to be sensitive (Cooling & Blundell, 1998), and valid subjective markers of the motivation to eat in both nutritional and pharmacological studies (Hill & Blundell, 1990; Jenkins *et al.*, 1992). Although De Graaf (1993) and Mattes (1994) argued that testing for subjective appetite ratings using these VAS data were are an unreliable technique, recent evidence has been reported where VAS data were a reliable means of assessing hunger and satiety in 55 healthy men (Flint *et al.*, 2000).

1.4.i The *pre-load – test meal* paradigm

Testing energy intake in humans can take a number of forms of varying degrees of accuracy. In the earlier years of appetite research, scientists requested subjects keep dietary records, and today, most of that data is meaningless in light of the gross under-, and mis-reporting that occurred. The most accurate manner of assessing energy dynamics in humans, is in a fully – functional metabolic chamber in which a subject can remain for an extended period. This allows the investigators total control over proceedings; however, even under such strict conditions, when the cognitive component of appetite research is taken into account, subject variability will always be an extraneous variable.

Caught in the middle of these two methods of assessment is the *pre-load – test meal* paradigm. Blundell & Green (1996) discussed this procedure within the confines of scientific 'cross contamination'. When a pre-load is given, and the subsequent test meal is what is being investigated, particularly when different pre-loads are given on the same day (ie testing foods of different sensory properties in the same day), the cumulative effects can contaminate the findings of the test meal. They advised scientists using this procedure to be extremely cautious of this possibility. Essentially, this procedure is uncomplicated: the pre-load (which is manipulated) is the independent variable and the test meal (which is measured) is the dependent variable. It was the period in-between these two variables that concerned these authors the most: they felt the validity only held if the two variables were protected from each other entirely (Blundell & Green, 1996). This method of assessment has been used thoroughly in the field of appetite research (Rolls *et al.*, 1988; Tournier &

Louis-Sylvestre, 1991; Hulshof *et al.*, 1993; Rolls *et al.*, 1994; Porrini *et al.*, 1995; Lavin & Read, 1995; Turconi *et al.*, 1995).

Another factor that needs to be taken into account is whether this procedure measures *satiety* or *satiation*. Blundell & Macdiarmid (1997) have argued that a procedure of such short duration and limited choice at the *ad libitum* test meal only measures satiation and not satiety. Rolls *et al.*, (1994) referred to the measurement of satiety using this exact procedure. The pre-loading technique has been found to be a sensitive indicator of the satiating efficiency of foods (Kissileff, 1984; Kissileff *et al.*, 1984). However, Spitzer & Rodin (1981) had previously reported that the cognitive and sensory cues associated with the pre-load may confound the outcome meal. A danger in testing *appetite* is that subjects should not be able to distinguish the foods used in the pre-loads (Rolls *et al.*, 1991).

The effect of a pre-load on subsequent intake may depend on a number of factors, such as the sensory properties of the pre-load (Rolls, 1986), satiety signals from the stomach or the small intestine (Rolls *et al.*, 1988), or postingestive changes (Friedman *et al.*, 1986). It has also been suggested that the timing between a preload and a test meal will determine which of these inhibitory factors will be exerting effects on subsequent intake (Rolls *et al.*, 1991). Macronutrient factors also contribute, and because fat and carbohydrate may stimulate different pre-absorptive inhibitory mechanisms, these effects need to be considered. Given the limitations of this technique, every effort should be made to control for sensory cross-contamination, that the timing of the pre-load is adequately spaced, and that the subjects are sufficiently ignorant of the methodology and required outcome of the experiment.

1.5 MACRONUTRIENT VALUE OF FOODS

1.5.i Definition of macronutrient value of food and the dietary guideline for intakes of foods with different macronutrient values.

The term "macronutrient" refers to the relative energy substrates that are in all food, of which there are fundamentally four: carbohydrate, protein, fat, and alcohol (Jéquier, 1999). The term 'macronutrient' part of the diet is different to the 'micronutrient' parts of the diet, which are vitamins and minerals (USDA, 1999)

Cellular energy metabolism utilises two main energy substrates: glucose and fatty acids (Rankle *et al.*, 1963; Friedman, 1999; Jéquier, 1999). The major determinants of the fuel mix that are oxidised are glucose availability and insulin secretion, both of which promote glucose oxidation. Fatty acid oxidation occurs mainly when glucose availability is reduced, for instance during the postabsorptive period, or when energy expenditure is increased (eg endurance exercise of long duration) (Vander *et al.*, 1997).

The importance of this cellular metabolic balance is important to the discussion on obesity insofar as, although eucaloric diets with high carbohydrate and low-fat content may induce *de novo* lipogenesis (in the long-term) in adults, the rate of conversion of glucose to fatty acids is low. Accordingly, carbohydrate intake does not have much influence on lipogenesis in the short-term (Jéquier, 1998, Jéquier & Tappy, 1999).

The United States Dietetic Association (USDA) has proposed that the amount of carbohydrate that is ingested should be primarily dependent on how much energy an individual requires. This association has suggested that the carbohydrate intake should be approximately 50-55% of the total daily intake (USDA, 1999).

The amount of an adult requires depends on three factors: the fat requirement to meet energy needs, the need for essential fatty acids, and the amount of fat in the diet that is necessary to absorb fat-soluble vitamins (Jéquier & Tappy, 1999). The lower limit of fat intake to meet the energy needs in adults is assumed to be between 10 and 15% of dietary energy, provided that enough carbohydrate is available. Saturated fatty acids should not exceed 10% of the energy intake (USDA, 1999).

The amount of protein that an individual ingests on a daily basis is dependent on a number of mitigating factors: growth, exercise and level of activity, stress, and disease, to name a few. Ideally, the protein content of a balanced diet should comprise of 20-25% of total intake.

1-5.ii The effects of fat and carbohydrate on satiety and their impact on obesity

Well, anyway, it is a matter of fat. The point is that we're getting obese. We can only get fat by eating fat, so I don't know how or where the evidence is that actual fat consumption per capita is going down.

Pi-Sunyer (1997)

On being told that the USDA's statistics show overall fat intake to be down.

It has been proposed that the metabolism of fat and carbohydrate fuels generate signals that the brain uses to control consequential food intake (Langhans & Scharrer, 1992; Friedman, 1995; Friedman, 1998). Human overfeeding studies that have measured the components of energy balance have found no experimental support for a metabolic off-loading in over-feeding (Ravussin *et al.*, 1986; Forbes *et al.*, 1986; Diaz *et al.*, 1992; Stubbs *et al.*, 1995). Thus, any major change in energy balance is most likely to be fundamentally due to differences in the regulation of energy intake (Flatt, 1987; Prentice *et al.*, 1989) together with alterations in physical activity. Flatt (1987) has proposed that the macronutrient content of the diet may be important in influencing the regulation of energy intake.

Fundamentals of nutritional physiology are based on the macronutrient groups, which are, as discussed previously, carbohydrates, protein and fat. Since fat is so energy-dense, providing 9 kcal·g⁻¹ compared with 4 kcal·g⁻¹ for carbohydrate or protein, it may be easy for high-fat foods to be over-eaten if we assume that there is a tendency for food intake to be regulated by the perceived amount consumed (volume and/or weight). Similarly fat is an efficient storage form of energy in the body (Jéquier, 1993). In addition

to its high energy density, fat is hydrophobic and therefore requires less water for storage than does either protein or carbohydrate.

1.5.ii.a The effect of carbohydrate on appetite and incidence rates of obesity

In accordance with the glucostatic theory of Mayer (1953), Carlson (1991) has suggested that since it is the brain which may control eating, it seems reasonable that hunger and appetite should be triggered by a decrease in the brain's primary fuel (ie glucose). If glucose were to play a significant role in appetite regulation, what then are the mechanism(s) in place in the human body to deal with consuming a meal containing copious amounts of carbohydrate?

The glycogenostatic model (Flatt, 1987; Flatt, 1993) predicts that when feeding *ad libitum* on a diet of a given composition, people will eat to maintain a fixed range of carbohydrate stores. He proposed a framework of the theory by which the composition of the food eaten can affect body weight through effects on food intake. Flatt's model incorporates the inability of the body's capacity for storage of body carbohydrate compared to that of body fat. Based on this premise, he argued for a tighter control over carbohydrate balance (compared to fat balance) and that metabolic mechanisms must be in place that are able to adapt to changes in carbohydrate intake (Flatt, 1993). This model implies that that high-carbohydrate diets would be more satiating in the long-term and less likely to cause obesity. Several studies have shown this to be true in rodents (Sclafani, 1989; Hill *et al.*, 1993) and humans (Lissner *et al.*, 1987; Kendall

et al., 1991; Stubbs *et al.*, 1993). However, others have shown that 300g of glycogen have no detectable effect on subsequent intake in lean men (Stubbs *et al.*, 1993; Shetty *et al.*, 1994). It would seem that although Flatt's hypothesis does have merit, it may be oversimplifying the complexity of energy regulation linked to appetite. Friedman & Tordoff (1986) and Hill & Prentice (1995) explain the physiological effects on appetite of fat versus carbohydrate as being dependent on the oxidative flux generated by each macronutrient, which itself depends on the energy level at which it is consumed. Needless to say, that this is an ongoing debate in the field of appetite research.

1.5.ii.b The effect of fat on appetite and incidence rates of obesity

There is a considerable body of evidence that diets high in fat are associated with weight gain and obesity (Lissner *et al.*, 1987; Dreon *et al.*, 1988; Romieu *et al.*, 1988; Tremblay *et al.*, 1989; Drewnowski, 1992). As an adjunct to this direct relationship, there is compelling evidence to suggest that there are energy homeostatic processes, which are disrupted by the excessive intake of fats (Duncan *et al.*, 1983; Lawton *et al.*, 1993; Blundell *et al.*, 1996; Blundell & King, 1996; Blundell & Macdiarmid, 1997). Furthermore, it appears that the autoregulatory adjustments in fuel selection that help to maintain carbohydrate, protein, and alcohol balance may be absent for fat (Tremblay & St. Pierre, 1996). It has been proposed that these mechanistic findings support the prevailing view that fat undermines the normal body-weight control systems and contributes specifically to the development of obesity in certain individuals (Prentice, 1998).

The hypothesis that there may be distinctive behavioural phenotypes for the consumption of fat has recently been investigated (Blundell & King, 1996; Cooling & Blundell, 1998). In a tightly controlled, fully repeated 2x2x2 measures design, Cooling & Blundell (1998) attempted to characterise the appetite control in habitual high-fat (46.7% ADER as fat) and low-fat (29.9% ADER as fat) phenotypes in eight lean men. The main finding was that the appetite control in habitual high-fat and low-fat consumers was different, which is in line with similar findings that people characterised by their morphology display differences in the central control of appetite (Krotkiewski *et al.*, 1997). It appeared that the high-fat eaters were insensitive to changes in energetic load, whereas those that preferred low-fat foods were more sensitive to fat content of food. In addition, these authors concluded that the different styles of appetite control could arise from intrinsic physiological differences or a system, which is adapted to deal with a particular type of diet (Cooling & Blundell, 1998). That these distinct phenotypes may exist may have implications for the relationship between diet and obesity, which needs further development through further research.

It is generally accepted that the more fat that is present in a diet, the more palatable it will be (Rolls *et al.*, 1985; Cotton *et al.*, 1994; Drewnowski & Greenwood, 1993; Drewnowski, 1993), and it has been suggested that some people may overeat high-fat foods simply because they are enjoyable (Drewnowski *et al.*, 1991; Golay & Bobbioni, 1997). De Graaf *et al.*, (1999) have recently investigated the effect that the palatability a food has on satiation and satiety. They used a within-subjects repeated measures design in 35 non-restrained subjects (26 female, 9 male), and employed the pre-load – test meal paradigm (discussed later). By

manipulating the palatability of foods and timing of pre-loads, and then evaluating the responses at a standard test meal (on its own) or with an added factor of the availability of other foods on subsequent intake, these authors found that the palatability of the food effects satiation, but there was no effect of the pleasantness on subsequent satiety (FIGURE 1-11). Furthermore, they reported that people eat more food when they know they have no access to other foods for a particular amount of time (De Graaf *et al.*, 1999).

1.5.iii Correlating obesity with high-fat diets

As obvious as the relationship may appear, providing evidence of causality between increased dietary fat causes obesity is more confounding. One of the principle obstacles in this regard is the gross degree of *dietary under-reporting* that occurs in obese subjects. Prentice *et al.*, (1986) asked obese subjects to keep a dietary record of their food intake over a 7-day period whilst in a metabolic chamber days (to determine metabolic requirements) at the Dunn Unit for Clinical Nutrition in Cambridge, UK. Their main finding was that these subjects under-reported intake up to 25%. Further studies have supported this type of concept (Bandini *et al.*, 1990; Poppitt *et al.*, 1998; Voss *et al.*, 1998). Various cross-sectional investigations of diet composition reveal that overweight persons consume a greater percentage of dietary energy from fat than do lean counterparts (Lissner *et al.*, 1987; Nelson *et al.*, 1996).

Data derived from another other diet-survey study suggests a positive correlation between energy intakes and BMI (Miller *et al.*, 1990). This

premise is supported by other studies, which have found a correlation between dietary fat intake and adiposity (Dreon *et al.*, 1988; Romieu *et al.*, 1988; Tremblay *et al.*, 1989). There has been a trend toward growing confusion over the role of sugar and fat in energy balance, and their influence on body weight. Drewnowski (1987) suggested that individuals actually *crave* these foods, which in turn would allow a basis for overeating. In support of his own hypothesis, Drewnowski *et al.*, (1992) found that obese women rank sweet-fat foods highly in their listing of preferences. Furthermore, Drewnowski & Greenwood (1983) studied 16 normal-weight subjects who rated the perceived intensity of sweetness and fatness of 20 different milks, creams and sugars. The subjects rated the pleasantness of increasing concentrations of sugar and fat, and the main findings were that ratings for sugar increased and then peaked; whereas those for fat continued to rise (Drewnowski & Greenwood, 1983). Other studies have suggested that obese individuals have an enhanced sensory preference for high-fat foods (Mela, 1991). Going one step further, Drewnowski (1993) found that appetite for fats were particularly pronounced in obese men (for fat-savory mixtures) and obese women (for fat-sweet mixtures). In contrast, it has been shown that lean individuals exhibit greater satiety when exposed similar stressors associated with high-fat foods (Rolls *et al.*, 1994; Blundell & Stubbs, 1999).

Different types of foods satisfy hunger to different extents (Kissileff, 1984; Rolls *et al.*, 1988; Rolls *et al.*, 1990). An interesting observation is that the pleasantness of a food does not remain constant, but instead decreases as the food is consumed. Further, this decline is specific to the food which has been eat, and that all other food left on the plate remains the same level of *pleasant* (Rolls *et al.*, 1981). High-fat foods have traditionally been

associated with rich flavour and high overall palatability (Lichtenstein *et al.*, 1998), and the removal of fat from the diet is at the concomitant expense of its palatability. To this end, in trying to educate the masses as to the benefits of low-fat cooking, that the majority of the populus considers such low-fat cooking as bland (Porter *et al.*, 1998).

In response to this, the food industry has researched and developed new ways of producing fat-modified products: the key objective of these products is to market foods that can facilitate a reduction in dietary fat intake without compromising palatability (Lichtenstein *et al.*, 1998). More than 6,000 new fat-modified foods have appeared on the global market in the last five years (Sigman-Grant, 1997), and yet most of the western world does not appear to be reducing its intake of dietary fat (Seidell, 1998).

1.5.iv Passive overconsumption

Using the procedure of *concurrent evaluation* (where individuals have free access to a wide range of foods for consumption, and have no limitations on choice and/or inhibitions on making those choices) Blundell *et al.*, (1994) were able to show that people consume much greater quantities of energy from a range of high-fat foods than from foods high in general carbohydrates (Blundell *et al.*, 1994) or sucrose (Green *et al.*, 1994). Furthermore, he noted that this effect was particularly strong in obese subjects (Lawton *et al.*, 1993) and the high-fat hyperphagia was referred to as *passive overconsumption* (Blundell *et al.*, 1993; Blundell *et al.*, 1995; Blundell & King, 1996; Blundell *et al.*, 1996; Blundell & Macdiarmid, 1997a; Blundell & Macdiarmid, 1997b; Prentice, 1998; James & Ralph, 1999).

Experiments with isoenergy-dense diets show that this high-fat hyperphagia is caused by the high-energy density of fatty foods rather than by their other attributes (Stubbs *et al.*, 1995). This excessive energy consumption occurs unintentionally, and without the ingestion of additional bulk (Stubbs *et al.*, 1995), so the individual doesn't feel that he/she is eating more than he/she should.

1.5-v The effect of varied duration of eating on satiety and obesity

The time that it takes for a meal to be consumed is normally indicative of the rate of gastric emptying (into the small intestine). It has been shown that the rate at which food is consumed decreases with the passing of the time taken to consume the meal (Westerterp *et al.*, 1990), and that the gradient of the slope indicating the rate at which food is consumed is a function of the palatability of the food (Bobroff & Kissileff, 1986), the type of food eaten, or macronutrient value (Kissileff *et al.*, 1984), and whether any foods were ingested prior to the consumption of the meal in question (the presence of any pre-loads) (Kissileff *et al.*, 1984). It has been suggested that these findings are the manifestation of an internal regulatory mechanism that allows for the retardation of -, and the eventual cessation of eating (Westerterp *et al.*, 1990). It appears that the duration of eating is a consequence rather than a cause in the process of satiation and satiety (Bobroff & Kissileff, 1986; Blundell *et al.*, 1994).

Relative overconsumption (to expenditure) induces weight gain has been viewed as a consequence of emotionality (Kaplan & Kaplan, 1957),

heightened external responsiveness (Schachter & Blundell, 1974), altered taste parameters (Cooling & Blundell, 1997; Dwyer & Blundell, 1992) and abnormal eating behaviour or obese eating style (Hill & Blundell, 1990). Differences in food intake between lean and obese populations have been difficult to establish as a function of dietary under-reporting, although subtle differences have been detected. Examples of these are the palatability of food appears to be more powerful determinant of food intake in the obese, the obese do not exhibit a slowing effect with increased passing of time at a meal (compared to lean subjects (Blundell & Hill, 1988)). Indeed it has been proposed by a number of authors that obese people have a specific defect in satiety (a weakness in the ability to bring a period of eating to an end) (Blundell, 1977; Hill & Blundell, 1990).

As far as investigating what the potential effects that altered frequency of feeding may have on appetite and satiety, a comprehensive search of the literature failed to produce any such data in obese individuals. Jenkins *et al.*, (1989) found that when lean individuals consumed smaller meals more regularly, their satiety indices were more controlled than when isocaloric meals were gorged less frequently.

In conclusion, the majority of work conducted in the effects of macronutrient and obesity have shown that when food choice is unrestricted and positive energy balance occurs, the gain in body weight is greater when the dietary intake is high in fat. This postulate which suggests that fat has a low satiating efficiency (the consumption of fat has a weak suppressive effect on subsequent food intake (Kissileff, 1984)), coupled to the relative hyperenergetic characteristics of dietary fat, indicates that overconsumption is a formality on a high-fat diet (Cotton *et*

al., 1994). As such, a number of authors have found that the consumption of a high-fat diet leads to passive overconsumption (Cotton *et al.*, 1994; Blundell & Macdiarmid, 1997), and further, Cooling & Blundell (1998) reported that there may be specific phenotypic behaviour that drive the consumption of high-fat foods, and that obese individuals may be more prone to this phenotypic behaviour compared to their lean counterparts.

1.6 THE OXIDATION OF FUELS

The cost of ingesting, absorbing and assimilating fuels is referred to as dietary induced thermogenesis (DIT), and gives an idea of total metabolic demand. Studies looking at DIT have found that after the ingestion of isoenergetic meals, protein elicits the greatest levels of thermogenesis, and fats the lowest. This specificity of the DIT, was formally referred to as the specific dynamic action (SDA) (Prentice, 1998). Studies using whole-body calorimetry have found that there are no real differences in the DIT values of food, irrespective of the macronutrient value of the food consumed (Prentice, 1995; Shetty *et al.*, 1994; Stubbs *et al.*, 1995).

Further evidence exists to show that the oxidation of carbohydrate is closely linked to the amount of carbohydrate consumed, whereas the opposite holds true for fat (Jéquier & Tappy, 1999). It has been shown that carbohydrate overfeeding produced progressive increases in carbohydrate oxidation and total energy expenditure resulting in 75-85% of excess energy being stored (Horton *et al.*, 1995).

Alternatively, fat overfeeding has been shown to exert minimal effects on fat oxidation and total energy expenditure, leading to storage of 90-95% of excess energy (Horton *et al.*, 1995). Clearly, the more fat that is consumed is closely associated to that which is stored (Jéquier, 1993). It has been suggested that those who become obese may be characterised by defects in the ability to oxidise fat (Lichtenstein *et al.*, 1998). Evidence for this is found in post-obese subjects, who appear to oxidise a lesser proportion of energy as fat compared to never-obese subjects (Hirsch *et al.*, 1998; Larson *et al.*, 1995). Horton *et al.*, (1995) found that excess dietary fat

leads to greater accumulation than does excess dietary carbohydrate, and the difference was greatest early in the overfeeding period.

Pre-obese individuals may underoxidise fat relative to other macronutrients, leading to gains in fat mass due to the imbalance between fat intake and oxidation. When fat mass reaches a point where fat oxidation can equal fat intake, stability in fat mass and hence weight can occur. Whether or not there are differences in the rates of fat oxidation have been explored previously: Owen *et al.*, (1992) found these rates to be similar in lean and obese populations, whereas Astrup *et al.*, (1992) found that obese groups had higher rates of fat oxidation reinforces the notion that obesity in some persons may be an adaptation to a high-fat diet in those who have a relative defect in fat oxidation.

The metabolism of glucose is linked to the metabolism of lipids in a reciprocal fashion. When insulin is low (corresponding to the post-absorptive metabolic phase), lipolysis and fatty acid oxidation are increased, whilst glucose uptake and oxidation are decreased (Cooney & Storlien, 1994). Conversely, the ingestion of energy (corresponding to the absorptive phase) increases the availability of glucose, which stimulates the release of insulin. Increased insulin levels inhibit lipolysis (thus activating PDHC (Pyruvate dehydrogenase complex)) and stimulates glucose uptake and conversion to glycogen, pyruvate and CO₂. In the appropriate tissues (liver and adipose tissue) insulin also stimulates conversion of glucose to fatty acids by activating acetyl CoA carboxylase (Randle *et al.*, 1963; Buechler *et al.*, 1984). In the glucose – fatty acid cycle, insulin promotes the use of glucose as the preferred fuel. This is achieved by increasing glucose oxidation in muscle and by inhibiting the

production of free fatty acids (FFA) in adipose tissue leaving fewer FFA's available for oxidation by muscle (Randle *et al.*, 1963). LPL activity declines in muscle in response to elevated insulin, further reducing the potential for utilising fat as an energy source. If left unchecked, reduced insulin sensitivity would promote continued fat accumulation. This would occur when either total energy intake exceeds energy expenditure over time, or when fat oxidation remains low relative to dietary fat intake (Flatt, 1987; Swinburn *et al.*, 1991; Hoag *et al.*, 1995).

1.7 INTEGRATING THE PERIODICITY OF EATING AND APPETITE CONTROL

1.7.i Inter-meal control

Meal pattern analysis has been a corner-stone in the field of metabolic and hormonal perspectives of feeding. In essence, the pattern in which meals are consumed throughout the day defines the relevant time domains of functional feeding behaviour in most animals and humans. Functional feeding behaviour through the day is clearly separated into the processes of meal initiation, maintenance and meal termination, and defined by a set inter-meal interval.

The concept for categorisation of eating episodes in dietary surveys was developed to compare meal patterns (Basdevant *et al.*, 1993; Andersson *et al.*, 1996; Drummond *et al.*, 1996; Bellisle *et al.*, 1997; De Castro, 1997), and a more recent categorisation has been developed that incorporates eating events as four types of 'meals' or four types of 'snacks' due to their combination of food categories, as well as including "quick-prepared" meals (Gatenby, 1997; Lennernäs & Andersson, 1999). The significance of this new format is derived from the increasing trend for western populations to be moving from regular, and planned meals, to more episodic eating "around the clock" (Lennernäs & Andersson, 1999). Such analyses are relevant from a bio-social perspective, and include the dynamics nature of changing feeding behaviour that is characteristic in humans.

FIGURE 1-8 (schematic representation of frequency of eating to rhythms) depicts the changes in appetite and satiety that would occur on a daily basis when a person eats 3 meals in a regulated pattern. This figure should be interpreted as an adjunct to FIGURE 1-7. FIGURE 1-7 depicts the factors leading to the acute eating episode (AEE) which lead to satiation and ultimately satiety. The timing between these AEE's is crucial, and, as a composite, is referred to as the periodicity, or frequency, of feeding.

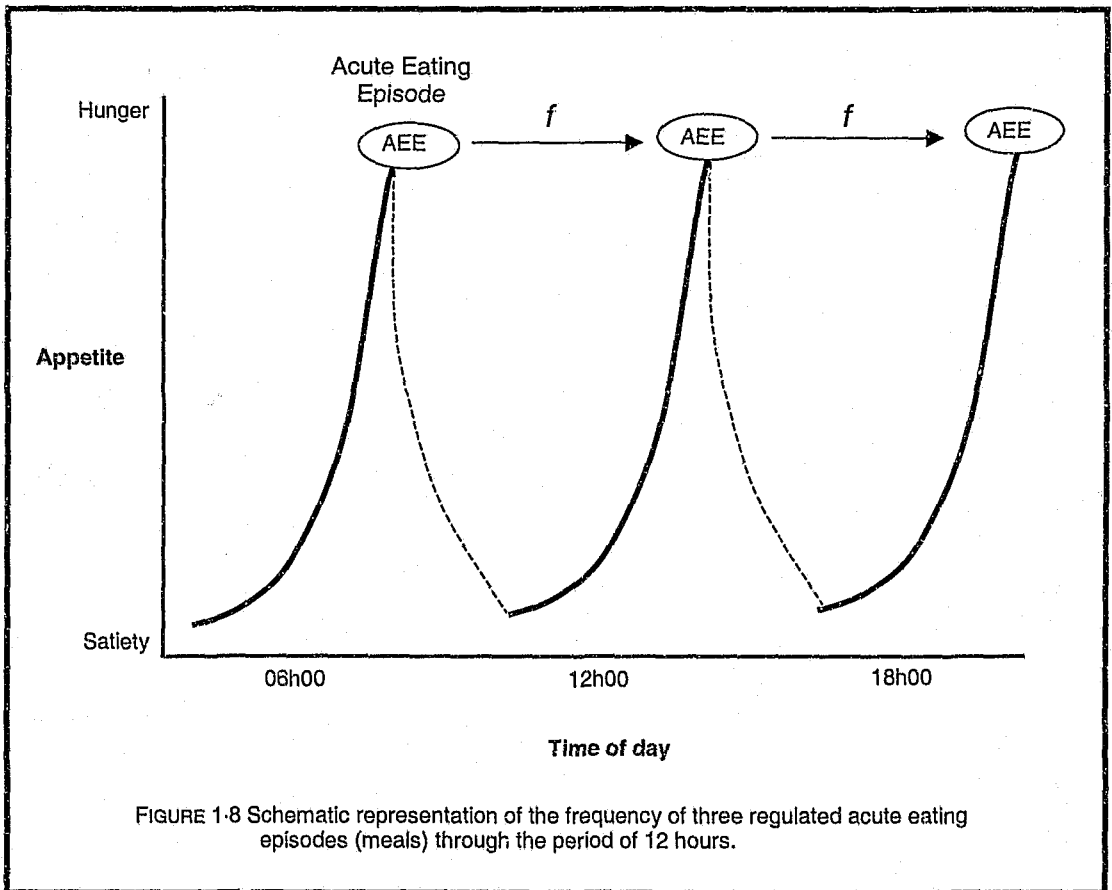


FIGURE 1-8 Schematic representation of the frequency of three regulated acute eating episodes (meals) through the period of 12 hours.

There has been very little work done to date on the periodicity of eating and its influence on the processes of satiation and satiety. It is an attractive, and plausible hypothesis that an increased feeding frequency may exert regulatory effects over these processes. Within this context, one of the key aims of this review was to explore the effects that an altered feeding frequency has over the control of appetite.

Viewed simply, an acute eating episode (AEE in FIGURE 1-7) is brought about by external and internal cues that stimulate appetite. As laid out in TABLE 1-1, there are external and internal cues driving appetite which initiate the eating process. The AEE is a function of the macronutrient value of the food that is being consumed (fat affects the palatability of the food, which affects the volume of the food that will be consumed), and the energetic density (amount of energy) that is consumed in that eating episode. I shall refer to the consumption of energy as an acute eating episode as opposed to a "meal", in that little consensus has been achieved with regards the definition of a *meal* (Gatenby, 1997). The termination of the AEE results in responses of a psychological and physiological nature. As Blundell *et al.*, (1994) point out in their *Cascade Theory of Satiety* (FIGURE 1-6), it is a sensory (both psychological and physiological) process that brings about satiation, and which is highlighted in the schematic representation in FIGURE 1-7. FIGURE 1-6 shows that this cascade is influenced by external, intermediate, and internal responses that bring on the cessation of the eating process when satiety occurs.

In their address to the international workshop convened to discuss the periodicity of eating and human health, Bellisle *et al.*, (1997) showed, with convincing clarity, that the frequency of eating has very little bearing on

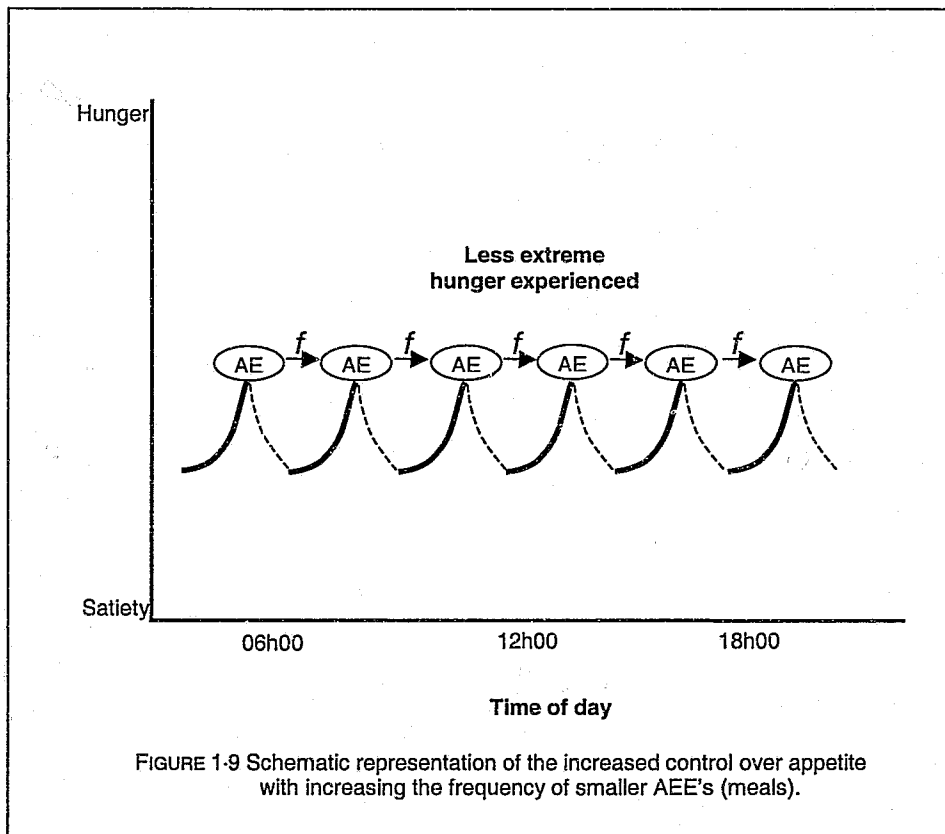
weight loss during intentional energy restriction, and the pattern in which one eats has no significant impact on the energy expenditure (thermogenic effect of food, or diet induced thermogenesis) of an individual as described above. Their final motivation was that any dietary advice pertaining to how often one should eat, should merely consider the effects on carbohydrate and lipid metabolism, as the pattern in which one eats has little or no effect on energy expenditure (Bellisle *et al.*, 1997). Since the general understanding of energy dynamics applied to weight fluctuations are consequential to both energy intake and energy expenditure (Swinburne *et al.*, 1995), the perspective highlighting intake of energy with emphasis on the periodicity of eating was omitted from their paper. Furthermore, the effect of frequency of feeding on appetite was also not addressed.

"The amount of food eaten from the onset to the termination of a meal is mainly determined by the peripheral, ie oral and gastrointestinal action of the ingested foods. Meal to meal intervals, and therefore the meal frequency are mainly dependent on post-absorptive and metabolic factors".

Le Magnien & Devos (1984)

FIGURE 1-8 shows the temporal swings in appetite and satiety ratings through the day when a person would eat on three occasions, with a set period, t , between each eating episode.

In addressing the hypothesis that the frequency of feeding, or altered f values, influence appetite, FIGURE 1-9 may be drawn to depict more frequent AEE's of smaller energy density (but in total isoenergetic), which may effect reduced oscillations in hunger and satiety (compared to fewer, larger meals of FIGURE 1-8). If this is indeed the case, the consuming isoenergetically-density meals that are divided into smaller, but more frequent, meals may enhance appetite.



The reality of meal consumption, however, does not occur in a vacuum where identical meals may be consumed in such a controlled manner. The analysis of meal ingestion needs to address a plethora of factors that

trigger the drive to eat. When the cues that induce appetite are considered, as TABLE 1.1 highlighted earlier, there are 'cues' that contribute to the induction of an eating episode (external and internal). These cues have been shown to be a function of a circadian rhythm (Waterhouse, 1994). Needless to say, any investigation into feeding patterns should address the circadian rhythms that influence behaviour.

1.7.ii Relating circadian rhythm to the periodicity of eating

The term *circadian rhythm* was first coined by Franz Halberg (1959) from the Latin *circa* = about, and *dies* = day, which was used to describe the approximately 24-hour cycles that are endogenously generated by an organism. The term *zeitgeber* is German and literally means, "time giver" – inferring it influences the circadian rhythm of physiological function.

The central issue linking circadian rhythm to nutritional physiology is whether a *zeitgeber* exists for the intake of food. It has been shown that there are circadian rhythms of food intake in humans, as well as patterns for gastrointestinal functions like gastrointestinal motility, the secretion of digestive juices and absorption of digested foods (Waterhouse *et al.*, 1997), and of concentrations of carbohydrates, amino acids and lipids in the bloodstream (Lavie, 1980; Mejean *et al.*, 1988). Essentially, the origins of these rhythms lie in the individual's set habits and social norms which are a function of the external cues depicted in TABLE 1.1. The essential question at this stage is whether these rhythms reflect endogenous oscillatory drives. When we eat, and the types of foods that we eat, are determined by conditioned reflexes, routine, and by a gut feeling that "it is

time to eat", rather than by biological need (Aschoff *et al.*, 1986; Waterhouse *et al.*, 1997).

Are the patterns of energy intake in adults intrinsically determined, are they learned, or are they conditioned? Feeding neonates on demand was associated with rises in their heart rate, blood pressure, and general behavioural activity (Weinert *et al.*, 1994). Based on these findings, we are left to ponder whether the baby's desire for food reflects the activity of some internal ultradian oscillator, which also influences cardiovascular system. It has been shown that the periodicity of feeding (± 4 hours) exists throughout the 24-hours, but that this could have reflected the accumulation of a drive for energy replacement rather than the cycling of some internal oscillator. It remains that if the drive for food were wholly due to energy replacement, then changing the energy value of the food eaten would produce a proportional change in the interval between feeds; but if the drive were due only to an oscillator then the timing of food intake would be unchanged, even though the amount taken would vary inversely with its energy content.

John de Castro found that there were intrinsic patterns of food consumption even in one-year old infants, he was unsure whether or not these were socially induced or whether they were intrinsic to their physiological function. He has further observed, however, that similar trends occur in free-living individuals (de Castro, 1990). These data however, need to be interpreted with caution due to the huge discrepancy based on dietary under-reporting, as depicted overleaf (Bandini *et al.*, 1990; Lichtman *et al.*, 1993; Prentice & Poppitt, 1996; Bellisle *et al.*, 1997, Poppitt *et al.*, 1998; Voss *et al.*, 1998).

1.7.iii Terminology and definitions on the frequency of feeding

The literature is filled with terminology's to describe the varied feeding frequencies that scientists have formulated according to their respective protocols. Hollifield & Parson (1962) referred to the two extreme feeding patterns as "stuff and starve" when describing their studies of adipose tissue and liver glycogen in rats that were limited to a short daily feeding period. When the terms "nibbling" and "gorging" are considered, the first refers to many smaller meals as opposed to fewer larger meals referred to by the latter. The terms first make their published debut's in *The Lancet* when Gwinup *et al.*, (1963) had individuals "gorge" on meals a day, compared to "nibble" on ten meals a day. As Southgate (1991) points out that it stretches the English language to some degree when one describes eating a meal of 750 – 1,000 kcal (3.14 – 4.18 MJ) over 3 sessions in a day as gorging. Further, Jenkins *et al.*, (1989) refer to "nibbling" as eating 17 meals in a 24-hour period, which is not the connotation one has of "nibble" in the strictest sense. Southgate (1990) proposes the use of the term "grazing" when one refers to an increased frequency of feeding.

The term "meal" requires further consideration: what constitutes a meal? How much food should be eaten before it is considered a meal? Should the food meet certain requirements in terms of macronutrient parameters, are liquid meals sufficient to meet these requirements? Should a meal have a minimum amount of fibre? This is of prime importance when one considers the absorption of nutrients into the system following varied meal patterns. Many studies addressing issues in appetite regulation have looked at altered meal frequency.

1.7.iv Historical perspective on the periodicity of eating

The early part of the 20th century saw the emergence of some understanding into the effects of eating patterns on the physiological responses to given meals. Hamman & Hirschman (1919) first showed that the prior ingestion of glucose resulted in an increased tolerance to a subsequent glucose load. This phenomenon, the "Staub Effect" since Staub (1921) postulated that if a normal healthy person is given two glucose loads 30 minutes apart, the blood sugar level at 60 minutes should be no higher than that obtained at 30 minutes. Staub noted that this phenomenon was due to the "priming effects" of the initial load: essentially, it is these "*priming effects*" of an initial dose that forms the basis of all periodicity of eating studies, since it is priming of the consequent meals after the initial meals that elicit the responses found in the literature. Ellis (1934) noted that when NIDDM patients are given 10-30g doses of glucose hourly with a constant infusion of insulin, they experienced reduced insulin requirements, with the corresponding increases in blood glucose control. It seemed, under those conditions, that carbohydrate utilisation is increased by mechanisms other than increased insulin secretion.

Although not specifically noted at the time, this is the first reference (albeit indirectly) to improved insulin sensitivity as a consequence of the related periodicity of eating. Over 60 years have passed since these findings, and the bulk of the work done to date has shown with remarkable consistency that a strong negative correlation relationship exists between the number of meals a person ingests through the day and the associated state of general health (Bray, 1970).

1.7.v Adaptations to the frequency of feeding

When considering the following physiological adaptations to the different frequencies of feeding in various species, it must be pointed out at this stage that the macronutrient composition of the diet, particularly the concentration and form of the carbohydrate, is a crucial factor in regard to the observed responses to meal-feeding relative to a nibbling diet.

TABLE 1-2 Physiological adaptations to different feeding frequency regimes.

Animal Model	Gorging	Nibbling
Morphological		
Rat		Increased absolute weight of stomach with hypertrophy of mucosa & musculature (Jackson, 1915).
Histological		
Rat		Intestinal tissue shows annular space increases by up to 18% (Lojda & Fábry, 1959). Enlargement of the intestine, and the associated increased surface area for absorption, appears to be a functional adaptation to increased frequency of feeding (Adams & Morgan, 1981).
Metabolic		
Rat		Enhanced rate of glucose absorption (Kujalova & Fábry, 1960) due to increased weight of intestine.

TABLE 1.2 Physiological adaptations to different feeding frequency regimes (continued).

Animal Model	Gorging	Nibbling
Metabolic		
Humans	Impaired glucose tolerance (Jenkins <i>et al.</i>, 1989; 1991). Enhanced phosphoenolpyruvate carboxylase to shunt gluconeogenesis in between meals (maintain glycaemic normality). Enhanced efficiency at converting carbohydrate to fat (Chakrabarty & Leveille, 1968). Enhanced activity of the pentose pathway enzymes (Hollifield & Parson, 1962). Hypercholesterolaemia (Jenkins <i>et al.</i>, 1989) Increased activities of hexokinase, pyruvate kinase, alpha-glycerophosphate, acetyl-CoA carboxylase in adipose tissue (Leveille (1970) Increased activity of enzymes (pyruvate carboxylase & malic enzyme), and the hexose monophosphate shunt (G-6-P dehydrogenase), that supply reduced NADPH necessary for FA synthesis. Higher rates of fatty acid synthesis (Leveille, 1967a; 1967b; Baker <i>et al.</i>, 1976) Accelerated lipogenesis (Leveille & O'Hea, 1967; Hollifield & Parson, 1965) Energy expenditure higher postprandially (possibly due to DIT) (Verboeket-van de Venne & Westerterp, 1991).	No difference in 24 hour energy expenditure as a function of frequency of eating (Dalloso <i>et al.</i>, 1982; Verboeket-van de Venne & Westerterp, 1991).
Weight		
Rat	No difference in weight when isoenergetic intake either as two meals or 12 meals (Hill <i>et al.</i>, 1988)	
Humans	119 obese subjects lose 78g per day eating 3 meals per day (Debrey <i>et al.</i>, 1973). No significant relationship between frequency of feeding and weight loss (Bortz <i>et al.</i>, 1966; Finkelstein & Fryer 1971; Young <i>et al.</i>, 1971; Verboeket-van de Venne & Westerterp, 1993). 86 obese men (BMI $37.7 \pm 4.4 \text{ kg}\cdot\text{m}^{-2}$) consume 5.3 meals per day compared to 5.3 meals per day in observed in lean men (BMI $23.0 \pm 1.9 \text{ kg}\cdot\text{m}^{-2}$) (Andersson & Rossner, 1996). 72 subjects (BMI $> 25 \text{ kg}\cdot\text{m}^{-2}$) consume 5.6 meals per day (Crawley & Summerbell, 1997).	119 obese subjects lose 142g per day eating 7 meals per day (Debrey <i>et al.</i>, 1973). 288 subjects (BMI $< 20 \text{ kg}\cdot\text{m}^{-2}$) consume 6.2 meals per day (Crawley & Summerbell, 1997).

1.7.vi Energy regulatory considerations

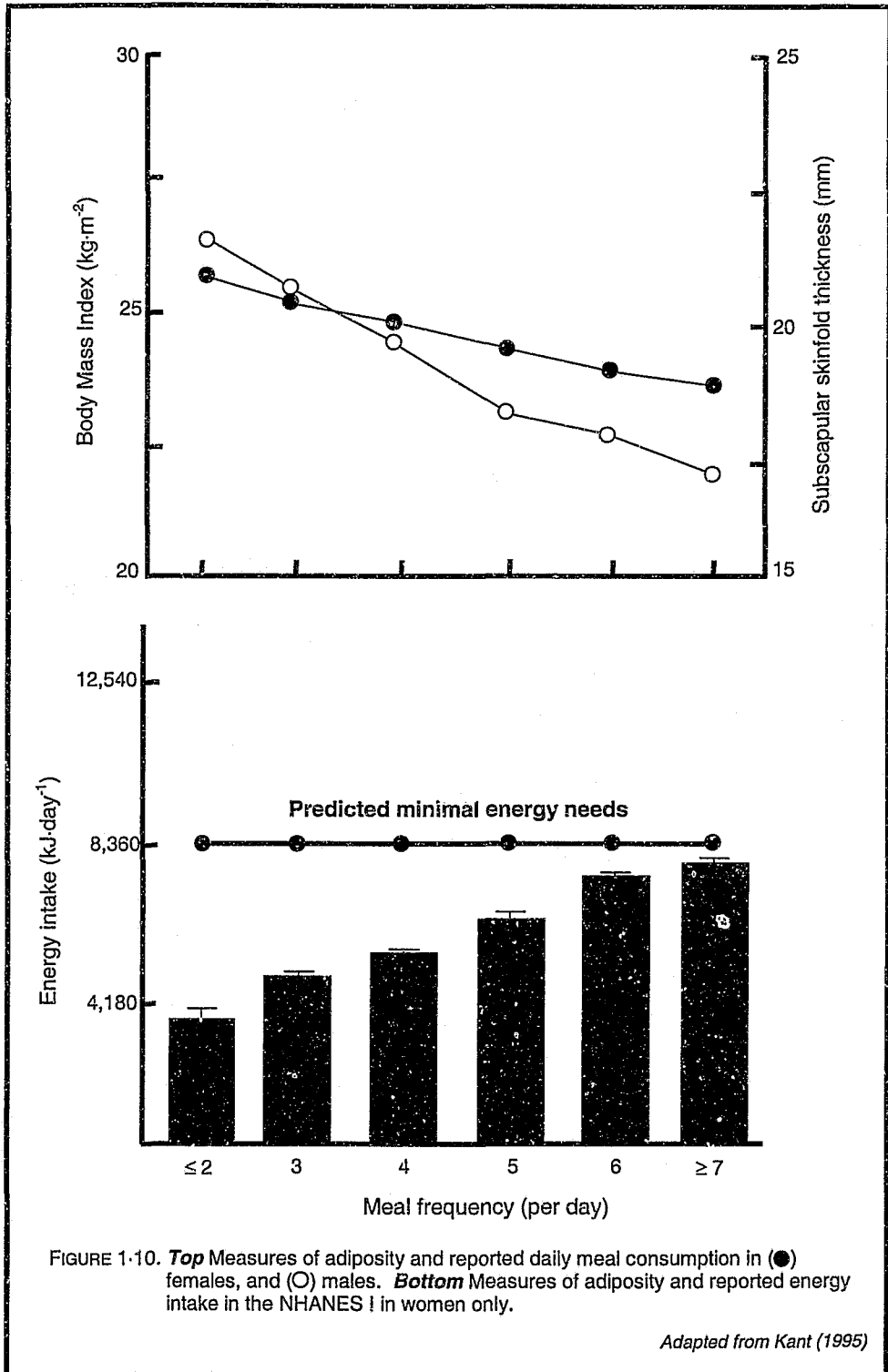
Most of the work done to date on the periodicity of eating suggests an inverse proportional relationship between the number of meals eaten and the relative amount of weight gained. It was shown that intermittently fasted rats had higher proportions of body fat compared to their controls (Fábry *et al.*, 1962; Fábry *et al.*, 1964a; Fábry *et al.*, 1964b). Adams & Morgan (1981) suggest that the force-fed animals more efficient in converting ingested calories to stored energy in the form of body fat. There have been numerous studies that have been done since to support these data. However, the results that have been published on the periodicity of eating (in particular to energy metabolism) has been criticised by Leveille, in that he suggests that three fundamental parameters should be considered before any validation of these studies can be made: firstly, amount of energetic restriction of diet; secondly, duration of the study; and thirdly, initial adaptation period (Leveille, 1970).

In their review on meal frequencies, Fábry & Tepperman (1970) listed some functional and morphological changes that occur due to more frequent intake of isoenergetic meals. Striking observations were that the GIT displays marked functional and morphological changes including increased activities of digestive enzymes of the pancreas, increased enzyme activities in the intestinal mucosa (Lojda & Fábry, 1959), and an increased rate of glucose absorption from the intestine. They found increased evidence to support the notion of habitual adaptation in various animals to feeding patterns: that adapted animals are well equipped to meet the increased needs of dissimulation, interconversion, and deposition of biological materials supplied by the periodic loads of food (FÁBRY &

Tepperman, 1970). Of particular importance was the observation that there is an increased capacity for fat formation from carbohydrate precursors - a complex set of changes referred to as "*adaptive hyperlipogenesis*" (Tepperman & Tepperman, 1964; Tepperman & Tepperman, 1965). Insulin seems to participate in the hyperlipogenesis in at least two ways: increased amount of carbohydrates ingested within a short period result in periodic short-termed hyperinsulinaemia; and secondly, the adipose tissue of intermittently-fed animals is more sensitive toward the lipogenesis-stimulating effect of insulin.

Furthermore, infrequent loads of food induce complex changes described as the *adaptive hyperlipogenesis*, which, together with a positive energy balance may play a part in the development of obesity (Tepperman & Tepperman, 1965). It has been postulated that infrequent food intake decreases the average energetic energy expenditure by decreasing voluntary activity - the activity that may be the essential portions of the energy homeostasis when considering the effect of infrequent meals in the development of obesity. When the effect of meal frequency on the adaptation to overeating was compared, it was found that if the hyperenergetic daily ration was served in two large meals, the subjects gained weight to the degree of their energetic excess, whereas subjects to whom the ration was served in either three meals with additional snacks making up 14 small portions were able to maintain weight (Miller *et al.*, In Fábry & Tepperman, 1970). These authors (Verboeket-van der Venne & Westerterp, 1991) place the emphasis on the diurnal consideration in that energy intake is only possible during the eating hours, and that energy expenditure is drastically reduced during sleeping hours.

Although Debrey *et al.*, (1973) found that 119 obese subjects lost 78g per day when they ate 3 meals per day compared to 142g per day eating 7 meals per day, the bulk of the literature suggests that no difference exists in weight change when isoenergetic diets are consumed either as two meals or 12 meals (Bortz *et al.*, 1966; Finkelstein & Fryer 1971; Young *et al.*, 1971; Hill *et al.*, 1988; Verboeket-van de Venne & Westerterp, 1993). Andersson & Rossner (1996) found that 86 obese men (BMI $37.7 \pm 4.4 \text{ kg}\cdot\text{m}^{-2}$) consume 5.3 meals per day compared to 5.3 meals per day in lean men (BMI $23.0 \pm 1.9 \text{ kg}\cdot\text{m}^{-2}$). In a similar trial, Crawley & Summerbell, (1997) found that 72 subjects (BMI $> 25 \text{ kg}\cdot\text{m}^{-2}$) consumed 5.6 meals per day compared to the 6.2 meals consumed per day by 288 subjects (BMI $< 20 \text{ kg}\cdot\text{m}^{-2}$).



1.7.vii Metabolic considerations

Hollifield and Parson (1962) investigated the effect of varied feeding-frequency patterns on metabolic profiles in experimental rats. Rats, which are essentially foragers (i.e. they eat consistently while awake), that were made to eat an unnatural one meal (of as much food as they could eat) in two-hours-a-day over a seven day period showed the following characteristics: 1. Initially lost weight in the first 3-4 days, but weight returned to within 10% of initial levels by day seven. 2. G-6-P d-ase, and 6-P-G d-ase activity in adipose tissue in these animals increased over 200% by day 5, as well as increases in acetate-1-C14 into lipids from liver slices. 3. The FFA content of adipose tissue fell as the rate of lipogenesis in adipose tissue increased.

This adaptation is understandable in view of the limited capacity of animals to store excess energy in any form other than fat. The periods of fasting between meals necessitate adaptation to the provision of a continued supply of glucose for metabolic processes in which carbohydrate is indispensable. Thus, during periods of fasting some of the enzymes concerned in the elaboration of blood-glucose are increased: G-6-P-ase in the liver is one such enzyme (Ashmore & Weber, 1959).

Individuals who "gorge" themselves are forced to endure periods of "mini-fasts" which actually mimic the physiological responses to fasting. Work done to date on this phenomenon reveals that the long "fasts" between "gorges" apparently leads to metabolic adaptation that encourages storage of energy both as fat and glycogen, with increased activity of the hexose monophosphate shunt enzymes glucose-6-phosphate dehydrogenase and

6-phosphogluconate dehydrogenase - both favour lipogenesis, and of many of the enzymes involved in hepatic gluconeogenesis (Cohn & Joseph, 1960; Tepperman & Tepperman, 1961; Bray, 1970).

When the enzyme responses to re-feeding after starvation are considered, it has been postulated that hepatic and skeletal muscle pyruvate dehydrogenases (PDH) are activated only after a latent period of a few hours (Sugden *et al.*, 1989). As a result, the consequences of such a phenomenon would favour glycogen deposition from dietary glucose, with the endogenous 3-carbon fragments would be enhanced by the failure of the immediate reversal of starvation-induced increases in the phosphoenolpyruvate carboxykinase activity. PDH and phosphofructokinase (PFK), enzymes that supply Acetyl CoA substrate for cholesterologenesis, are insulin-sensitive (Kellet *et al.*, 1986). Insulin release post-feeding therefore serves to promote the biosynthetic enzyme activity directly, and the movement of carbon substrate through glycolysis toward biosynthesis. Furthermore, it appears that fatty acid oxidation may continue in the early hours of re-feeding after starvation when fed eucaloric meals (Kellet *et al.*, 1986).

When rats are forced-fed 100% ADER only once a day, they became obese, hypercholesterolaemic, and hyperglycaemic (Adams & Morgan, 1981). Furthermore, when the nitrogen metabolism is considered, rats that eat once a day were shown to excrete 37% more urea than those rats that nibble *ad lib* through a day. In considering the Staub effect (Staub, 1921) where it was shown that one glucose load has an impact on how well the body tolerates the next glucose load, and further the resultant increased insulin sensitivity. The literature is filled with the beneficial factors of increased meal frequency on hormonal profiles (Jenkins *et al.*, 1989; 1992), enhanced control over blood metabolites (Fábry & Tepperman, 1970; Bray, 1970), and triglyceride concentrations (Bray, 1970; Jenkins *et al.*, 1989). Although a few have investigated the effects of periodicity of eating and energy metabolism (Cohn, 1961; Leveille & Chakrabarty, 1968a; Cohn & Joseph, 1970; Young *et al.*, 1972; Wadha *et al.*, 1973; Schoenborne & Canolty, 1980), the results have revealed contradictory findings. In 1961, Cohn found that the carcasses of rats fed twice daily via stomach-tube contained almost twice the amount of body fat as those of animals freely eating the same amount of food (Cohn, 1961). He was able to reinforce this finding in the following years with by tightly controlled studies investigating the effects of altered feeding frequencies on body composition in rats (Cohn 1963; Cohn & Joseph, 1968). Furthermore, higher concentrations of lipids and glucose were noted in the serum of the two-meal animals, and their urine contained more nitrogen (Cohn, 1961). Using energetic intakes that were required to maintain body weight, Gwinup *et al.*, (1963) postulated that the process of "nibbling" results in frequent stimulation of the pancreatic islets, which might result in an increased capacity to synthesise and/or release insulin in response to glucose.

1.7.viii Periodicity of eating and the Thermic Effect of Food (TEF)

Adams & Morgan (1981) suggested that meal frequency might be a possible factor in the regulation of energy balance both in experimental animals, and in humans. The thermic effect of food (TEF), or postprandial thermogenesis, has been defined as the increase in heat production that occurs 0-8 hours after the ingestion of a meal. The TEF represents that amount of energy that is required for the transformation of dietary substrates into usable metabolites and for storage of excess fuel (Woo, 1985). Having studied the effects of the size of a meal on the TEF, and having found that TEF increases a linearly with meal size, Hill *et al.*, (1988) further suggested that meal frequency might be an important determinant influencing energy expenditure. Three years later, Hill *et al.*, (1988) tested that hypothesis: the results indicated that continuous enteral administration of a diet at very low rates produced little or no effect.

The findings with regards to the effects of variable frequencies of feeding on the TEF are somewhat precarious to say the least. Nacht *et al.*, (1986) found that when an enterally administered liquid meal is given either as a single-bolus dose or during 3 hours of nasogastric feeding in healthy humans, the single dose elicited a greater TEF compared to the continuous administration. Contrary to these findings were those of Belko *et al.*, (1988) who compared 10-hour TEF's of two isoenergetic dietary trials either given as two meals, or as four meals: there were no differences in the TEF's between the two feeding patterns. Tai *et al.*, (1991) showed that when seven healthy normal-weight young females are given an isoenergetic energy intake in one meal, or the same energetic ingestion

over five smaller meals (every 30 minutes for 3 hours), the single large-meal costs more to digest and absorb.

The effects of meal frequency on TEF remain uncertain: the bulk of the evidence though rests with the notion that the more frequent the meals, the lower the TEF. Furthermore, it remains to be seen what these effects are when the aspect of obesity are taken into account (i.e. how do lean individuals deal with their meals as opposed to obese individuals?).

DIT is a measure of the heat energy produced as a by-product of the digestion and metabolism of food. It has been reported that one or two hours after ingestion of a meal, the thermogenic effect can increase the metabolic rate by as much as 40% above the basal rate (Drummond *et al.*, 1996; Carrow, 1978). Most others however, have failed to find a relationship similar to this, in fact most reports suggest that the frequency of feeding has no effect at all on metabolic rate (Belko & Barbieri, 1987; Verboeket van de Venne *et al.*, 1993, Prentice *et al.*, 1994). In those that have found a relationship, report it to be so small that it may not necessarily contribute significantly in the control of body weight (Dalloso *et al.*, 1982, Belko *et al.*, 1987; Kinabo & Durnin, 1990).

1.7.ix Cardiovascular considerations

When the cardiovascular consideration is taken into account, it has been shown above that serum cholesterol concentrations are inversely related to the frequency of the meals. In addition to this, is the finding that the percentage of subjects in which ischaemic heart disease (IHD) was diagnosed (subjects with angina pectoris of grades I or II or a history of pain compatible with myocardial infarction or with electrocardiographic changes pointing to a probable ischaemic lesion of the myocardium) decreased significantly with the increased meal frequency from 30.4% in the subgroup with an infrequent meal pattern to 19.9% in the sub-group taking five or more meals per day (Fábry *et al.*, 1968).

The concern over serum cholesterol was investigated in 1965 where Kudicka *et al.*, (1966) found that the decline of serum cholesterol in obese patients treated with a 1,000kcal diet was greater during periods when they ingested the daily food in eight meals of different size, than in periods when it was served in two 500-kcal meals. These findings were reinforced by Gwinup *et al.*, (1963); Jagannathan & Gopalan (1963); and Irwin & Feely (1967).

In a landmark study, Jenkins *et al.*, (1989) compared the difference between nibbling and gorging: they found that when seven normal men nibbled 17 meals through the day, they had reduced serum concentrations of total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B compared to when they ate the same amount and quality of food only three times per day. Although there were no significant differences in

hormonal profiles, these authors noted that the effect of nibbling reduced insulin concentrations by 27.9% (Jenkins *et al.*, 1989).

Jones *et al.*, (1993) reinforced these findings when they reported that when 6 healthy non-diabetic men were subjected to six evenly spaced liquid meals per day over a three-day trial period, their circulating glucose-dependent insulintropic polypeptide (GIP) and insulin levels reduced, with the concomitant drop in serum cholesterol compared to 6 control individuals who received the same amount of food three times per day over the same period. It was speculated by these authors that the small meals consumed by the more frequent-feeding group may have suppressed glucose release into the portal circulation, thereby blocking the stimulation of insulin, either directly by not achieving the threshold required for release or indirectly through suppression of GIP (Jones *et al.*, 1993).

Having reported this finding, they set out to determine the effect of continual nibbling (as opposed to eating 3-meals per day) on circulating insulin levels in NIDDM patients: the key finding was similar to that found previously with a 9.2% reduction in insulin concentrations (Jenkins *et al.*, 1992). This reduction in insulin concentration with nibbling implies an increased insulin sensitivity which prompted them to conclude that spreading the nutrient load over the day would be beneficial in the treatment of NIDDM (Jenkins *et al.*, 1992).

There are other physiological benefits to spreading the nutrient load, and although the cholesterol-lowering mechanism as a function of meal frequency is beyond the scope of this review, it may have significance in this discussion since cholesterologenesis has been shown to be hormonally regulated (Williamson *et al.*, 1985). Chait *et al.*, (1979) showed that insulin enhances the action of [^{14}C]acetate incorporation into cholesterol in skin fibroblasts. Since we have noted that increased meal frequency reduces serum cholesterol, it follows that increased meal frequency should result in reduced serum cholesterol concentrations. Only one investigation has shown that meal frequency has no impact on mean diurnal triglyceride levels and cholesterol (Terpstra *et al.*, 1978). These authors did find an overall lowering effect in both TG and cholesterol levels after the last meal of the day, however, this was not a function of meal frequency but rather a function of diurnal fluctuations.

1.8 ENDOCRINOLOGICAL CONSIDERATIONS ASSOCIATED WITH OBESITY

1.8.1 Endocrinological considerations and adipose tissue

The excessive adipose tissue of obesity may be stored either in the abdominal area (upper body or *android* obesity) or in the gluteal-femoral region (lower body or *gynoid* obesity) (Vague *et al.*, 1971; Vague *et al.*, 1974; Kopelman, 1994). Differences have been found in rates of lipolysis in adipose tissue fragments obtained from different anatomical sites (Vague *et al.*, 1974). Human adipose tissue is richly endowed with α and β adrenoreceptors – binding of agonists to β receptors enhances lipolysis whereas agonists that bind α_2 receptors inhibit lipolysis (Kopelman, 1994). A detailed analysis in men and women suggests that the usual pattern of male fat distribution reflect greater α_2 activity in the abdominal tissue of men (LaFontan *et al.*, 1975). There is evidence that lipoprotein lipase (LPL) plays a controlling role in the regional distribution of fat (Cigolini & Smith, 1979). The biochemical mechanisms regulating adipose tissue LPL activity are not completely understood, but insulin is permissive for LPL synthesis and the glucocorticoids enhance the activity of LPL when added *in vitro* with insulin (Cigolini & Smith, 1979).

The '*abnormal*' state of obesity calls for physiological adaptation to facilitate normal physiological function. These adaptations are fairly well documented: increased muscle mass to carry the excess adipose tissue (Jéquier & Schutz, 1983), and altered states of cardiovascular function, an example of which is the increased blood pressure required to drive blood

volume over an increased area (Mikhail *et al.*, 1999). Although the pathologies and their aetiologies are beyond the scope of this review, the manifestations of an endocrinological dysfunction are most pertinent in the understanding of energy dynamics linked to obesity. The hormones most closely associated with energy dynamics are insulin, glucagon, leptin, and cholecystokinin (CCK). These hormones are linked primarily to the feeding process, and upon ingestion of energy, serum insulin is the first of the hormones secreted to allow for the transport of glucose from the blood into the cells (Vander *et al.*, 1997). This hormone is released from the β cells of the pancreas in response to elevated concentrations of glucose in the blood. The half-life of insulin is 6-7 minutes (Kostecka & Blahovec, 1999), and its function is to transport glucose into the cell from the blood stream. It does this by binding to specific sites on the cell membrane and allows for the active transport of glucose into the working cell. It was earlier postulated that glucose did not need insulin to move into the brain cells (Horn *et al.*, 1984; Vander *et al.*, 1997), but it has recently been suggested that it does, (Woods, 1997).

1.8.ii Hyperinsulinaemia and Obesity

Serum insulin is often chronically elevated raised in the fasting state, which has been referred to as the functional *hyperinsulinaemia* of obesity (Kolterman *et al.*, 1980; Kopelman, 1994; Koyama *et al.*, 1997; Ferrannini *et al.*, 1999; Jéquier & Tappy, 1999). In an epidemiological study into hyperinsulinaemia, the San Luis Valley Diabetes Study, where the average BMI study was $25 \text{ kg}\cdot\text{m}^{-2}$ at baseline, and the average weight gain was 1.1kg over 4.3 years (Hoag *et al.*, 1995), this study revealed "high" fasting

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levels of 89.3 ± 51.4 pM in males and 85.3 ± 55.1 pM in females. Franssila-Kallunki *et al.*, (1991) found that 12 pre-menopausal obese subjects ($\text{BMI} = 33.4 \pm 1.1 \text{ kg}\cdot\text{m}^{-2}$) had fasting insulin levels of 137 ± 22 pmol/l. These authors found that these elevated insulin concentrations could be reduced with weight loss: upon reduction of BMI's to $29.9 \pm 1.0 \text{ kg}\cdot\text{m}^{-2}$, their insulin levels fell to 79 ± 14 pmol/l.

The cause of basal hyperinsulinaemia is still unclear: it may be due to overstimulation of the β cells of the pancreas or to the reduced hepatic clearance, or a combination of both. Morrel *et al.*, (1988) presented findings after exposing obese and lean mice to a conditioned olfactory stimulus prior to and during eating, and showed that a state of hyperinsulinaemia could be induced via classical conditioning in the ob/ob mouse. They further observed that although hyperinsulinaemia is induced in the "trained conditioned" animals, it is more marked in the obese animals, and that a cholinergic mechanism may have been involved in the characteristic of this animal. These results suggest that hyperinsulinaemia may be a trained phenomenon, and that the chronic positive energy balance which induces the state of obesity, may inevitably force the body to deal with these excesses by reducing insulin action (Eckel, 1992).

The significance of hyperinsulinaemia in obesity needs to be addressed on two integrated levels. Firstly, as the pathological adaptation to the chronic excessive energy intake that is representative of the obesity syndrome, and secondly, the possible disruption that such a reduced insulin sensitivity (as a manifestation of the insulin resistant state) could have on appetite function.

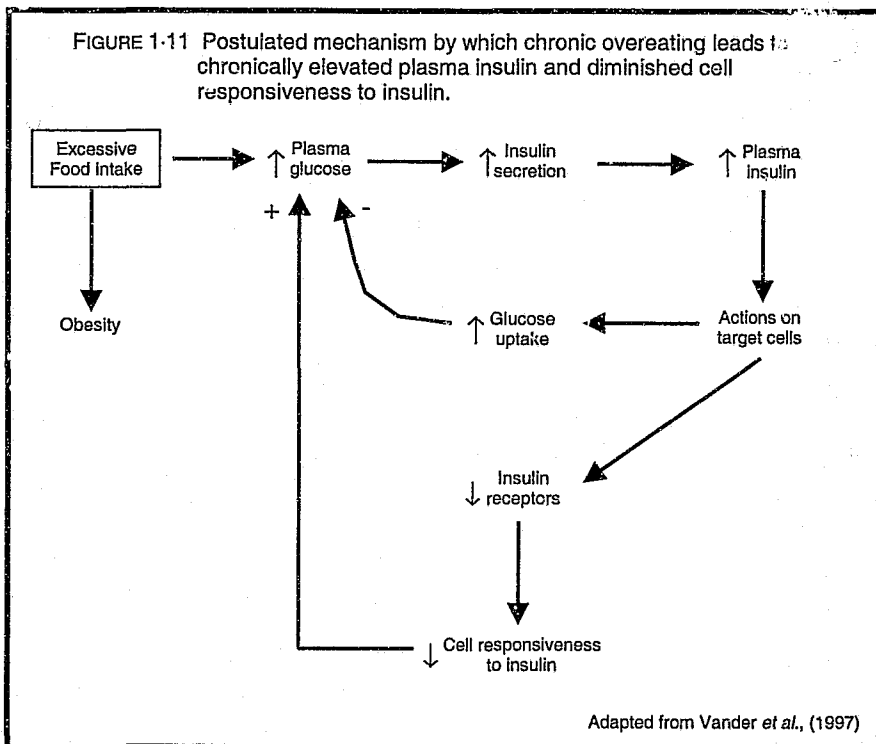
1.8.ii.a Hyperinsulinaemia as the *functional adaptation* to obesity

Obesity itself leads to an increased demand for insulin, and induces a diabetic-like state (Karam *et al.*, 1968; Faber *et al.*, 1981; Ferrannini *et al.*, 1991; Skelton & Skelton, 1992; Koyama *et al.*, 1997). The ingestion of food invokes the response of an acute insulin secretion from the β -cells of the pancreas to enable the transportation of glucose from the ECV into the working cell (Vander *et al.*, 1997). Using this premise, it may be plausible that frequent over-eating may result in a chronically elevated blood insulin level. This is a consequence of the fact that insulin levels do not have sufficient time to return to basal values after the post-meal insulin spike. (Bjorntorp, 1995).

Hyperinsulinaemia is a common feature in patients with upper-body obesity compared to lower-body obesity (Ferrannini *et al.*, 1991; Ferrannini *et al.*, 1999; Gould *et al.*, 1999). Unlike peripheral fat, which is mobilised during such states as pregnancy and lactation, abdominal fat is in a constant state of lipolysis, during which it is broken down into fatty acids and then reconstituted (Skelton & Skelton, 1992). Released free fatty acids enter the portal circulation and are deposited in the liver where they interfere with hepatic insulin extraction (Bjorntorp, 1995). The fasting state of hyperinsulinaemia in obese individuals is characterised by elevated basal insulin secretion (Kolterman 1980; Faber *et al.*, 1981). Normally, 50-80% of insulin secreted by the pancreas undergoes first-pass extraction by the liver (Vander *et al.*, 1997). Infiltration of the liver by fatty acids interferes with this process, and the net result being that insulin enters the systemic circulation, and hyperinsulinaemia occurs. Faber *et al.*, (1981) have shown

that the fasting hyperinsulinaemia observed in obesity is solely the result of the hypersecretion of insulin from the β cells.

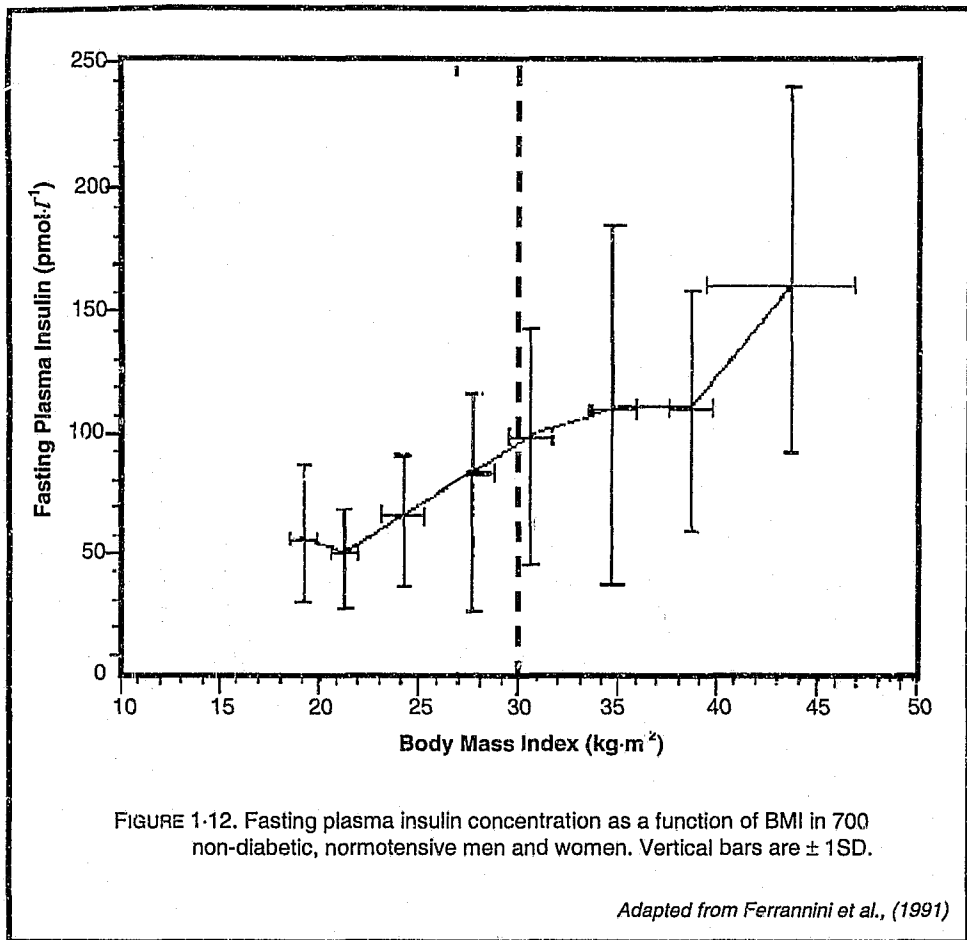
Vander *et al.*, (1997) have proposed the mechanism of insulin insensitivity as follows: insulin secretion is increased during ingestion and absorption of food, and the chronic overeating causes an increased secretion, on average, of insulin (Vander *et al.*, 1997). The resulting elevation of plasma insulin over time induces a reduction in the number of insulin receptors; thus, insulin itself, is responsible for the decrease in target-cell responsiveness.



As described above, and represented in FIGURE 1-11, insulin has specific receptors on peripheral tissue cells, and it is the *down-regulation* of the insulin-receptor function in obesity that may lead to a reduced receptor-

dependent insulin degradation (Vander *et al.*, 1997). These authors proposed that it is this receptor-degradation that may contribute to the relative increase in the incremental insulin area in obese subjects (Faber *et al.*, 1981; Ferrannini *et al.*, 1999).

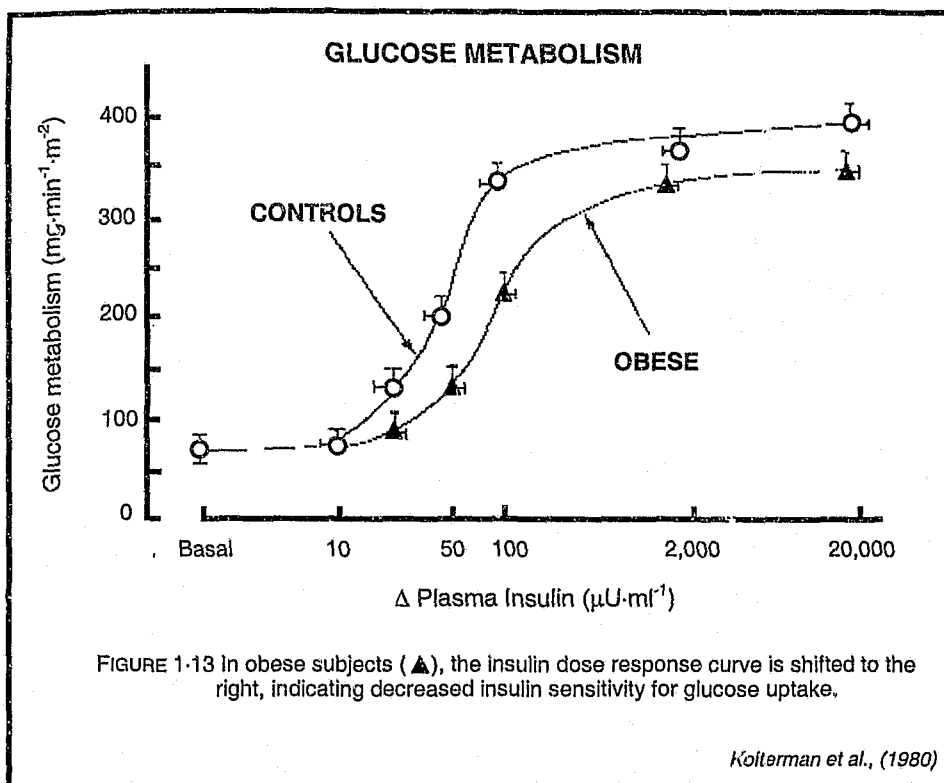
In an attempt to examine whether fasting insulin concentrations were associated with weight gain, Gould *et al.*, (1999) conducted a longitudinal (over 4.4 years) population-based cohort study in 767 subjects. These authors found the mean weight gain in this population was 2.17kg for men, and 2.49kg for women, and that fasting insulin levels correlated with baseline weight ($r = 0.32$, $P < 0.001$). Furthermore, the baseline fasting insulin was positively correlated with percentage increase in the waist-hip ratio ($r = 0.12$, $P < 0.01$). These authors concluded that the fasting hyperinsulinaemia was associated with an increase in waist-hip ratio over time (Gould *et al.*, 1999), which reinforce the relationship between fasting insulin concentration and BMI reported by Ferrannini *et al.*, (1991) (FIGURE 1.12).



1.8.ii.b Insulin dynamics and the metabolic responses to feeding

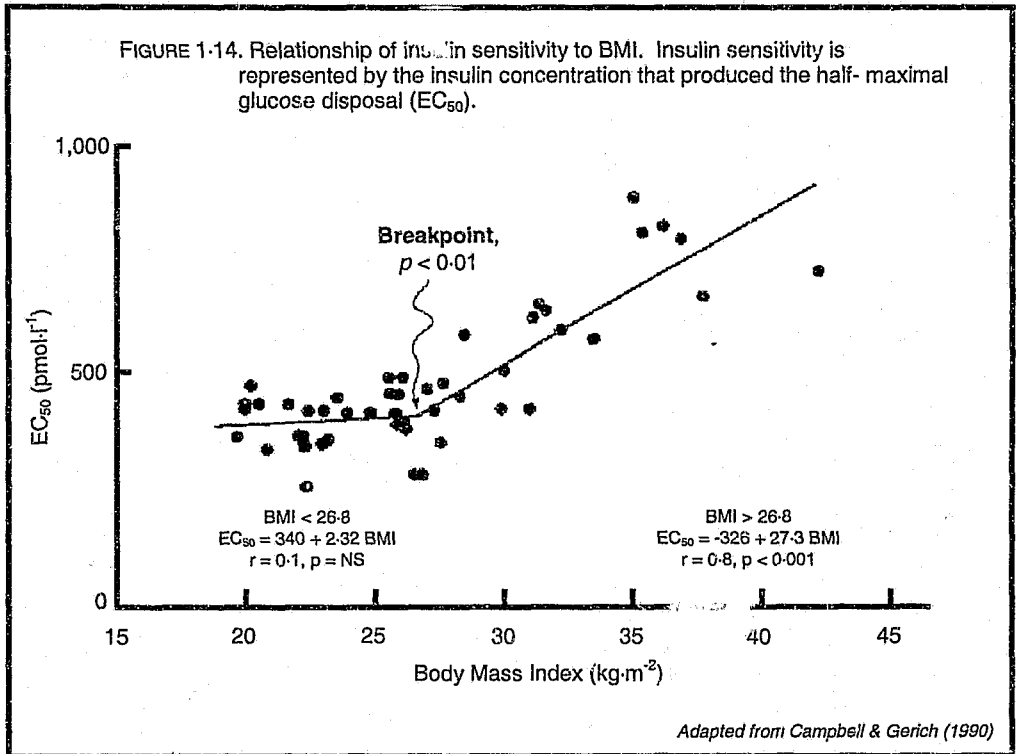
The postprandial rise in insulin concentrations are influenced by the following factors: the combination of carbohydrate-rich and protein-rich foods in the same meal increases the postprandial insulin response (Nuttall & Gannon, 1991); factors that affect the rate of carbohydrate digestion and absorption, e.g. chemical composition and physical form of ingested starch, the processing method, the presence of viscous fibres, and anti-nutrients in the food ingested.

As depicted in FIGURE 1-13, when the effect of hyperinsulinaemia on glucose metabolism is compared between lean and obese individuals via the insulin dose-response curve, the curve assumes a sigmoidal function (Kolterman *et al.*, 1980). Using the same euglycaemic clamp technique during sequential insulin infusion as Campbell & Gerich (1990), these authors found that the change in serum insulin concentration that is required for maximal glucose uptake shifts to the right in obese subjects; indicating decreased insulin sensitivity for glucose uptake (Kolterman *et al.*, 1980). Effectively, in obese individuals, more insulin (compared to lean individuals) is required to ensure a given rate of glucose metabolism ($\text{mg} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$). The amount of insulin required to ensure a given rate of glucose metabolism is the *insulin sensitivity*, and as more insulin is required to sustain the same glucose metabolism, so the insulin sensitivity is reduced. The mechanism for this reduction in insulin sensitivity has been described in FIGURE 1-11.



In an attempt to explore this phenomenon, FIGURE 1-14 depicts the relationship that exists between insulin sensitivity, represented by the insulin concentration that produced half-maximal glucose disposal (EC_{50}) and BMI category (Campbell & Gerich, 1990). 49 healthy volunteers (with a range of ideal body weight of 80-240%, and with normal glucose tolerance) underwent euglycaemic clamps during sequential insulin infusion of 0.4, 1.0, and 10 $mU \cdot kg^{-1} \cdot min^{-1}$. Insulin sensitivity was assessed by estimation of the plasma insulin concentration that produced half-maximal glucose disposal (EC_{50}). Glucose disposal during the highest insulin infusion was used as an index of maximal insulin responsiveness. These authors found a significant correlation between BMI and insulin sensitivity, best fitted by a straight line that broke at a BMI of 26.8 $kg \cdot m^{-2}$, where there

were dramatic reductions in insulin sensitivity, which consequently led to insulin resistance. This relationship is highlighted in FIGURE 1-14, below.



1.8.ii.c Insulin Insensitivity linked to the disruption of appetite control in obesity

Since insulin plays such a significant part in the control over nutrient dynamics, it has been proposed that it may form an integral part of the appetite-satiety process (Campfield, 1997). This hypothesis has its origins in the *glucostatic theory*, which proposed a *feedback effect* where peripheral metabolic dynamics (specifically plasma glucose levels) influence subsequent intake of food (Mayer 1953; 1955). The glucostatic theory suggested that transient declines in blood glucose initiated hunger, and the restoration of these levels to a pre-set concentration would induce satiety. It has been shown that the transient decline in blood glucose immediately prior to feeding may be internally mediated. Further, the observation that it was a spontaneous event suggested its causal-relation to the onset of feeding, but not to the size nor the duration of the meal (Campfield & Smith, 1990). Interestingly, this drop in blood glucose has been shown to occur equally in both lean and obese Zucker rats (Russel *et al.*, 1999).

Given that hyperglycemia may influence satiety, one mechanism by which glucose could influence food intake is through hyperinsulinaemia. Based on this premise, Gluckens *et al.* (1996) investigated the short-term effects of acute hyperglycemia and euglycaemic hyperinsulinaemia on satiety. Using six healthy volunteers (aged 20 to 26 years) who were studied for 240 minutes on three separate occasions in random order during i. intravenous saline; ii. acute hyperglycemic hyperinsulinaemia with plasma glucose at $15 \text{ mmol}\cdot\text{l}^{-1}$; and iii. euglycaemic hyperinsulinaemia with plasma insulin at $80 \text{ }\mu\text{U}\cdot\text{ml}^{-1}$ and glucose at 4 to $5 \text{ mmol}\cdot\text{l}^{-1}$. These authors found

that subjective appetite ratings (using VAS techniques) gradually increased over basal levels during control conditions and hyperinsulinaemia. In contrast, however, appetite and hunger gradually decreased during hyperglycaemia. Their findings suggested that hyperglycaemia induced satiety in humans, and that this effect did not appear to be mediated by insulin, since hyperinsulinaemia had no effect on appetite (Gielkens *et al.*, 1998). However, they did acknowledge that a potentiating effect of endogenous insulin on the satiating effect of high blood glucose levels could not be excluded. This study reinforces that glucose – insulin dynamics are strongly linked to ingestive behaviour, and there are numerous glucose-dependent processes that are important in the regulation of metabolism occur in many peripheral organs (Woods, 1997). Several brain areas receive direct and/or synaptic inputs relating to the dynamic changes in blood glucose concentrations (Oomura, 1983) on a continuing basis through a whole set of peripheral and central receptors which provide crucial information on blood glucose throughout the day.

Rather than a mere facilitative role in the control of energy intake, it has been proposed that insulin has a more direct effect in the regulation of appetite, and the ingestion of energy (Jenkins *et al.*, 1989; Woods, 1997; Ellis, 1934; Hejda & Fábry, 1964; Bray, 1972). It is logical that both the regulator of appetite and satiety should be regulated by stimuli linked to feeding, with regulatory signals emanating from either the gastrointestinal tract to the central nervous system or from post-ingestive chemical signals in the blood. These regulating stimuli would have to meet three criteria: firstly, it would have to exist in amounts proportional to the body and circulate in the blood; secondly, it would have to acquire access to the CNS and be able to interact with the brain; and finally, it

would have to have predictable effects on behaviour and metabolism (Woods, 1997). These putative mechanisms in satiety regulation are at variance with some studies which report that increased insulin responses to ingested foods are associated with lessened satiety (Woods, 1997; Lavin & Read, 1995; Holt *et al.*, 1992; Holt & Brand Miller, 1992), but support the finding in primates that were given $0.33\mu\text{U}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of exogenous insulin posted 13% suppression of energy intakes (Holt & Brand Miller, 1995). This finding is compatible with the hypothesis that circulating endogenous or exogenous insulin is a signal for appetite suppression.

The negative effects of increased insulin on food intake have been shown to be true using non-human primates (baboons) (Woods *et al.*, 1984; Woods *et al.*, 1979) and rats (Vander Weele *et al.*, 1980; Steffens *et al.*, 1988). Thus, insulin meets the three criteria to act as a feedback signal from the gut to the CNS in the regulation of adiposity and weight. There is consensus at present that peripheral information provides crucial information to the central nervous system to control food intake (Woods, 1997; Campfield, 1997).

These works suggest that the *decreased insulin sensitivity* observed in obese individuals may contribute to the disruption of the appetite-satiety mechanism responsible for maintaining energy balance. Since there is a great deal of uncertainty that exists in the literature on the role that insulin plays in meal initiation and termination, if indeed it contributes at all, a priority of future research into ingestive behaviour should incorporate the potential "insulin effect" to determine any causal link between appetite control and insulin concentrations.

1.8.ii.d The reversibility of hyperinsulinaemia associated with obesity

Investigating the reversibility of hyperinsulinaemia, Slabber *et al.*, (1994) proposed that the reduction of the basal insulin levels may occur through dietary intervention and that this would pose as a simple economic way to reduce the risk of degenerative metabolic diseases in the hyperinsulinaemic subset of an obese population. Their study considered the effects of two low-energy diets on serum insulin concentrations and weight loss in 30 obese hyperinsulinaemic females during a 12-week period. The 30 subjects were divided into two groups of 15 each; each group was put onto a diet designed either to invoke a low-insulin response, or a high-insulin response. The fundamental finding in this work was that a low-insulin¹ response diet resulted in a significant reduction in the fasting and 120-minute stimulated insulin concentrations whereas the high-insulin response diet had no effect on plasma insulin concentrations. It has also been reported that elevated insulin concentrations ($137 \pm 22 \text{ pmol.l}^{-1}$) could be reduced with weight loss: upon reduction of BMI's to $29.9 \pm 1.0 \text{ kg.m}^{-2}$, 12 pre-menopausal obese women elicited reduction in insulin levels to $79 \pm 14 \text{ pmol.l}^{-1}$ (Franssila-Kallunki *et al.*, 1991). More recently, Sjostrom *et al.*, (1999) using data from the Swedish Obesity Study (SOS), compared 845 surgically treated obese patients ($\text{BMI } 41.0 \pm 4.6 \text{ kg.m}^{-2}$) with matched control obese individuals for two years. After losing $28 \pm 15 \text{ kg}$, the surgically treated group had shown a significant recovery from hypertension, diabetes, hypo-HDL, and hypertriglyceridaemia compared to the control group (whose weight had maintained at $0.5 \pm 8.9 \text{ kg}$ over the 2 y. period) (Sjostrom *et al.*, 1999).

¹ Slabber *et al.*, (1994) designed their experiment to investigate two dietary regimes: one was a conventionally balanced diet (ND) and the second was a diet designed to invoke a low-insulin response, or what they called a "low insulin" diet (LD).

1.8. iii. Other Hormones implicated with energy regulation

Although not the focus of the current study, other hormones are also implicated with energy regulation, and these are dealt with in brief.

1.8. iii. a Leptin

Leptin was identified by Zhang *et al.* (1994) at Rockefeller University, as the product of a gene that was known to be defective in an obese strain of

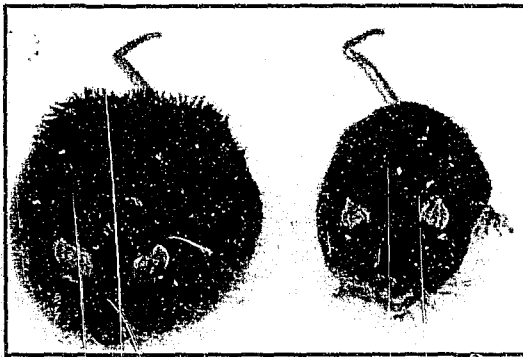


Photo by John Sholtis, The Rockefeller University, New York, N.Y. Copyright ©1995 Amgen Inc.

mice. It is a 167 amino acid protein transcribed from the *ob* gene, and was originally cloned in the mouse during research directed at identifying the

molecular defect in an obesity-prone strain, the

ob/ob mouse. The human leptin gene is on chromosome 7q31; its DNA has more than 15,000 base pairs, and there are 3 exons, the major coding sites driving protein synthesis (Tartaglia *et al.*, 1995). Leptin is produced mainly in white adipose tissue, and very small amounts are found in brown adipose tissue.

Leptin was first described as an adipocyte-derived signalling factor, which after interaction with its receptors induced a complex response including control of body weight and energy expenditure (Friedman, 1998). Investigators have suggested that leptin's principal role is to suppress the

body's response to starvation. When leptin is injected into rats, it is thought to alter the mouse's "set point" for body weight, bringing it down to a lower level, by reducing the food intake without an accompanying decrease in energy use (Chen *et al.*, 1996). A variety of investigations has been carried out in the past few years in order to examine the relationship between leptin and obesity. Kennedy *et al.*, (1997) characterised 116 subjects (62 men and 54 women) with regard to body composition, glucose intolerance, insulin sensitivity, energy expenditure, substrate utilisation and blood pressure. Their data pointed towards the concept that there are important gender-based differences in the regulation and action of leptin in humans. It was established that serum leptin levels increase with progressive obesity in both men and women, consistent with a state of relative leptin resistance. They concluded that these findings implicated differences in body composition between men and women; their observation that serum leptin levels are not related to energy expenditure suggested that leptin can regulate body fat predominantly by altering eating behaviour rather than calorogenesis, as suggested earlier.

In addition to these functions, it seems that leptin has a role in metabolism, reproduction, and neuroendocrine signalling (Auwerx & Staels, 1998). Since human obesity is a complex disorder with many factors playing a part, the jury is still out on the role that leptin has to play in the explanation and understanding of obesity. Ongoing research shows that weight loss is a complex process, involving weakened responses to leptin and its receptor (Muzzin *et al.*, 1996; Gainsford *et al.*, 1996; Chen *et al.*, 1996).

1.8. iii. b Glucagon

Glucagon is a protein pathway hormone produced by the α -cells of the pancreatic islets² (Vander *et al.*, 1997). The major effects of glucagon on organic metabolism are all opposed to those of insulin: whilst insulin may be viewed as the "hormone of plenty", glucagon is the "hormone of famine". Both are affected by the plasma glucose concentration, but the secretion of glucagon is not only stimulated by hypoglycaemia, it is also stimulated by exercise and any mechanism that induces the mobilisation of energy stores for coping with a "fight-or-flight" situation.

The overall result of glucagon's effects is to increase the plasma concentrations of glucose and fatty acids: both important events of the post-absorptive period. Based on this premise, the glucagon concentration may correlate quite strongly with hunger and appetite; however, very little research has been conducted in this area (Vander *et al.*, 1997).

1.8. iii. c Cholecystokinin (CCK)

Cholecystokinin (CCK) is a prototypic satiety peptide. It has been reported to affect stomach fullness by constricting the pyloric valve and initiating signals to the brain via the vagus nerve (Gibbs *et al.*, 1993; Moran *et al.*, 1996). It also acts as a neurotransmitter that promotes satiety within the medial hypothalamus (Schwartz *et al.*, 1988). The central nervous system may regulate appetite by means of signals triggered by dietary breakdown

² Glucagon and glucagon-like substances are also secreted by cells in the lining of the gastrointestinal tract morphologically identical to the α cells; the significance of this nonpancreatic glucagon is unclear (Vander *et al.*, 1997).

products and by autonomic signals produced by distension of the stomach and intestines (Jack, 1996; Vander *et al.*, 1997; Greenough *et al.*, 1998). It has been proposed that the multiple signals generated are processed by complex interactions between neuronal networks and neurotransmitters, and among the neurotransmitters are the cholecystokinins, a family of hormonal and neuronal peptides that act on the gut and brain. Cholecystokinin 8 (CCK-8), the sulphated carboxy-terminal octapeptide of cholecystokinin, has been postulated to be one of the main neurotransmitters involved in appetite control (Jack, 1996; Ebenezer, 1999).

Whether or not food intake is controlled through the CCK-8 system is yet to be determined, although there is evidence to support this hypothesis in mice (Bhavsar *et al.*, 1998) rats (Ebenezer, 1999), dogs (Simmons *et al.*, 1998), and humans (Greenough *et al.*, 1998). Work is currently being done to design a receptor agonist with acceptable bioavailability (Ebenezer, 1998; Simmons *et al.*, 1999), with new attempts to suppress appetite by supplying exogenous CCK-8 (subcutaneously and peritoneally) in rats (Ebenezer, 1999). Since CCK-8 is a peptide, orally administered CCK-8 is rapidly broken down in the gastrointestinal tract before it can be absorbed. Even when given intravenously, CCK-8 has a very short-lived effect because of peptidase digestion. Clearly, there is great potential for this in the field of appetite control, and it warrants closer investigation in the future.

1.9 Pathologies associated with obesity

Obesity is associated with a greatly increased likelihood of diabetes (Reaven, 1988), hypertension (Karason *et al.*, 1998; Mikhail *et al.*, 1999; Himeno *et al.*, 1999), hyperlipidaemia, and heart disease as well as increased rates of breast, colon, and uterine cancer. Zamora-Gonzalez *et al.*, (1996), found in a Mexico City trial, that IR related-metabolic disorders were high. These disorders included the increased cardiovascular risk factors associated with the IR syndrome (hypertension and hypercholesterolaemia), and apolipoprotein(a) was inversely and significantly related to insulin. It is well documented that blood pressure is primarily determined by the cardiac output and the peripheral vascular resistance (Himeno *et al.*, 1999) - both are dependent on the total body sodium content and neurohormonal factors. Very little is known about the contribution of the sympathetic nervous system and the renin-angiotensin-aldosterone system to the hypertension observed in obesity (Reaven, 1988; Després, 1994). Recently, it has been suggested that hyperinsulinaemia, perhaps acting by enhanced renal sodium absorption, plays a causative role in the hypertension observed in obese individuals (Mikhail *et al.*, 1999). The overwhelming majority of the studies investigating hypertension as a function of weight have shown that weight loss has a beneficial effect on hypertension (Reisein *et al.*, 1978; Reaven *et al.*, 1988; Karason *et al.*, 1998; Mikhail *et al.*, 1999; Himeno *et al.*, 1999), however, one study (Dahl *et al.*, 1958) showed that salt restriction from a dietary perspective is the major drive in reducing hypertension, with energetic restriction playing a minor role (if any at all) and consequently, that weight reduction had very little effect (Dahl *et al.*, 1958).

Other investigators have found contrasting outcomes: Reisein *et al.*, (1978) postulated that it was weight reduction *per se* that is the most important component in controlling hypertension in obese subjects (Mark *et al.*, 1999). Recently, in a carefully designed study to investigate the effects of weight loss on hypertension, Himeno *et al.*, (1999) found that when 14 obese individuals with mild hypertension were subjected to a 12-week mild exercise and hypoenergetic diet, weight reduced by 5.8kg ($P < 0.01$) and blood pressure was reduced significantly.

It has been shown that following energetic restriction and weight loss, there is a marked reduction in fasting and glucose-stimulated insulin levels (Beck-Nielsen *et al.*, 1979; Franssila-Kallunki *et al.*, 1991). When plasma insulin levels are increased by as little as $30 \mu\text{IU}\cdot\text{ml}^{-1}$, significant sodium retention occurs (De Fronzo, 1982). Based on this, it is possible that the insulin resistance with respect to glucose metabolism leads to hyperinsulinaemia, which subsequently stimulates sodium reabsorption by the kidney, and expands the intracellular volume (DeFronzo, 1981). Continued expansion of the ECV by salt and water will lead to an increased cardiac output and the eventual rise in blood pressure (Guyton *et al.*, 1974). As the perfusion pressure to which the kidney is exposed increases, eventually the antinatriuretic effect of insulin will be overcome and a natriuresis will occur. Consequently, there is maintenance of an elevated blood pressure to prevent the excessive sodium retention that would otherwise occur with hyperinsulinaemia. There follows an increase in the peripheral vascular resistance, which is then responsible for the sustained elevation in blood pressure.

1-10 CONCLUSION

This review has shown that obesity manifests from a chronic positive energy balance, which seems more likely to result from a dysfunction in the control of energy ingestion than a dysfunction in energy metabolism. Most studies contained in the literature which have investigated obesity, report the disruption to the appetite-satiety complex as the significant contributing factor in the increased energy intake that contributes to the increased incidence and prevalence of obesity in the western world.

The assessment of the putative mechanisms regulating the physiology of appetite include the frequency with which meals are consumed, the macronutrient content of the food that is consumed, and its associated energy density. Although the majority of studies in this field have shown that the frequency in which an individual eats plays a large part in the metabolic profile of the individual, this review has shown mixed results. As such, there is conflicting evidence on the effects that meal frequency has on weight regulation, metabolism, and health profiles. Most studies reviewed here have suggested that eating more regularly (compared to eating isoenergetic amounts of food less frequently) exerts a positive effect on hormonal profiles (reduction in hyperinsulinaemia associated with NIDDM), enhanced control over blood metabolites (glucose and FFA), and hyperlipidaemia (decreased serum cholesterol). The most plausible reason for these conflicting findings may be in the different testing circumstances in the duration of the studies, the accommodation of an adaptation phase, and the macronutrient value of the foods used in the respective studies (Leveille, 1970); it is clear that for more comparable

assessment of the field linked to the periodicity of feeding, these need to be more tightly controlled.

There have been surprisingly few studies that have explored whether satiety is affected by the periodicity of eating; and those that have, have not measured this relationship directly (Jenkins *et al.*, 1989; 1992). The small bit of evidence suggests that eating smaller more frequent meals would maintain a more balanced appetite control, and less extreme oscillations in hunger ratings, which may exert a positive effect on energy ingestion. This, coupled to the physiological adaptations to frequent feeding, is the rationale behind the hypothesis that proposes that more frequent meals correlate with reduced adiposity in humans. However, due to the small number of studies performed in this area, these postulates are limited in showing causality in the relationship between the frequency of feeding, appetite control, and the incidence of obesity. Accordingly, it follows that a great deal of work is required in this area of feeding frequency linked to obesity and appetite research which would enable a more comprehensive understanding of feeding behaviour, and its potential contribution toward obesity.

The macronutrient value of dietary intake is an integral part assessing obesity: fat intakes across the world have increased which coincide with the increasing prevalence of obesity, particularly in children. The energy density of the diet plays a large part, and it is also linked to the *passive overconsumption* of the obese. In several studies which have evaluated the effects of fat on satiety and satiation, fat differed little from carbohydrate when both the palatability and energy density of the test foods were matched. As such, it is unlikely that the effects of fat on satiety

or satiation provide the primary explanation for why it is overeaten. Support for the view that the high energy density of fat facilitates its overconsumption comes from recent studies in which energy density significantly influenced intake when both the macronutrient content and palatability of the test foods were matched (Rolls & Bell, 1999).

It has been proposed that insulin has a more direct effect in the regulation of appetite, and the ingestion of energy. It seems logical that both the regulator of appetite and satiety should be regulated by stimuli linked to feeding, with regulatory signals emanating from either the gastrointestinal tract to the central nervous system or from post-ingestive chemical signals in the blood. This work suggests that these regulating stimuli meet the three requisite criteria to exert predictable effects on behaviour and metabolism (Woods, 1997), and that circulating endogenous or exogenous insulin is a signal for appetite suppression.

In conclusion, this review has shown that the three fundamental putative factors controlling the ingestion of energy all contribute to appetite control: they are key determinants of energy intake insofar as cognitive, behavioural, and sensory cues related to the volume, or weight, of food consumed can interact with physiological cues associated with food intake. What follows is a work designed primarily to investigate how these relationships interact to effect appetite control in humans, and further, to explore their potential dysfunction, as an aetiological contributor toward the obese state.

Chapter Two

The association of hyperinsulinaemia with obesity in South African Caucasian adults

INTRODUCTION

The incidence of obesity (body mass index or BMI $> 30\text{kg}\cdot\text{m}^{-2}$) in the western world is increasing with each passing year (Kuczmarski, 1992; Flegal *et al.*, 1998). Epidemiological evidence, to date, suggests that the South African population is following this trend (Walker, 1998). South African men appear to be leaner than their female counterparts, in the age category of 35 – 54 year-olds, 10.7% of black, and 16.7% of white men had BMI in the *obese* range as indicated by values exceeding $30\text{kg}\cdot\text{m}^{-2}$, whereas 54% of black women, and 20% of white women attained *obese* status (Steyn *et al.*, 1991; Jooste *et al.*, 1988). The incidence of obesity within developing countries of the African continent is of even greater concern since body mass index (BMI) appears to be a good indicator of the standard of living, with the higher the BMI, the higher the status associated with the individual (Nube *et al.*, 1998).

Since there is an element of uncertainty surrounding the field of obesity as to which factors contribute to the aetiological induction of obesity, it follows that therapeutic intervention for the obese population is embarrassingly poor (Hyman *et al.*, 1993). Furthermore, obesity is associated with a number of chronic pathologies, which include increased risk of hypertension (Modan *et al.*, 1985; Ferrannini *et al.*, 1987; Ferrannini *et al.*, 1999), dyslipidaemia (Laakso *et al.*, 1987a; 1987b; Haffner *et al.*, 1992; Percheron *et al.*, 1998), cardiovascular disease (Ducimetiere *et al.*, 1980; Ferrannini *et al.*, 1991; Ferrannini *et al.*, 1996; Ferrannini *et al.*, 1999), and non-insulin-dependent diabetes mellitus (DeFronzo & Ferrannini, 1982). Hyperinsulinaemia and insulin

resistance are another interesting metabolic perturbation associated with obesity (Ferrannini *et al.*, 1996; Reaven, 1988). Hyperinsulinaemia has further been recognised as a risk factor for hypertension (Modan *et al.*, 1985) coronary heart disease (Ducimetiere *et al.*, 1980; Ferrannini *et al.*, 1991; Ferrannini *et al.*, 1996), and Type 2 diabetes (DeFronzo & Ferrannini, 1982).

The metabolic effects of hyperinsulinaemia have been well documented (Kolterman *et al.*, 1980; DeFronzo, 1982; Caro, 1991; Kopelman, 1994; Koyama *et al.*, 1997; Jéquier & Tappy, 1999; Ferrannini *et al.*, 1999). It has been shown quite convincingly, that the hyperinsulinaemic state can be reversed through weight loss (Slabber *et al.*, 1994; Sjostrom *et al.*, 1999), suggesting that the hyperinsulinaemia associated with obesity is more consequential to the condition rather than a causal factor.

The incidence of hyperinsulinaemia in a South African population has not been investigated previously. The primary aim of this investigation was to determine whether a relationship between fasting insulin levels and a) BMI and b) various anthropometric variables does indeed exist in a group of Caucasian South African adults.

MATERIALS AND METHODS

Subjects

Seventy-nine (34 male and 45 female) non-diabetic individuals were recruited to participate in this study, characteristics of whom are presented in TABLE 2-1. This study was approved by the Ethics Committee of the University of the Witwatersrand (Ethical clearance number: M980624).

Examination Procedure

Subjects were examined for weight, height, and waist and hip circumferences at the initial test. They were all instructed to report to a local commercial pathology laboratory (Lancet Laboratories) in the fasted state having had nothing to eat or drink since 22h00 the previous evening. Venous blood was drawn and treated for plasma (test for glucose) and serum (insulin) extraction. The assays for plasma and serum insulin were conducted by specialist pathologists on the same day of blood being drawn (Lancet, Johannesburg). The assays used in the determination of serum insulin and plasma glucose are included in Appendix A.

Statistical methods

Differences between the male and female groups were assessed using an unpaired *t*-test. Pearson's correlation analyses were used to determine the correlation between insulin and BMI values. All statistical tests were conducted using the software package STATISTICA, developed by Statsoft, Tulsa, OK, USA. In the text, Tables, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

Fasting Insulin concentrations

Fasting insulin concentrations for the male cohort showed the insulin concentration to be $24.4 \pm 12.2 \mu\text{U}\cdot\text{ml}^{-1}$, and the plasma glucose concentration was $6.0 \pm 1.8 \text{ mmol}\cdot\text{l}^{-1}$. Females' insulin concentrations were $15.2 \pm 11.2 \mu\text{U}\cdot\text{ml}^{-1}$, with concomitant plasma glucose concentrations of $5.2 \pm 1.4 \text{ mmol}\cdot\text{l}^{-1}$. There were however no significant gender differences. The average insulin concentration for the whole group was $19.2 \pm 12.4 \mu\text{U}\cdot\text{ml}^{-1}$, and plasma glucose $5.5 \pm 1.6 \text{ mmol}\cdot\text{l}^{-1}$.

There was no gender difference ($P > 0.05$) in the BMI values ($33.16 \pm 7.85 \text{ kg}\cdot\text{m}^{-2}$ vs $29.31 \pm 7.07 \text{ kg}\cdot\text{m}^{-2}$ for males vs females respectively): BMI for the whole group was $30.97 \pm 7.62 \text{ kg}\cdot\text{m}^{-2}$.

TABLE 2.1

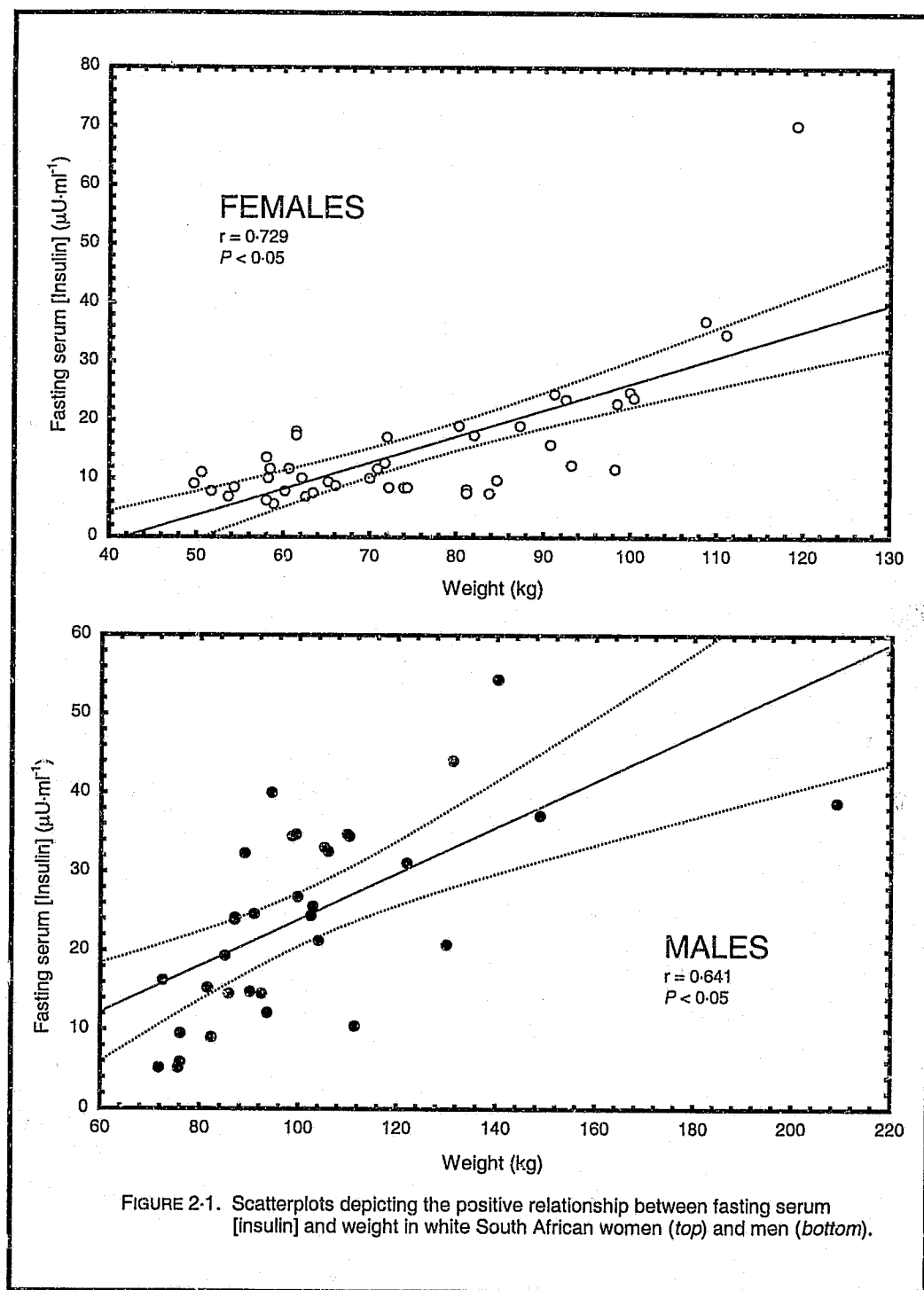
Characteristics of male and female subjects

		MALES	FEMALES	SIGNIFICANCE
N		34	45	
Age	(Years)	42.1 ± 13.07	37.2 ± 11.82	NS
Weight	(kg)	101.7 ± 26.7	75.5 ± 18.1	$t = 2.76 \ P < 0.01$
Height	(m)	1.75 ± 0.07	1.61 ± 0.06	$t = 4.57 \ P < 0.01$
BMI	($\text{kg}\cdot\text{m}^{-2}$)	33.16 ± 7.85	29.31 ± 7.07	NS
WHR		1.09 ± 0.89	0.83 ± 0.06	$t = 5.36 \ P < 0.01$
Waist	(cm)	113.4 ± 23.3	98.5 ± 23.8	$t = 3.07 \ P < 0.01$
Hip	(cm)	103.6 ± 14.5	104.3 ± 16.9	NS
Insulin	($\mu\text{U}\cdot\text{ml}^{-1}$)	24.4 ± 12.2	19.2 ± 12.4	$t = 2.06 \ P < 0.05$
Glucose	($\text{mmol}\cdot\text{l}^{-1}$)	6.0 ± 1.8	5.2 ± 1.4	NS

The female group had a lower ($P < 0.05$) waist to hip ratio (WHR) (0.831 ± 0.006 cm) compared to the male group (1.086 ± 0.89 cm). The average WHR for the whole group was 0.940 ± 0.150 cm.

Serum insulin and body weight and body mass index

When the serum insulin was correlated with body weight in the two groups, (FIGURE 2.1), the two variables correlated significantly ($P < 0.05$) in both the male ($r = 0.641$) and the female ($r = 0.729$) cohort. The correlation between fasting insulin levels with body mass index between the two gender-groups also showed significant ($P < 0.05$) positive correlations (FEMALE: $r = 0.729$; MALE: $r = 0.645$; FIGURE 2.2). Gender differences in these correlations ($P < 0.05$) were evident with *flatter* slopes in the relationship between serum insulin and BMI in females compared to that of the males (FIGURE 2.2).



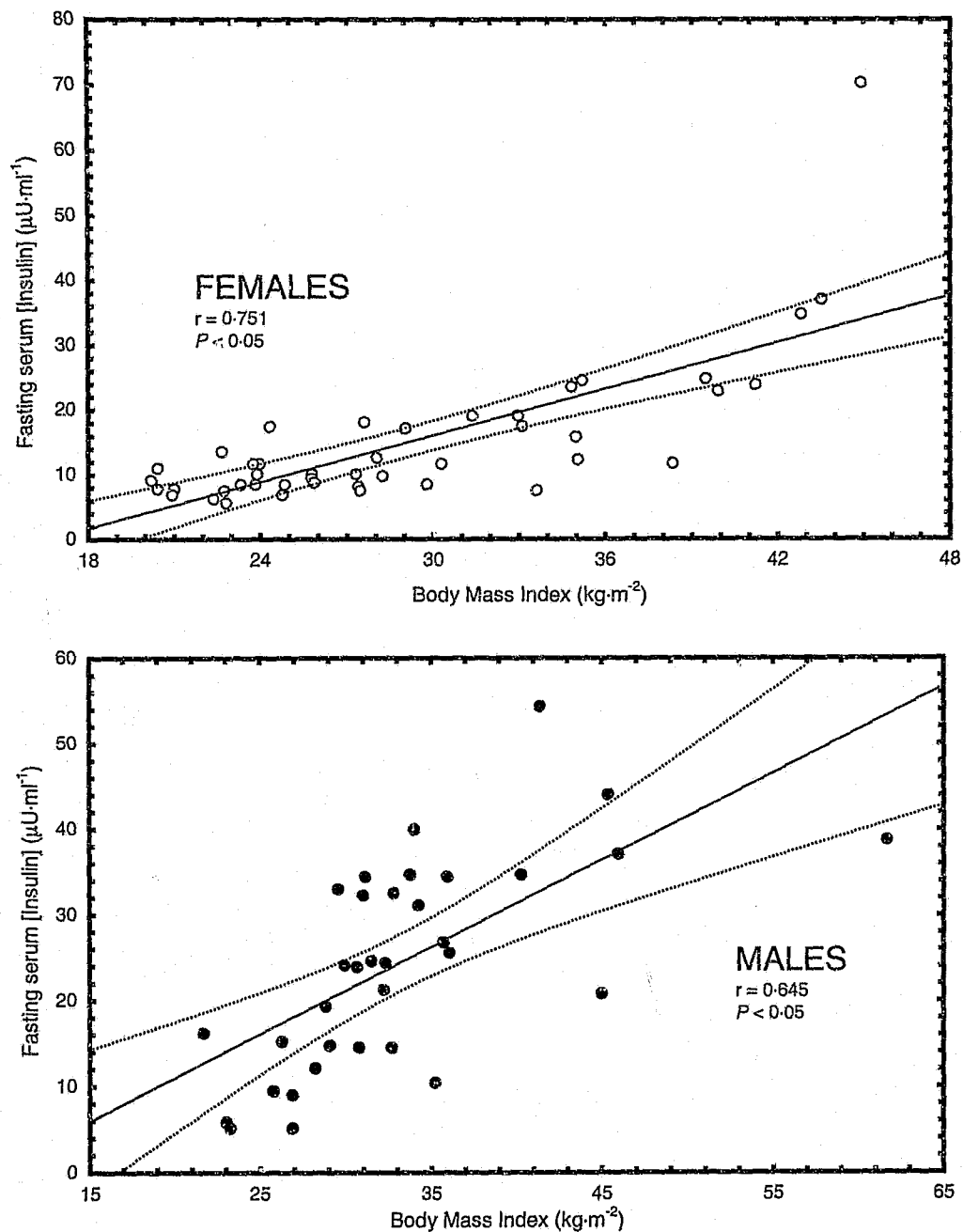


FIGURE 2.2. Scatterplots depicting the positive relationship between fasting serum [insulin] and BMI in white South African women (top) and men (bottom).

Serum insulin and plasma glucose

When fasting serum insulin concentrations was plotted against fasting plasma glucose concentrations (FIGURE 2.3) no relationship ($P > 0.05$) in either males ($r = -0.328$) or females ($r = 0.282$) was evident. As serum insulin concentration increases (with BMI), the blood glucose concentration is nevertheless, maintained within euglycaemic conditions. However, two female subjects, and one male subject had fasting plasma [glucose] over $7.8 \text{ mmol} \cdot \text{l}^{-1}$, and two other female subjects were on the border with fasting plasma [glucose] $\sim 7.8 \text{ mmol} \cdot \text{l}^{-1}$.

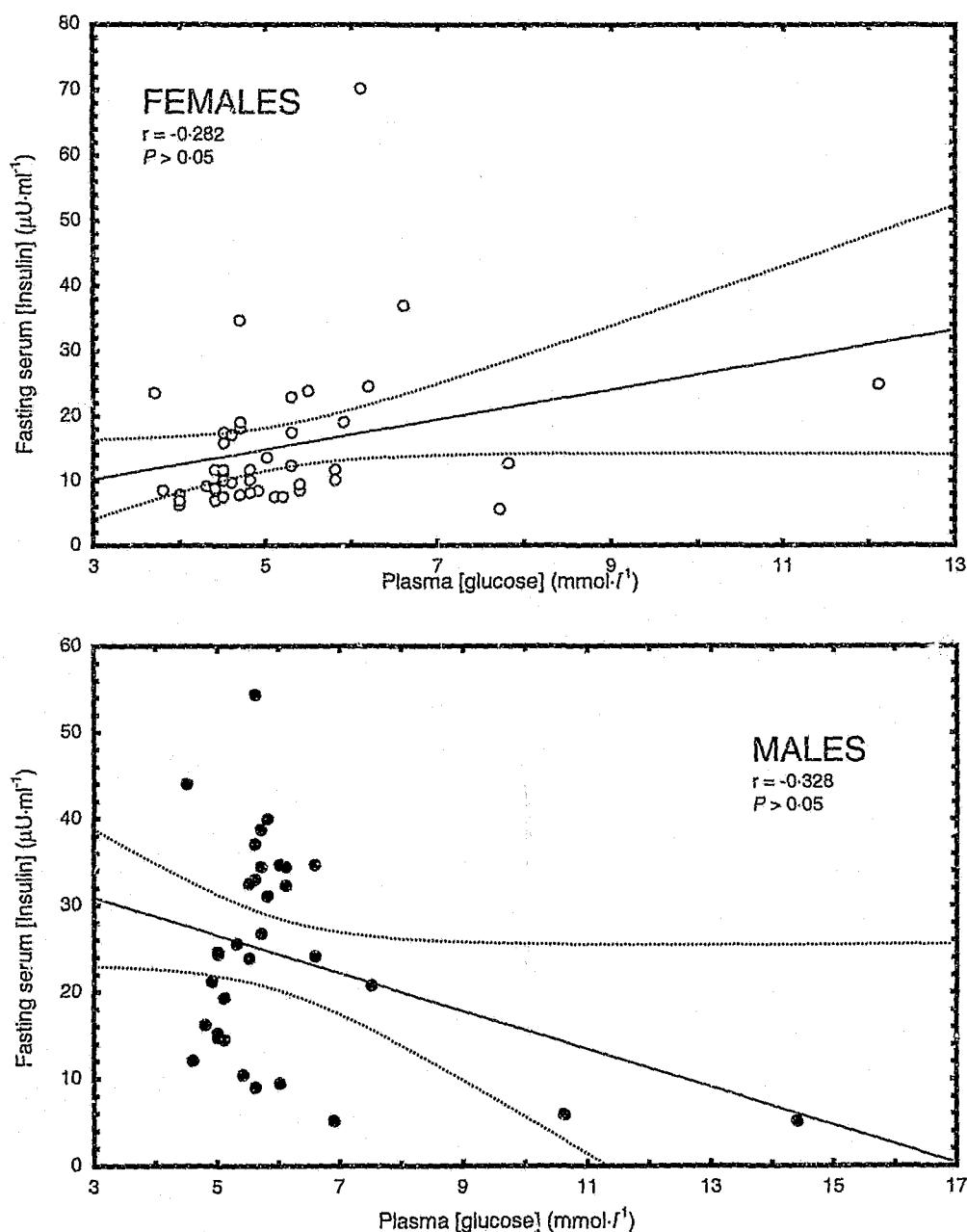


FIGURE 2.3. Scatterplots depicting no relationship between fasting serum [insulin] and plasma glucose concentrations in white South African women (top) and men (bottom).

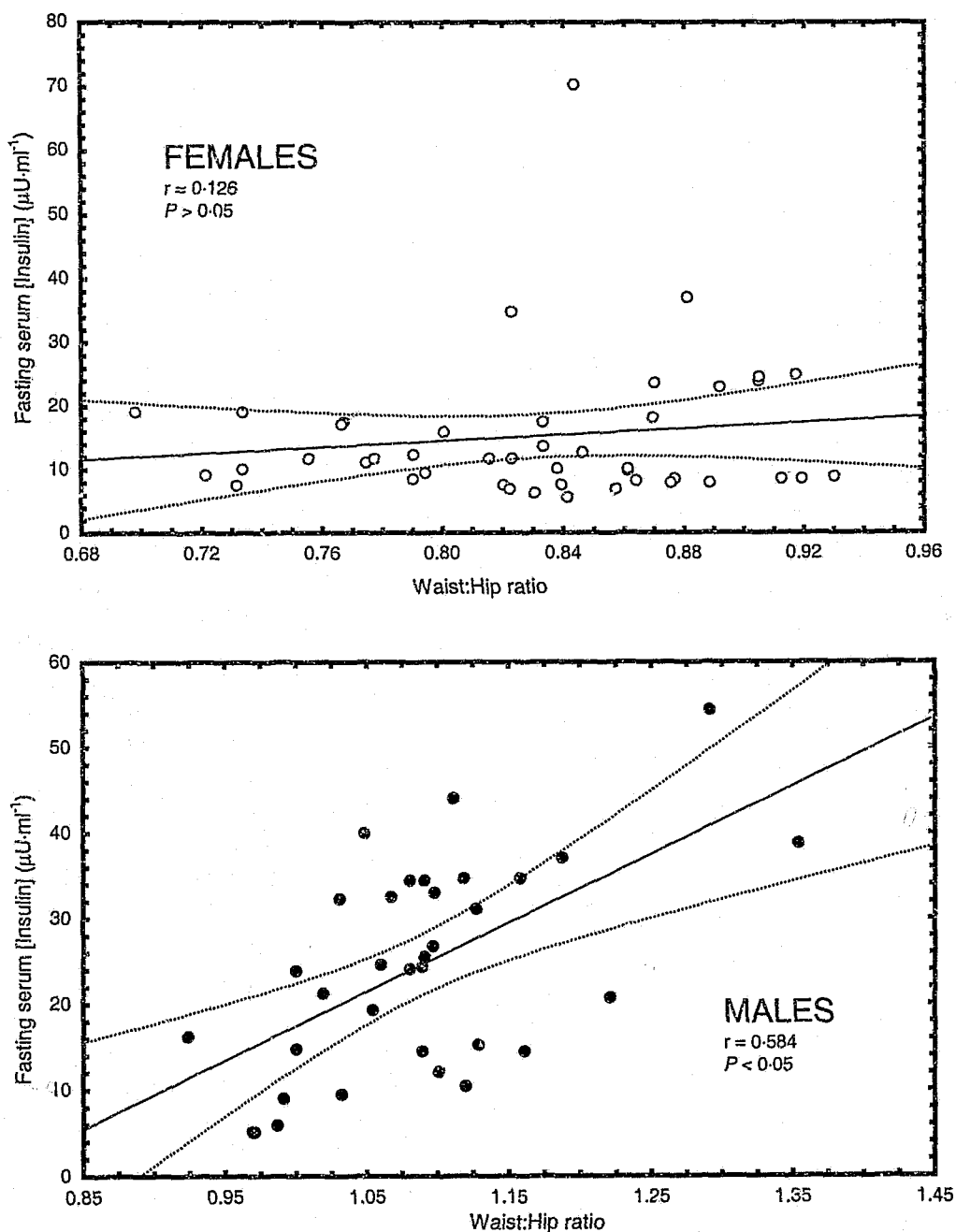


FIGURE 2.4. Scatterplots depicting the positive relationship between fasting serum [insulin] and the waist:hip ratios in South African men (*bottom*), and no relationship in women (*top*).

Serum insulin and WHR

Fasting serum insulin concentrations correlated significantly ($P < 0.05$) positively ($r = 0.584$) with waist/hip ratios (WHR) in the male group (FIGURE 2.4). However, the female group failed to produce such a response ($P > 0.05$; $r = 0.126$).

When the fasting serum insulin concentration was correlated with hip measurements alone, it was found that both the male ($r = 0.772$) and the female group ($r = 0.670$) exhibited significant ($P < 0.05$) positive correlations between serum insulin and hip circumference (FIGURE 2.5).

FIGURE 2.6 shows the scatterplots testing for a relationship between fasting serum insulin concentration and waist circumferences, and shows that both female ($r = 0.671$) and male ($r = 0.752$) groups were positively related to the circumference of their waists ($P < 0.05$).

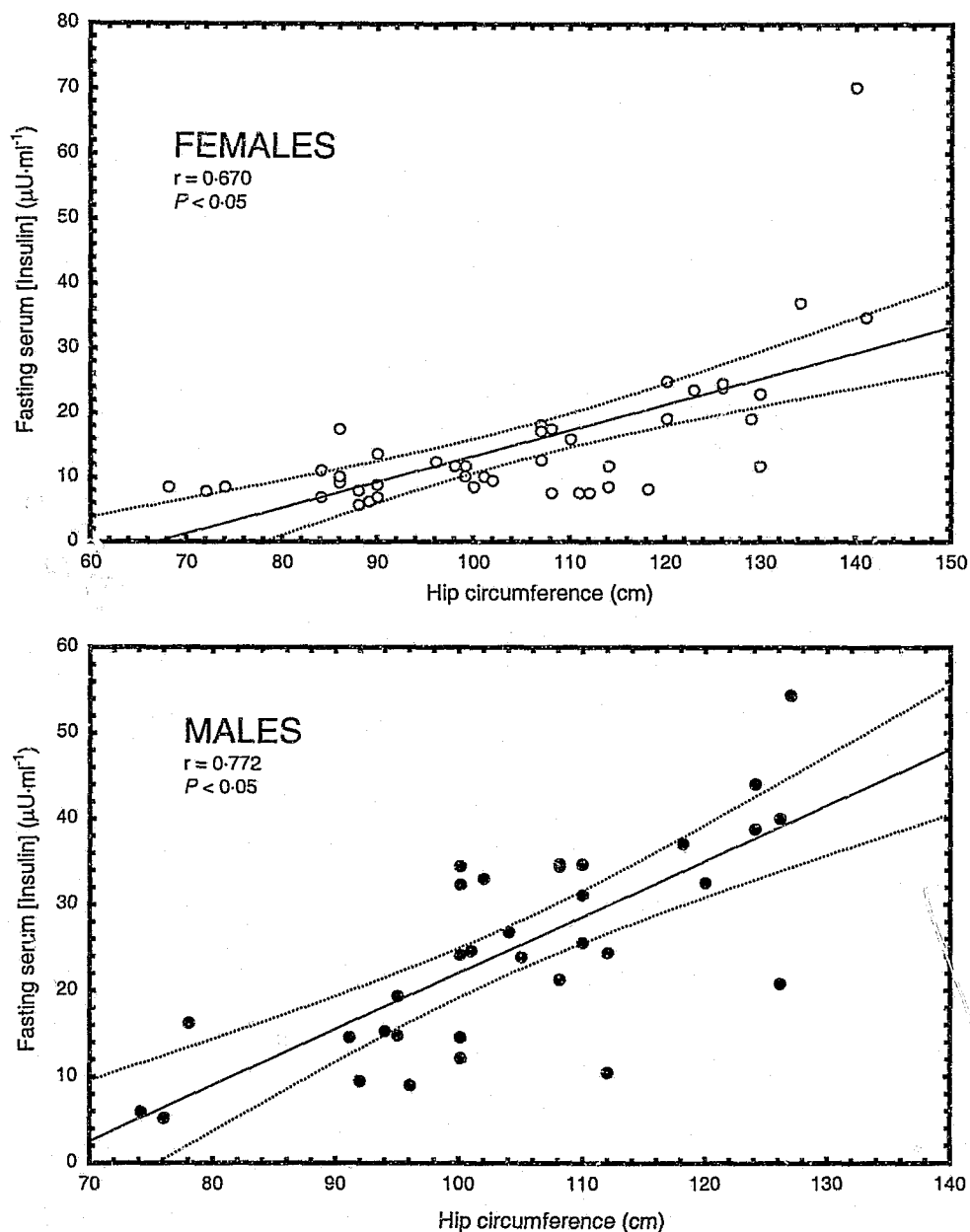


FIGURE 2-5. Scatterplots depicting the positive relationship between fasting serum [insulin] and waist circumference measurements in white South African women (top) and men (bottom).

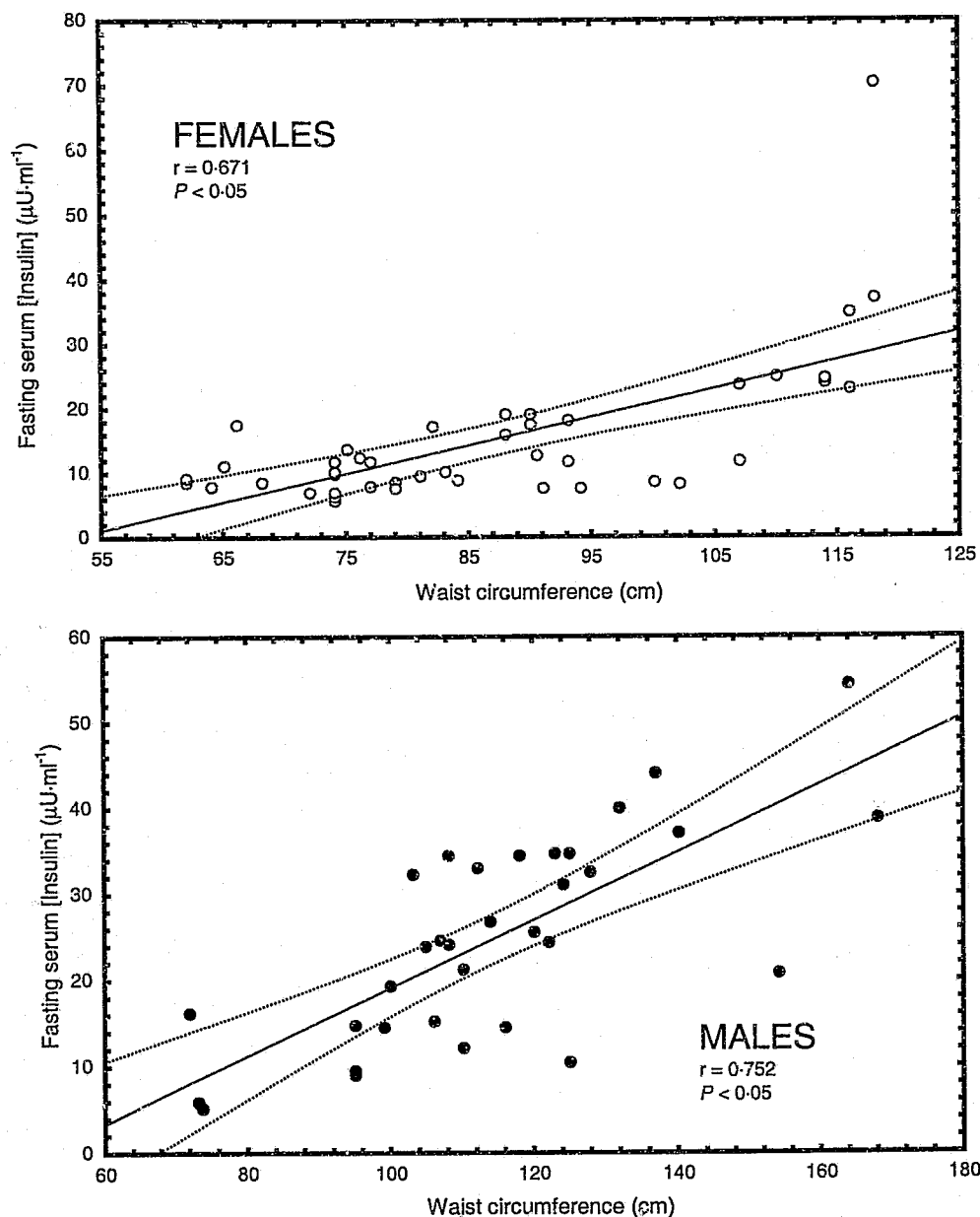
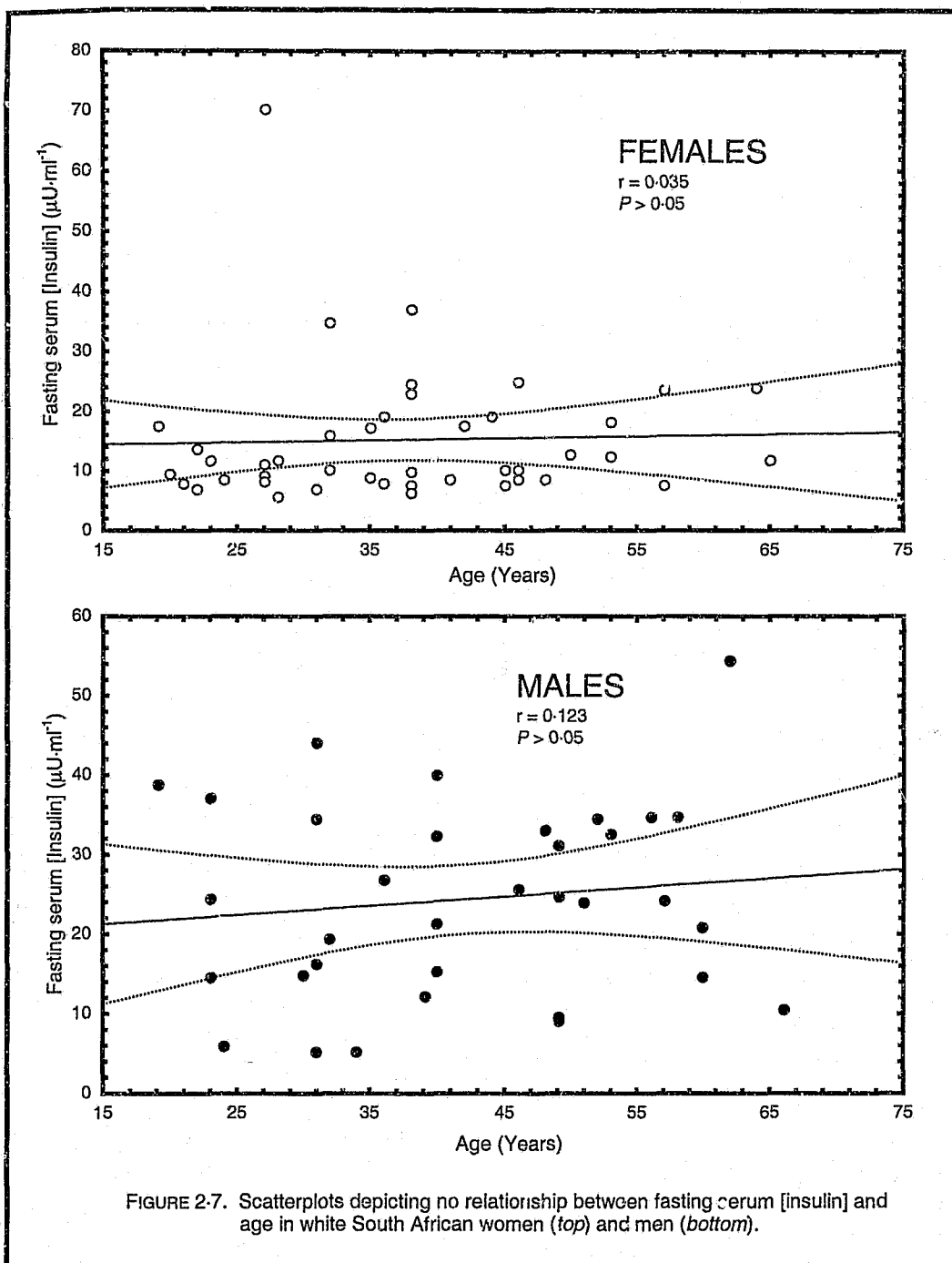


FIGURE 2-6. Scatterplots depicting the positive relationship between fasting serum [insulin] and waist circumference in white South African women (*top*) and men (*bottom*).

Serum insulin and age

FIGURE 2-7, shows the scatterplot depicting no relationship in neither female ($r = 0.035$, $P > 0.05$) nor male groups ($r = 0.123$; $P > 0.05$) between fasting serum insulin concentration and age.

When the BMI values were plotted against waist circumferences, there was a strong correlation in both female ($r = 0.895$, $P < 0.05$) and male groups ($r = 0.886$, $P < 0.05$) (FIGURE 2-8) between the two variables. This suggests that waist measurements (or central adiposity) is a strong indicator of BMI, and accordingly, may be a good predictor of pathologies associated with obesity.



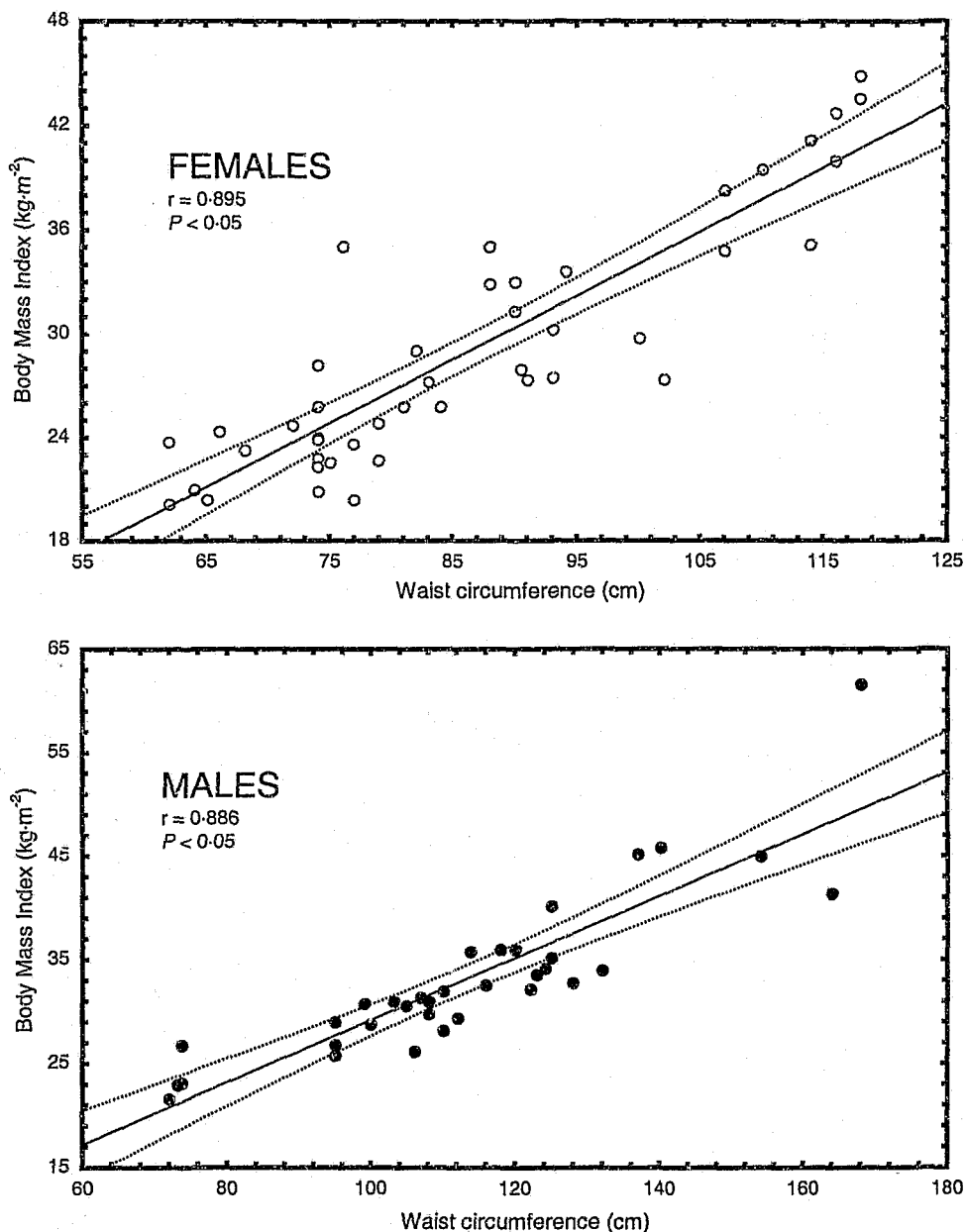


FIGURE 2-8. Scatterplots depicting the positive relationship between body mass index and waist circumference in white South African women (*top*) and men (*bottom*).

DISCUSSION

There was a positive correlation between fasting insulin concentrations and the BMI's and WHR's in Caucasian South African male and female adults. Elevated fasting insulin concentrations have been documented in obese subjects (Slabber *et al.*, 1994; Campbell & Gerich, 1990; Niskanen *et al.*, 1996; Meyian *et al.*, 1987), and have further been interpreted as demonstrating insulin resistance (Reaven, 1988; Caro, 1991).

Comparison of the findings in a South African sub-population (in the current study) with those in the literature, revealed a lack of congruency in the actual insulin values as based on body mass index category (TABLE 2.2). In the current study, those male subjects who qualified for the obese category with a BMI > 30kg·m⁻² exhibited fasting insulin concentrations ~ 31µU·ml⁻¹ while that of lean males were under 15µU·ml⁻¹. Those female subjects who qualified for the obese category with a BMI > 30kg·m⁻² exhibited fasting insulin concentrations ~ 26µU·ml⁻¹ while that of lean females were under 10µU·ml⁻¹. These results are higher than those found in the literature although there is clearly a lack of consensus when it comes to a clear and common understanding of the relationship between BMI and the normal range of fasting insulin concentrations. Much of the discrepancies in insulin concentrations of the various studies may be due to the different assays for insulin yielding different results of serum insulin concentration (Hales *et al.*, 1985; Gould *et al.*, 1999)

Subtle differences in the collection and handling of insulin samples may contribute to the variability of responses given the dynamics of insulin

and its short half-life (Kostecka & Blahovec, 1999). Inconsistency of results is further exacerbated by the different conventions used in reporting insulin concentration. While some studies report insulin concentration as $\text{pmol}\cdot\text{l}^{-1}$, it is also reported as $\mu\text{U}\cdot\text{ml}^{-1}$ or $\mu\text{U}\cdot\text{ml}^{-1}$. A universal convention is strongly needed to enable the accurate reporting of data (in insulin-related studies). This will further facilitate and enhance the field of insulin dynamics related to obesity (Temple *et al.*, 1992; Hales *et al.*, 1996).

TABLE 2.2
References of fasting insulin concentrations according to body mass index

	BODY MASS INDEX (BMI) CATEGORY				REFERENCE
	20 – 25	25 – 30	30 – 35	> 35	
Insulin Concentration ($\mu\text{U}\cdot\text{ml}^{-1}$)	18.0 $\pm$ 1.0		38.0 $\pm$ 3.0		Evans <i>et al.</i> , 1984
	13.0 $\pm$ 1.0			22.0 $\pm$ 1.0	DeFronzo, 1982
				13.2 $\pm$ 2.5	Gumbiner <i>et al.</i> , 1996
				23.2 $\pm$ 4.5	Niskanen <i>et al.</i> , 1996
		12.4 $\pm$ 7.1			Hoag <i>et al.</i> , 1995
				14.3 $\pm$ 4.6	Hale <i>et al.</i> , 1985
			13.4 $\pm$ 0.2		Meylan <i>et al.</i> , 1987
		17.6 $\pm$ 7.7			Ascaso <i>et al.</i> , 1997
	8.8 $\pm$ 2.4		25.3 $\pm$ 11.3	25.8 $\pm$ 9.0	Campbell & Gerich, 1990
			12.4 $\pm$ 7.1		Ferrannini <i>et al.</i> , 1996
	6.0 $\pm$ 1.0			14.0 $\pm$ 1.0	Elahi <i>et al.</i> , 1982
	8.2 $\pm$ 0.1		15.1 $\pm$ 0.6		Ferrannini <i>et al.</i> , 1991
Females			10.7 $\pm$ 6.0		Percheron <i>et al.</i> , 1998
	14.1 $\pm$ 11.1				Speechly & Buffenstein, 1999
Males				36.2 $\pm$ 9.0	Speechly <i>et al.</i> , 1999
	5.9 $\pm$ 1.7	11.1 $\pm$ 3.4	17.7 $\pm$ 6.2		Gould <i>et al.</i> , 1999
	9.9 $\pm$ 3.3	12.6 $\pm$ 4.7	26.0 $\pm$ 8.9	35.7 $\pm$ 18.8	Current study
	14.4 $\pm$ 8.6	26.4 $\pm$ 8.5	31.5 $\pm$ 14.7	39.1 $\pm$ 12.4	Current study

The normal range of fasting insulin concentrations in different ethnic population groups remains to be elucidated. In addition, the relationship between insulin concentration and physiological fitness in both athletes and sedentary sub-populations requires further investigation. Gender-related differences in fasting insulin concentration, however, have been

previously reported although results of these findings are at variance. For instance, Yki-Järvinen (1984) examined 13 women and 11 men (mean age 22 years), and showed that when adjusted for body fat or lean body mass, women had greater insulin sensitivity than men. Similarly, in a population-based trial, Donnahue *et al.*, (1996) found that females exhibit an intrinsic '*insulin advantage*' over males, despite the higher percent body fat, and that female muscle tissue is more insulin sensitive compared to their male counterparts. On the other hand, in a study of 12 people, Hale *et al.*, (1985) concluded that men were more insulin sensitive than women following an incremental low-dose insulin infusion. Based on the premise that hyperinsulinaemia is linked to the aetiology of cardiovascular diseases, that women are less prone to CVD's would mean that they are more likely to be insulin sensitive than men are (Donnahue *et al.*, 1987).

Data in the present study support the 'female insulin advantage' hypothesis proposed by Donnahue *et al.*, (1996) as indicated by the steeper male slope (FIGURE 2-1). It has been reported that IR is closely related to intra-abdominal fat mass (Kopelman, 1980). In this light, it is possible that if males have relatively more intra-abdominal fat compared to their female counterparts for a given BMI, it would contribute to higher fasting insulin concentrations in men.

Hyperinsulinaemia reflects the reduced insulin sensitivity at the level of the cell, with (more insulin is required to achieve the same effect (Campfield & Gerich, 1990). Increased insulin secretion in obesity may serve to compensate for insulin resistance (Meylan *et al.*, 1987), yet the processes that trigger both hyperinsulinaemia and insulin resistance are

not fully understood. Histological findings have shown that obesity is characterised by hypertrophic pancreatic β -cells which hypersecrete the hormone (Ogilvie, 1933). *In vitro* studies in human obesity have demonstrated tissue-specific impairment of both α -subunit and β -subunit of the insulin receptor (Caro *et al.*, 1989) leading to insulin resistance. These data suggest that in obese individuals, circulating insulin concentrations do not regulate insulin secretion to those concentrations that prevail in the lean subjects.

CONCLUSION

In conclusion, there was a direct relationship between fasting serum insulin and BMI with normo-, or euglycaemia, in Caucasian South African adults. Females exhibited greater insulin sensitivity than men at similar BMI values. The findings contained in this study support the positive correlation between BMI and fasting insulin concentrations previously reported.

Chapter Three

**Greater appetite control associated with an
increased frequency of eating in lean males**

INTRODUCTION

Both epidemiological and experimental studies have shown that an inverse relationship exists between the number of meals consumed per day and the general health status of an individual (Bray, 1972; Fábry & Tepperman, 1970). An increase in the daily number of meals consumed results in an improved carbohydrate metabolism (Fábry *et al.*, 1964a; Jenkins *et al.*, 1989; Wolever, 1990), enhanced lipid metabolism (Irwin & Feely, 1967; Fábry & Tepperman, 1970), greater insulin sensitivity (Jenkins *et al.*, 1989; Jenkins *et al.*, 1990), reduced adiposity (Fábry *et al.*, 1964a; Fábry *et al.*, 1964b) and improved weight control (Debry *et al.*, 1973; Young *et al.*, 1971). Consequently, greater meal frequency has been prescribed in diabetes care, cardiovascular treatment, and in the prescription of energy-restricted dietary programmes in the treatment and management of obesity.

The effects of feeding frequencies have revealed that greater fluctuations in hormone and metabolite levels occur following larger meals (gorging), than after smaller, more frequent meals (Jenkins *et al.* 1989, Jenkins *et al.* 1992, Wolever, 1990). These studies however, have focused primarily upon the effects of altered meal frequency on intermediary metabolism, and not on the issue pertaining to energy regulation. Although numerous studies have investigated the effects of the periodicity of eating on energy expenditure (Tai *et al.*, 1991, Verboeket-van de Venne & Westerterp, 1993, Young *et al.*, 1971), none have considered the direct effects on varied frequencies of eating on subsequent energy intake.

It is within this context that the following experiment has been designed: a degree of uncertainty exists as to the role that the periodicity of eating plays in the regulation of energy intake; and whether the body is easier able to detect a large meal more readily than it can detect many small meals, and compensate accordingly. It was therefore the primary aim of this investigation to consider the effects that manipulating eating frequencies would have on the physiological profile and the associated effects on appetite and satiety mechanisms. Accordingly, the null hypothesis tested in this experiment was that there was no difference in non-obese males' energy consumption at an ad libitum test meal after different feeding frequencies at the pre-load period.

MATERIALS AND METHODS

Subjects

Eight healthy, non-obese young men were recruited to participate in this study (TABLE 3.1). All subjects were screened for eating restraint via the Dutch Eating Questionnaire (Van Strien *et al.*, 1986) which is aimed at three basic categories, viz external motivation to eat, emotional eating, and restrained eating patterns. The test was done "blindly", and only subjects who scored normal scores in all three categories were used (TABLE 3.1). This study was approved by the Ethics Committee of the University of the Witwatersrand (Ethical clearance number: M960425), and informed consent was obtained from all volunteers.

TABLE 3.1.

Characteristics of subjects.

		MEAN $\pm$ SD	RANGE
N		8	
Age	(Years)	22.9 $\pm$ 4.2	19 - 29
Weight	(kg)	73.3 $\pm$ 11.5	58.3 - 90.8
Height	(m)	1.78 $\pm$ 0.07	1.70 - 1.90
BMI	(kg·m ²)	23.11 $\pm$ 2.84	18.8 - 29.1
WHR		0.978 $\pm$ 0.062	0.878 - 1.036
DREQ			
External		3.313 $\pm$ 0.963	
Emotional		2.413 $\pm$ 1.230	
Restraint		2.125 $\pm$ 1.315	

Protocol

The effect of meal frequency on appetite, perception of hunger, glucose and insulin concentrations was assessed by randomly assigning subjects on their first visit to one of two feeding regimes. Each subject took each eating pattern in random order with 50% of the study cohort taking the *nibbling* diet first. One-third of their average daily energy requirement (ADER) (as determined by the Harris-Benedict equation (Van Way, 1992)) was either given as a single meal (SINGLE) or divided into five equal portions given at hourly intervals (MULTI).

Testing Considerations

All subjects reported to the Sleep and Metabolic Laboratory at the University of the Witwatersrand Medical School, Johannesburg, at 07h00 in the morning. The laboratory is at an altitude of 1,800m (625 torr) and regulated at a room temperature of 20-22°C. The subjects were monitored throughout the testing period, and they were not allowed to leave the laboratory for any reason. Subjects were asked to refrain from eating and drinking anything other than that prescribed in the testing protocol. The first pre-load meal was given at ~07h30. Irrespective of whether a single meal or one of the multi-meals was given, the men were subjected to identical treatments and identical nutrient intake (TABLE 3-2): visual analogue scales (VAS) and blood measurements were collected hourly for 5 hours. After five-and-a-half hours (~13h00), subjects were given an *ad libitum* lunch (of pre-prepared cottage pie and orange juice, TABLE 3-2). This was eaten in isolation. Subjects were told to eat as

much as they wanted and for as long as they desired. They were unaware that the quantity of food ingested was being monitored in any way. The amount of food (and energy) intake at the *ad libitum* lunch was measured by weighing the cottage pie (in its container) and the orange juice carton, immediately before presenting it to the subjects, and then weighing the whole container after completion of the test lunch meal.

TABLE 3-2

The nutritional information of foods (per 100g) of the breakfast pre-load and the ad libitum lunch.

	Carbohydrate (grams)	Protein (grams)	Fat (grams)	Energy (kJ)
BREAKFAST³				
Pasta	71.0	11.6	1.5	1,430
Pasta sauce	13.0	1.8	0.2	245
Orange juice	12.1	0.3	0.1	209
Ice-cream	23.9	3.6	10.8	847
LUNCH⁴				
Orange juice	12.1	0.3	0.1	209
Cottage Pie	5.1	9.2	8.8	572

Timing of the time taken to consume the *ad libitum* lunch meal began immediately as the meal was presented to the subject. Subjects were instructed to start eating when they were presented with the food, and then to leave the table upon completion. Accordingly, each subject was monitored covertly throughout the eating process.

³ Although pasta is not a traditional breakfast in a South African context, it was decided to use this food as the pre-load since it was required to constitute 33% ADER with "prudent" amounts of carbohydrate and protein. It would have been unrealistic to achieve this energetic value using breakfast cereal as a pre-load.

⁴ Cottage Pie is composed of beef mince mixed with vegetables (carrots and peas), and baked mashed potato on top. It is consumed as either lunch or dinner in South Africa. Nutritional information contained in TABLE 3-2.

Food considerations

Every effort was made to ensure that the food presented to all subjects on all feeding treatments was identical (relative to each specific subject) in relative weight and energetic value, texture, temperature, and appearance. Although the temperature of the food was not measured, the preparation in all foods was sufficiently identical (storage, and cooking time) to satisfy us that the foods were similar when they were presented to the subjects.

The *ad libitum* meal (cottage pie) was considerably different in macronutrient value and energetic value to the pre-load meal(s) (pasta) (FIGURE 3-2) to ensure that the results obtained in this study were not the affects of sensory specific satiety rather than the frequency of feeding.

Body mass (accurate to 0.05kg, Mettler TE/J, Zurich, Switzerland) and height (accurate to 0.1cm, SECA, Germany) were measured at the start of the experiment.

Scores for satiety

Subjective satiety and hunger were assessed through means of six VAS scales that accounted for the degree of changes in the following factors: hunger; thoughts of the amount of food that could be eaten; and the urge to eat (Hill *et al.*, 1984). The VAS scales were all 100mm in length, which were anchored at either extremity with terms indicating extremes as depicted below.

An example of the visual analogue scale (VAS) used to assess hunger.

How hungry do you feel?

Not at all hungry | | As hungry as I have ever felt

In analysis of the VAS data, the mid-point of the 100mm line (ie 50mm) was arbitrarily assigned the neutral line (Jenkins *et al.*, 1992). The value determined in this manner was then converted into a percentage of the extreme being measured, and subsequently analysed as such.

Plasma glucose and serum insulin analyses

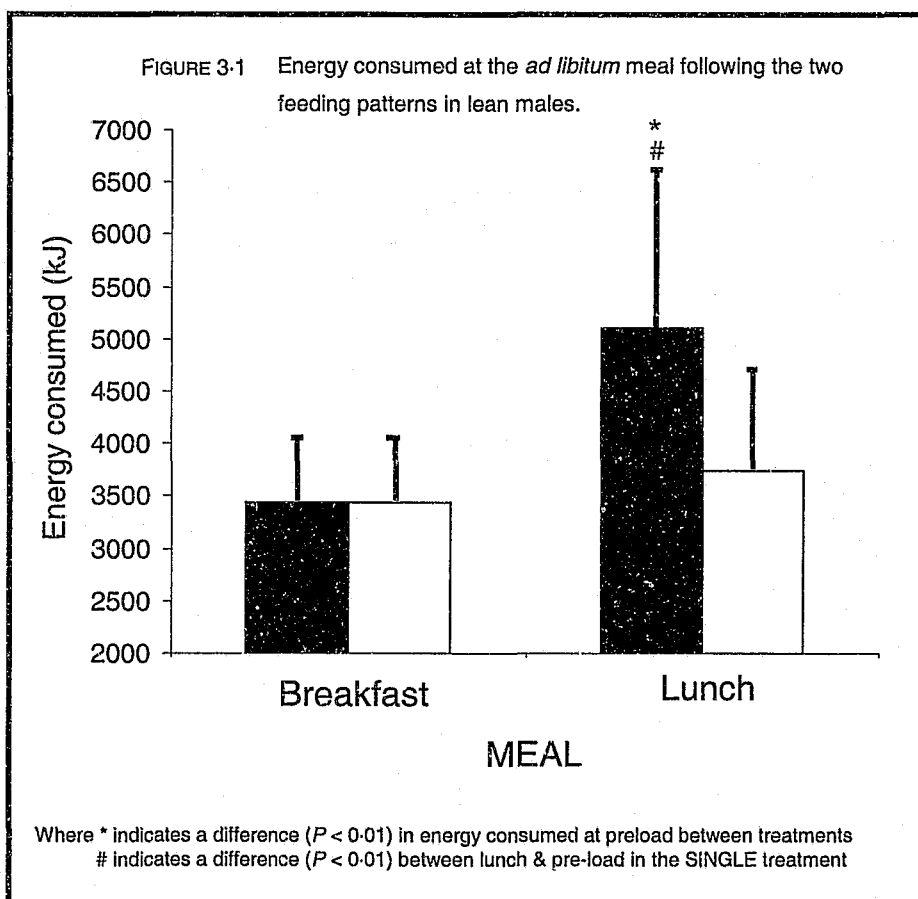
Venous blood was drawn into prepared tubes and centrifuged at 5 000 rpm for 15 minutes for plasma and serum collection. These samples were stored at -70°C until assayed. The plasma samples were assayed for blood glucose levels using the GLUCOSE (Glu-cinet®) Technicon method ID-2G46-E94 (Sclavo Spa. Siena, Italy). The serum samples were assayed for insulin levels using the Coat-A-Count® method 91145 via the ¹²⁵I radiolimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The precise techniques and test principles are given in Appendix A.

Statistical methods

Changes in blood glucose and insulin over time, and changes in VAS ratings were statistically tested using the multiple analysis of variance (MANOVA) for repeated measures, and the Scheffé Test was used as an *ad hoc* test for differences between means. The data on time taken to consume the *ad-libitum* meal, the weights of food consumed, and the amounts of energy consumed at that meal were compared using the paired *t*-test. Multiple regression (TABLE 3-6) analyses were used to determine the correlation between VAS scores completed immediately before lunch and energy intake during lunch (VAS ratings and treatment were the independent variables). All statistical tests were conducted using the software package STATISTICA, developed by Statsoft, Tulsa, OK, USA. In the text, Tables, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

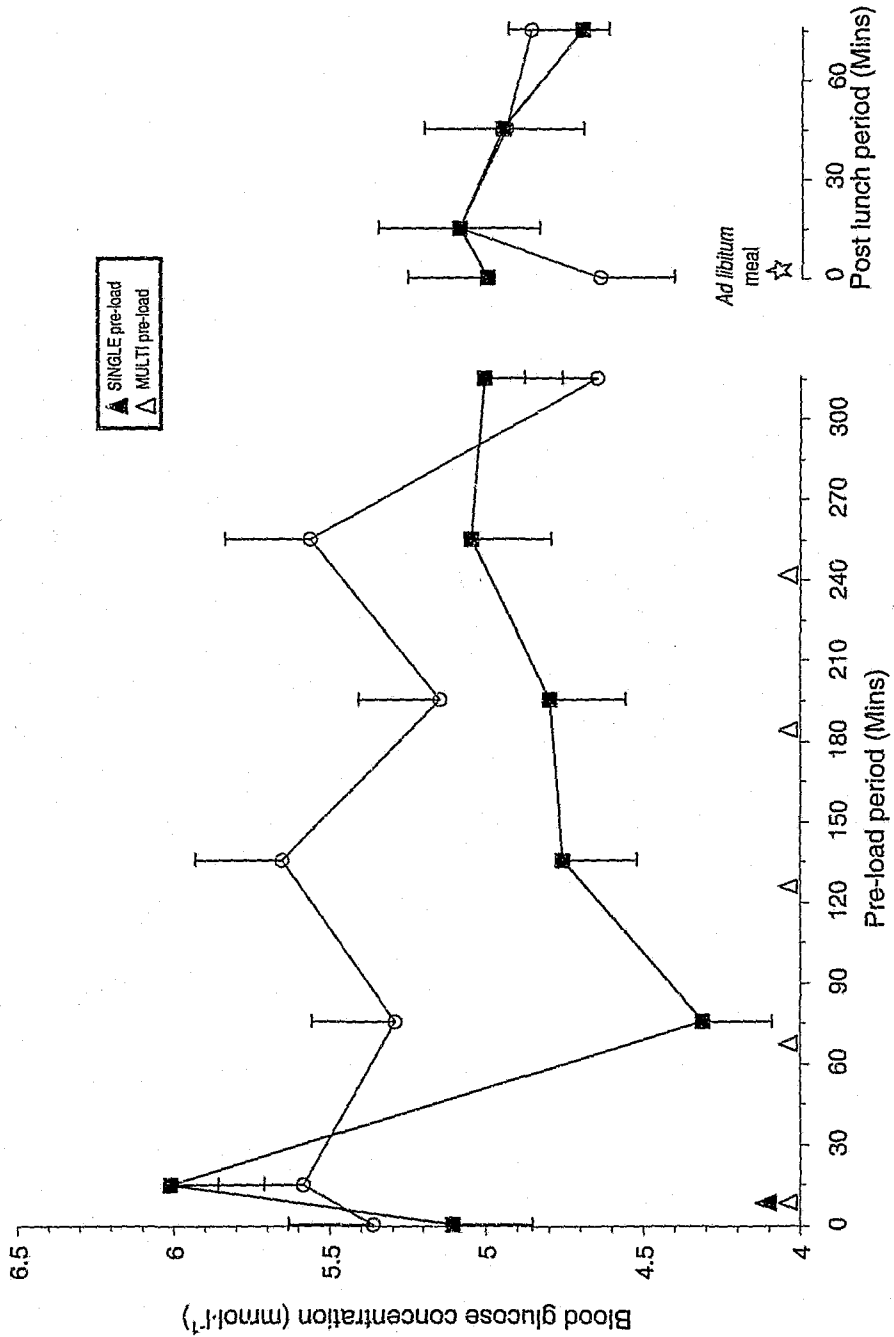
All subjects complied with the treatments and followed the protocol precisely. On both experimental treatments, fasting (baseline) blood glucose and insulin concentrations were in the normal range (FIGURE 3-2). Differential effects ($P < 0.05$) of meal frequency were evident, with significant changes in serum insulin (FIGURE 3-3), and in the amount of food consumed in the *ad libitum* meal (FIGURE 3-1).



Blood Glucose

Mean blood glucose rose 15 minutes after completing the first meal on both treatments (FIGURE 3-2). The SINGLE treatment's mean blood glucose declined to under the initial basal levels one hour after the initial spike where they remained until the *ad libitum* meal. Blood glucose concentrations following the MULTI regime were maintained at basal levels throughout the treatment, and there were no changes ($P > 0.05$) in blood glucose concentrations on either treatment following the *ad libitum* lunch.

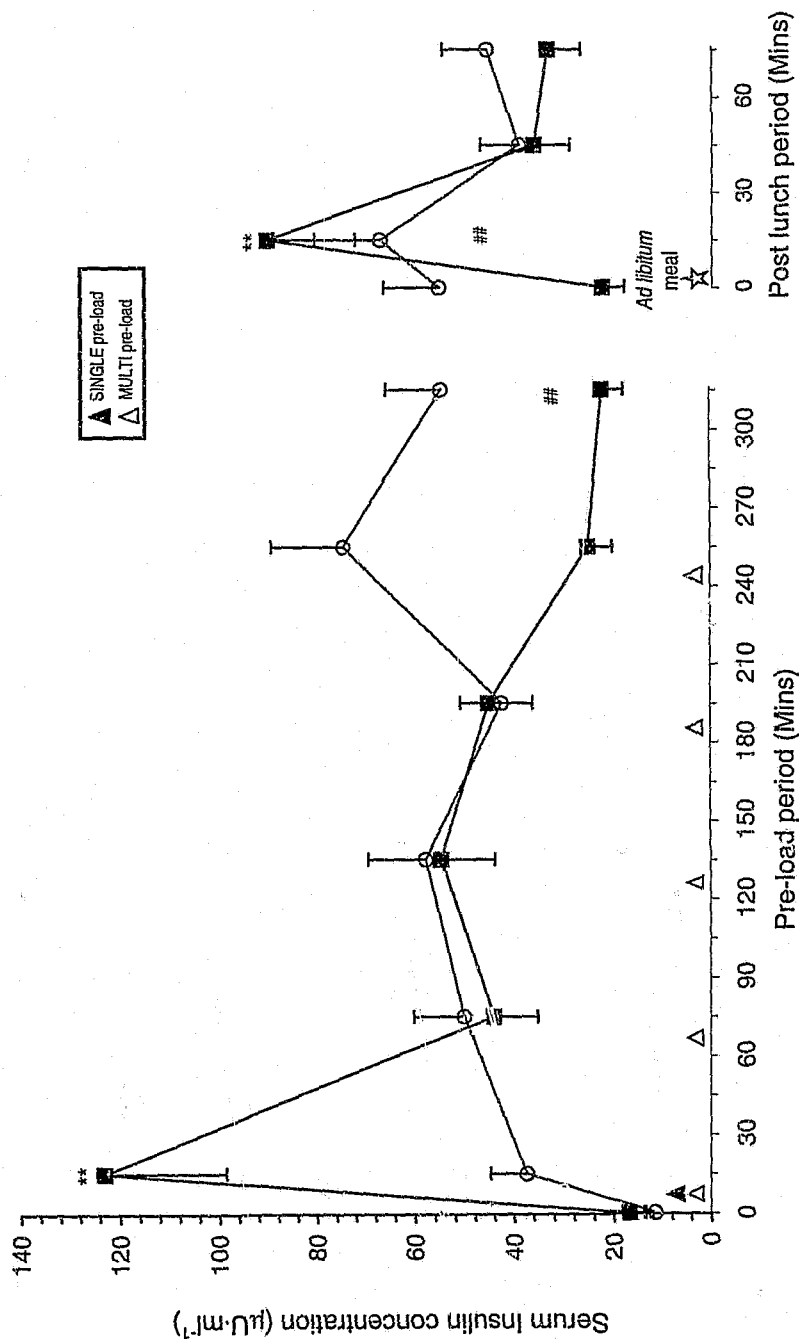
FIGURE 3-2 Changes in plasma glucose concentrations between SINGLE (■) and MULTI (○) Feeding patterns in lean males.



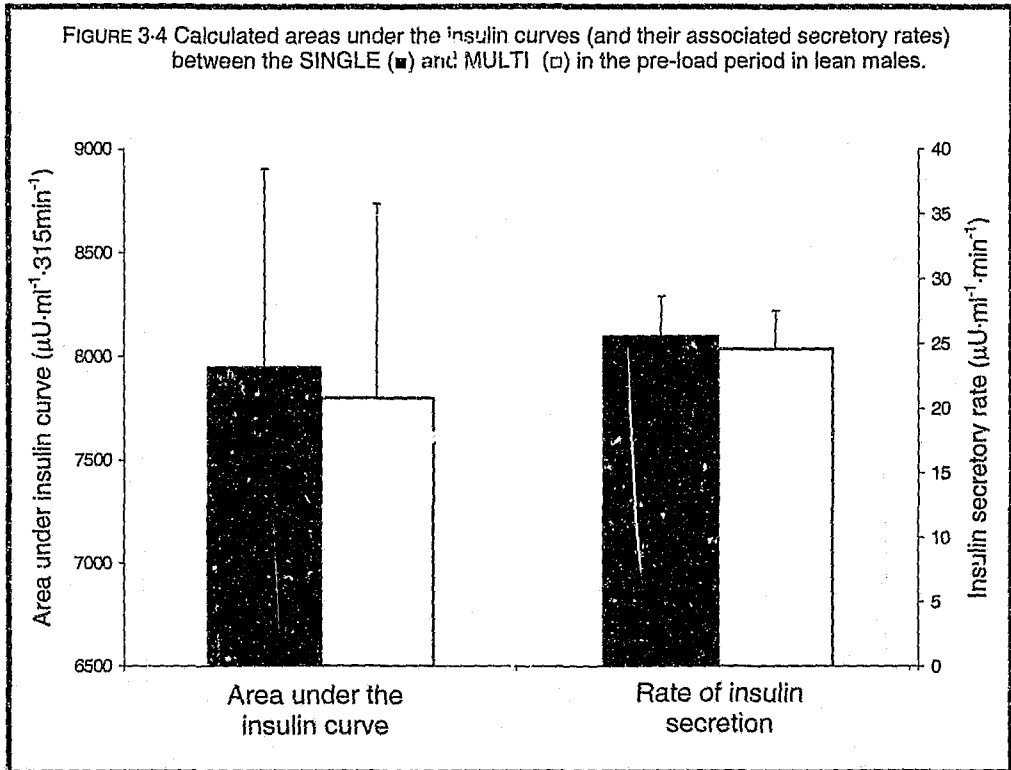
Serum Insulin

The two baseline readings in serum insulin concentrations were similar ($P > 0.05$) (FIGURE 3-3) and both treatments showed a significant variation with time ($F_{8,96} = 10.86$, $P < 0.01$). When the two eating patterns were compared, the SINGLE treatment had significantly higher ($P < 0.01$) insulin concentrations 15 minutes after the initial meal, and 15 minutes following cessation of the *ad libitum* meal ($P < 0.01$) compared to the MULTI treatment. The insulin responses on the two feeding patterns were significantly different over both the pre-load and the post-lunch courses.

FIGURE 3.3 Changes in serum insulin concentrations between SINGLE (■) and MULTI (○) Feeding patterns lean males.



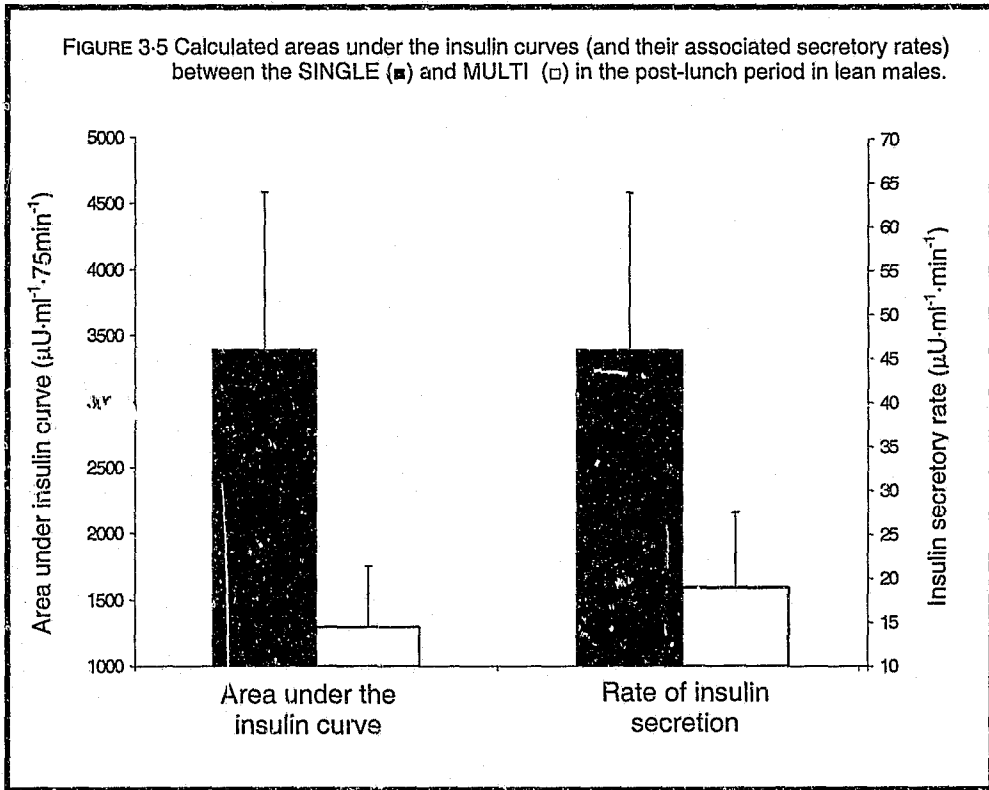
Where ** indicates significant difference ($P < 0.01$) between SINGLE and MULTI treatments
indicates difference ($P < 0.01$) between feeding patterns over the feeding period



Areas under the insulin curves (FIGURE 3-4)

In the 315-minute pre-load period, the areas under the insulin curves were calculated using a mean-model of calculation according to time intervals of serum insulin assessment. There were no differences in the amounts of insulin that were released over the full period (FIGURE 3-4, *left*), and consequently, the rates of insulin secretion were similar through the pre-load period (FIGURE 3-4, *right*).

As in FIGURE 3-5, the calculated areas under the insulin curves in the 75-minute period after lunch were similar ($P > 0.05$) (SINGLE; $3,397 \pm 1,254 \mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$ vs MULTI; $1,197 \pm 737 \mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$), and accordingly, the rate of insulin secretion was also similar in this post-lunch period.



Visual Analogue Scales (VAS)

In the three factors that constituted the VAS tests, the two treatments yielded similar values at baseline ($P > 0.05$) (FIGURES 3-6, 3-7, and 3-8). Furthermore, the three individual VAS factors, while generally indicating the same perception of satiety, showed slight nuance differences depending on the phrase used to describe hunger, the amount that each subject felt that they could eat, and the urge to eat. In all terms used to describe subjective hunger, there were two-way interactions ($P < 0.01$) between the two feeding patterns over both the pre-load, and the post lunch period: the MULTI treatment elicited a *flatter* (exhibiting less variance and amplitude in ratings) subjective hunger rating compared to those (subjective hunger ratings) when they ate their pre-load meals in a MULTI fashion.

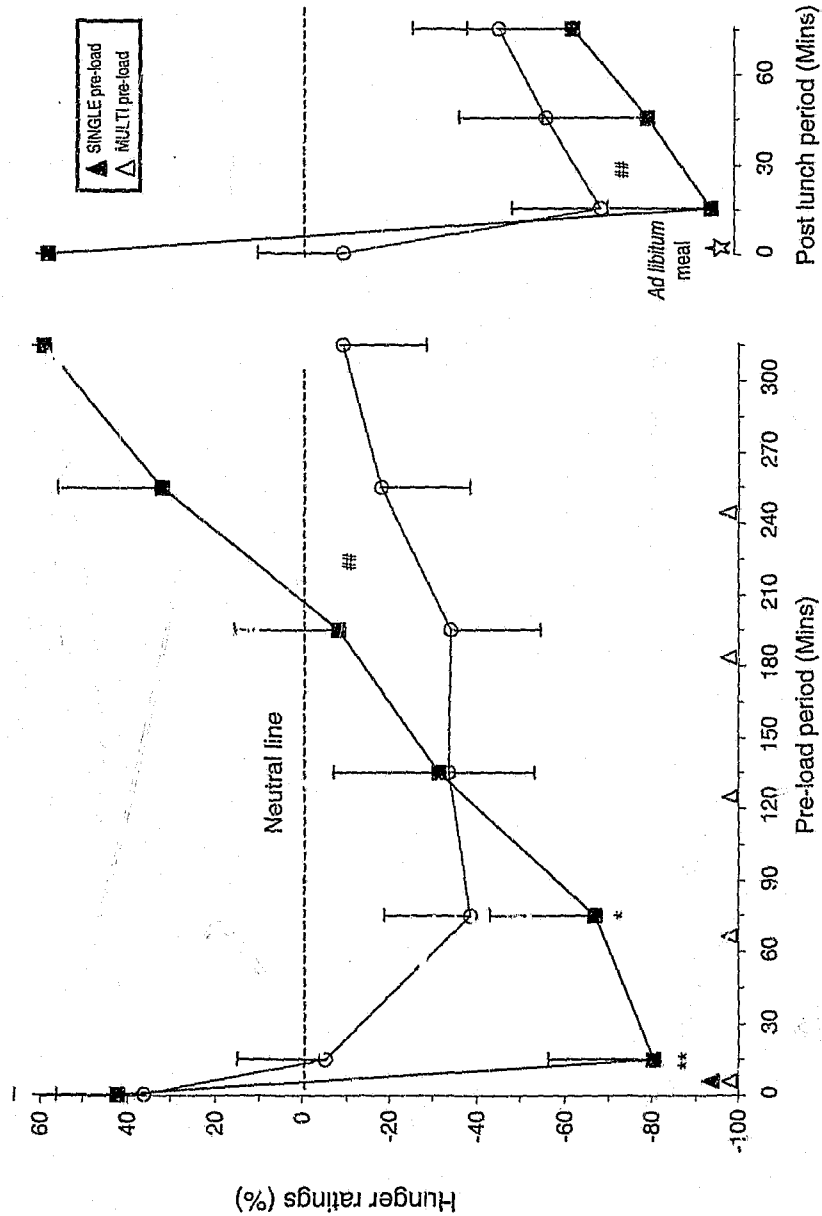
The SINGLE treatment wanted to eat less ($P < 0.01$) food, showed a weaker ($P < 0.01$) urge to eat, and their hunger ratings were less ($P < 0.01$) than their baseline scores in the thirty-minute period following the pre-load meal. After 90 minutes, the SINGLE group still had a weaker ($P < 0.01$) urge to eat than, and reduced ($P < 0.05$) hunger rating and felt they could eat less than when they started the trial. In the hour after the *ad libitum* lunch, both treatments' urge to eat was weaker ($P < 0.01$), and the amount that could be eaten was less ($P < 0.01$) than at the start of the experiment. The MULTI treatment had a reduced ($P < 0.05$) hunger rating 15 minutes after lunch, whilst the SINGLE treatment had a lower hunger rating for the 30 minutes ($P < 0.01$), and 60 minutes ($P < 0.05$) following the *ad libitum* lunch, compared to their subjective feelings at the start of the trial.

TABLE 3-3

Characteristics of the ad-libitum meal consumed on the two different eating regimes.

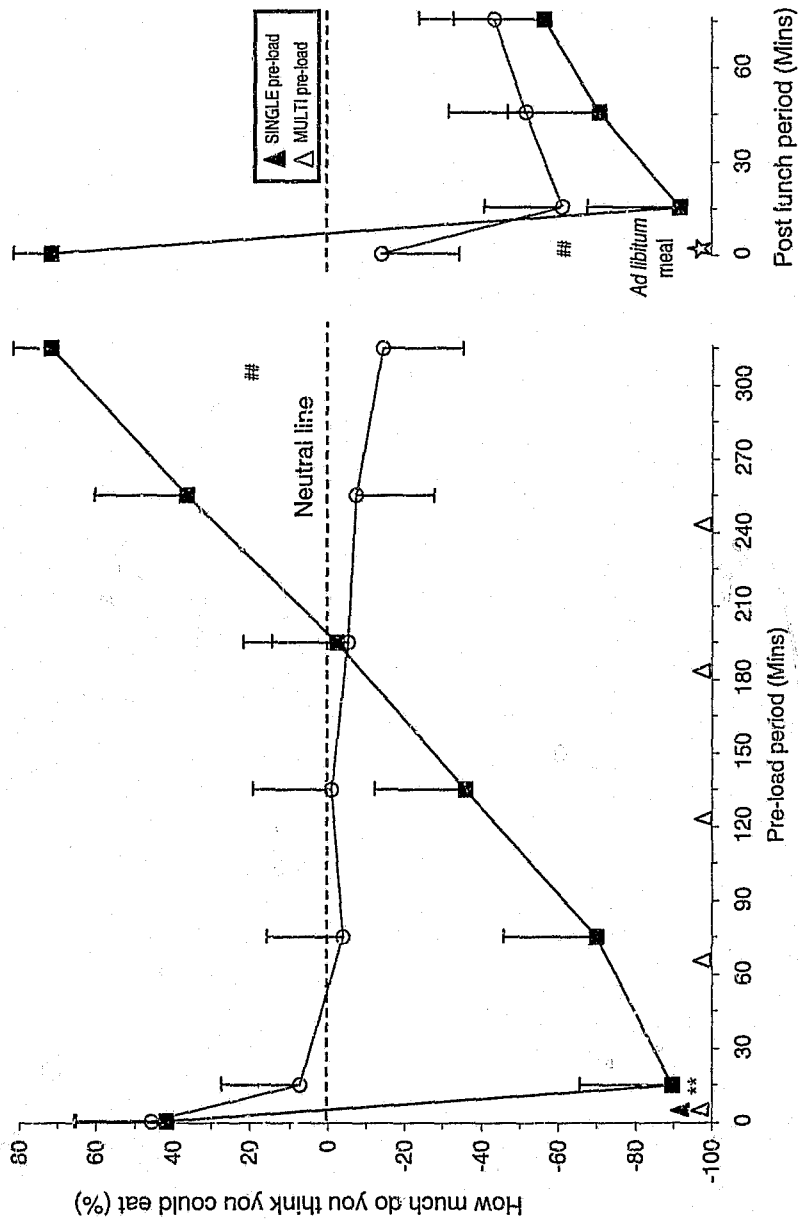
		SINGLE MEAL PROTOCOL	MULTIPLE MEALS PROTOCOL	LEVEL OF SIGNIFICANCE
Time to consume meal	(min)	17.1 ± 2.2	16.4 ± 3.4	NS
Weight consumed				
Cottage pie	(g)	652 ± 232	484 ± 148	$t = 2.8, P < 0.05$
Liquifruit	(g)	658 ± 226	473 ± 161	NS
Total	(g)	1,311 ± 357	957 ± 217	$t = 3.0, P < 0.05$
Energy consumed				
Cottage pie	(kJ)	3,734 ± 1,330	2,770 ± 847	$t = 2.8, P < 0.05$
Liquifruit	(kJ)	1,377 ± 474	988 ± 338	NS
Total	(kJ)	5,111 ± 1,502	3,753 ± 893	$t = 3.0, P < 0.05$

FIGURE 3-6 Changes in subjective hunger ratings between SINGLE and MULTI feeding patterns in lean males.



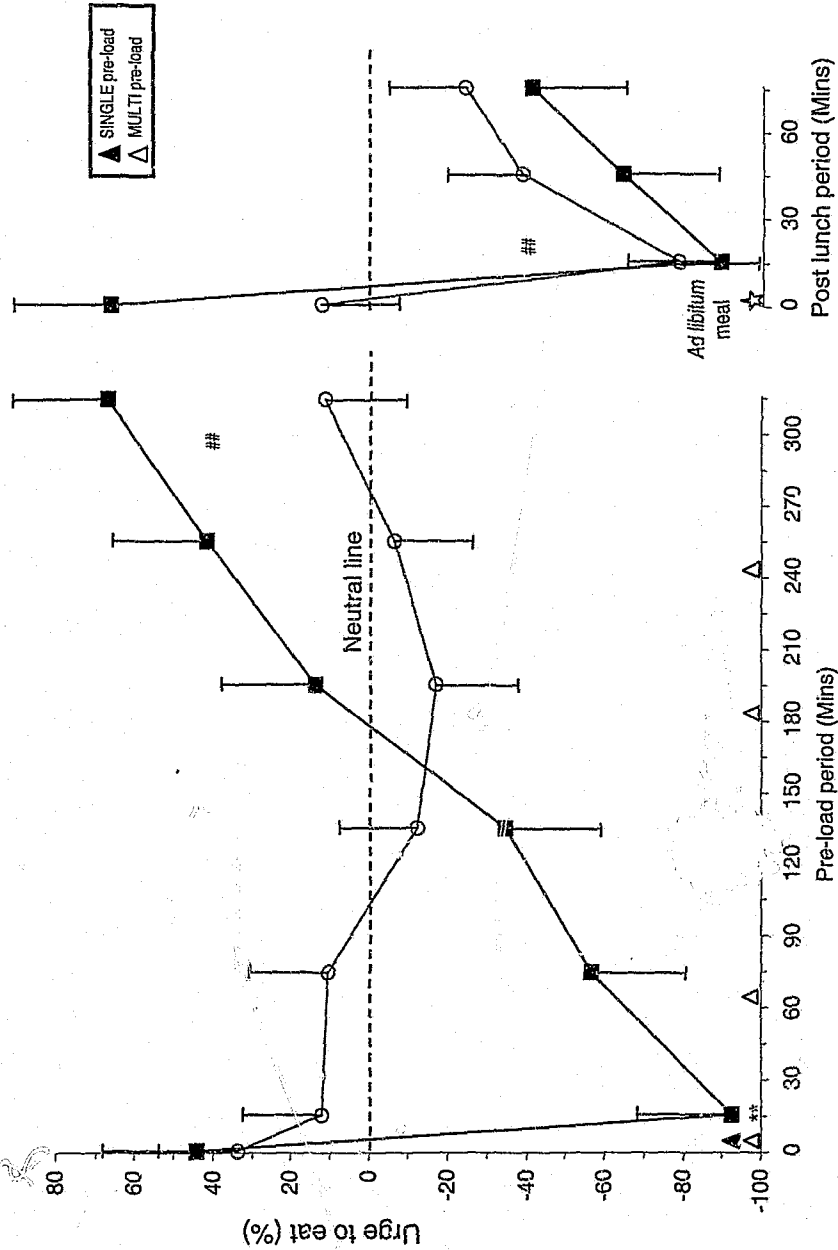
Where * indicates significant difference ($P < 0.05$, $P < 0.01$) between SINGLE and MULTI treatments
 ## indicates difference ($P < 0.01$) between feeding patterns over the feeding period

FIGURE 3-7 Changes in subjective ratings of how much the lean males felt they could consume between SINGLE (■) and MULTI (○) feeding patterns.



Where ** indicates significant difference ($P < 0.01$) between SINGLE and MULTI treatments
indicates difference ($P < 0.01$) between feeding patterns over the feeding period

FIGURE 3.8 Changes in subjective urge to eat between SINGLE (■) and MULTI (○) feeding patterns in lean males



Where ** indicates significant difference ($P < 0.01$) between SINGLE and MULTI treatments

indicates difference ($P < 0.01$) between feeding patterns over the feeding period

While the time taken to eat the *ad libitum* meal was similar ($P > 0.05$) following the two treatments, 1.2 times more food was consumed in the *ad libitum* meal when the subjects ate one large breakfast pre-load compared to that consumed following the MULTI meal treatment (TABLE 3.1).

As a direct consequence thereof, more ($t = 3.63$, $P < 0.01$) energy was consumed in the SINGLE treatment, accounting for over 50% of the recommended ADER in this one *ad libitum* meal, compared to 36% ADER in the *ad libitum* meal following the MULTI regime (TABLE 3.4). Consequently, the excess that the SINGLE treatment consumed was greater ($P < 0.01$) than the (33.3% of ADER) expected value, whereas the excess consumed by the MULTI treatment was not.

TABLE 3.4

Comparison of macronutrient compositions between breakfast and the *ad-libitum* lunch meal.

		BREAKFAST	SINGLE LUNCH	MULTI LUNCH
Weight consumed	(CHO)	(g) 145 ± 15.87	113 ± 32.1	81.9 ± 20.8
	(Protein)	(g) 20.0 ± 1.7	62.0 ± 21.5	46.0 ± 13.7
	(Fat)	(g) 20.3 ± 4.82	58.1 ± 20.5	43.1 ± 13.0
% consumed	(CHO)	(%) 78.3 ± 1.0	48.2 ± 9.0	47.4 ± 7.9
	(Protein)	(%) 10.9 ± 0.4	26.2 ± 4.6	27.2 ± 4.0
	(Fat)	(%) 10.8 ± 1.3	25.1 ± 4.5	24.5 ± 4.7

TABLE 3.5

Analysis of the ad-libitum meal consumed on the two different eating regimes.

		SINGLE MEAL PROTOCOL	MULTI MEALS PROTOCOL	LEVEL OF SIGNIFICANCE
Rate of energy consumption	(kJ·min)	320 ± 107	285 ± 153	NS
Expected energy consumption	(kJ)	3,404 ± 441	3,404 ± 441	---
Actual energy consumption	(kJ)	5,111 ± 1,502	3,753 ± 893	$t = 3.0, P < 0.05$
Difference (actual - expected)	(kJ)	1,898 ± 1,479	639 ± 986	$t = 3.0, P < 0.05$

When the pre-*ad libitum* lunch VAS scores were compared with the amount of energy consumed at the *ad libitum* lunch, there were significant correlations between the MULTI treatment's self-assessment on the amount that they thought they could eat at the next meal ($P < 0.05$), their urge to eat ($P < 0.05$), and their preoccupation with food ($P < 0.01$). There were no significant correlations in any of the SINGLE treatment's VAS scores and the amount of energy consumed.

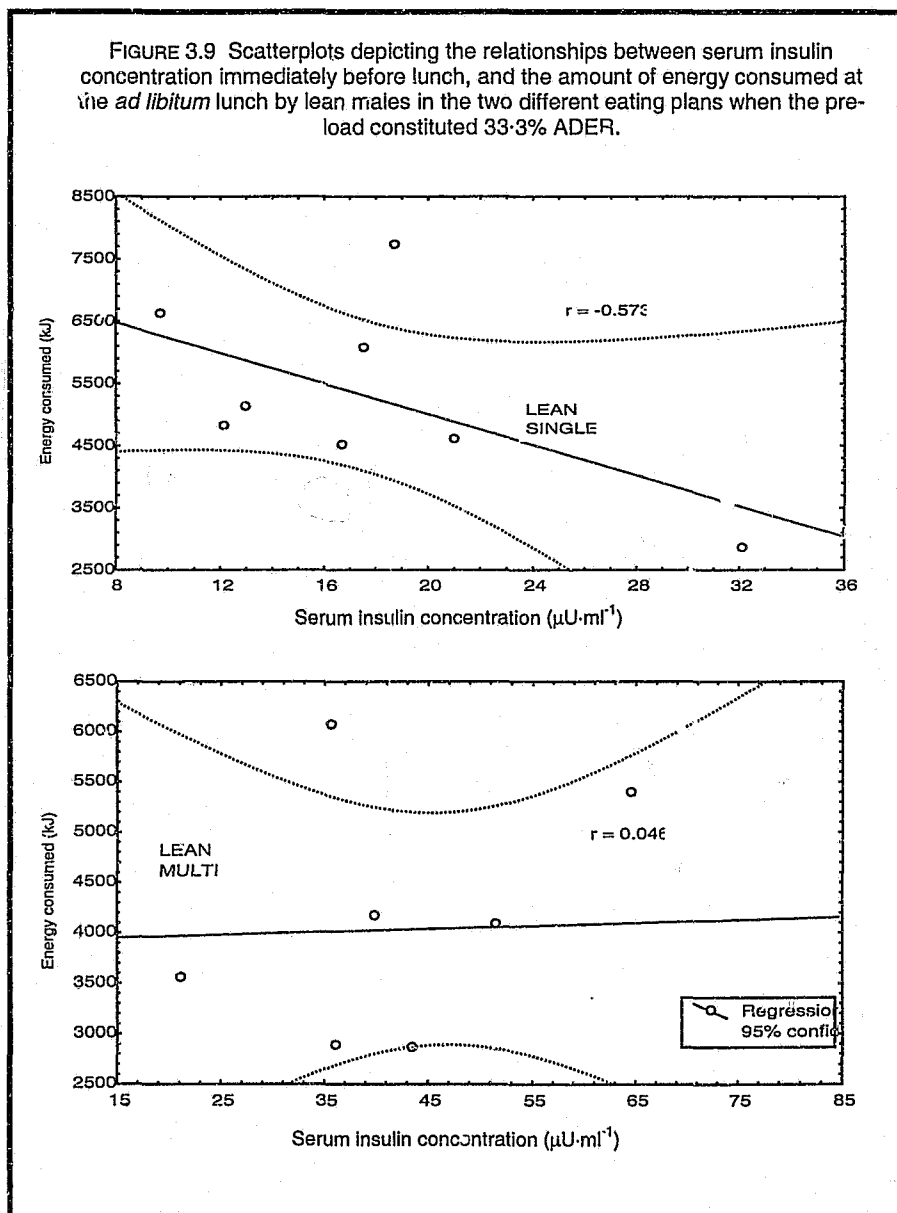
TABLE 3.6

Correlation data between VAS data and the energy consumed at the ad libitum lunch

	SINGLE MEAL R	LEVEL OF SIGNIFICANCE	MULTIPLE MEALS R	LEVEL OF SIGNIFICANCE
Hunger ratings	0.204	NS	0.404	NS
Amount could eat	0.257	NS	0.740	0.036
Urge to eat	0.327	NS	0.723	0.043

FIGURE 3.9 shows the relationships in these non-obese males between their insulin levels immediately consuming the *ad libitum* lunch, and the amount of energy consumed at the test lunch meal. Although the SINGLE pattern of eating the pre-load appears to result in an inverse

relationship between the two variables, it is not significant ($r = -0.573$, $P > 0.05$). As can be seen in FIGURE 3-9 *Bottom*, the effect of insulin concentration on energy intake is very weak ($r = 0.046$, $P > 0.05$).



DISCUSSION

When a group of young non-obese males were fed an isoenergetic pre-load that was spread out over the course of a morning (as opposed to a single breakfast), they consumed less energy at a subsequent *ad libitum* lunch. Moreover, they felt equally satiated despite eating significantly less food. Although blood glucose was unchanged with the frequency with which meals were consumed (FIGURE 3-2), insulin concentrations, the VAS scores, and the amount eaten in a subsequent *ad libitum* meal were significantly altered with feeding pattern. Furthermore, there was a close correlation between appetite ratings and the amount of food eaten the *ad libitum* meal in the MULTI treatment. These findings suggest better intuitive assessment of appetite and energy intake with more frequent, smaller meals than with one larger meal, and accordingly, the null hypothesis was rejected.

Although the direct effects of meal frequency on subsequent energy intake have not been previously measured, Hejda & Fábry (1964) reported an indirect relationship where they showed that individuals who consumed 3-4 meals per day were more overweight and had thicker skinfolds than those who consumed 5-6 meals per day. Indirectly, the current data substantiate their postulate that more frequent feeding was negatively associated with adiposity, and show greater appetite control with increased meal frequency. Reports from animal studies also imply that mice, which have been intermittently-fed more frequently, have decreased food consumption relative to *ad libitum* intake levels (Van Potten *et al.*, 1955).

Better control of energy regulation with increased meal frequency may be attributed to differentiating effects on energy expenditure; however, no consensus has been reached in this regard. Many studies suggest that the pattern in which an individual eats has no effect on the energetic cost of digesting, absorbing, and utilising the nutrients. Similarly the frequency of eating has no effect on 24h energy metabolism, and its components sleeping metabolic rate and dietary-induced thermogenesis (Verboeket-van de Venne & Westerterp, 1991; 1993; Tai *et al.*, 1991). Furthermore, the inverse relationship between the number of meals consumed per day and the degree of adiposity (as postulated by Fábry *et al.*, (1964a) have since been questioned within the confines of *dietary under-reporting* and reverse causality on the parts of the subjects who participated in studies of this nature (Bellisle *et al.*, 1997; Poppitt *et al.*, 1998; Voss *et al.*, 1998). The report of Bellisle *et al.*, (1997) placed particular emphasis on the energy expenditure side of the energy-balance equation, and ignored the role of periodicity of feeding in relation to appetite control, and the associated energy intake.

The VAS scores showed a close correlation between the factors "*How much do you think you could eat now?*", and "*urge to eat*", and the amount of energy consumed at the *ad libitum* lunch in the group that consumed a greater number of meals (TABLE 3-6). These findings suggest that there was greater control over appetite sensations when the same (high-carbohydrate) pre-load was consumed over smaller, more frequent meals. Using the *pre-load – test-meal* paradigm, when satiety sensations and food intake were evaluated after different preloads, Porrini

et al., (1995) found that subjects consumed more food following a carbohydrate-rich preload, compared to a protein-rich preload.

Hunger ratings, depicted from the VAS results, appeared to be effective tools for measuring appetite and satiety in the current study. However, these results should be assessed with an element of caution, as numerous reports have criticised the reproducibility of subjective appetite scores on the basis of i. subjects' prior experience of the test meals (Raben *et al.*, 1995), and ii. lack of correlation between hunger ratings and an increased food intake in certain test situations (Mattes, 1990; Friedman *et al.*, 1999). In the current study, subjective VAS ratings showed consistently that subjects on the SINGLE treatment experienced greater fluctuations in hunger/appetite assessment than did those on the MULTI meal treatment. There were no differences between the two groups in their pre - *ad libitum* measure for the subjective degree of fullness, yet the SINGLE treatment consumed 26.6% more energy than the MULTI group did. Furthermore, despite consuming less energy at the *ad libitum* meal, the MULTI treatment exhibited a similar post-prandial satiety index as the SINGLE treatment did.

The interaction between the blood glucose and insulin indicated that the amount of insulin that was secreted allowed for sufficient removal of glucose, resulting in the overall maintenance of blood glucose homeostasis, irrespective of feeding pattern in these non-obese men. Immediately following the single pre-load, the insulin concentration elicited in the SINGLE treatment rose over 3x that was experienced following the first meal of the MULTI group (FIGURE 3-3). Schierf & Rietzer (1972)

noted individual insulin spikes twice as great in a three-meal per day regimen as compared to a six-meal per day regimen. These authors concluded from their observations that the insulin response was a function of the load of calories requiring deposition, and that there was no relation to the frequency with which the calories were ingested (Schierf & Raetzer, 1972). While the initial 3-fold response following the larger energetic bolus of the SINGLE meal compared to the 20% calorie intake of the first meal of the MULTI regime could concur with these earlier reports on energetic loads and insulin spikes. These findings were similar to those found by Jenkins *et al.*, (1992) who reported that when non-insulin dependent diabetics nibbled the same amount and type of food (as opposed to gorging in larger meals) over a 9.5 hour period, the nibbling group had reduced mean blood glucose and serum insulin concentration by $\pm 12.7\%$ and $\pm 20.1\%$ respectively.

Since there were no differences in blood glucose concentrations between the two feeding regimens prior to the *ad libitum* meal, the difference in energy consumption at the *ad libitum* meal cannot be explained within the confines of the glucostatic theory (Mayer 1955). Despite consuming more energy in the *ad libitum* lunch compared to the sum of all the five smaller "breakfast pre-load" meals, the MULTI group's serum insulin concentration failed to rise significantly from its pre-lunch value. The SINGLE group's serum insulin response to the *ad libitum* meal was higher ($P < 0.01$) than at baseline, but was not different ($P > 0.05$) at the same time period in the MULTI trial.

The difference between the two insulin responses (on each specific eating pattern, FIGURE 3.3) over the pre-load period may have had a bearing on

appetite. However, the lack of a difference in the areas under the insulin curves (and the associated lack of any difference in the rates of insulin secretion) suggests that if insulin exerts an effect on appetite it would be due to differences in temporal secretion, and not due to the total sum of the insulin secreted. The finding that the MULTI treatment elicited a 26.6% reduction in energy content consumption at the *ad libitum* lunch with an associated absence of acute hyperinsulinaemia may have been the result of retarding glucose delivery and therefore glucose absorption, through the duration of the morning, which enhanced their satiety scores in the period before the *ad libitum* lunch. This finding is in line with numerous studies that have shown improved glucose tolerance and enhanced insulin sensitivity with increased frequency of feeding (Ellis, 1934; Hollifield & Parson, 1962; Gwinup *et al.*, 1963). This phenomenon was further reinforced by the finding that slowing the absorption of carbohydrate by *guar gum* (a highly viscous solution that delays absorption by slowing access to the absorptive intestinal epithelium) increases satiety through the recruitment of glucose receptors in the small intestine (Lavin & Read, 1995). The spreading of the energetic load in the current study, may mimic the effects of *guar gum* in-so-much that the multi-meal pattern may have a cumulative *priming effect* on the overall handling of the carbohydrate delivery into the intestine, possibly allowing for an increased insulin sensitivity as reported by Jenkins *et al.*, (1989, 1992). Another factor that may play a part in these findings was the increased satiety via delayed gastric emptying, as has been noted with a guar gum-induced gastric distension (Krotkiewski, 1984).

CONCLUSION

This study has shown that increasing the frequency of the meals in a group of non-obese males, results in a greater control of satiety, with a subsequent reduction in energy intake at an *ad libitum* meal. This greater control of satiety could not be attributed to changes in plasma glucose concentration. However, eating more frequently may have a *priming effect* (or adaptative effect) on serum insulin concentrations as these levels were maintained on a steady level throughout the trial and failed to rise following a substantial *ad libitum* test meal. Although the dynamics of insulin secretion were different between the two feeding patterns, the total amount of insulin that was secreted were similar, indicating that the timing of insulin secretion according to feeding patterns may play a significant role. Accordingly, it may be the *primed effect* on the metabolic responses to an increased frequency of eating, that may explain the greater control over appetite and subsequent energy intake.

In contrast to the hypothesis postulated by Booth (1988), that the more frequent consumption of meals and snacks is the major aetiological factor toward the rising prevalence of obesity in the modern society, these data suggest that eating smaller meals more frequently has an positive effect on appetite control in non-obese males. Further, if such a phenomenon held true in the obese population, it may allow the obese an effective method of implementing greater control over their appetite and satiety mechanisms in combating the chronic excess in energy intake that is symptomatic of their condition. It has been shown that weight loss among

obese women on a weight-reducing diet was greater if isoenergetic feeds were given five times a day compared to three times or once a day (Debry *et al.*, 1973). Furthermore, for any weight loss to occur, an individual must reduce energy intake (Booth, 1988), and these results merely suggest that improved appetite control may facilitate a reduction in energy intake required for this weight loss.

The current study suggests a psycho-physiological basis for this phenomenon in a group of non-obese males. Whether indeed such a phenomenon exists in an obese group of males remains to be tested in an obese group. This shall be addressed in the next chapter.

Chapter Four

**Greater appetite reduction associated with an increased
frequency of eating in obese males**

INTRODUCTION

Excessive energetic ingestion relative to energetic requirements is considered to be the main reason behind obesity in normal populations (Prentice & Poppitt, 1996). Hyperphagia in the obese has been attributed for the disruption of the appetite – satiety regulatory mechanism (Ramirez *et al.*, 1989; Blundell & King, 1996; Blundell & Macdiarmid, 1997), the physiological mechanisms regulating appetite control, however, remain unclear. Satiety and hunger are influenced by the quantity (energy content and density (Prentice & Poppitt, 1996), and quality (macronutrient composition) of the diet (Prentice & Poppitt, 1996; Blundell & King, 1996; Hill *et al.*, 1984; Blundell *et al.*, 1994; Rolls *et al.*, 1988; Rolls *et al.*, 1990; Porrini *et al.*, 1995), and may also be regulated by the periodicity of eating (Durrant *et al.*, 1978; Jenkins *et al.*, 1989).

Epidemiological studies have reported that smaller more frequent meals are associated with reduced body fat in humans (Fábry *et al.*, 1964a; Fábry *et al.*, 1970; Kudicka *et al.*, 1966). These investigations were based primarily on dietary reports on the parts of the respondees, and thus uncertainty surrounds the validity of this inverse relationship since the entire spectrum of responses to meal frequency has been previously reported: one study has shown that snacking contributes to the increased energy consumption in the obese state (Basdevant *et al.*, 1993), others have shown no relationship at all (Edelstein *et al.*, 1992; Summerbell *et al.*, 1996), and a third cohort report an inverse relationship between meal frequency and energy intake (Drummond *et al.*, 1996; Drummond *et al.*, 1998; Metzner *et al.*, 1977). These inconsistencies may be partially explained by reliance on the accurate reporting of foods eaten and the

common finding that under-reporting is rife in the obese group (Bellisle *et al.*, 1997; Poppitt *et al.*, 1998; Blundell *et al.*, 1996).

If such an inverse relationship between number of meals and the incidence of obesity was valid, this would imply that those individuals who eat more frequently would be leaner than those who eat less frequently. Using the fundamental premise that obesity is based on appetite dysfunction, one may deduce that non-obese individuals enjoy a state of ideal energy regulation facilitated by the periodicity of their ingestive behaviour. A recent study from our laboratory showed as much: by increasing the frequency of eating an isoenergetic pre-load resulted in a greater appetite control at a subsequent test meal in lean males (Speechly & Buffenstein, 1999).

If altering the frequency of eating were able to improve appetite control in an obese population, the practicalities of such a phenomenon would allow an improved probability for those individuals who wish to lose weight on a restrictive dietary regimen. In light of the paucity of work on the periodicity of eating in relation to appetite control in the obese the null hypothesis tested in this experiment was that there was no difference in obese males' energy consumption (and the corresponding appetite ratings) at an *ad libitum* test meal after different feeding frequencies at the pre-load period, and that there was no difference to that observed in lean men in the previous chapter.

MATERIALS AND METHODS

Subjects

Seven healthy, non-diabetic obese men were recruited from the general population by means of community advertisements to participate in this study (TABLE 4.1). Of the fifteen respondents, eight men were either not comfortable with the venipuncture, or were unavailable on the days of testing, and consequently 53% were rejected. All subjects were screened for eating restraint via the Dutch Eating Questionnaire (Van Strien *et al.*, 1986), which is aimed at three basic categories, viz external motivation to eat, emotional eating, and restrained eating patterns. The test was done "blindly", and only subjects who scored normal scores in all three categories were used (TABLE 4.1). This study was approved by the Ethics Committee of the University of the Witwatersrand (Clearance number: M960425), and informed consent was obtained from all volunteers.

TABLE 4.1.

Characteristics of nonobese and obese subjects.

		Obese	Lean	Level of significance
N		7	8	
Age	(Years)	37.4 ± 18.5	22.9 ± 4.2	<i>P</i> < 0.05
Weight	(kg)	130.0 ± 40.9	73.3 ± 11.5	<i>P</i> < 0.01
Height	(m)	1.80 ± 0.07	1.78 ± 0.07	NS
BMI	(kg·m ⁻²)	40.02 ± 10.93	23.11 ± 2.84	<i>P</i> < 0.01
WHR		1.17 ± 0.08	0.978 ± 0.062	<i>P</i> < 0.01
DRQ				
External		3.27 ± 1.06	3.31 ± 0.97	NS
Emotional		2.87 ± 1.59	2.41 ± 1.23	NS
Restraint		2.97 ± 0.95	2.13 ± 1.32	NS

Where BMI = body mass index; WHR = waist:hip ratio;
and DRQ = Dutch Restrained Questionnaire.

Protocol⁵

The men reported to the laboratory on one occasion when they were weighed, screened for eating restraint, and informed partially of the nature of the experiment. They were not informed that the study would examine appetite control, but merely that the effect of meal frequency on blood variables and perceived mood and hunger would be assessed. Body mass (accurate to 0.1kg, Mettler TE/J, Zurich, Switzerland) and height (accurate to 0.1cm, SECA, Germany) were measured at the start of the experiment. The men were randomly divided into two treatment groups determining the order of their experimental meal treatment, such that meal treatment was randomised and all subjects received both meal treatments.

On both meal regimes, subjects received 33.3% of their calculated average daily energy requirement (ADER) on the experimental pre-load. This was determined using the equation of Harris-Benedict (Van W. *et al.*, 1992) as follows:

$$\text{ADER (kcal)} = \{66 + [(13.8 W) + (5 H)] - (6.8 A)\} P$$

where A is age (years), W is weight (kg), and H is height (cm). The ADER (in kcal) was multiplied by 4.18 for the conversion to Joules. The men reported to the laboratory in the fasted state at 07h00, having been asked to complete their dinner meal the night before no later than 22h00,

⁵ An identical protocol was used in this experiment to that described in the previous chapter, and every effort was made to ensure that subjects' behaviour was controlled and complied with the protocol exactly. Measurements of energy intake, the time of feeding, VAS measurements were identical to those described in the previous chapter.

and to restrict alcohol consumption for 24 hours beforehand. The pre-load meal (TABLE 4.2) was either given as a single meal (SINGLE) or divided into 5 equal portions and given hourly over a five-hour period (MULTI). The subjects were asked to consume the entire amount of food presented in the pre-load meal. The pre-load meal consisted of pasta and tomato sauce, ice-cream and orange juice (TABLE 4.2), and was made-up according to each individual's ADER. The pre-load comprised 70% carbohydrate, 15% protein, and 15% fat).

TABLE 4.2

The nutritional information of foods (per 100g) of the breakfast pre-load and the ad libitum lunch.

	Carbohydrate (grams)	Protein (grams)	Fat (grams)	Energy (kJ)
BREAKFAST³				
Pasta	71.0	11.6	1.5	1,430
Pasta sauce	13.0	1.8	0.2	245
Orange juice	12.1	0.3	0.1	209
Ice-cream	23.9	3.6	10.8	847
LUNCH⁴				
Orange juice	12.1	0.3	0.1	209
Cottage Pie	5.1	9.2	8.8	572

Five-and-a-half hours after consuming the first pre-load meal, a test meal (TABLE 4.3) was supplied *ad libitum*. The test meal was eaten in isolation, and subjects were informed to eat as much as they wanted for as long as they wanted. They were unaware that the quantity of food ingested was being monitored in any way. The *pre-load – test meal* paradigm has been criticised on the basis of cross-contamination (Blundell & Green, 1996) of foods given in a set sequence, and that certain foods given before another had different effects on the test meal (Porrini *et al.*, 1995). In the

current study, however, this was not the case as the food in the pre-load was all of the same composition.

Throughout the five-hour pre-load period, blood samples were collected and visual analogue scores (VAS) on appetite and hunger ratings were completed at hourly intervals. These same variables were collected and recorded at 30-minute intervals for 90 minutes after completion of the *ad libitum* test meal.

TABLE 4-3
Characteristics of the ad-libitum meal consumed by the obese group on the two different eating regimes.

		Single meal protocol	Multiple meal: protocol	Level of Significance
Time to consume meal	(min)	18.3 ± 3.7	21.0 ± 5.6	NS
Weight consumed Cottage pie	(g)	653 ± 182	554 ± 191	$P < 0.05$
Liquifruit	(g)	728 ± 383	564 ± 111	NS
Total	(g)	1,382 ± 418	1,119 ± 215	$P < 0.01$
Energy consumed Cottage pie	(kJ)	3,740 ± 1,039	3,172 ± 1,095	$P < 0.05$
Liquifruit	(kJ)	1,521 ± 801	1,180 ± 232	NS
Total	(kJ)	5,261 ± 1,289	3,763 ± 1,986	$P < 0.01$

Blood collection

Prior to starting each trial, an on-line catheter was inserted into the antecubital vein for the drawing of blood samples: the catheter was kept patent by the constant infusion of 0.9% sodium chloride (normal saline) (Sabax, Johannesburg). In order not to interfere with thirst mechanisms, not more than 100ml of saline was infused throughout the period on either trial. 5ml of whole blood was drawn at each period and put into prepared tubes (plasma had potassium oxalate as a metabolic inhibitor, and serum had a normal clotting factor) and centrifuged at 5,000 rpm for 20 minutes for plasma and serum collection. These samples were stored at -70°C

until assayed. Plasma samples were assayed for blood glucose levels using the Glucose (Hexokinase) method Cat.-No.0SR6121 (Sclavo Spa. Siena, Italy). Serum samples were assayed for insulin levels using the Coat-A-Count® method 91145 via the ¹²⁵I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Free fatty acid (FFA) or non-esterified fatty acids (NEFA) concentrations were measured by extraction from serum as chloroform soluble copper salts, and the concentration determined photometrically (Lancet Laboratories, Johannesburg, South Africa).

Scores for satiety

Subjective satiety and hunger were assessed through means of three VAS scales that accounted for the degree of changes in the following factors: hunger; thoughts of the amount of food that could be eaten; and the urge to eat. The VAS scales were all 100mm in length, which were anchored at either extremity with terms indicating extremes. In analysis of the data, the mid-point of the 100mm line (ie 50mm) was arbitrarily assigned the neutral line (Jenkins *et al.*, 1989; Porrini *et al.*, 1995). The value determined in this manner was then converted into a percentage of the extreme being measured, and subsequently analysed as such.

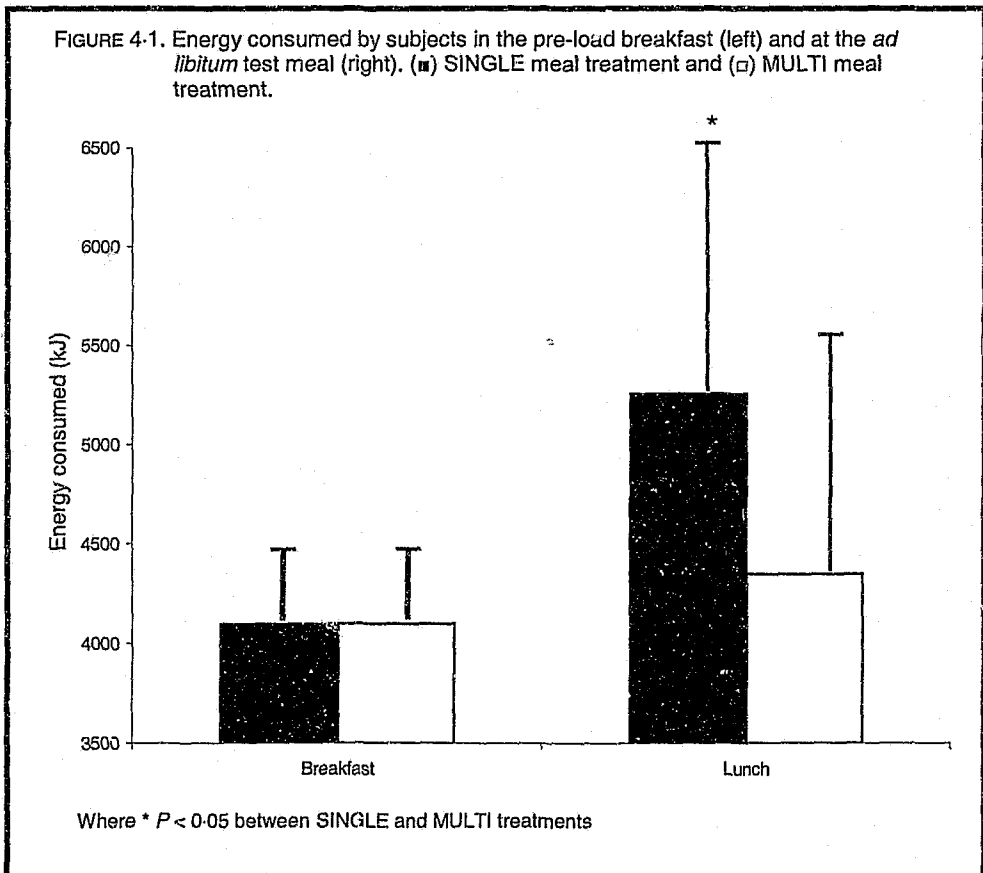
Statistical methods

Changes in plasma glucose, serum FFA, insulin and VAS ratings over time were statistically tested using the multiple analysis of variance (MANOVA) for repeated measures, and the Scheffé Test was used as an *ad hoc* test for differences between means. The data on time taken to consume the *ad-libitum* meal, the weights of food consumed, the amounts of energy consumed at that meal, and the areas under the respective insulin graphs were compared using the paired *t*-test. Pearson's correlation analyses were used to determine the correlation between insulin and VAS scores completed immediately before lunch and energy intake during lunch (energy consumed was the dependent variable; insulin and VAS ratings were the independent variables). All statistical tests were conducted using the software package STATISTICA, (Statsoft, Tulsa, OK, USA). In the text, tables, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

Food, energy and macronutrient intakes in test meal

When given a SINGLE pre-load, significantly more ($t = 2.651$; $P < 0.05$) energy was consumed in the *ad libitum* test meal ($5,261 \pm 1,289\text{kJ}$) than consumed in the *ad libitum* meal following the MULTI treatment ($3,763 \pm 1,986\text{kJ}$) (FIGURE 4.1). Neither the time taken to consume (SINGLE; $18.4 \pm 3.7\text{min}$ vs MULTI; $21.0 \pm 5.6\text{min}$) nor in the rate of energy consumption (SINGLE; 286.4 ± 47.5 vs MULTI $211.0 \pm 16.8\text{kJ}\cdot\text{min}^{-1}$) at the *ad libitum* test meal differed with the periodicity of test meals.

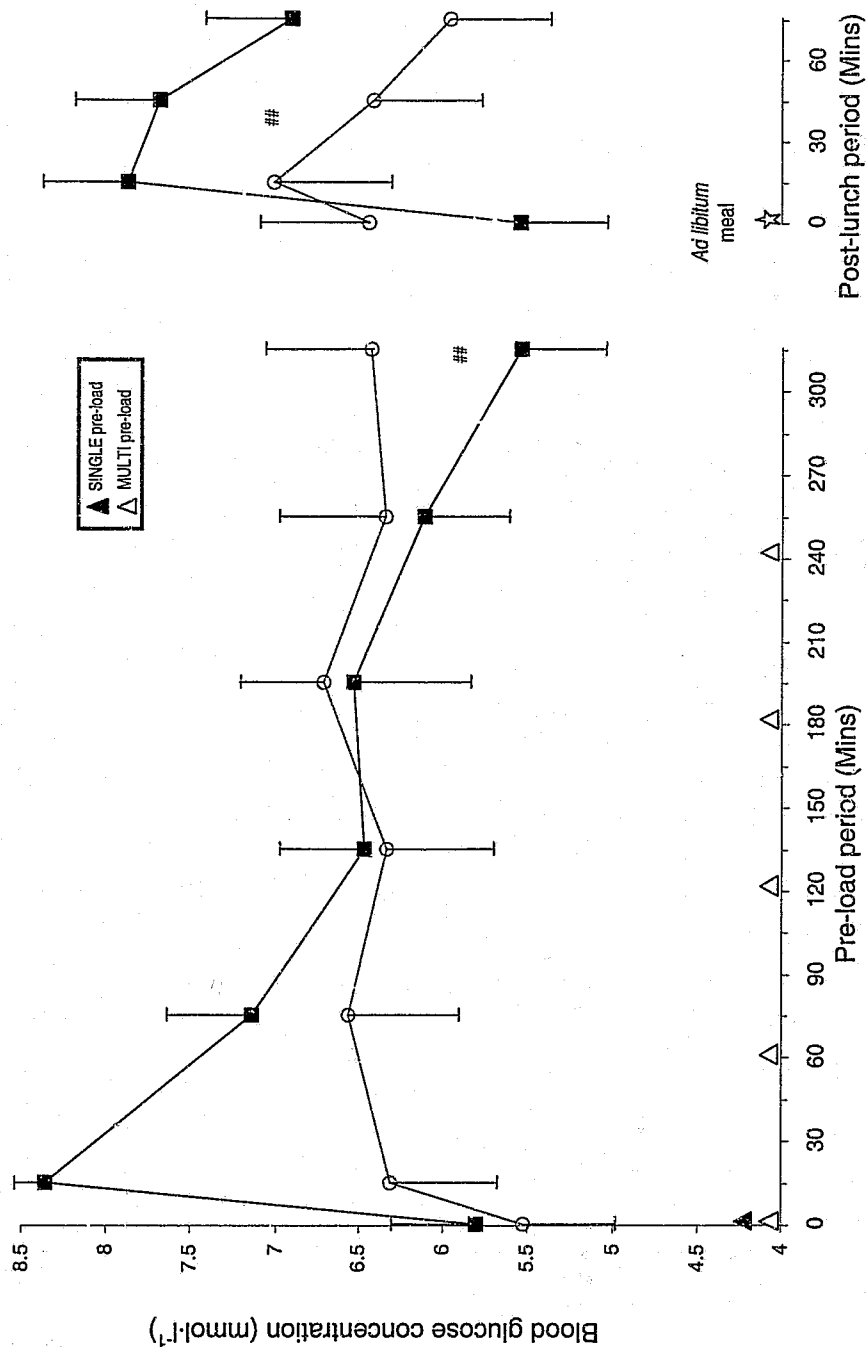


Pre-load period

Plasma glucose concentration responses

Baseline glucose concentrations on both trial days were within normal ranges (SINGLE; 5.8 ± 0.4 vs MULTI $5.5 \pm 0.3 \text{ mmol} \cdot \text{l}^{-1}$). Within 15 minutes of consuming the larger SINGLE meal, blood glucose of the subjects was 1.5x that of the baseline level ($8.4 \pm 1.1 \text{ mmol} \cdot \text{l}^{-1}$), which was significantly higher ($P < 0.05$) compared to the response noted in the MULTI treatment ($6.3 \pm 0.8 \text{ mmol} \cdot \text{l}^{-1}$) at the same time period. Thereafter blood glucose returned to 1.2x baseline 75 minutes into the experiment, and remained at approximately the same as baseline values in all the last readings prior to the test meal. When subjects were on the MULTI-meal treatment, blood glucose increased to 1.1x baseline, and remained unchanged at this elevated level for the duration of the pre-load period of 315 minutes. As such, statistical analyses revealed marked intergroup differences in glucose concentration over the experimental period [$F_{6,72} = 3.74$, $P < 0.01$] (FIGURE 4-2, *left*).

FIGURE 4.2 Changes in plasma glucose concentrations between SINGLE (■) and MULTI (○) feeding patterns in obese males.

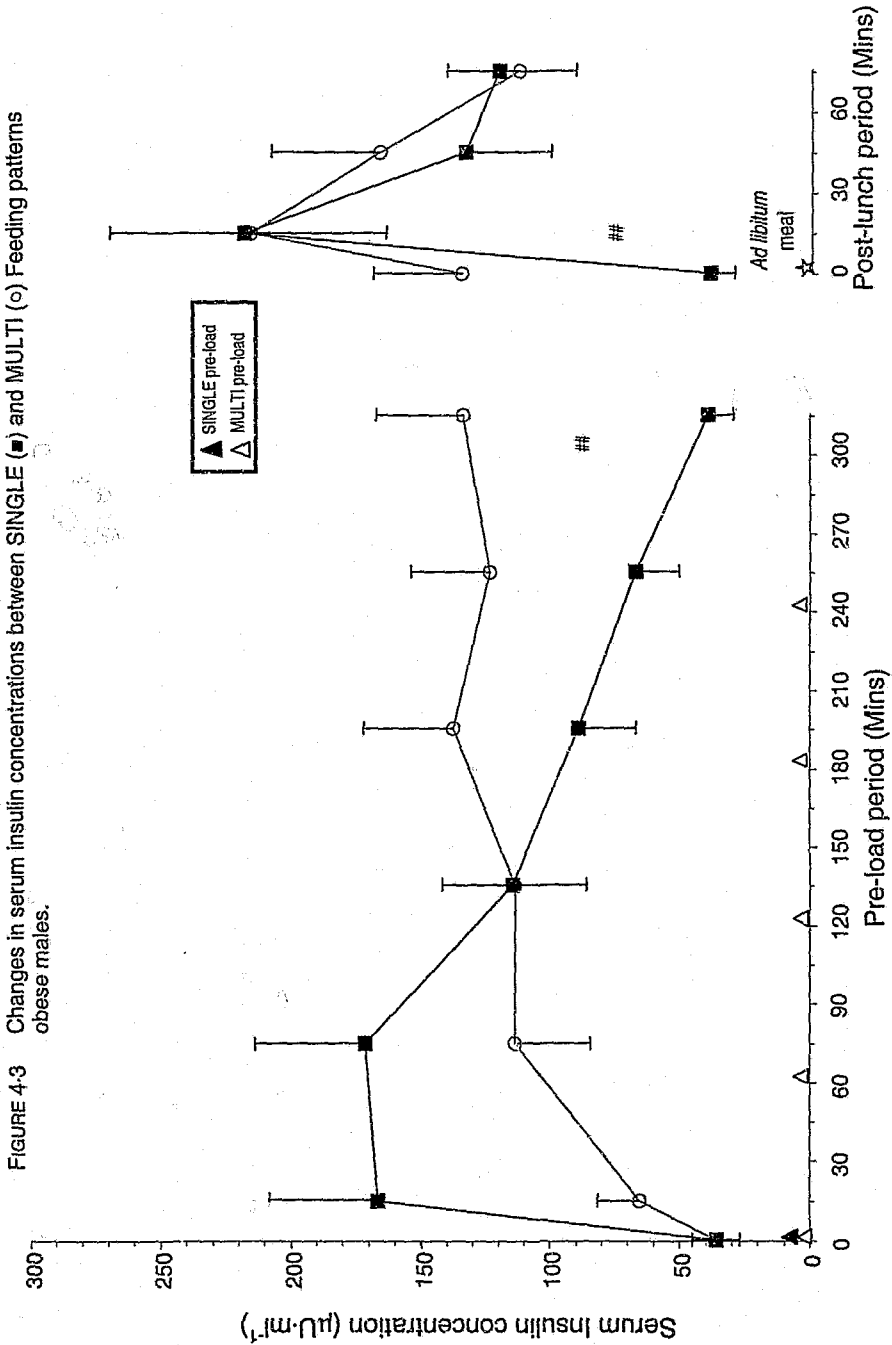


Where ## indicates difference ($P < 0.01$) between feeding patterns over the feeding period

Serum insulin responses

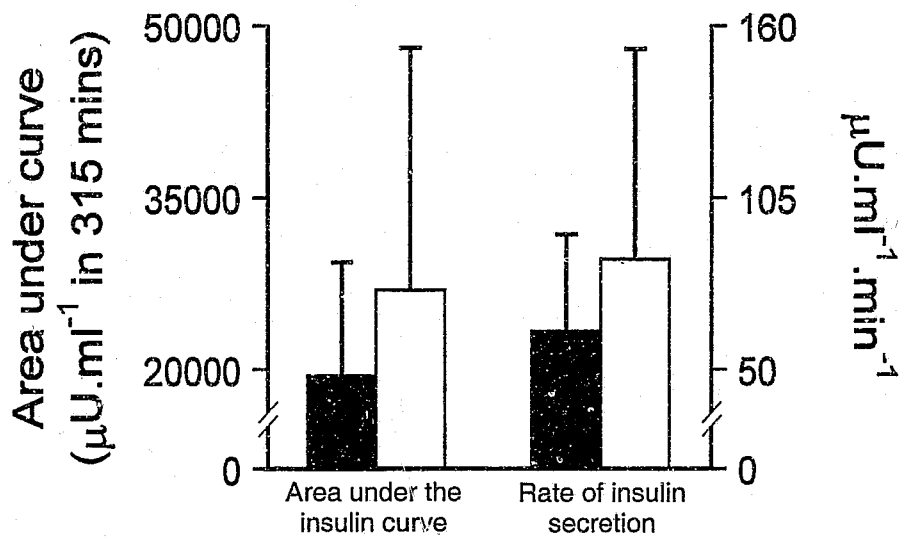
There was no difference between either treatments' baseline serum insulin concentrations on either of the trial days (SINGLE; $35.8 \pm 7.5 \mu\text{U}\cdot\text{ml}$ vs MULTI; $36.2 \pm 9.0 \mu\text{U}\cdot\text{ml}$). The frequency with which the pre-load was consumed affected serum insulin concentrations [$F_{6,72} = 7.95$, $P < 0.01$ (baseline to 315 minutes), however, *post-hoc* statistical testing could not reveal where the difference occurred (FIGURE 4-3, *left*).

Fifteen minutes after completing the SINGLE pre-load, serum insulin concentrations rose to 5x ($P < 0.05$) that of its baseline level ($166.6 \pm 54.0 \mu\text{U}\cdot\text{ml}^{-1}$). Insulin remained elevated ($P < 0.01$) for a further 75 minutes ($171.2 \pm 129.8 \mu\text{U}\cdot\text{ml}^{-1}$) thereafter 5-hours after consuming the SINGLE pre-load, insulin concentration had returned to baseline values. On the MULTI meal feeding pattern insulin levels rose slowly with each hourly pre-load until 195 minutes into the experiment where they peaked ($137.5 \pm 85.1 \mu\text{U}\cdot\text{ml}^{-1}$) and plateaued until immediately before the *ad-libitum* meal at 315 minutes.



When the areas under the insulin curves for the two feeding treatments were compared over the 315 minute pre-load period, no differences ($P > 0.05$) were observed ($19,484 \pm 9,913 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$ vs $26,934 \pm 21,137 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$, SINGLE vs MULTI respectively (FIGURE 4.4). This translates to an average rate of insulin secretion in the SINGLE group of $61.9 \pm 31.4 \mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$, compared an average rate of $85.5 \pm 67.1 \mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$ observed in the MULTI group (FIGURE 4.4).

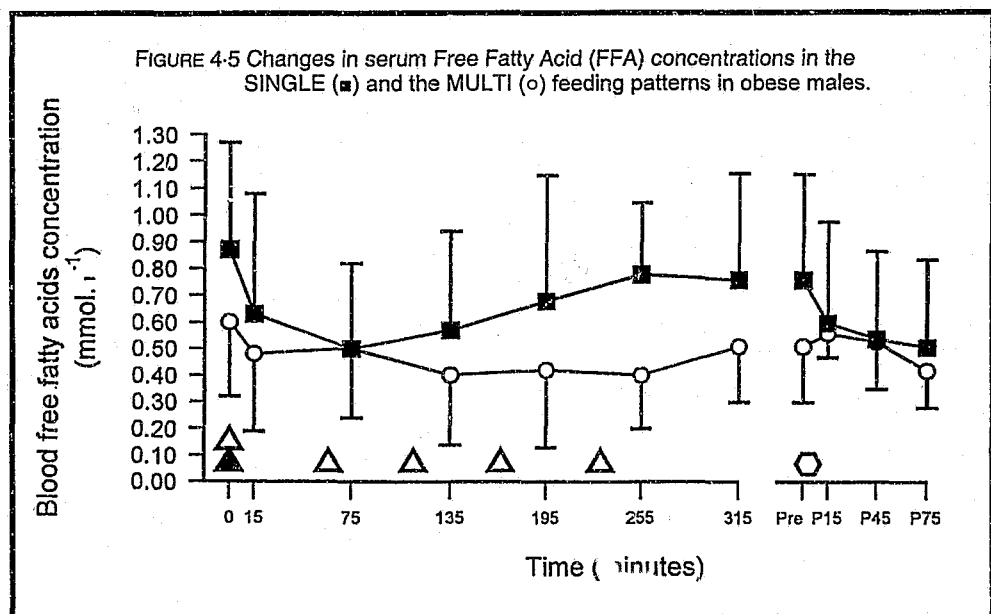
FIGURE 4.4 Calculated areas under the insulin curves (and their associated secretory rates) between the SINGLE (■) and MULTI (□) in the pre-load period in obese males.



There was a positive correlation ($r = 0.8676$) between the amount of energy consumed at the *ad libitum* lunch and the insulin concentration immediately before the lunch in the SINGLE group ($r = 0.9642$, $P < 0.001$), and although no such difference existed in the MULTI group, there was a tendency for a negative correlation between the insulin concentration and the energy consumed at the test meal in the MULTI group ($r = 0.7143$, $P < 0.071$) (FIGURE 4-10).

Serum free fatty acid responses

There were no differences between the groups' baseline serum free fatty acid (FFA) concentrations on either of the treatment days ($0.87 \pm 0.40 \text{ mmol} \cdot \text{l}^{-1}$ vs $0.60 \pm 0.28 \text{ mmol} \cdot \text{l}^{-1}$ SINGLE vs MULTI respectively). No differences ($P > 0.05$) in interaction of FFA responses between the two feeding regimes over time were noted in neither the 315-minute pre-load period, nor in the 75-minute post *ad libitum* test meal period.



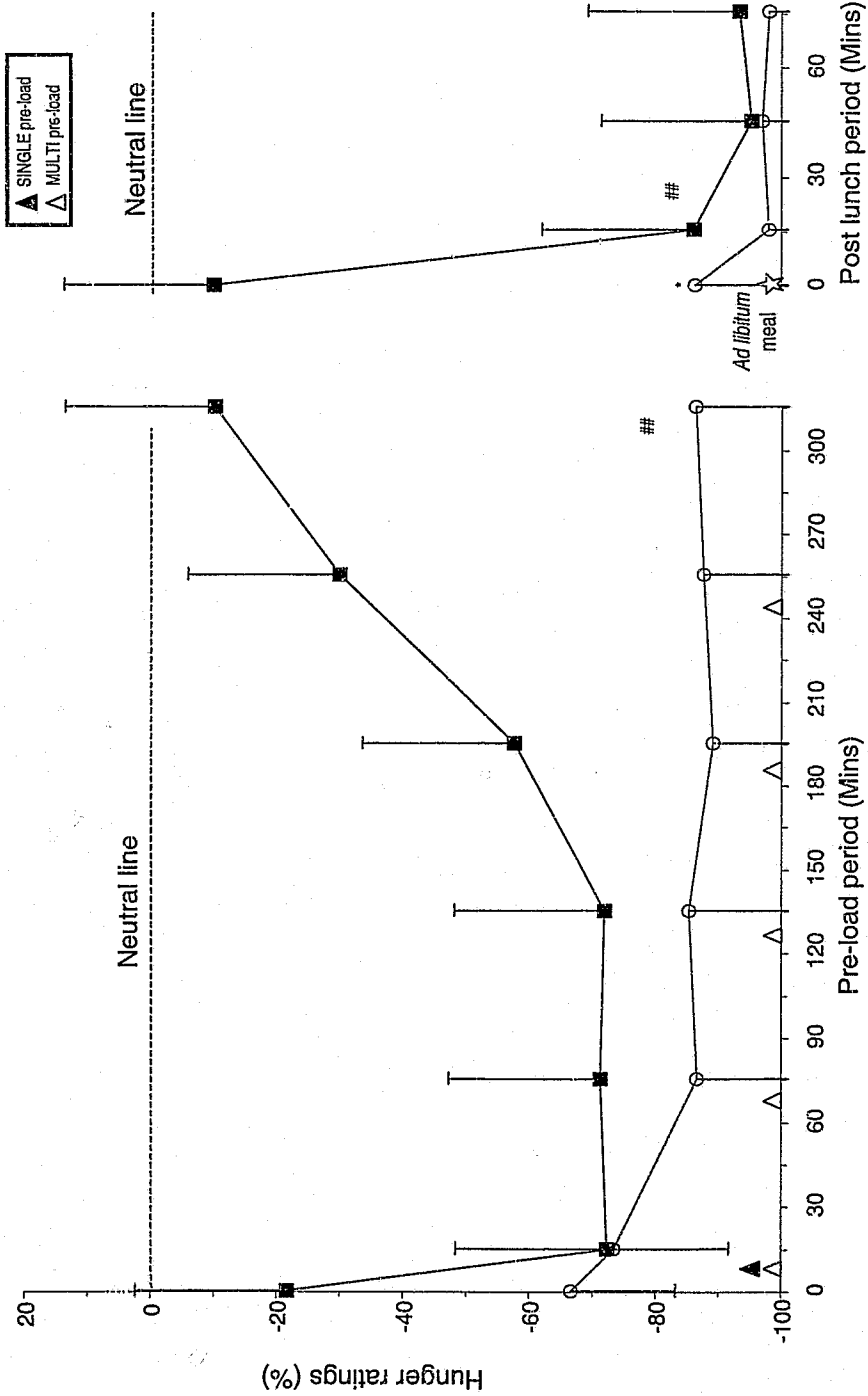
Visual Analogue Scales (VAS) for appetite ratings

Baseline subjective hunger ratings, using three different statements on both experimental days gave similar results. There were however, significant changes over the 315-minute pre-load time period between the two feeding patterns (SINGLE vs MULTI) in all three cases used to determine subjective appetite scores. Subjective hunger ratings between the two groups (FIGURE 4-7 *left*) showed a significant difference [$F_{6,72} = 3.99$, $P < 0.01$], which was shown to occur 315 minutes into the trial immediately prior to eating the *ad libitum* meal ($P < 0.01$), where the SINGLE treatment elicited a greater hunger response ($-10.3 \pm 69.8\%$) than when these same individuals ate the MULTI pre-load pattern ($-86.4 \pm 15.5\%$).

TABLE 4.4.
Characteristics of the ad-libitum meal consumed by the lean group on the two different eating regimes.

		Single meal protocol	Multiple meals protocol	Level of Significance
Time to consume meal	(min)	17.1 $\pm$ 2.2	16.4 $\pm$ 3.4	NS
Energy consumed Cottage pie	(kJ)	3,734 $\pm$ 1,330	2,770 $\pm$ 847	$P < 0.05$
Liquifruit	(kJ)	1,377 $\pm$ 474	988 $\pm$ 338	NS
Total	(kJ)	5,111 $\pm$ 1,502	3,753 $\pm$ 893	$P < 0.01$

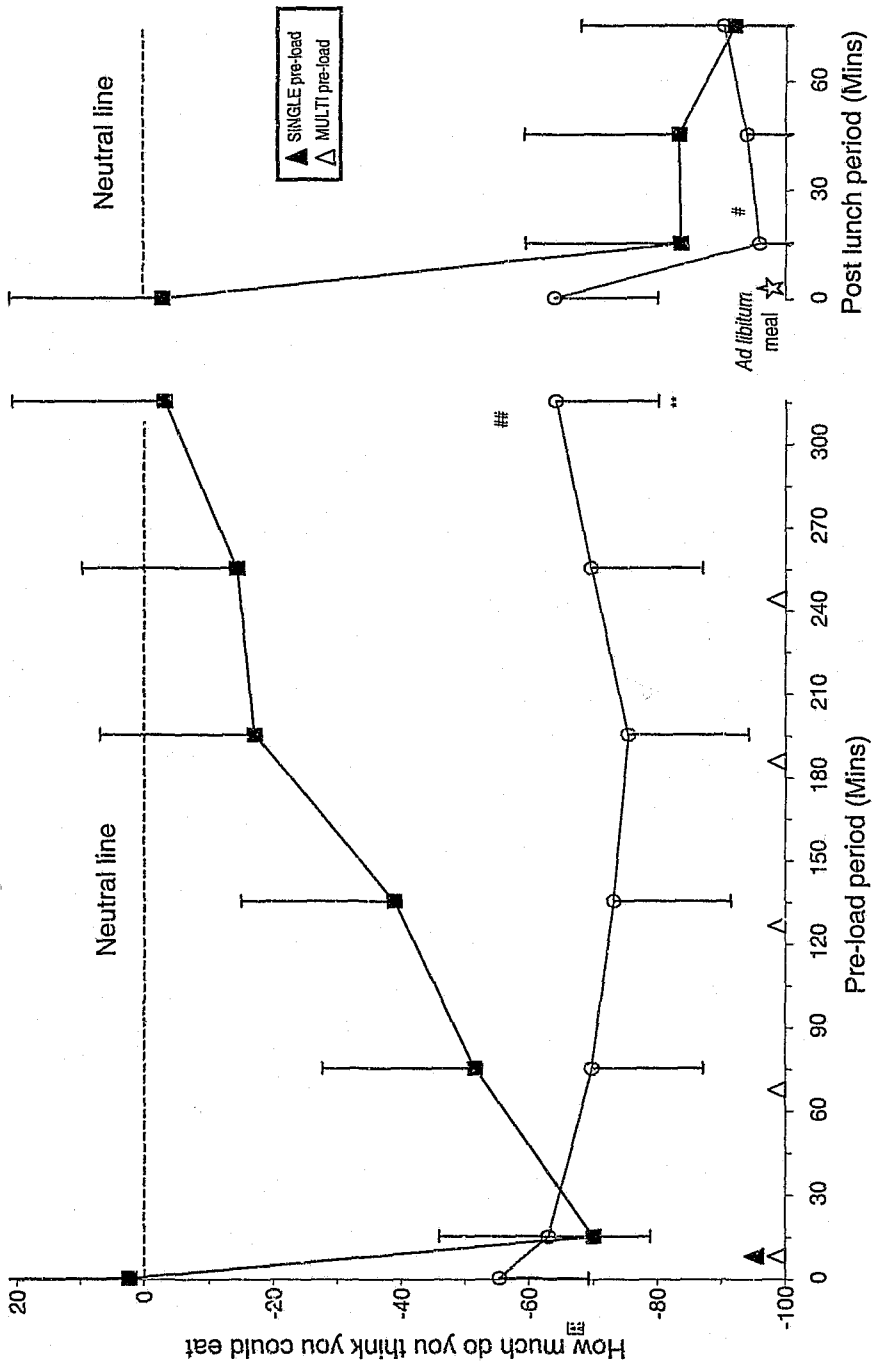
FIGURE 4-6 Changes in subjective hunger ratings between SINGLE and MULTI feeding patterns in obese males.



Where * indicates significant difference ($P < 0.05$) between SINGLE and MULTI treatments

indicates difference ($P < 0.01$) between feeding patterns over the feeding period

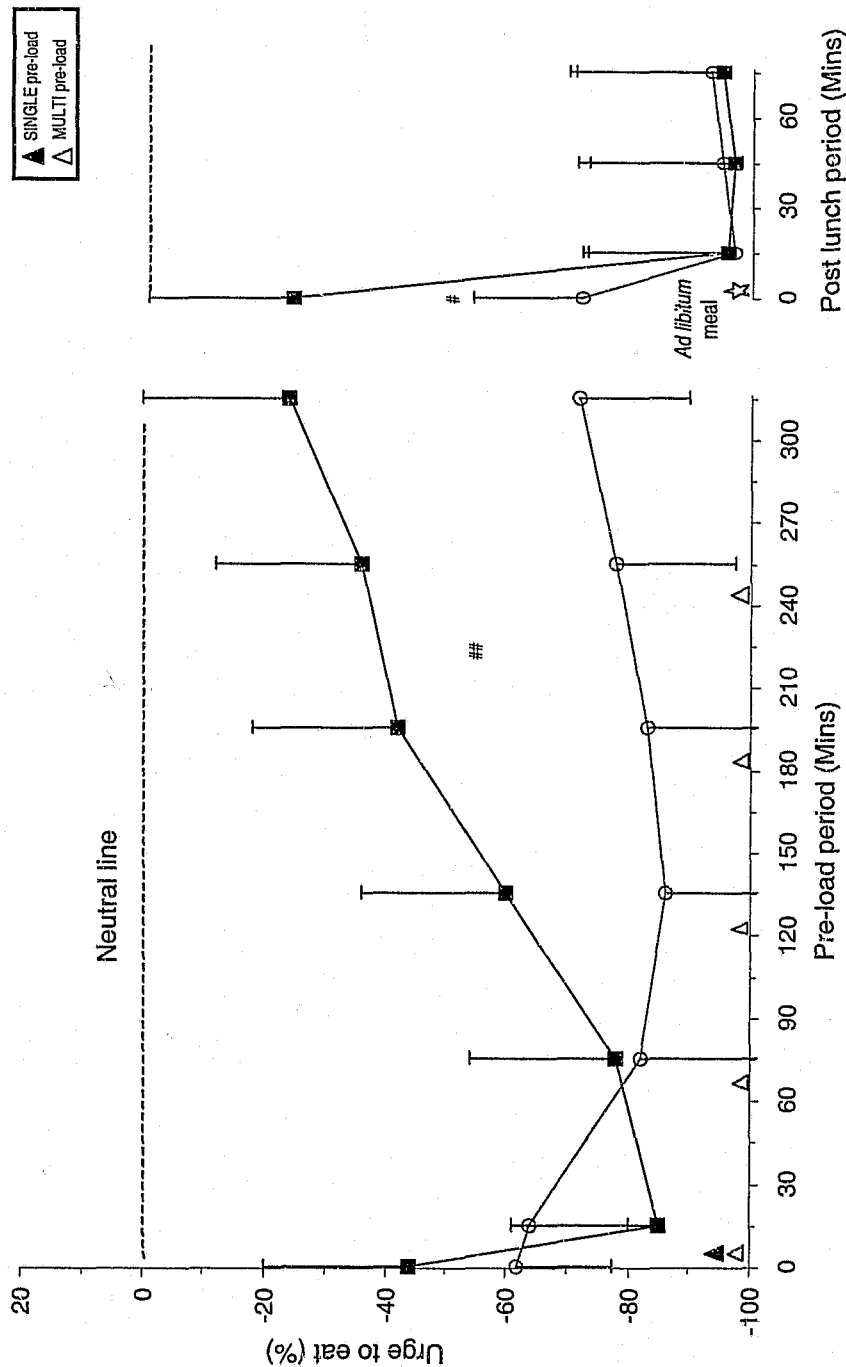
FIGURE 4-7 Changes in subjective ratings of how much each obese male felt they could eat between SINGLE (■) and MULTI (○) feeding patterns.



Where ** indicates significant difference ($P < 0.01$) between SINGLE and MULTI treatments

indicates difference ($P < 0.05$, $P < 0.01$) between feeding patterns over the feeding period

FIGURE 4-8 Changes in subjective urge to eat between SINGLE (■) and MULTI (○) feeding patterns.



Where #, ## indicates difference ($P < 0.05$, $P < 0.01$) between feeding patterns over the feeding period

Post-lunch period

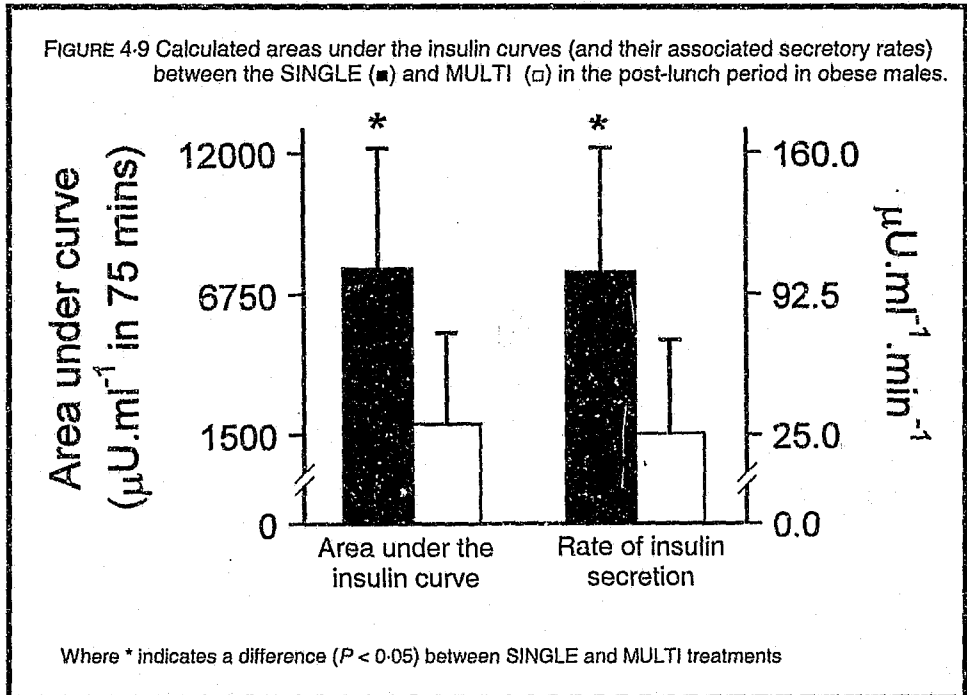
Blood glucose concentration

Changes in the blood glucose responses to the test meal was more pronounced following the SINGLE pre-load over the 75-minute post-meal period [$F_{3,36} = 6.64$, $P < 0.01$] (FIGURE 4-2, *right*). Fifteen minutes after the outcome meal, blood glucose concentrations of the SINGLE treatment meal was 1.4x that immediately prior to the meal. This remained higher ($P < 0.01$) than baseline for 45 minutes after completion of the meal. Blood glucose concentrations on the SINGLE treatment increased more markedly and peaked 15-minutes after completing lunch (compared to those responses following the MULTI trial). On the MULTI pre-load programme, 15 minutes after consumption of the *ad libitum* meal changes in blood glucose were markedly attenuated (1.2x that immediately prior to the test meal). These had returned to baseline within 45 minutes of the test meal.

Serum insulin concentration

Serum insulin concentrations increased immediately after the *ad libitum* meal to similar levels with both treatment groups (FIGURE 4-3, *right*). In the 75-minutes following completion of the test meal, the MULTI group's insulin levels declined to similar levels to those noted in the period before lunch. In contrast however, the SINGLE treatment's insulin levels remained elevated ($P < 0.01$) at both P45 ($38.6 \pm 8.8 \mu\text{U} \cdot \text{ml}^{-1}$) and at P75 ($38.6 \pm 8.8 \mu\text{U} \cdot \text{ml}^{-1}$) compared to pre-lunch values. The SINGLE

treatment's area under the insulin concentration curve ($7,738 \pm 4,441 \mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$) for the period immediately before consuming lunch to 75 minutes after completion of lunch was less ($P < 0.05$; $t = 2.5$) than that area under the MULTI group's curve ($1,907 \pm 3,404 \mu\text{U}\cdot\text{min}^{-1}\cdot\text{ml}^{-1}$) (FIGURE 4.9).

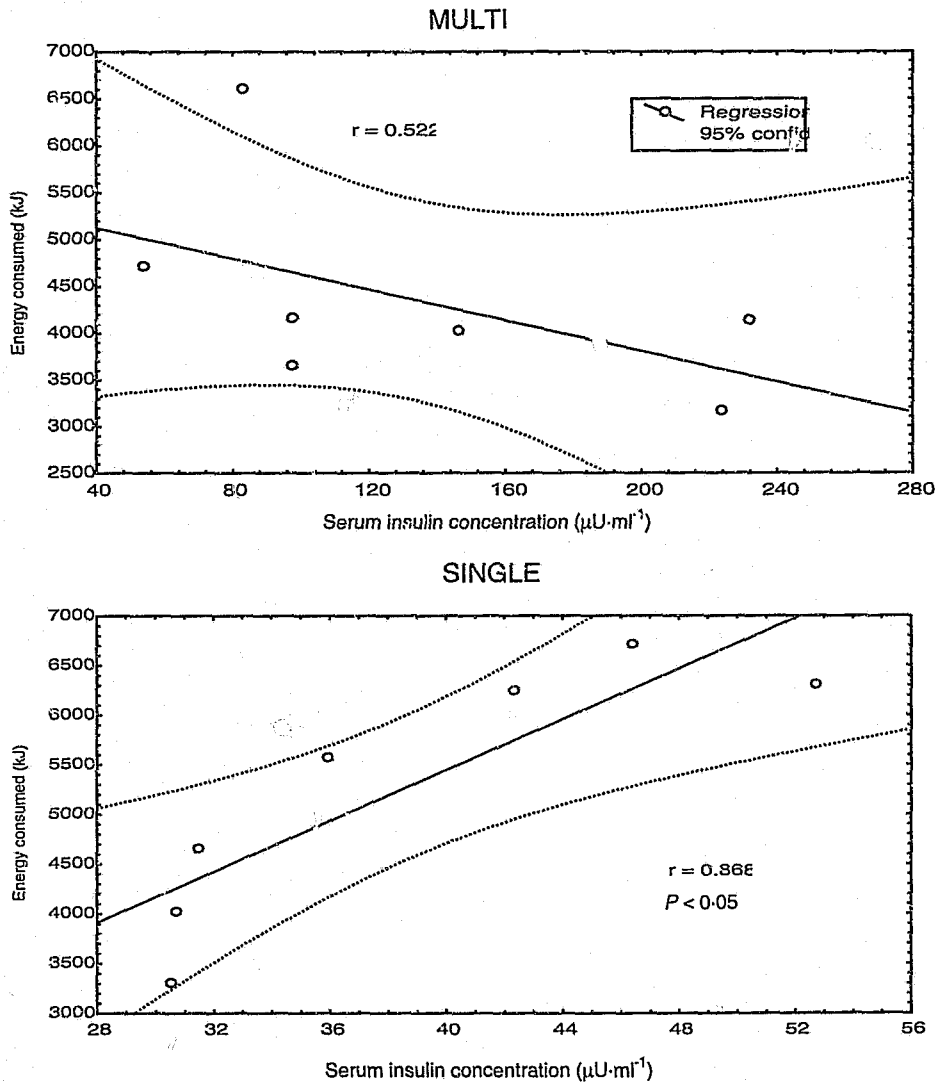


Visual Analogue Scores

As with the VAS ratings in the pre-load period, consuming the *ad libitum* meal had significantly different effects on the two groups' subjective appetite scores 75 minutes after completing the test meal (*hunger rating*: [$F_{3,36} = 6.13, P < 0.01$]; *amount that could be eaten*: [$F_{3,36} = 3.95, P < 0.05$]; and the *urge to eat*: [$F_{3,36} = 3.33, P < 0.05$]). For all cases, the SINGLE treatment's ratings were lower ($P < 0.01$) at all times after completion of the test meal compared to the rating immediately before eating the *ad libitum* meal. Conversely, there were no intra-group differences in the MULTI group's appetite scores.

There was a positive correlation ($r = 0.868; P < 0.05$) between the amount of energy consumed at the *ad libitum* lunch and the serum insulin concentration immediately before the lunch when the pre-load was consumed as a SINGLE meal. Although no such difference existed in the MULTI trial, there was tendency for a negative correlation between the serum insulin concentration and the energy consumed at the *ad libitum* meal ($r = 0.522; P < 0.071$) FIGURE 4-10 overleaf.

FIGURE 4.10 Scatterplots depicting the relationships between serum insulin concentration immediately before lunch, and the amount of energy consumed at the *ad libitum* lunch in the two different eating plans when the pre-load constituted 33.3% ADER.



DISCUSSION

Data obtained in this study show that when a group of obese males were fed an isoenergetic high carbohydrate pre-load in a more frequent pattern, they consumed 26.8% less energy at the test meal (FIGURE 4-1) and also exhibited smaller fluctuations in blood glucose, insulin (FIGURES 4-2 and FIGURE 4-3), and perceived hunger (FIGURE 4-7), than when the pre-load was consumed in one sitting. Increased meal frequency thus induced better appetite control at the subsequent meal and reduced perceived feelings of hunger. This response in an obese group of otherwise healthy men were similar to that previously reported in both the obese group as well as non-obese "normal" young men, (Speechly & Buffenstein, 1999).

These findings are contrary to what we had hypothesised for we had speculated that dysfunctional appetite regulation was a characteristic of the obese, and that the periodicity of eating would have no appetite-regulating effect in this population group.

Most studies that have addressed appetite – satiety mechanisms have focused specifically on lean individuals and their response to sweet, and/or high-fat foods (Rolls *et al.*, 1988; Rolls *et al.*, 1990; Crovetti *et al.*, 1997; Westcombe & Wardle, 1997) or dietary fibre on appetite (Rigaud *et al.*, 1998). While these studies performed in a population group that does indeed regulate appetite to maintain energy balance might confirm the mechanism, studies employing an obese population cohort may better elucidate dysregulation. Recent reports show obese women are less

sensitive to the satiety effects of bombesin (tetradecapeptide neurotransmitter that influences gastric function) (Lieveise *et al.*, 1998) than lean women are. Furthermore, in a 15-day study using a milk formula diet, obese subjects became increasingly hungry throughout the study whereas non-obese subjects did not (Wooley, 1971). Blundell *et al.*, (1994) proposed that the *satiety cascade* operates as efficiently in obese people as in lean individuals: thus a normal appetite response to a reduced energy intake is evident in obese subjects. Similarly, the data in this study show no differences between lean and obese males when the variable under study is the periodicity of meals. Indeed, increasing feeding frequency enhances appetite – satiety control mechanisms in both non-obese (Speechly & Buffenstein, 1999) and obese males.

These data may support epidemiological studies, where an inverse relationship exists between the number of meals consumed in a day and the degree of adiposity (Fábry *et al.*, 1964a; Fábry & Tepperman, 1970; Kudicka *et al.*, 1966; Metzner *et al.*, 1977). However, caution must be taken in the interpretation of these data, as they may provide only a possible explanation for obesity insofar as *fewer* meals may mean *bigger* meals as well. However, the epidemiological studies done thus far have been criticised for the large reliance on subjective ratings on the parts of the respondents' lack of credible and accurate reporting of meal frequency and content. *Dietary under-reporting* may undermine the interpretation of these types of studies (Summerbell *et al.*, 1996; Bellisle *et al.*, 1997; Poppitt *et al.*, 1998). Furthermore, a number of studies report contradictory findings with increased snacking resulting in increased energy consumption (Basdevant *et al.*, 1993) while other studies report

no relationship, be it positive or negative, between the periodicity of eating and obesity.

For the obese population group to reach BMI's in excess of $30 \text{ kg}\cdot\text{m}^{-2}$, energy intake must exceed energy requirements. Clearly, therefore, appetite – satiety mechanisms must be dysfunctional and it may be that obese subjects may not necessarily eat in a regular, structured, and frequent manner. Rather, they may undergo cyclic starvation (reverse causality), followed by over-eating, and it may be that during the latter activity, within which at any one meal, their satiating mechanistic regulation may then be over-ridden. This hypothesis requires further investigation.

Visual Analogue Scales

The hunger ratings obtained from the visual analogue scores in this trial were ambiguous. It appeared that increased frequency of feeding had a positive effect on maintaining satiety ratings compared to the gradual increase in hunger observed on the SINGLE treatment (Figures 4-6, 4-7, and 4-8). Evidence for the enhanced effect of increased frequency of feeding was apparent immediately prior to the *ad libitum* meal on the MULTI meal who were *not at all hungry* (-67%) and the SINGLE treatment posted ratings of -22% (relatively hungrier). However, both treatments were able to consume more than 33.3% of ADER in the subsequent eating session, despite posting hunger ratings less than the neutral line for hunger. It is possible that the obese group could not truly

perceive hunger. Alternately the subjective completion of the VAS scales may rather reflect what they anticipate should be a correct and/or desired answer.

In contrast, the VAS data from lean subjects (as shown in Chapter 3), was better correlated (Speechly & Buffenstein, 1999). There are major limitations with subjective VAS scores for hunger (Wooley, 1971; Mattes, 1990, Friedman *et al.*, 1999). Nevertheless the data obtained in the current study are useful insofar as the ratings given on the respective eating plans are relative to each other, and further indicate an element of accuracy toward the occurrence of intrinsic regulation.

Changes in blood variable concentrations with meal frequency.

Marked differences in serum insulin concentrations were observed with both meal frequency and also between the lean (Speechly & Buffenstein, 1999) and obese population groups. Both lean and obese groups' serum insulin concentrations responded to increased meal frequency in a cumulative effect, and in the SINGLE pre-load exhibited a dramatic rise 15 minutes after consuming the entire pre-load aliquot. Since the half-life of insulin is between 6-10 minutes, and the energy content of the pre-load substantial, this response and the time frame thereof would be expected.

A major difference between the lean and obese groups was observed 75 minutes after the SINGLE meal. Here, insulin concentrations of the obese group have continued to rise slightly ($171\mu\text{U}\cdot\text{ml}^{-1}$) whereas insulin concentrations of the lean group had peaked and dropped off fairly

quickly. This may reflect a degree of hyperinsulinaemia in this obese group, which reinforces the finding in Chapter 2.

Hyperinsulinaemia in an obese group (compared to lean) may also be indicated by the significantly higher ($t = 6.28$; $P < 0.01$) fasting insulin levels, which are supported by similar reports in the literature (DeFronzo, 1982; Faber *et al.*, 1981). Serum fasting insulin levels in the obese group on the morning of both experiments were approximately three times greater (SINGLE; $35.8 \pm 7.5 \mu\text{U}\cdot\text{ml}^{-1}$, and MULTI; $36.2 \pm 9.0 \mu\text{U}\cdot\text{ml}^{-1}$) than those measured in non-obese men ($\text{BMI} = 23.1\text{kg}\cdot\text{m}^{-2}$; $11.5 \pm 5.5\mu\text{U}\cdot\text{ml}^{-1}$) (Speechly & Buffenstein, 1999) under similar conditions. These data are at variance with those previously reported; in a group of 35 obese non-diabetic adults ($170 \pm 13\%$ of ideal weight), fasting insulin levels had a mean of $22 \pm 1\mu\text{U}\cdot\text{ml}^{-1}$ (DeFronzo, 1982). Other studies have shown fasting insulin levels in obese males not to be as high: in 10 obese ($\text{BMI} = 38\text{kg}\cdot\text{m}^{-2}$) Caucasians, Elahi *et al.*, (1982) found basal levels of insulin $14 \mu\text{U}\cdot\text{ml}^{-1}$. Ferrannini *et al.*, (1991) found fasting insulin concentrations of $15.1\mu\text{U}\cdot\text{ml}^{-1}$ in 852 obese ($\text{BMI} = 31.1\text{kg}\cdot\text{m}^{-2}$) men and women. These discrepancies may be a function of the different assay methodologies used to measure serum insulin (Temple *et al.*, 1992; Hales *et al.*, 1996).

When the obese group consumed five smaller meals hourly, insulin concentrations increased continuously albeit by smaller amounts over the entire 315-minute experimental pre-load period. In other words, the rate of secretion exceeded the rate of removal. This increase in insulin over time in our study is in direct contrast to the findings of Ellis (1934) who reportedly found 10-30g glucose doses given hourly with small doses of

insulin, decreased insulin requirements in insulin-dependent diabetes mellitus. Differences between that (Ellis, 1934) and this study might reflect the relatively high carbohydrate content of the diet in this study with concomitant cumulative effects on the insulin concentrations.

When the two feeding regimes of this study were compared over the five-hour experimental period, the areas under the curve show that similar amounts of insulin were secreted. Nevertheless, the obese group given the MULTI frequency pre-load exhibited greater control of appetite in the *ad libitum* meal. It may be inferred from this, that the total amount of insulin secreted in response to the feeding pattern does not influence eating behaviour. Rather, it is the peak response or the timing in which the insulin is secreted that plays a role in appetite control (Jenkins *et al.*, 1992). It would appear from these data that increasing meal frequency results in prolonged elevated, but not maximal, insulin levels ($\sim 130 \mu\text{U}\cdot\text{ml}^{-1}$) and that this acute elevation in serum insulin has a positive effect over appetite control. When the subjects were given a SINGLE pre-load meal followed by a five-hour fast, initial serum concentrations of insulin were significantly higher than those of the MULTI frequency pre-load. However, immediately prior to the test meal, insulin concentrations were higher on the MULTI frequency treatment.

A negative correlation between serum insulin and food consumption was observed in the MULTI feeding treatment in the obese group of males tested here, suggesting that insulin concentration may play a pivotal role in energy consumption. Although this relationship was not observed in the lean males tested previously (FIGURE 3-9), the significance of it

requires an understanding of the endocrinological control over fuel utilisation and the homeostasis thereof. Elevated serum insulin levels are indicative of the absorptive state, whilst low insulin concentrations indicate a post-absorptive state of fuel utilisation: the fluctuations reflect the swing of the body's metabolic system from storing to using fuels. It appears that increasing the frequency with which exogenous fuels are delivered maintains the body in an absorptive state for longer, with a direct effect on appetite. This postulate is based on the positive correlation found between the amount of energy that the SINGLE treatment consumed at the *ad libitum* test meal and the insulin concentration immediately before the meal (FIGURE 4-10, *top*), and an almost negative correlation between these variables in the MULTI feeding pattern. However, it must be pointed out that the precise quantity of circulating insulin that would exert a positive effect on appetite remains unclear in the current study, as these data cannot suggest a proportional relationship between insulin and satiety. Further research in this area is required before any suggestion can be made.

A suggested role for insulin in the regulation of energy intake has been previously considered (Jenkins *et al.*, 1989; Woods, 1997; Ellis, 1934; Hejda & Fábry, 1964; Bray, 1972). It is logical that both the regulator of appetite and satiety should be controlled by stimuli linked to feeding. The regulatory signals may emanate from either the gastrointestinal tract to the central nervous system, or from post-ingestive chemical signals in the blood. These regulating stimuli would have to meet three criteria: firstly, it would have to exist in amounts proportional to the body size and circulate in the blood; secondly, it would have to acquire access to the CNS and be able to interact with the brain; and finally, it would have to have

predictable effects on behaviour and metabolism (Woods, 1997). These putative mechanisms in satiety regulation are at variance with some studies that report increased insulin responses to ingested foods are associated with lessened satiety (Woods, 1997; Lavin & Read, 1995; Holt *et al.*, 1992; Holt & Brand Miller, 1992). However these support the finding that primates given $0.33\mu\text{U}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of exogenous insulin posted 13% suppression of energy intakes (Holt & Brand Miller, 1995). This finding is compatible with the hypothesis that circulating endogenous or exogenous insulin is a signal for appetite suppression.

The negative effects of increased insulin on food intake have been shown to be true using non-human primates (baboons) (Woods *et al.*, 1984; Woods *et al.*, 1979) and rats (Vander Weele *et al.*, 1980; Steffens *et al.*, 1988). Thus, insulin meets the three criteria to act as a feedback signal from the gut to the CNS in the regulation of adiposity and weight. There is consensus at present that peripheral information provides crucial information to the central nervous system to control food intake (Woods, 1997; Campfield, 1997). Whilst this hypothesis appears to be reinforced in the MULTI feeding pattern in the current trial (FIGURE 4-10, *bottom*), there is also evidence in direct contrast to this hypothesis in obese males on the SINGLE trial: the higher the serum insulin concentrations were (prior to consumption of the next meal), the more energy was consumed at that meal (FIGURE 4-10, *top*).

Having consumed 26.8% more energy in the SINGLE meal protocol compared to the MULTI meal pattern, both serum insulin concentrations rose to the same elevated point of $\sim 215\mu\text{U}\cdot\text{ml}^{-1}$. Interesting to note

further, is that both trends continued to decline after the lunch meal, at similar rates. Other studies have shown relative (from baseline) increases in serum insulin $\sim 100\mu\text{U}\cdot\text{ml}^{-1}$ following glucose loads (Elahi *et al.*, 1982). From these data, the frequency of eating in the pre-load period appear to have no different metabolic effects after consumption of the lunch meal in that all variables noted changed in similar fashions for the 75 minutes after the meal. In this period immediately prior to -, and until 75-minutes after completion of the *ad libitum* lunch meal, there was a significant difference in the areas under the insulin curves between the two eating patterns.

CONCLUSION

In this experiment, I have shown an inverse relationship between the number of meals and the amount consumed at a subsequent test meal: when obese males eat smaller quantities more regularly, they exhibit reduced appetite in the short-term. This finding concurs with the majority of epidemiological studies (Fábry *et al.*, 1964a; Fábry *et al.*, 1970; Kudicka *et al.*, 1966; Metzner *et al.*, 1977; Hejda & Fábry *et al.*, 1964; Bray 1970; Bray, 1972). In this current study, fluctuations in serum insulin may be implicated in the regulation of appetite, where the maintenance of serum insulin slightly above basal levels (ie acute absorptive metabolic state) may be a contributing factor in enhancing appetite control. Furthermore, these data suggest a similar mechanism in the regulation of appetite in both lean and obese via altered patterns of meal frequency. Indeed, increased meal frequency appears to reduce appetite in the short-term.

These data suggest a similar mechanism in the regulation of appetite in both lean and obese via altered patterns of meal frequency. It would seem that the consumption of smaller regular meals enhances appetite control. Finally, a worthwhile point for the reader to bear in mind in the analysis and interpretation of these data, is that macronutrient value of the food used thus far in the pre-load, has been of fairly high carbohydrate value. The responses noted in this experiment should be considered within this context: under these conditions, a crucial trial would be the investigation of altered feeding patterns in an obese group using

pre-loads of varying energetic content and macronutrient value. This is investigated in the next chapter.

Chapter Five

**Appetite dysfunction in obese males:
evidence for the role of hyperinsulinaemia
in passive overconsumption with a high-fat diet**

INTRODUCTION

Obesity is currently exploding into a chronic epidemic in the modern world (Popkin & Doak, 1998), with more than one-third of Americans currently classified as overweight (Flegal *et al.*, 1998), and the incidence of obesity continues to increase throughout the developed world (Popkin & Doak, 1998; Maillard *et al.*, 1999; NAO, 1999). The fundamental aetiology of the obese state occurs as a consequence of excessive energetic ingestion relative to energetic expenditure (Lissner *et al.*, 1987; Blundell, 1991; Blundell *et al.*, 1994; Hill & Prentice, 1995; Bouchard, 1996; Blundell & King, 1996). Based on this premise, it may be assumed that lean individuals enjoy a state of ideal energy regulation. Coupled to this is that the regulation of energetic intake in humans lies in the intrinsic control of appetite (Blundell, 1991; Verboeket-van de Venne & Westerterp, 1996). Similarly, it may be inferred that the chronic positive energy balance of the obese state is primarily the result of appetite dysfunction.

The notion of dysfunctional appetite control as a factor contributing toward obesity has gained momentum over the past 45 years (Mayer 1955; Spiegel, 1973; Kissileff *et al.*, 1984; Rolls *et al.*, 1991; Cotton *et al.*, 1994; Blundell & Macdiarmid, 1997). Factors influencing the control of appetite include the frequency with which foods are eaten (Wooley, 1971; Drummond *et al.*, 1998; Speechly & Buffenstein 1999; Speechly *et al.*, 1999), as well as the macronutrient value of foods that yield specific sensory responses (Drewnowski *et al.*, 1983; Rolls *et al.*, 1991; Drewnowski *et al.*, 1992; Blundell *et al.*, 1996, Poppitt *et al.*, 1998a).

Whilst the vast majority of epidemiological studies performed have linked high-fat diets with the development of obesity (Golay & Bobbioni, 1997; Lissner *et al.*, 1987; Blundell & Macdiarmid, 1997; Ravussin & Tataranni, 1997; Seidell, 1998). Willett, (1998) provides substantial evidence that this may not necessarily be the case. The direct relationship between dietary fat consumption and the incidence of obesity may be a consequence of the relative ease with which excessive energetic intake on a high-fat diet occurs. This is primarily due to the high energy density of fat, but also can be attributed to its increased palatability and hedonistic desire to over-consume these tasty, high-density foods. The latter is referred to as *passive overconsumption* (Lawton *et al.*, 1993; Prentice & Poppitt, 1996; Blundell & Macdiarmid, 1997).

In the cascade of satiety theory (FIGURE 1.10), Blundell *et al.*, (1994) postulated that appetite regulatory mechanisms worked as well in both lean and obese population groups, which would suggest it may only be their preference for foods that differentiates lean from obese groups. Recent evidence has shown however, that lean and obese people may not necessarily self-select diets with different perceived sensory or hedonic attributes (Cox *et al.*, 1999). Accordingly, in an attempt to determine whether indeed any physiological difference(s) exists in the appetite regulatory mechanisms between lean and obese males in their responses to variable macronutrient foods, the current experiment was designed to determine whether lean and obese males physiologically recognise pre-loads of different energetic and macronutrient content and compensate appropriately at the next meal. I hypothesised that obese males are less able to differentiate covert manipulation of energy content and control appetite than lean counterparts. As a corollary of testing this

hypothesis, there was the ability to investigate possible metabolic and endocrinological factors contributing to the passive overconsumption observed with high-fat diets in the aetiology of appetite dysfunction leading to obesity (Rolls *et al.*, 1994; Blundell & Macdiarmid, 1997). Although components of this question (i.e. the role of macronutrients on appetite regulation) have been previously addressed (Wooley *et al.*, 1975) in lean females (Poppitt *et al.*, 1998a), and in both lean male (Rolls *et al.*, 1991; Stubbs *et al.*, 1996) and lean female study participants (Rolls *et al.*, 1991; Rolls *et al.*, 1994) as well as obese females (Rolls *et al.*, 1994), to our knowledge, no study has combined these simultaneously with endocrine assessments in both lean and obese males.

MATERIALS AND METHODS

Study participants

Twelve healthy, non-diabetic men were recruited from the general population by means of community advertisements to participate in this study. Of the twenty respondents, eight men were either not comfortable with the venipuncture, or were unavailable on the days of testing, and consequently 40% were rejected prior to commencing the study. All study participants were screened for eating restraint via the Dutch Eating Questionnaire (Van Strien *et al.*, 1986) which is aimed at three basic categories, viz external motivation to eat, emotional eating, and restrained eating patterns. There were two groups of study participants: lean and obese, and the men were characterised as non-obese if their mean BMI < 23kg·m⁻² and obese if their mean BMI > 30kg·m⁻². The characteristics of the study participants are shown in TABLE 5-1.

This study was approved by the Ethics Committee of the University of the Witwatersrand (Clearance number M960425), and informed consent was obtained from all volunteers.

TABLE 5.1

Characteristics of study participants.

	Nonobese	Obese	Significance
N	6	6	
Age (years)	26.67 ± 5.47	39.83 ± 19.03	NS
Weight (kg)	77.13 ± 5.60	125.88 ± 43.82	* t = 2.76
Height (m)	1.85 ± 0.61	1.79 ± 0.075	NS
BMI (kg·m ⁻²)	22.50 ± 1.08	39.05 ± 11.63	** t = 3.45
WHR	1.01 ± 0.04	1.17 ± 0.09	** t = 3.91
ADER (kJ)	12,098 ± 1,103	12,225 ± 3,543	NS
20% ADER (kJ)	2,419 ± 220	2,445 ± 709	NS
55% ADER (kJ)	6,562 ± 583	6,723 ± 1,948	NS
Relative energy (kJ·kg ⁻¹)	156.8 ± 6.38	98.1 ± 7.66	** t = 14.4
DRQ			
External	2.65 ± 0.88	3.18 ± 1.05	** t = 3.08
Emotional	1.62 ± 0.77	2.65 ± 1.53	** t = 5.29
Restraint	2.30 ± 1.29	3.08 ± 0.96	** t = 3.77

Where BMI = body mass index; WHR = waist:hip ratio and DRQ = Dutch Restrained Questionnaire.

Protocol⁶

The experiment was designed to address two principle objectives: firstly, whether lean and obese males physiologically recognise pre-loads of different energetic and macronutrient content and compensate appropriately at the next meal; and secondly whether metabolic and endocrinological factors contribute to the passive overconsumption as an aetiological factor in appetite dysfunction leading to obesity.

The men reported to the laboratory on one occasion when they were informed partially of the nature of the experiment, and screened for eating

⁶ An identical protocol was used in this experiment to that described in the previous chapters, except for the obvious difference in the types of foods used in the pre-load, and that the pre-load was presented as a SINGLE meal. As in the previous experiments, every effort was made to ensure that subjects' behaviour was controlled and complied with the protocol exactly. Measurements of energy intake at the *ad libitum* meal, the time of feeding, VAS measurements were identical to those described in the previous chapters.

restraint. They were not informed that the study would examine appetite control, but merely that the effects of eating on blood variables and perceived mood and hunger would be assessed. Body mass (accurate to 0.1kg, Mettler TE/J, Zurich, Switzerland) and height (accurate to 0.1cm, SECA, German) were also measured.

The effects of altering energy content and macronutrient composition of the pre-load on subsequent appetite was assessed by covertly monitoring subsequent food intake at the *ad libitum* test meal. Putative physiological mechanisms were explored by also monitoring changes in plasma glucose and serum insulin concentrations at regular intervals throughout the treatment period. In addition perceived hunger was also monitored at these times.

The men were randomly divided into two treatment groups (LF and HF) determining the order of their experimental meal treatment. As such, meal treatment was randomised and all study participants received the two meal treatments over the next two visits to the laboratory. Upon each visit, the men reported to the laboratory in the fasted state at 07h00. They were asked to complete their dinner meal the night before no later than 22h00, and to abstain from all alcohol consumption for 24 hours beforehand. They were given a pre-load meal of covertly manipulated variable fat and energy intake.

In the underfeeding trial⁷, (LF) study participants received 20% of their average daily energy requirement (ADER) as a pre-load meal; whereas in the overfeeding trial (HF), study participants were covertly given 55% of their ADER as the pre-load.

The ADER for each individual was determined using the equation of Harris-Benedict (Van Way, 1992) as follows:

$$\text{ADER (Joules)} = \{66 + [(13.8 W) + (5 H)] - (6.8 A)\} P^{4.18}$$

where A is age (years), W is weight (kg), and H is height (cm).

The underfeeding energetic value of 20% ADER was selected on the rationale of a prudent dietary guideline suggests five meals per day, with each meal composing 20% ADER (Niklas *et al.*, 1998). As such, we felt that the physiological responses to meals with this energy content were relevant. The 55% ADER selected in the HF pre-load treatment was chosen because it represented an intake of more than half daily requirements at one meal. We predicted that lean study participants would regulate subsequent energy intake in response to the different pre-loads to maintain intake at ~ 50-60% of ADER. Using the premise of appetite dysfunction, we anticipated that the obese group would consume more energy than the lean group; due to the paucity of data in this field, we were uncertain of the exact quantity of the excessive intake.

⁷ Several epidemiological studies have shown that breakfast does usually represent 20% of daily intake (Zoet Nederland, 1992; De Castro, 1997), and that 20% ADER may not necessarily be a restrictive pre-load. Although no such epidemiological evidence exists for this in a South African context, anecdotal experience would suggest this is indeed the case in South Africa. In testing the hypothesis of altered energetic loads of varied macronutrient content on appetite control, 20% of ADER was referred to as "Underfeeding" relative to the 55% ADER "Overfeeding" treatment.

Five hours after the pre-load meal, an *ad libitum* test meal was given and the amount of food consumed and the time taken to eat were covertly monitored. At hourly intervals throughout the five-hour period between the two meals, and for 30 minutes after the test outcome meal, blood samples were collected and visual analogue scores (VAS) on appetite and hunger ratings were completed. These same variables were collected and recorded at 30-minute intervals for 90 minutes after completion of the *ad libitum* test meal.

Pre-load meals⁸

Study participants were told to consume the entire pre-load given, as a single meal over no more than a 20-minute period. In both the HF and LF pre-loads, the meal consisted of a broccoli and chicken bake, fruit juice, ice cream and a chocolate snack. The LF pre-load, used low-fat "Slimmer's Choice" ice-cream and a carob rice cake and fruit juice diluted with water, whereas in the HF pre-load cream was added to the entrée, full-cream ice-cream was used and a high-fat "Mars" chocolate bar was given as the snack (TABLE 5-2).

The Test Meal

Five hours after consuming the first pre-load meal, a test meal (TABLE 5-2) was supplied *ad libitum*. The test meal composed of a prepared cottage

⁸ Although baked chicken & broccoli, ice-cream and chocolate are not a traditional breakfast in a South African context, it was decided to use this food combination as the pre-load meal as it allowed for the easy covert manipulation of macronutrient sources in the pre-load. Using more traditional breakfast foods would have made it difficult for covert manipulation of foods.

pie and orange juice, of which the initial weights were known. The test meal was eaten in isolation, and study participants were informed to eat as much as they wanted for as long as they wanted. They were unaware that the quantity of food ingested was being monitored in any way.

TABLE 5.2
The nutritional information of foods (per 100g) served in the two pre-loads and test meal.

	Carbohydrate (grams)	Protein (grams)	Fat (grams)	Energy (kJ)
Overfeeding trial				
Chicken & broccoli	8.2	11.4	41.5	1,860
Orange juice	12.1	0.3	0.1	209
Ice-cream	23.9	3.6	10.8	847
Chocolate snack	68.4	5.1	18.0	1,847
Underfeeding trial				
Chicken & broccoli	5.2	9.0	2.5	327
Orange juice	12.1	0.3	0.1	209
Ice-cream (Slim)	16.4	4.5	2.5	431
Chocolate snack	6.2	6.1	0.4	204
Test meal				
Orange juice	12.1	0.3	0.1	209
Cottage Pie	5.1	9.2	8.8	572

Blood collection

Prior to starting each trial, an on-line catheter was inserted into the antecubital vein for the drawing of blood samples. The catheter was kept patent by the constant infusion of 0.9% sodium chloride (normal saline) (Sabax, Johannesburg), not more than 100ml of saline was infused on either trial. 5ml of whole blood was drawn at each period and put into prepared tubes (plasma had potassium oxalate as a metabolic inhibitor, and serum had a normal clotting factor) and centrifuged at 5 000 rpm for 20 minutes for plasma and serum collection. These samples were immediately frozen and stored at -70°C until assayed. Plasma samples were assayed for blood glucose levels using the Glucose (Hexokinase)

method Cat.-No.0SR6121 (Sclavo Spa. Siena, Italy). Serum samples were assayed for insulin levels using the Coat-A-Count® method 91145 via the ^{125}I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA).

Scores for satiety

Subjective satiety and hunger were assessed by three VAS scales. These accounted for the degree of changes in the following factors: hunger; thoughts of the amount of food that could be eaten; and the urge to eat (Hill *et al.*, 1984). The VAS scales were all 100mm in length, which were anchored at either extremity with terms indicating extremes. In analysis of the data, the mid-point of the 100mm line (ie 50mm) was arbitrarily assigned the neutral line (Jenkins *et al.*, 1992). The value determined in this manner was then converted into a percentage of the extreme being measured, and subsequently analysed as such.

Statistical methods

Changes in plasma glucose, serum insulin over time, and changes in VAS ratings were statistically tested using the multiple analysis of variance (MANOVA) for repeated measures, and the Tukey Honest Significant Difference (HSD) Test was used as an *ad hoc* test for differences between means. The data on time taken to consume the *ad-libitum* meal, the weights of food consumed, the amounts of energy consumed at that meal, and the areas under the respective insulin graphs were compared by means of a two-way ANOVA. Pearson's correlation (FIGURE 5-10) analyses were used to determine the correlation between serum insulin concentration immediately before the test meal and the amount of energy consumed at the meal (energy consumed was the dependent variable; insulin was the independent variables). All statistical tests were conducted using the software package STATISTICA, (Statsoft, Tulsa, OK, USA). In the text, TABLES, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

Subject characteristics

The average weights of study participants in the two groups were significantly different (TABLE 5-1) ($t = 2.8$, $P < 0.05$) and these differences were also reflected in measurements of both BMI ($t = 3.5$, $P < 0.01$) and in waist:hip ratios ($t = 3.91$, $P < 0.01$) (TABLE 5-1). When the ADER values were calculated, no differences ($P > 0.05$) were noted in the absolute values for ADER, however, when these values were corrected for body weight, the lean group had a higher ($P < 0.01$) energy requirement relative to body weight (Lean; $156.8 \pm 6.4 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ vs Obese; $98.1 \pm 7.7 \text{ kJ}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$).

The lean group had significantly lower ($P < 0.01$) scores on the Dutch Restrained Questionnaire in all three factors: the lean group scored an average of 2.65 ± 0.88 on the external motivation ($t = 3.1$) toward eating compared to the 3.18 ± 1.05 posted by the obese group. The lean group also scored lower ($t = 5.29$) on their emotional responses to eating (1.62 ± 0.77) compared to the obese group (2.65 ± 1.53), and finally, the lean group was less restrained ($t = 3.8$) in their eating habits and attitudes (2.30 ± 1.29) compared to the obese group (3.08 ± 0.96) (TABLE 5-1).

Food, energy and macronutrient intakes in test meal

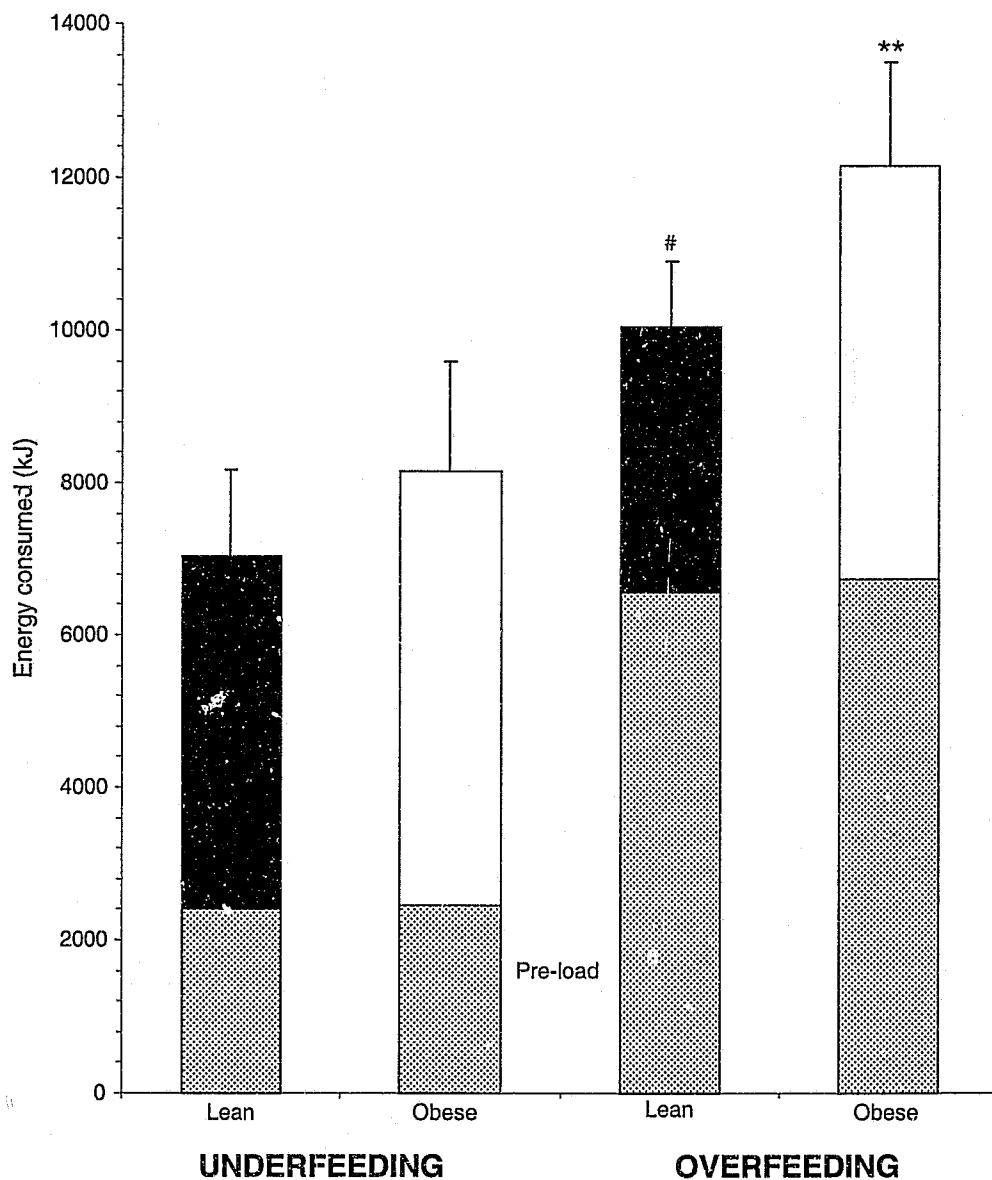
When given 20% of their ADER in the pre-load meal, there were no differences ($P > 0.05$) in the amount of energy consumed by the two groups at the test meal, despite significant differences in ADER (FIGURE 5.1). However, in the overfeeding HF pre-load with 55% ADER was given, the obese group consumed 1.6x more energy ($5,426 \pm 1,126\text{kJ}$) [$F_{1,20} = 11.45$, $P < 0.01$], than the lean group did ($3,473 \pm 1,114\text{kJ}$) (FIGURE 5.1). The lean group consumed 28% of their ADER at the test meal whereas the obese group consumed 44.4% of their predicted daily requirements at the test meal.

TABLE 5.3
Analyses of consumption (weight and energetic) at the ad libitum test meal in the lean and obese groups after served LF and HF pre-load treatments.

	Underfeeding (LF)		Overfeeding (HF)	
	Lean	Obese	Lean	Obese
Cottage Pie (g)	624 $\pm$ 177	757 $\pm$ 192	471 $\pm$ 115 ##	724 $\pm$ 206
Cottage Pie (kJ)	3,569 $\pm$ 1,011	4,328 $\pm$ 1,179	2,694 $\pm$ 659 ##	4,143 $\pm$ 1,179 *
Juice (ml)	495 $\pm$ 249	661 $\pm$ 244	373 $\pm$ 246 #	614 $\pm$ 481 *
Juice (kJ)	1,035 $\pm$ 520	1,381 $\pm$ 510	798 $\pm$ 476 #	1,283 $\pm$ 281 *
Total energy (kJ)	4,604 $\pm$ 1,183	5,709 $\pm$ 923	3,473 $\pm$ 1,114 #	5,426 $\pm$ 1,186 **
Time of lunch (minutes)	16.5 $\pm$ 0.1	19.7 $\pm$ 5.5	14.5 $\pm$ 3.4	17.7 $\pm$ 4.2
Consumption rate (kJ.min ⁻¹)	299 $\pm$ 95.6	306 $\pm$ 81.1	237 $\pm$ 44.2	313 $\pm$ 68.3

Where *, ** indicate $P < 0.05$ and 0.01 respectively between lean and obese in HF treatment
#, ## indicate $P < 0.05$ and 0.01 respectively between the underfeeding and overfeeding trials in the lean groups.

FIGURE 5-1 Energy consumed at the *ad libitum* meal between lean (■) and obese (□) males in the underfeeding and overfeeding trials.



Where ** indicates a difference ($P < 0.01$) between groups in the HF treatment

indicates a difference ($P < 0.05$) between LF and HF treatments in the lean group

The Pre-load period

Plasma glucose responses

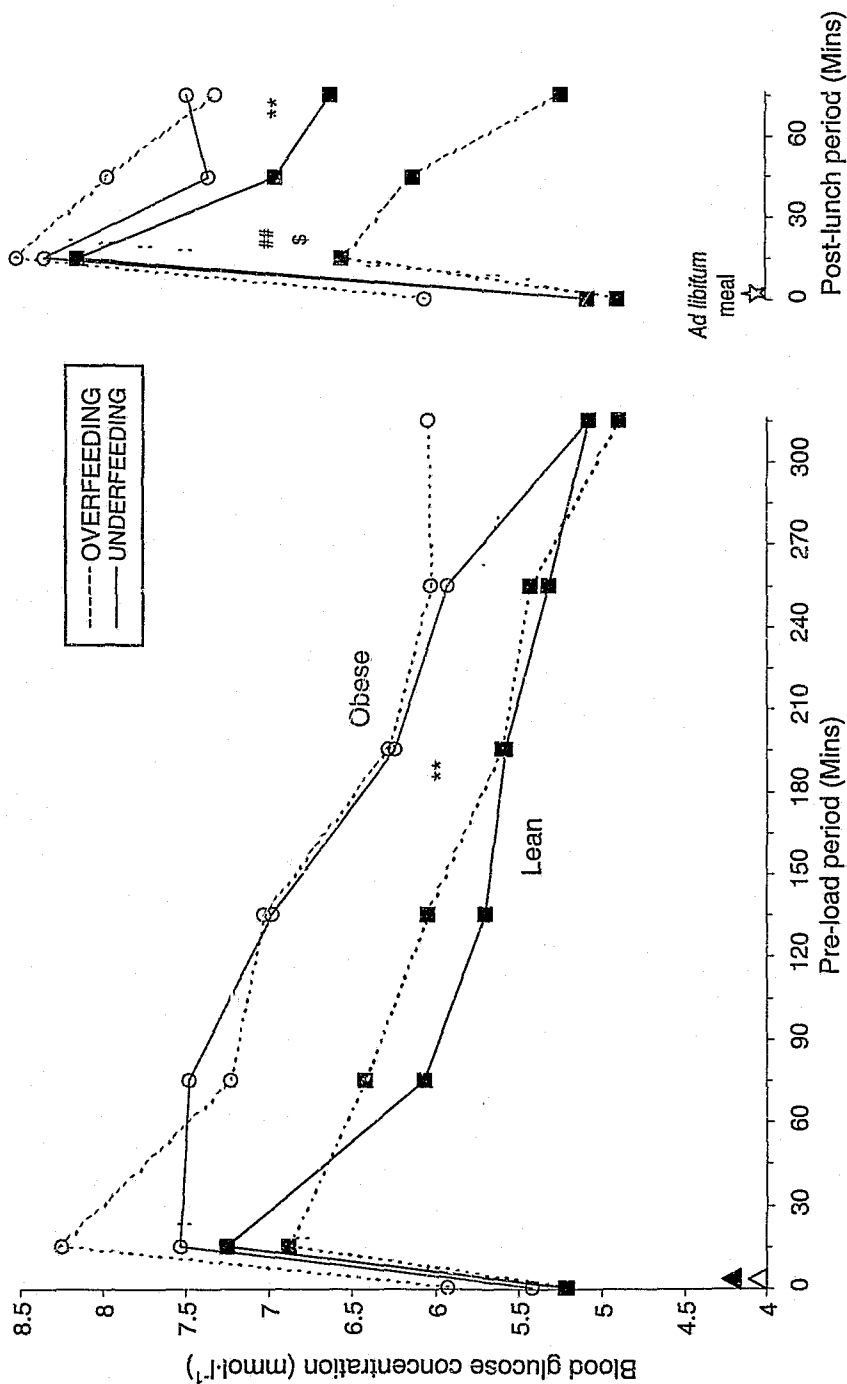
Although baseline glucose concentrations on all trial days for the two groups were similar and were within normal ranges ($5.2 \pm 0.4 \text{ mmol}\cdot\text{l}^{-1}$ and $5.2 \pm 0.6 \text{ mmol}\cdot\text{l}^{-1}$ in the lean group, and $5.4 \pm 0.7 \text{ mmol}\cdot\text{l}^{-1}$ and $5.9 \pm 0.5 \text{ mmol}\cdot\text{l}^{-1}$ in the obese group), plasma glucose concentrations after the preload were significantly higher in the obese group and remained elevated for a longer period than those of lean participants [$F_{1,20} = 13.6$, $P < 0.01$] (FIGURE 5.2). Elevation in glucose concentration was substantially greater on the HF diet (FIGURE 5.2)

Serum insulin responses

The lean group's fasting insulin concentrations ($9.9 \pm 2.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $12.4 \pm 4.3 \mu\text{U}\cdot\text{ml}^{-1}$ for LF and HF treatments respectively) were lower ($P < 0.01$) than those at the start of both trials in the obese group ($34.9 \pm 12.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $32.7 \pm 12.0 \mu\text{U}\cdot\text{ml}^{-1}$ for LF and HF treatments respectively) (FIGURE 5.3).

Insulin responses to the pre-load meals were significantly different [$F_{1,20} = 43.38$, $P < 0.01$] insofar as the HF treatment induced a greater response in serum concentrations compared to the LF treatment in both lean and obese men. In the obese group, however, the insulin response to the HF treatment was considerably greater and remained elevated over the 315-minute period between meals [$F_{6,120} = 11.07$, $P < 0.01$] whereas in the

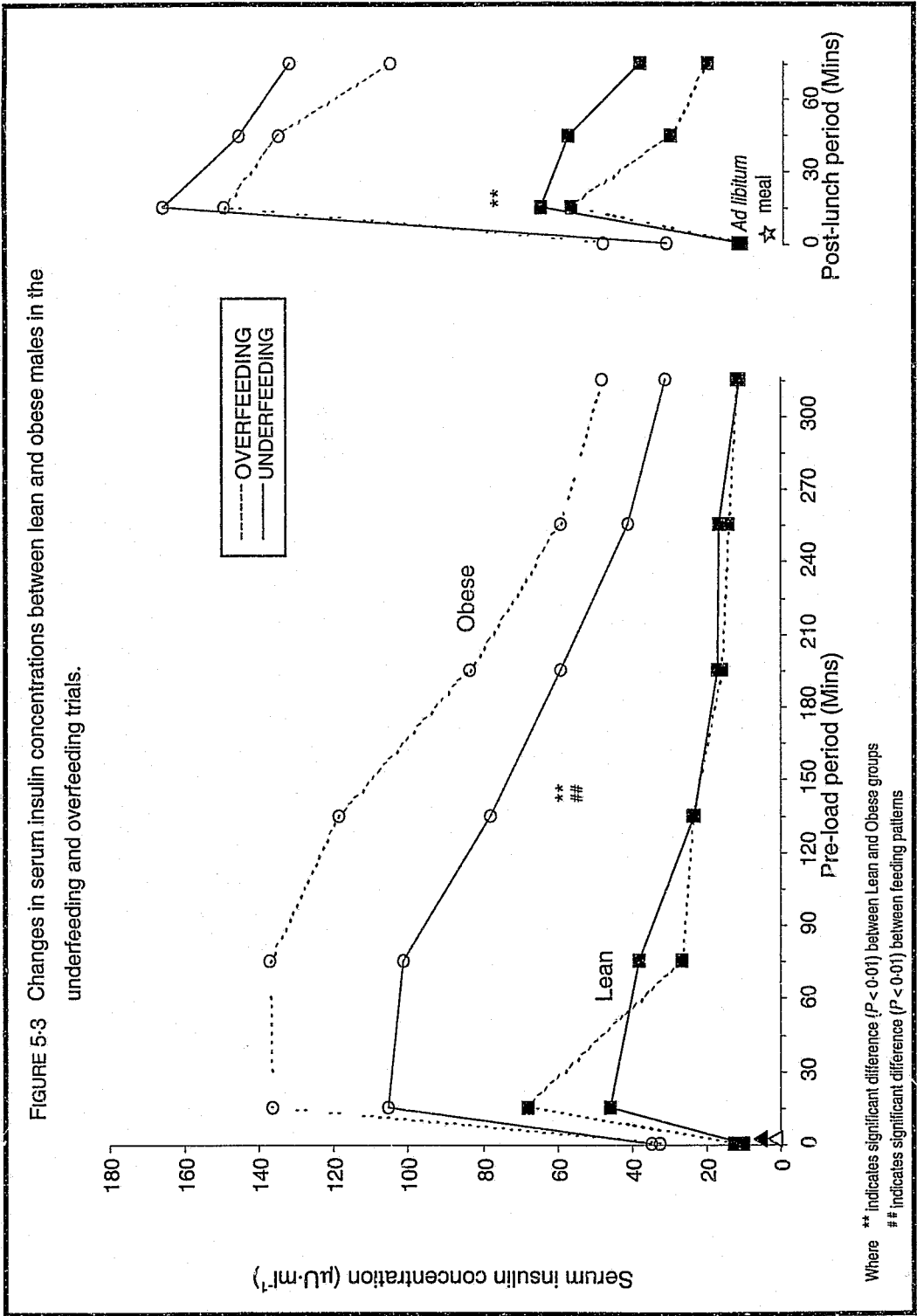
FIGURE 5.2 Changes in plasma glucose concentrations between lean and obese males in underfeeding and overfeeding trials.



Where ** indicates significant difference ($P < 0.01$) between Lean and Obese groups

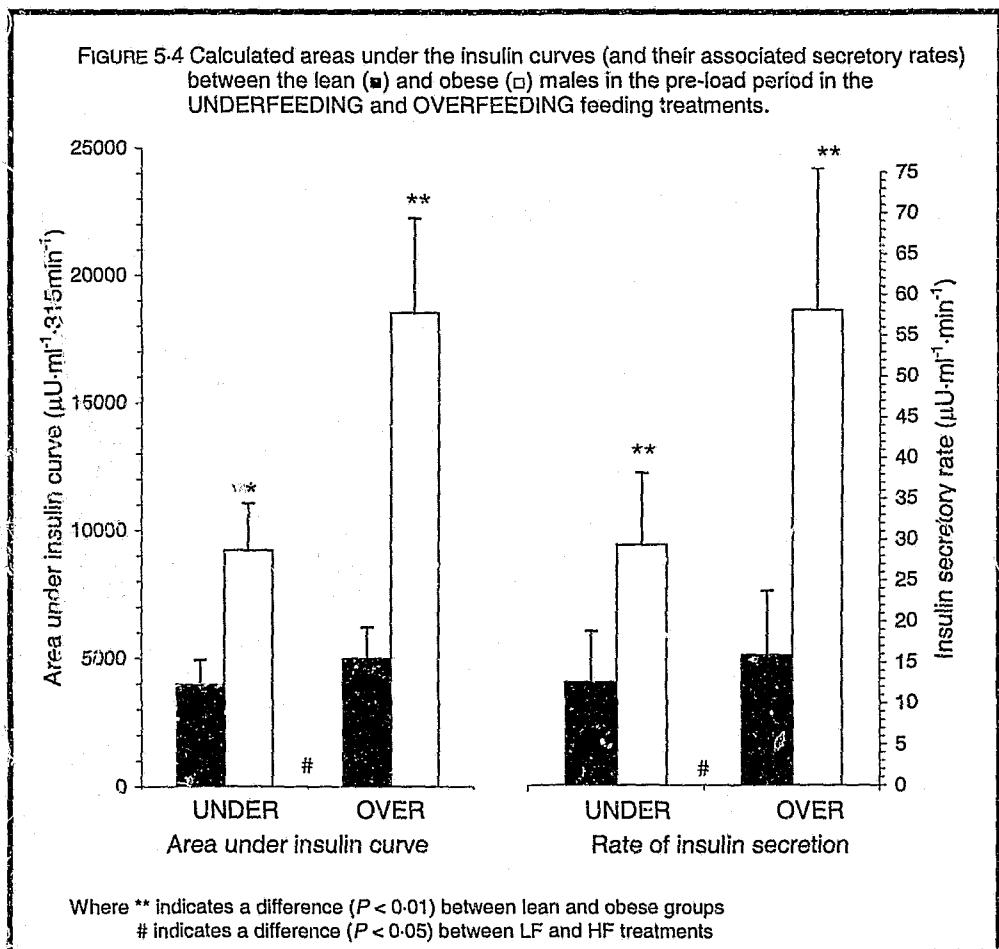
indicates significant difference ($P < 0.01$) between feeding patterns

\$ indicates non-significant difference ($P > 0.05$) between treatments as a function of time



lean group insulin was elevated above baseline for only the first hour after consumption of the pre-load.

When the areas under the insulin curves were calculated, the lean group's area ($3,945 \pm 2,450 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$ and $4,970 \pm 2,134 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$ for the LF and HF treatment pre-load periods respectively) was shown to be less [$F_{1,20} = 23.36$, $P < 0.01$] than that of the obese ($9,215 \pm 6,790 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$ and $18,524 \pm 5,862 \mu\text{U}\cdot\text{ml}^{-1}\cdot 315\text{min}^{-1}$ for the LF and HF treatment pre-load periods respectively), and further, the area under the insulin curve in the LF trial was less [$F_{1,20} = 7.04$, $P < 0.05$] than that in the HF trial (FIGURE 5.4).

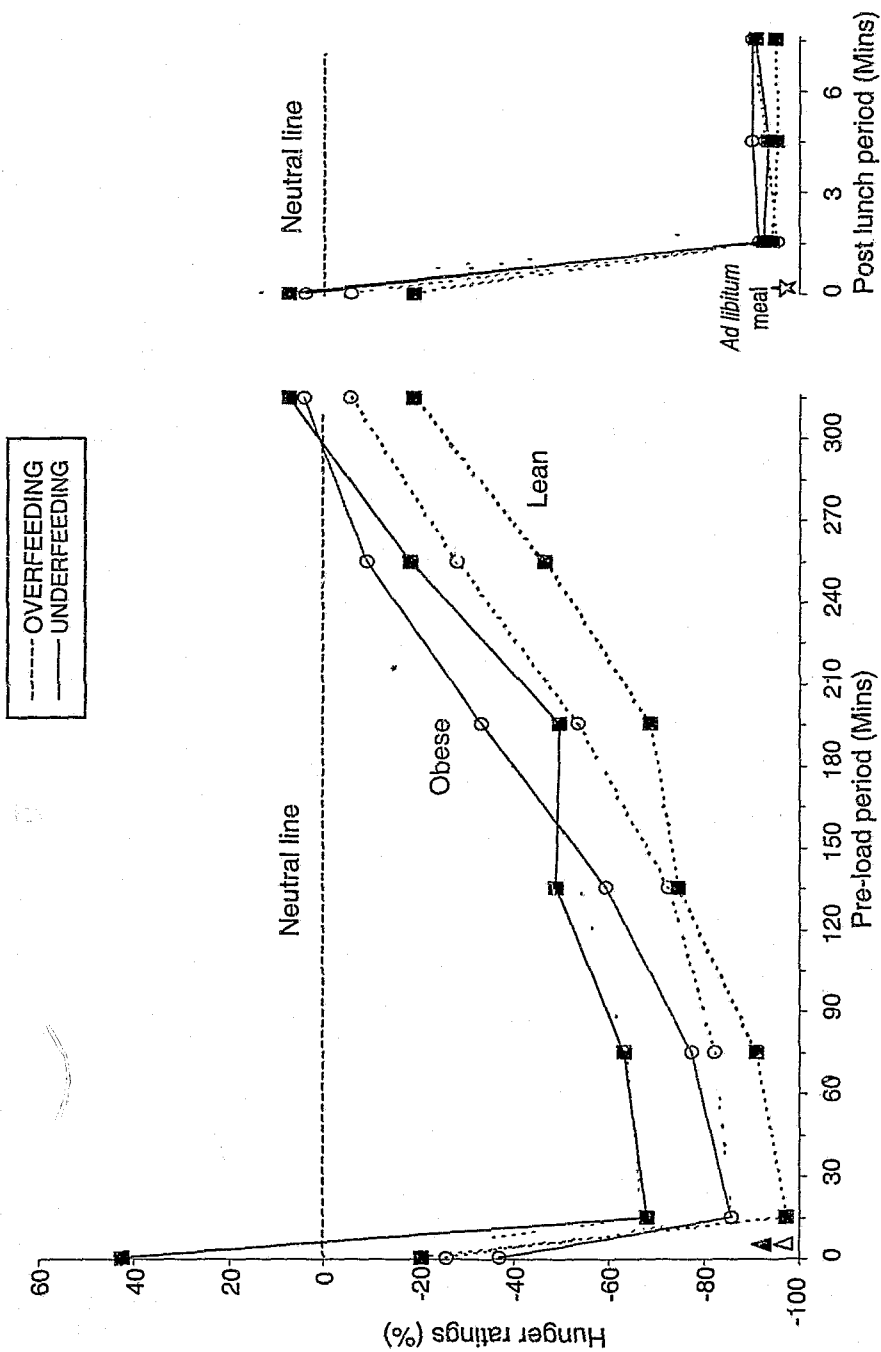


Visual Analogue Scales

All baseline hunger rating scores between the two groups or: the two energetic eating patterns were similar ($P > 0.05$) (FIGURES 5-5, 5-6, 5-7). However, over the pre-load period (on both treatments), the obese group showed signs of becoming hungrier [$F_{6,120} = 2.42$, $P < 0.05$] relative to those *hunger ratings* recorded by the lean group (Figure 5-5).

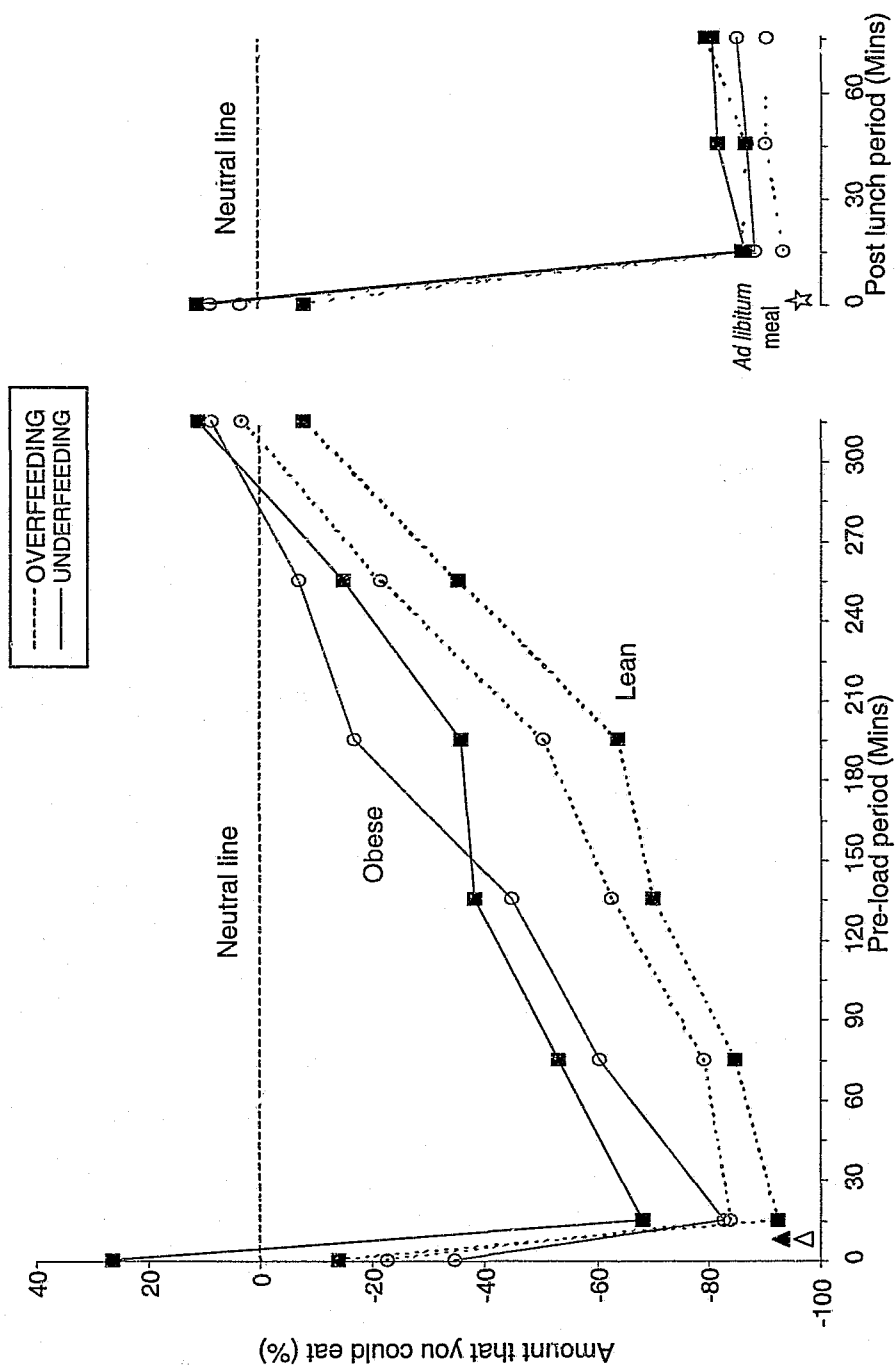
Both groups reported similar responses ($P > 0.05$) in their VAS scores to both the question on how much they felt they could eat at that instant and also on their *urge to eat* at the start of the experiment. Similarly the responses were the same ($P > 0.05$) throughout the pre-load period from both groups, however in both groups these responses changed over time [$F_{6,120} = 23.41$, $P < 0.01$] (FIGURE 5-7).

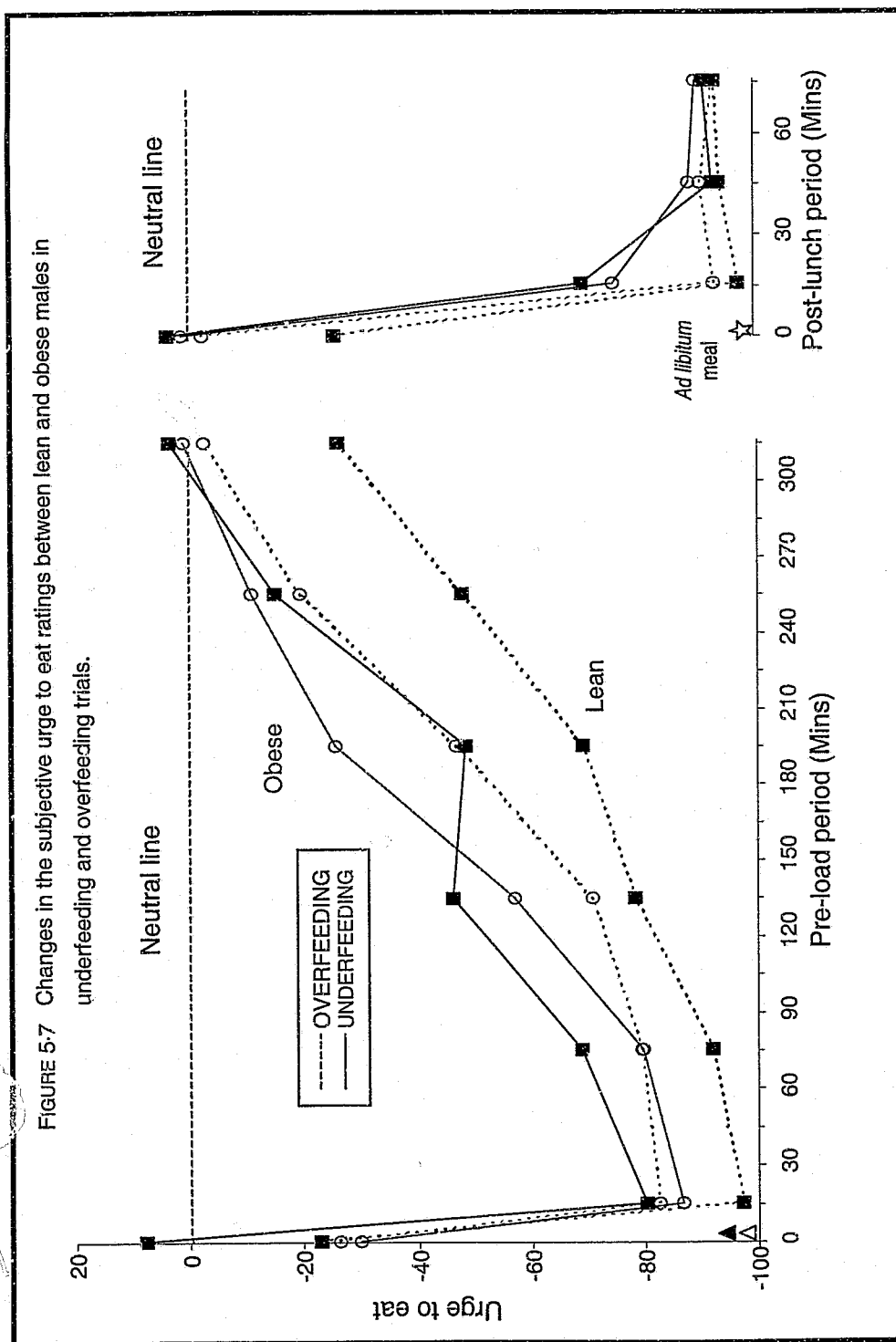
FIGURE 5.5 Changes in subjective hunger ratings between lean and obese males in underfeeding and overfeeding trials.



Where * Indicates significant difference ($P < 0.05$) between the lean and obese groups

FIGURE 5.6 Changes in perception in amount that subjects felt they could eat in lean and obese males in underfeeding and overfeeding trials.





Analysis of the meals taken to consume the *ad libitum* test meals revealed no differences ($P > 0.05$) between the lean and the obese groups on either energetic treatment. Further statistical analysis failed to reveal a difference ($P > 0.05$) in the rate of consumption of the meals between the groups (TABLE 5.3).

The post – *ad libitum* test meal period

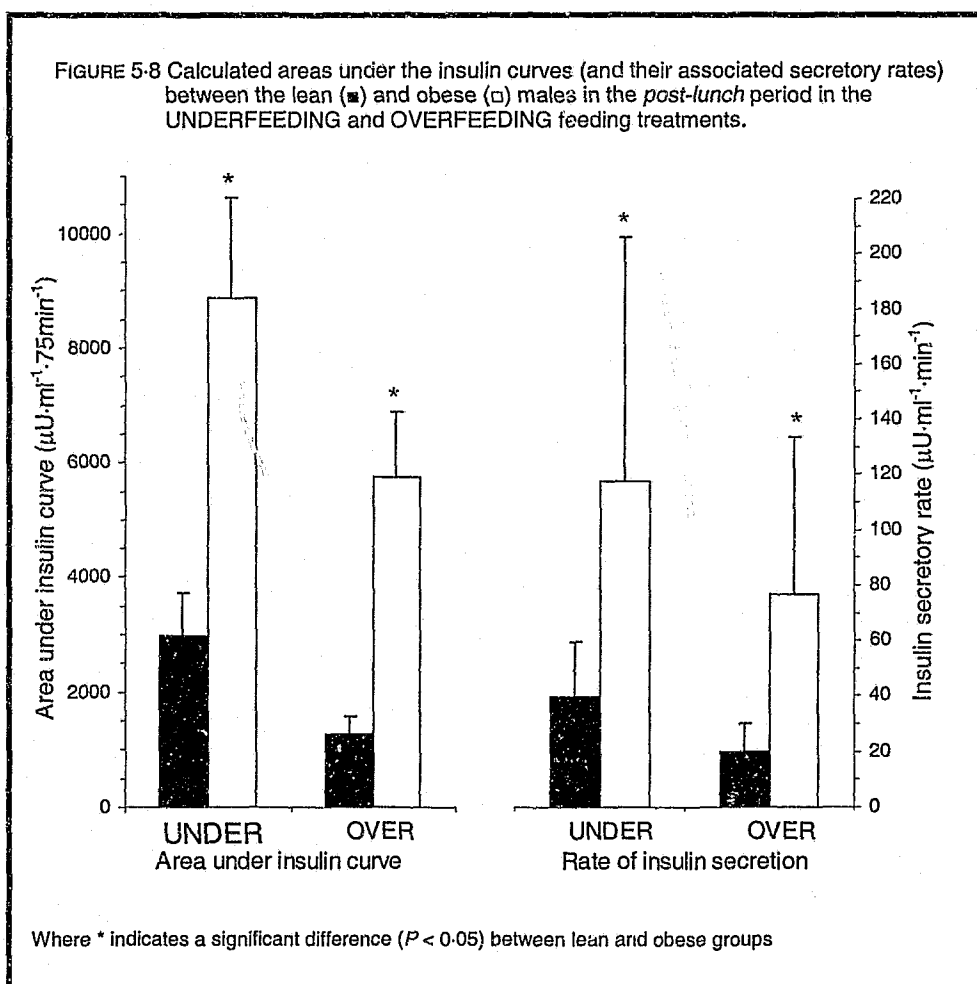
Plasma glucose responses

There were no differences in glucose concentrations immediately before the consumption of the test meal, but the obese group's plasma blood glucose response to the test meal was greater than that observed in the lean group [$F_{1,20} = 21.51$, $P < 0.01$]. There was an interaction in glucose responses between the LF and HF patterns insofar as the intervention of different meals (energetic and macronutrient based) affected plasma glucose concentrations differently in the lean and obese groups [$F_{1,20} = 9.38$, $P < 0.01$]. Furthermore, the blood glucose responses in the underfeeding LF trial were *flatter* than those in the overfeeding HF trial [$F_{1,20} = 3.12$, $P < 0.05$] (FIGURE 5.2, *right*).

Serum Insulin concentrations

In both treatments, the obese group's insulin concentration rose higher [$F_{1,20} = 19.51$, $P < 0.01$] than that noted in the lean group. Accordingly, the areas ($8,875 \pm 7,836 \mu\text{U}\cdot\text{ml}^{-1}\cdot 75\text{min}^{-1}$ and $5,739 \pm 3,504 \mu\text{U}\cdot\text{ml}^{-1}\cdot 75\text{min}^{-1}$ for LF and HF respectively) under the obese group's insulin curves in this

75-minute period were greater [$F_{1,20} = 8.51$, $P < 0.01$] to those areas under the lean group's insulin curve ($2,980 \pm 1,067 \mu\text{U}\cdot\text{ml}^{-1}\cdot 75\text{min}^{-1}$ and $1,269 \pm 980 \mu\text{U}\cdot\text{ml}^{-1}\cdot 75\text{min}^{-1}$ for LF and HF respectively). As with the plasma glucose responses, the *ad libitum* meal induced an associated interaction effect [$F_{3,60} = 4.64$, $P < 0.01$] in the insulin responses between the two groups over the 75-minute post-lunch period: the difference in response to eating the lunch (FIGURE 5-3, right).



Visual Analogue scores

In all three factors used to determine subjective appetite and satiety, there were no differences between lean and obese noted at any of the time intervals at which these ratings were assessed. However all VAS responses changed ($P < 0.01$) over the course of the 75 minutes after lunch (FIGURES 5-5; 5-6; 5-7).

Relating serum insulin concentration to energy consumption

There was a significant inverse correlation ($r = -0.628$; $P < 0.05$) between the serum insulin concentration in the lean group immediately before lunch and the amount of energy consumed at the *ad libitum* meal. Although there was a tendency for the obese group to show a negative correlation ($r = -0.314$), this was insignificant ($P > 0.05$) (FIGURE 5-10).

FIGURE 5.9 Scatterplots depicting the relationship between serum insulin concentration immediately before lunch and the amount of energy consumed at the *ad libitum* lunch in the OVERFEEDING (Top) and UNDERFEEDING (Bottom) trials.

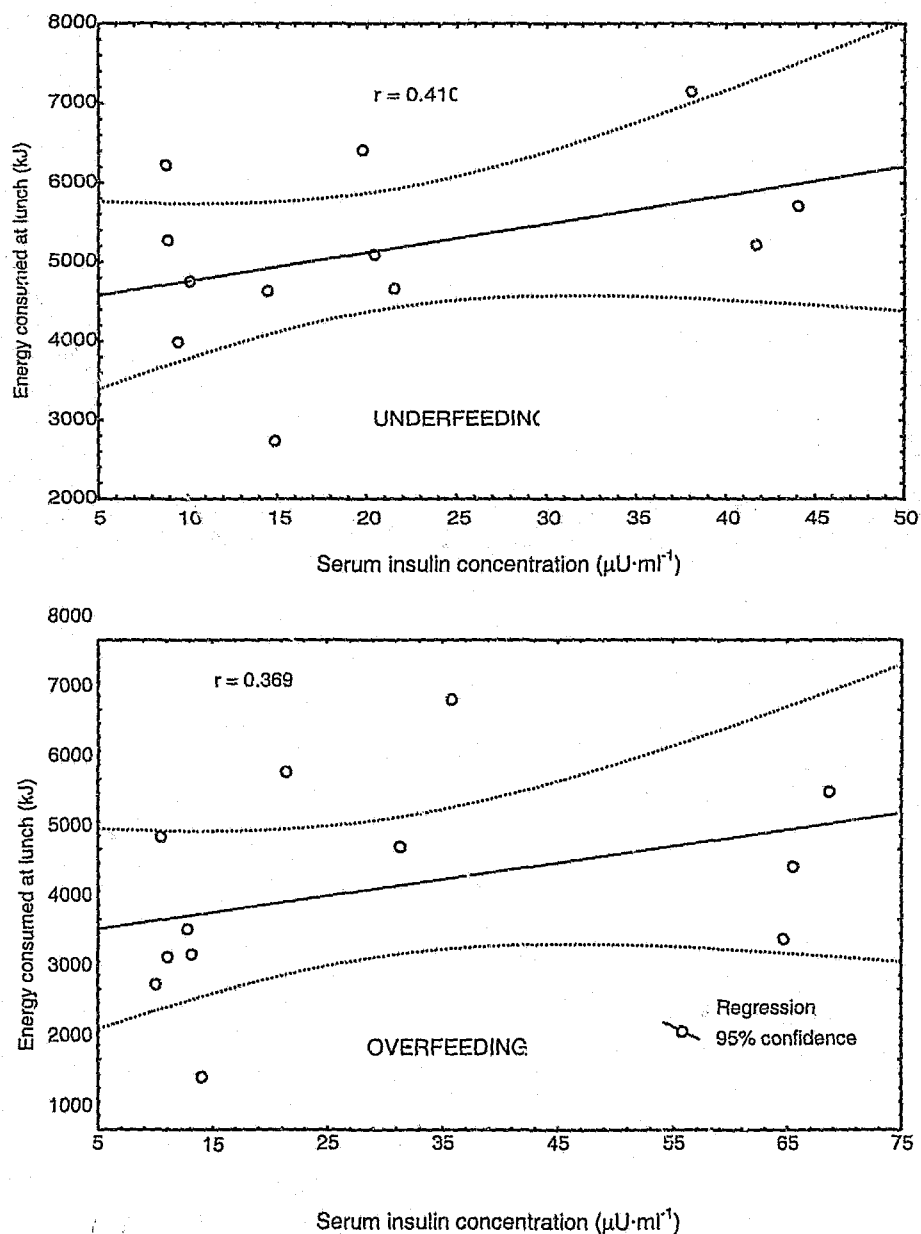
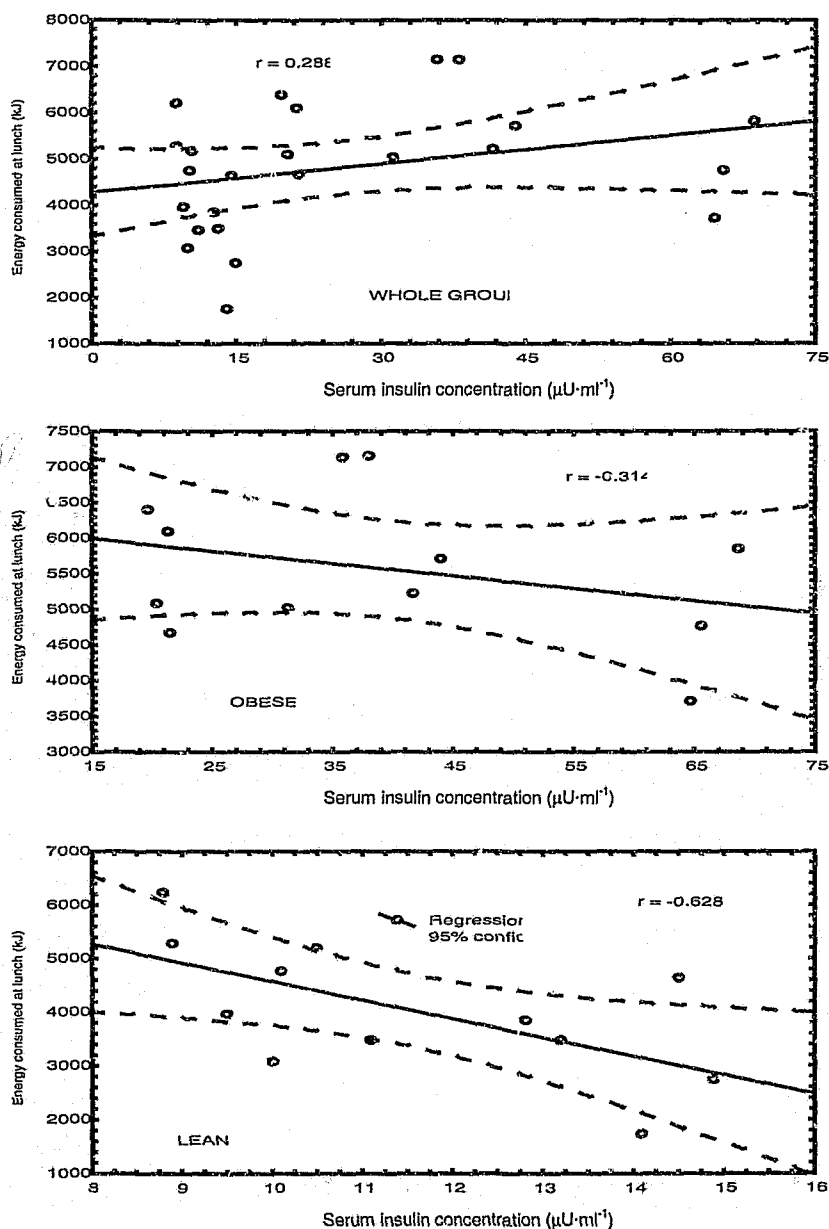


FIGURE 5.10 Scatterplots depicting the relationship between serum insulin concentration immediately before lunch and the amount of energy consumed at the *ad libitum* lunch by groups: WHOLE GROUP (*Top*); OBESE (*Middle*); and LEAN (*Bottom*).



DISCUSSION

Significant differences were evident in the ability of the lean and obese group to detect the energetic changes between the two pre-load treatments (FIGURE 5-1). The lean group regulated their intake at the test meal such that it inversely corresponded with the energy content of the pre-load. The obese group, on the other hand, showed no indication of any ability to detect energetic differences in the pre-load, and therefore did not compensate accordingly at the next meal. The subsequent hyperphagia observed in the obese group on the HF treatment resulted in the consumption of 56.4% more than their lean counterparts. Furthermore, the obese group consumed this excess without subjectively regarding themselves as particularly hungry immediately prior to the test meal (FIGURES 5-5; 5-6; 5-7) and despite their greater restraint with regards to their eating pattern (TABLE 5-1). This finding suggests, if the obese group had similar DRQ profiles to the lean group, the obese group may have consumed even more food at the test meal than they actually did.

A key observation was the hyperglycaemia noted in the obese group in the pre-load period (FIGURE 5-2) compared to the lean group in the same period. This hyperglycaemia remained despite the dramatically elevated serum insulin levels throughout the 315-minute pre-load period, thus suggesting reduced insulin sensitivity on the part of the obese group. The hyperinsulinaemia observed in the obese group in response to both HF and LF pre-loads has been previously widely reported (Eckel, 1992; Friedman, 1998). Eckel (1992) postulated that the insulin resistance

noted in an obese population is an adaptation for weight maintenance in so far as the reduction in insulin action affects metabolic fuel partitioning that would prevent further weight gain. The differences noted in metabolic and endocrinological function between the lean and the obese group in the current study suggest that one, or both of these variables may be linked to ingestive behaviour.

Any system that regulates itself within the confines of control requires a negative feedback of a variable that is linked to the function requiring the control (Friedman, 1998). In the current example of feeding behaviour as food is consumed, nutrients are absorbed into the blood. Although a degree of these nutrients diffuse into the working cells, the majority of these nutrients require insulin for their active transport into the cells (Randle *et al.*, 1963). It is an attractive theory to suggest that blood glucose fluctuations exert control over appetite. It has been shown convincingly that high carbohydrate diets ensure well-regulated blood glucose concentrations, with the concomitant control over appetite (Mayer, 1955; Blundell *et al.*, 1994; Raben *et al.*, 1996). Serum insulin is strongly implicated in the control over blood glucose and consequentially appetite, and is only ever elevated in the absorptive state, indicating post-prandial conditions. Yet, the obese group exhibits an elevated basal insulin concentration and reduced insulin sensitivity. This reduced insulin sensitivity in obese males is highlighted when the areas under the insulin curves are considered (FIGURE 5-3). Although both lean and obese groups were fed similar energetic pre-loads of similar macronutrient content (TABLE 5-2), the obese group produced more insulin over the pre-load period than the lean group did. The effect that the dramatic rise in

serum insulin, with the concomitant delay in its return to baseline values, has on subsequent food intake in a group of obese males may interfere with energetic regulatory mechanisms. The lean group exhibited initial rises in serum insulin following the pre-load bolus, and then returned to close to baseline fairly quickly, and they consumed less energy which is further reinforced by the inverse correlation. An interesting point to note was the absence of concomitant appetite differences before the test meal between the lean and obese groups (FIGURES 5-5; 5-6; 5-7), yet the lean group exhibited greater control over appetite in terms of consequential food consumption at the test meal.

The role that insulin plays in appetite and satiety has received considerable attention in the past few years. Some investigators report greater satiety sensations post-prandially with a lower insulin level (Holt *et al.*, 1992; Holt & Brand-Miller, Lavin & Read, 1995), while others describe opposite effects. In the latter studies, higher serum insulin concentrations are linked to heightened satiety (baboons (Steffens *et al.*, 1988; Woods *et al.*, 1979; 1984); rats (Woods, 1997); humans (Speechly & Buffenstein, 1999; Speechly *et al.*, 1999)). In studies investigating insulin treatment in rats, insulin replacement reportedly induces hyperphagia, and counteracts the satiating effect of a high-fat meal in both normal and diabetic rats (Friedman, 1977). My findings concur with those reported in Friedman's (1977) rat model and may indicate that insulin alters the behavioural response in humans to a high-fat meal through its effects on the metabolism of the ingested fat. Furthermore, these findings suggest that hyperinsulinaemia could foster hyperphagia, especially when a high-fat diet is consumed.

The glucose-fatty acid cycle (Randle *et al.*, 1963) suggests that either glucose or free fatty acids are being oxidised at any one time, and the absence of glucose oxidation would indicate a post-absorptive state (Friedman, 1998). Normally, the post-absorptive state would coincide with increased appetite, with a concomitant drive to eat, and it follows that the swing between the absorptive and post-absorptive state would suggest the swing between hunger and satiety. Within this model, insulin may play an integral role as a regulating *on/off* switch in this pathway and thus regulate feeding behaviour according to its intrinsic link to the feeding process. The findings in our current study suggest that lean males may possess a mechanism, like that described above, which incorporates insulin in the regulation of energy intake. Furthermore, this mechanism may have become dysfunctional in our obese study participants. Evidence supporting this premise includes reduced insulin sensitivity with the hyperinsulinaemic state further exacerbating this dysfunction. Clearly this is highly speculative and further research is required in this field.

The finding that the obese group consumed more energy at the test meal than the lean group, both in terms of absolute energy intake and also as a % of their ADER at first would appear blatantly obvious. Based on the premise of overconsumption as an inductive factor in obesity, this would have to be the case. However, the majority of studies that have investigated dietary intake and obesity show weak, or no correlations between energy intake and body weight. This lack of a relationship in intake and obesity may be ascribed to *dietary under-reporting* (Bellisle *et al.*, 1997; Poppitt *et al.*, 1998b; Voss *et al.*, 1998). In this study although there was no change in how the obese group recorded their subjective hunger ratings, considerably more was eaten at the test meal when given

a energetically-loaded pre-load. This could be attributed to the difference in macronutrient content on the two pre-loads.

A full understanding of the differences in the macronutrient values of the pre-loads should be considered within the concept of the "sugar-fat see-saw" (Blundell & Macdiarmid, 1997): a situation where the manipulation of either carbohydrate or fat affects the other proportionately. Thus, the LF trial could just as easily been referred to as the high-carbohydrate trial, and the HF trial the low-carbohydrate trial. A great deal of work has gone into the understanding of the effect of altered macronutrient value on energy intake and consequently, the induction and maintenance of obesity. Some studies in rats have shown that glucose- and sucrose-induced overeating have led to obesity (Hirsch & Walsh, 1982; Kanarek & Marks-Kaufman, 1979) as a result of the appetite-stimulant effect of sucrose. However, the full extent of *de novo* lipogenesis from carbohydrate and its impact upon energy intake is yet to be confirmed (Hill & Prentice, 1995; Friedman, 1998). However, the majority of the evidence supports the notion that increased carbohydrate ingestion enhances carbohydrate oxidation preventing lipogenesis (Randle *et al.*, 1963; Friedman & Tordoff, 1986; Raben *et al.*, 1996). When the study participants in the current study were given the low-fat, high-carbohydrate pre-load of only 20% of their ADER, there were no differences between the lean and obese groups in the amount of energy consumed at the test lunch meal (FIGURE 5.1). The prevalent use of carbohydrate fuels rather than fat substrates (or lipogenesis) may contribute to the observed enhanced control over appetite with the higher carbohydrate diet, in keeping with previous reports of better control of appetite on a high carbohydrate diet (Rolls, 1991; Raben *et al.*, 1996; Poppitt *et al.*, 1998a).

Although the lean participants in the current study did not adequately compensate for the increased energy intake on the HF treatment, they did consume less than when they were consumed only 20% of their ADER. Furthermore, they did exhibit greater control in consequent energy intake compared to their obese counterparts (TABLE 5-3). This finding is reinforced by the finding of Rolls *et al.*, (1994) which showed that lean individuals exhibit a weak regulation in response to the ingestion of high-fat foods.

That obese males consume more than their lean counterparts after a high-fat energetically-overloaded pre-load (FIGURE 5-1) was expected in the light of previous reports of passive overconsumption following covert manipulation of dietary fat (Blundell *et al.*, 1994; Stubbs *et al.*, 1995; Stubbs *et al.*, 1996). In addition, the obese population exhibit an elevated preference for fat in foods (Drewnowski & Greenwood, 1983; Mela & Sacchetti, 1991; Drewnowski *et al.*, 1992; Drewnowski 1997; Cooling & Blundell, 1998). Blundell & Macdiarmid (1997) proposed the "fat paradox" where increased fat ingestion potentiates the perpetual increased intake of fat, even though it has an ability to invoke strong satiety signals at an intestinal level (Welch *et al.*, 1985). These authors postulate that such a paradox is the "fat-induced satiety and high-fat hyperphagia". Lawton *et al.*, (1993) found that the effect of obese individuals eating high-fat foods dramatically increased the size of the meals (increased energy content).

In conclusion, the data obtained in the current study suggest that obese males are more prone to the appetite-potentiating effects of a high-fat diet,

and lean males are not. It would appear that the mechanism responsible for the regulation of ingestive behaviour appears to work equally well in both lean and obese groups with low-fat foods, and breaks down on a high-fat / high energy diet. Furthermore, our observations suggest that insulin plays a significant part in the control over appetite and satiety in lean males, whereas this relationship is absent in obese males. The latter may be a direct consequence of chronic hyperinsulinaemia observed in obese individuals. However, further investigation is required to confirm the mechanisms behind the observed response.

Chapter Six

**Obese males fail to detect energetic changes
in an overfeeding pre-load, yet increased
frequency of feeding enhances appetite control**

INTRODUCTION

Control over appetite has become a highly sought after effect in the field of obesity research: what makes a person eat more than he/she needs? Definite energy sensors have been proposed by some authors (Ramirez *et al.*, 1989; Friedman, 1997; Friedman, 1998), but these have never been detected. Jéquier (1993) however, proposed that the body has no receptors with which to measure energy, and that people eat nutrients, and not energy *per se*. Accordingly, understanding the regulation of macronutrient intake and the differential impact of the various macronutrients upon energy intake may elucidate the aetiology of obesity (Blundell, 1990; Rolls & Shide, 1992; Blundell *et al.*, 1994; Rolls *et al.*, 1994; Hill & Prentice, 1995; Rolls *et al.*, 1995; Blundell *et al.*, 1996; Blundell & Macdiarmid, 1997b; Poppitt *et al.*, 1998; Rolls & Bell, 1999).

Many studies support the premise that obesity results primarily from a chronic imbalance between the intake of fat, and the rate of fat oxidation (Giusti *et al.*, 1996; Jéquier, 1998; Hellerstein, 1999). While the ingestion of carbohydrate promotes carbohydrate oxidation, it would appear that the carbohydrate stores are regulated by negative feedback from the rate carbohydrate oxidation. In contrast, fat intake has no influence on fat oxidation (Jéquier, 1993; Jéquier, 1998; Blundell & Stubbs, 1999; Friedman *et al.*, 1999a). In addition, the hedonistic, orosensory, and more palatable features of a high-fat diet contribute to passive overconsumption on a high-fat diet. Thus obesity may more easily occur on high-fat rather than high-carbohydrate diets (Thomas *et al.*, 1992; Blundell &

Macciarmid, 1997; Rolls *et al.*, 1988; Lewinowski, *et al.*, 1991; Speechly & Buffenstein, 2000).

Increasing the frequency of eating episodes when consuming a prudent diet enhances appetite control at the next meal (Speechly & Buffenstein, 1999; Speechly *et al.*, 1999). I questioned whether similar improved appetite control would occur if the frequency of eating high-fat foods, rather, was increased. Therefore, I tested the null hypothesis that an increased meal frequency on a high-fat diet would induce a similar test meal outcome to that of a single meal of similar macronutrient and energy value.

MATERIALS AND METHODS

Subjects

The same twelve (six lean men with BMI < 23kg·m⁻², and six obese men with BMI > 32kg·m⁻²) healthy, non-diabetic obese men that were used in chapter five were used in this experiment. The details of the subject characteristics are contained in Chapter 5, but are tabulated below for convenience.

TABLE 6-1.

Characteristics of study participants.

	Lean	Obese	Significance
N	6	6	
Age (years)	26.67 ± 5.47	39.83 ± 19.03	NS
Weight (kg)	77.13 ± 5.60	125.88 ± 43.82	* t = 2.76
Height (m)	1.85 ± 0.61	1.79 ± 0.075	NS
BMI (kg·m ⁻²)	22.50 ± 1.08	39.05 ± 11.63	** t = 3.45
WHR	1.01 ± 0.04	1.17 ± 0.09	** t = 3.91
ADER (kJ)	12,098 ± 1,103	12,225 ± 3,543	NS
20% ADER (kJ)	2,419 ± 220	2,445 ± 709	NS
55% ADER (kJ)	6,562 ± 583	6,723 ± 1,948	NS
Relative energy	156.8 ± 6.38	98.1 ± 7.66	** t = 14.4
DRQ			
External	2.65 ± 0.88	3.18 ± 1.05	** t = 3.08
Emotional	1.62 ± 0.77	2.65 ± 1.53	** t = 5.29
Restraint	2.30 ± 1.29	3.08 ± 0.96	** t = 3.77

Where BMI = body mass index; WHR = waist:hip ratio
and DRQ = Dutch Restrained Questionnaire.

And * $P < 0.05$, and ** $P < 0.01$ between lean and obese groups

It must be pointed out that the SINGLE treatment data contained in this experiment have been presented in the previous chapter (Chapter 5).

Protocol

In this study, I investigated the effects of meal frequency on appetite control and serum insulin concentration when the pre-load meal was energetically dense (55% ADER) and high in fat (35% carbohydrate; 22% protein; 43% fat. 55% of their average daily energy requirement (ADER) (as determined by the Harris-Benedict equation (Van Way, 1992)) was either given as a single meal (SINGLE) or divided into five equal portions given at hourly intervals (MULTI). The effect of increased frequency of eating high-fat meals on appetite, perception of hunger, glucose and insulin concentrations was assessed by randomly assigning subjects on their first visit to one of two feeding regimes.

Testing Considerations

All subjects reported to the Sleep and Metabolic Laboratory at the University of the Witwatersrand Medical School, Johannesburg, at 07h00 in the morning. The laboratory is at an altitude of 1,800m (625 torr) and regulated at a room temperature of 20-22°C. The subjects were monitored throughout the testing period, and they were not allowed to leave the laboratory for any reason. Subjects were asked to refrain from eating and drinking anything other than that prescribed in the testing protocol. The first pre-load meal was given at ~07h30. Irrespective of whether a single meal or one of the multi-meals was given, the men were subjected to identical treatments and identical nutrient intake (TABLE 6-2): visual analogue scales (VAS) and blood measurements were collected hourly for 5 hours. After five-and-a-half

hours (~13h00), subjects were given an *ad libitum* lunch (of pre-prepared cottage pie and orange juice, TABLE 6-2). This was eaten in isolation. Subjects were told to eat as much as they wanted and for as long as they desired. They were unaware that the quantity of food ingested was being monitored in any way. The amount of food (and energy) consumed at the *ad libitum* lunch was measured by weighing the cottage pie (in its container) and the orange juice carton, immediately before presenting it to the subjects, and then reweighing the whole container after completion of the test lunch meal. Although baked creamed chicken & broccoli, ice-cream and chocolate are not a traditional breakfast in a South African context, it was decided to use this food combination as the pre-load meal as it allowed for the convenient covert manipulation of macronutrient sources in the pre-load. Subjects appeared to like the food, and did not question why this unusual meal was chosen.

TABLE 6-2.
The nutritional information of foods (per 100g) served in the overfeeding pre-load and test meal.

	Carbohydrate (grams)	Protein (grams)	Fat (grams)	Energy (kJ)
Overfeeding trial				
Chicken & broccoli	8.2	11.4	41.5	1,860
Orange juice	12.1	0.3	0.1	209
Ice-cream	23.9	3.6	10.8	847
Chocolate snack	68.4	5.1	18.0	1,847
Test meal				
Orange juice	12.1	0.3	0.1	209
Cottage Pie	5.1	9.2	8.8	572

Timing of the time taken to consume the *ad libitum* lunch meal began immediately as the meal was presented to the subject. Subjects were instructed to start eating when they were presented with the food, and then to leave the table upon completion. Accordingly, each subject was monitored covertly throughout the eating process.

Food considerations

Every effort was made to ensure that the food presented to all subjects on all feeding treatments was identical (relative to each specific subject) in relative weight and energetic value, texture, temperature, and appearance. Although the temperature of the food was not measured, the preparation in all foods was sufficiently identical (storage, and cooking time) to satisfy us that the foods were similar when they were presented to the subjects.

The *ad libitum* meal (cottage pie) had very different macronutrient and energy contents to that of the pre-load meal(s) (TABLE 6.2). This ensured that the results obtained in this study were not the affects of sensory specific satiety rather than the frequency of feeding.

Body mass (accurate to 0.05kg, Mettler TE/J, Zurich, Switzerland) and height (accurate to 0.1cm, SECA, Germany) were measured at the start of the experiment.

Scores for satiety

Subjective satiety and hunger were assessed by three VAS scales assessing the degree of changes in the following factors: hunger; thoughts of the amount of food that could be eaten; and the urge to eat (Hill *et al.*, 1984). The VAS scales were all 100mm in length, which were anchored at either extremity with terms indicating extremes as depicted below.

An example of the visual analogue scale (VAS) used to assess hunger.

The diagram shows a visual analogue scale (VAS) for hunger. It consists of a horizontal line with vertical end caps. Above the left end cap is a box containing the text "How hungry do you feel?". Below the left end cap is the text "Not at all hungry". Below the right end cap is the text "As hungry as I have ever felt".

In analysis of the VAS data, the mid-point of the 100mm line (ie 50mm) was arbitrarily assigned the neutral line (Jenkins *et al.*, 1992). The value determined in this manner was then converted into a percentage of the extreme being measured, and subsequently analysed as such.

Plasma glucose and serum insulin analyses

Venous blood was drawn into prepared tubes and centrifuged at 5,000 rpm for 15 minutes for plasma and serum collection. These samples were stored at -70°C until assayed. The plasma samples were assayed for blood glucose levels using the GLUCOSE (Glu-cinet®) Technicon method ID-2G46-E94 (Sclavo Spa. Siena, Italy). The serum samples were assayed for insulin levels using the Coat-A-Count® method 91145 via the ¹²⁵I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The precise techniques and test principles are given in Appendix A.

Statistical methods

Changes in blood glucose and insulin over time, as well as changes in VAS ratings were statistically tested using the multiple analysis of variance (MANOVA) for repeated measures. The Scheffé Test was used as an *ad hoc* test for differences between means. The data on time taken to consume the *ad-libitum* meal, the weights of food consumed, and the amounts of energy consumed at that meal were compared using the paired t-test. Multiple regression (FIGURE 6-9) analyses were used to determine the correlation between VAS scores completed immediately before lunch and energy intake during lunch (VAS ratings and treatment were the independent variables). All statistical tests were conducted using the software package STATISTICA, developed by Statsoft, Tulsa, OK, USA. In the text, tables, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

When the energetically-overloaded pre-load was consumed as a SINGLE meal, the obese group consumed 56.2% more energy [$F_{1,20} = 11.45$, $P < 0.01$] at the test meal compared to the lean group ($5,426 \pm 1,126\text{kJ}$ vs $3,473 \pm 1,114\text{kJ}$, obese vs lean respectively). A similar test meal energy consumption was observed when the pre-load was consumed as 5 smaller meals: the obese group consumed 66.7% more energy [$F_{1,20} = 5.74$, $P < 0.01$] at the test meal compared to the lean group ($4,138 \pm 1,491\text{kJ}$ vs $2,482 \pm 748\text{kJ}$, obese vs lean respectively).

When the obese group consumed the isoenergetic pre-load as 5 smaller meals, they consumed 23.7% less energy at the test meal compared to the single pre-load treatment ($4,138 \pm 1,491$ vs $5,426 \pm 1,126\text{J}$ MULTI vs SINGLE respectively). When the lean group consumed the isoenergetic pre-load as 5 smaller meals, they consumed 28.5% less energy at the test meal compared to that consumed on the single pre-load treatment ($2,482 \pm 748$ vs $3,473 \pm 1,114\text{J}$ MULTI vs SINGLE respectively).

TABLE 6-3

Energy intake between lean and obese males consuming an energetically-overloaded pre-load either as a SINGLE or as a MULTI meal pattern

	SINGLE		MULTI	
	Lean	Obese	Lean	Obese
Cottage Pie (g)	471 ± 115 #	724 ± 208 **	287 ± 107	549 ± 242 **
Juice (ml)	373 ± 246	614 ± 481	402 ± 254	481 ± 138
Cottage Pie (kJ)	$2,694 \pm 659$ #	$4,143 \pm 1,179$ **	$1,640 \pm 613$	$3,134 \pm 1,384$ **
Juice (kJ)	779 ± 513	$1,283 \pm 281$	841 ± 530	$1,005 \pm 288$
Total energy (kJ)	$3,473 \pm 1,114$ #	$5,426 \pm 1,186$ **	$2,482 \pm 748$	$4,138 \pm 1,491$ **
Time of lunch (min)	14.5 ± 3.39	17.7 ± 4.23	9.07 ± 3.39	16.2 ± 5.88
Rate of consumption ($\text{kJ} \cdot \text{min}^{-1}$)	237 ± 44.2	313 ± 68.3	264 ± 67.7	255 ± 74.6

Where ** $P < 0.01$ between lean and obese groups within SINGLE or MULTI feeding treatments;

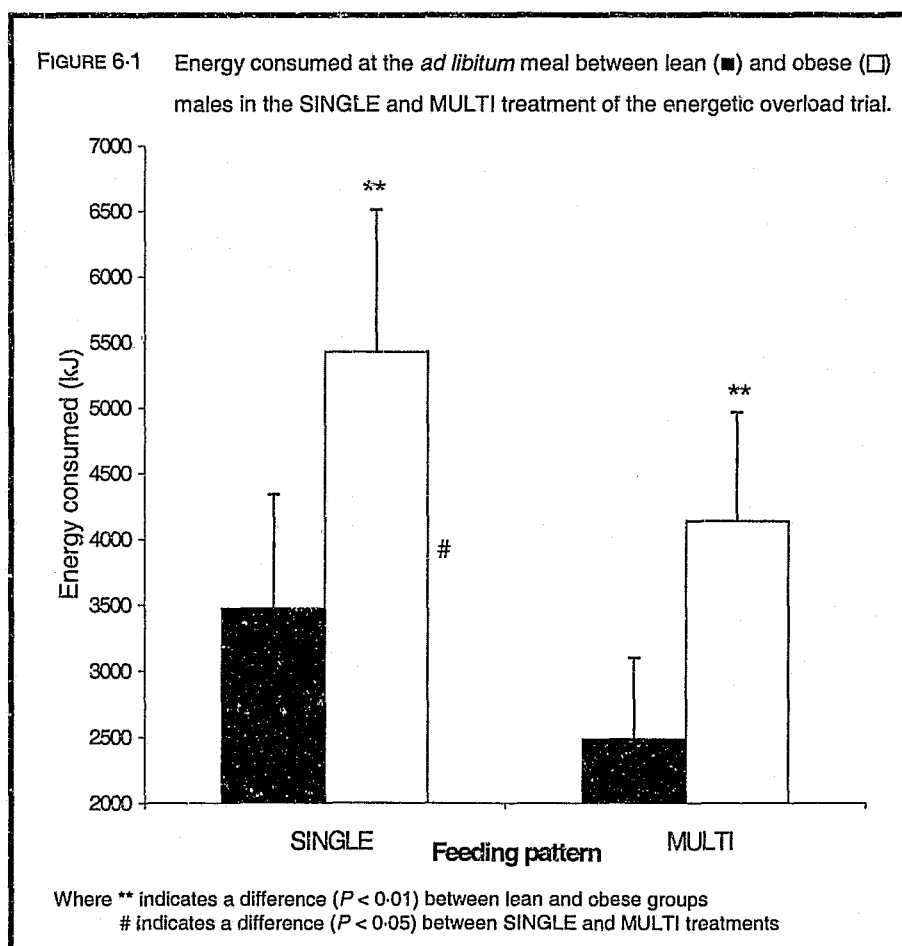
$P < 0.05$ between SINGLE or MULTI feeding treatments within lean and obese groups.

Energy consumption and feeding pattern

When the energetically-overloaded pre-load was consumed as a SINGLE meal, 34.4% more energy [$F_{1,20} = 5.74$; $P < 0.05$] was consumed at the test meal compared to that amount consumed when an isoenergetic pre-load was consumed over 5 smaller meals (4,450 vs 3,310kJ, SINGLE vs MULTI treatments respectively).

Energy consumption between groups.

The obese group consumed 60.6% more energy [$F_{1,20} = 14.4$, $P < 0.01$] than their lean counterparts at the *ad libitum* meal (4,783 vs 2,978kJ, for obese vs lean respectively) on both feeding treatments.



The pre-load period

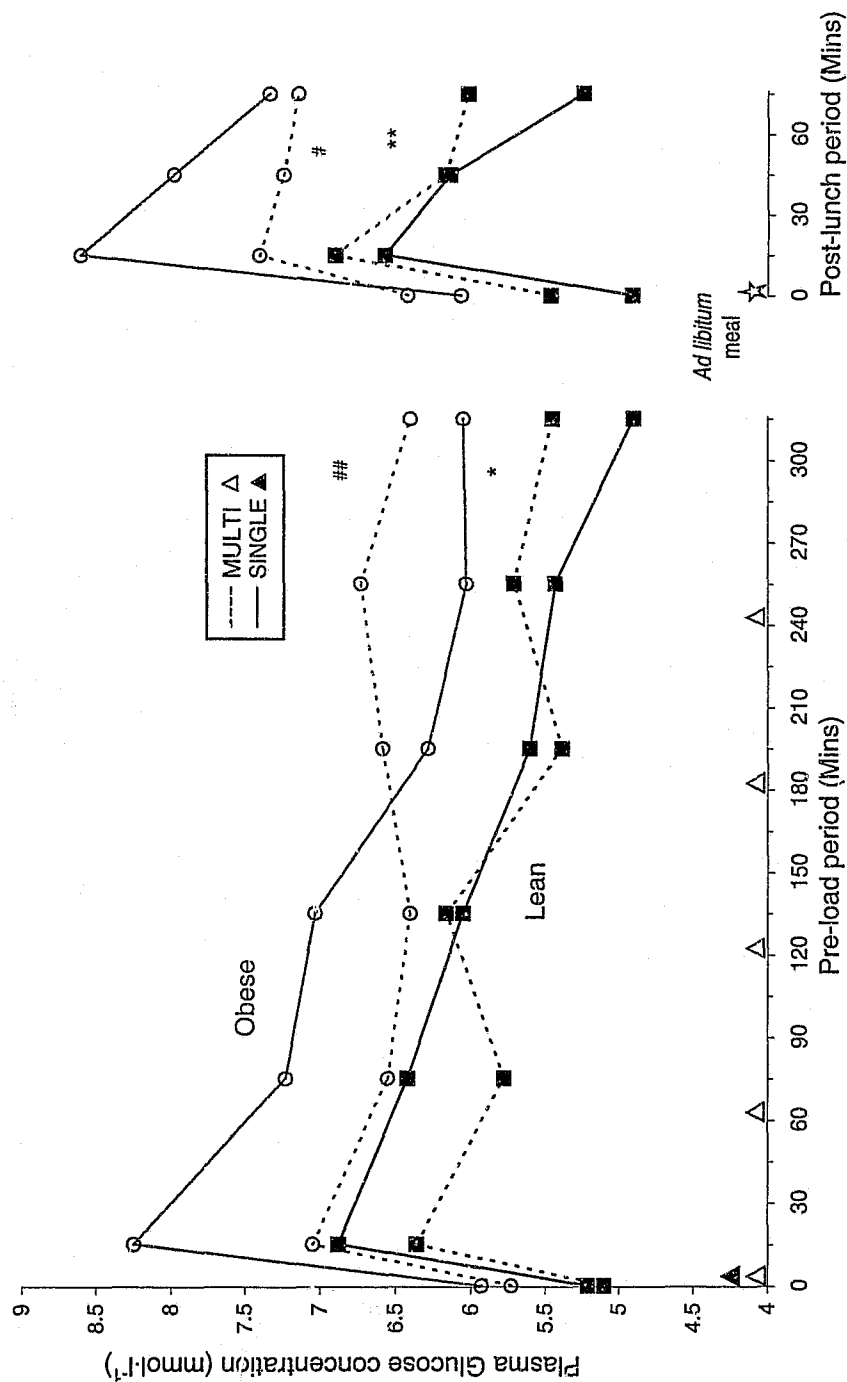
Plasma glucose responses

Fasting plasma glucose concentrations of both lean and obese subjects, on all trial days, were similar ($P > 0.05$) and within normal ranges (5.2 ± 0.6 and $5.1 \pm 0.3 \text{ mmol}\cdot\text{l}^{-1}$ in the lean group, and 5.9 ± 0.3 and $5.7 \pm 0.5 \text{ mmol}\cdot\text{l}^{-1}$ in the obese group). Overall, the obese group's blood glucose was higher [$F_{1,20} = 6.8$, $P < 0.05$] than the lean group's was. When the blood glucose responses over the course of the pre-load period were analysed, the SINGLE trial revealed a greater attenuation [$F_{6,120} = 3.27$, $P < 0.01$] in responses compared to those of the MULTI trial (FIGURE 6-2).

Serum insulin responses

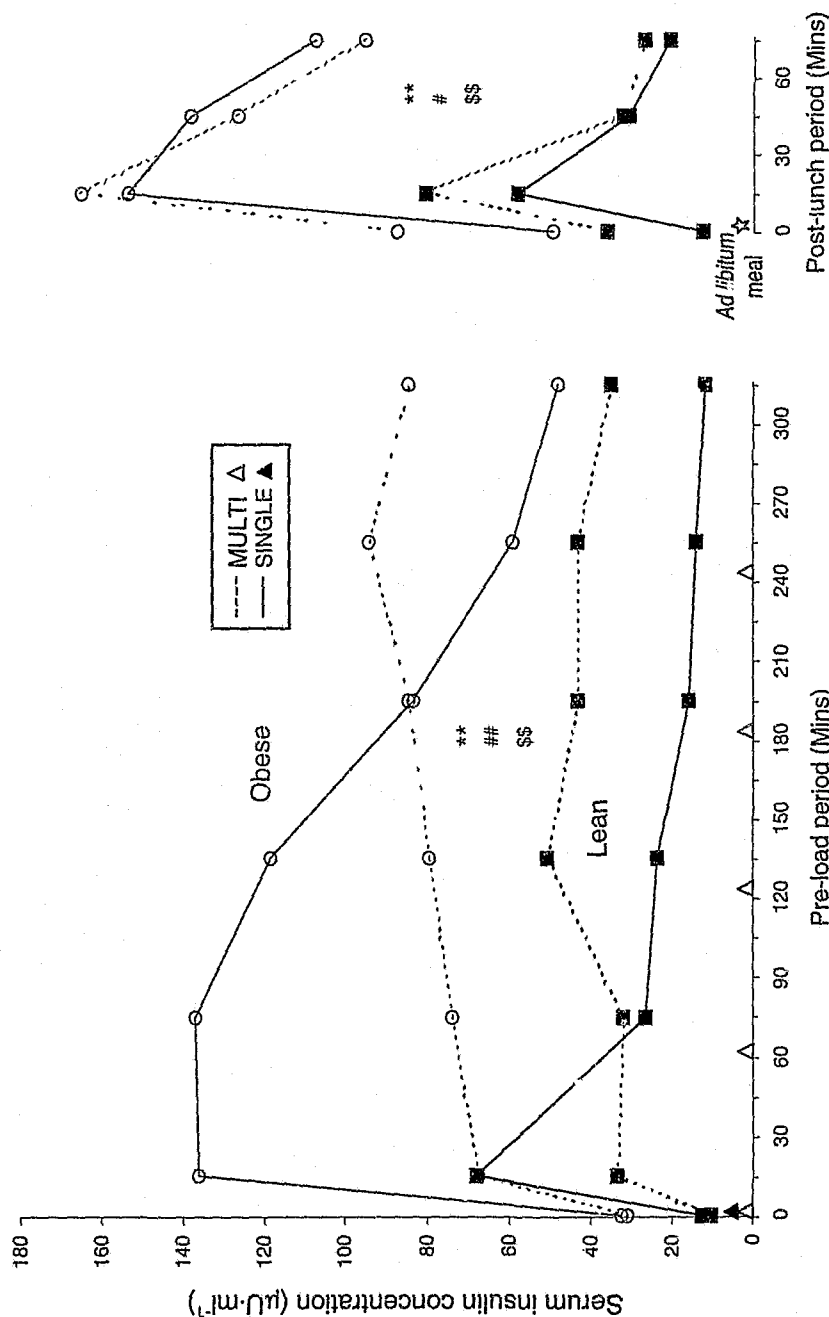
Regardless of pre-load meal frequency, the obese group's fasting serum insulin concentration was higher ($P < 0.01$) than that measured in the lean group ($34.9 \pm 12.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $33.3 \pm 16.5 \mu\text{U}\cdot\text{ml}^{-1}$ vs $9.9 \pm 2.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $9.3 \pm 3.0 \mu\text{U}\cdot\text{ml}^{-1}$ in obese vs lean respectively). Additionally, the obese group's insulin concentration was higher [$F_{1,20} = 45.0$, $P < 0.01$] than that measured in the lean group throughout the pre-load period. The two feeding treatments elicited different [$F_{6,120} = 30.6$, $P < 0.01$] insulin responses over the 315-minutes pre-load period; and further, on both treatments, the obese group's serum insulin responses were greater [$F_{6,120} = 9.7$, $P < 0.01$] than those serum insulin responses observed in the lean group (FIGURE 6-3).

FIGURE 6-2 Changes in plasma glucose concentrations between lean (■) and obese (○) males in the SINGLE (—) and MULTI (-----) treatments in the OVERFEEDING trial.



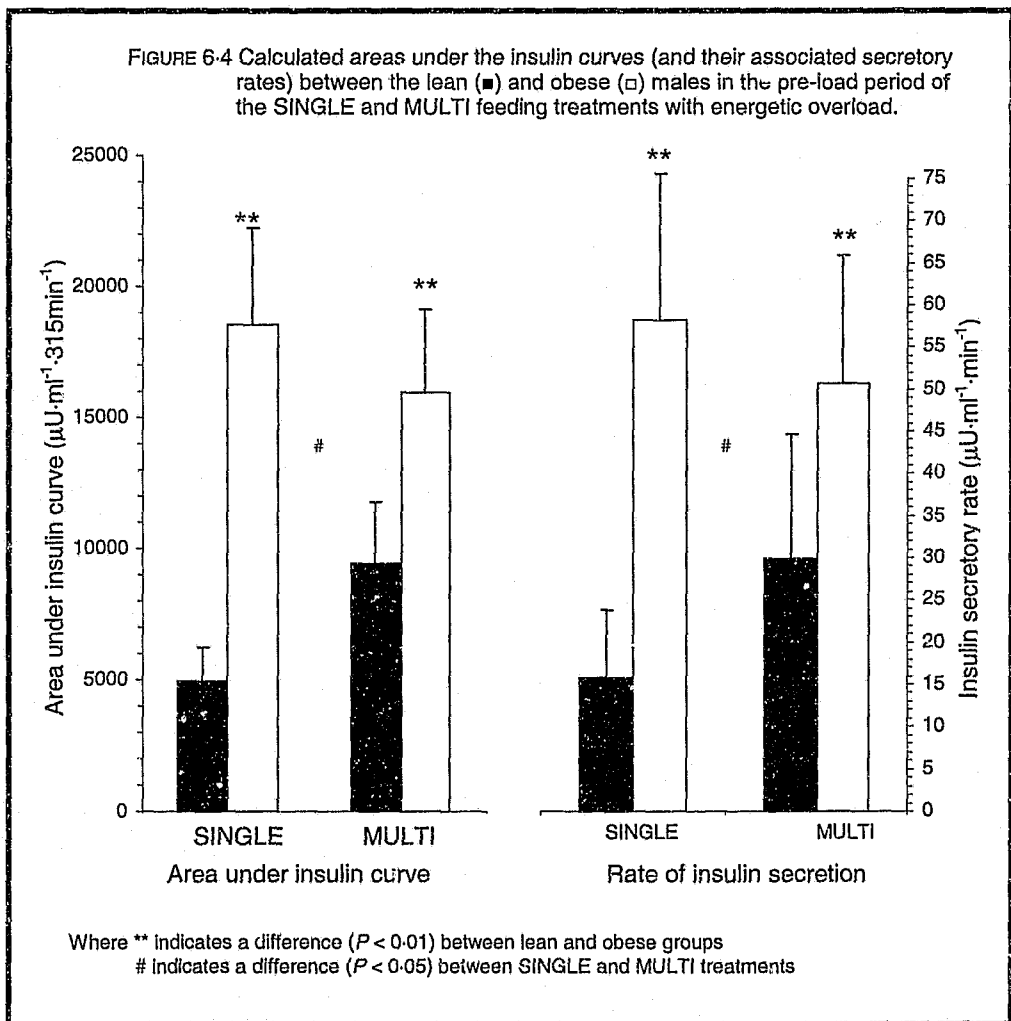
When: * indicates significant difference ($P < 0.05$, $P < 0.01$) between Lean and Obese groups
indicates significant difference ($P < 0.05$, $P < 0.01$) between feeding patterns

FIGURE 6-3 Changes in serum insulin concentrations between lean (■) and obese (○) males in the SINGLE (—) and MULTI (-----) treatments in the OVERFEEDING trial.



Where ** indicates significant difference ($P < 0.01$) between Lean and Obese groups
 # indicates significant difference ($P < 0.05$, $P < 0.01$) between feeding patterns
 \$, \$\$ indicates significant difference ($P < 0.05$, $P < 0.001$) between groups and feeding treatments as a function of time

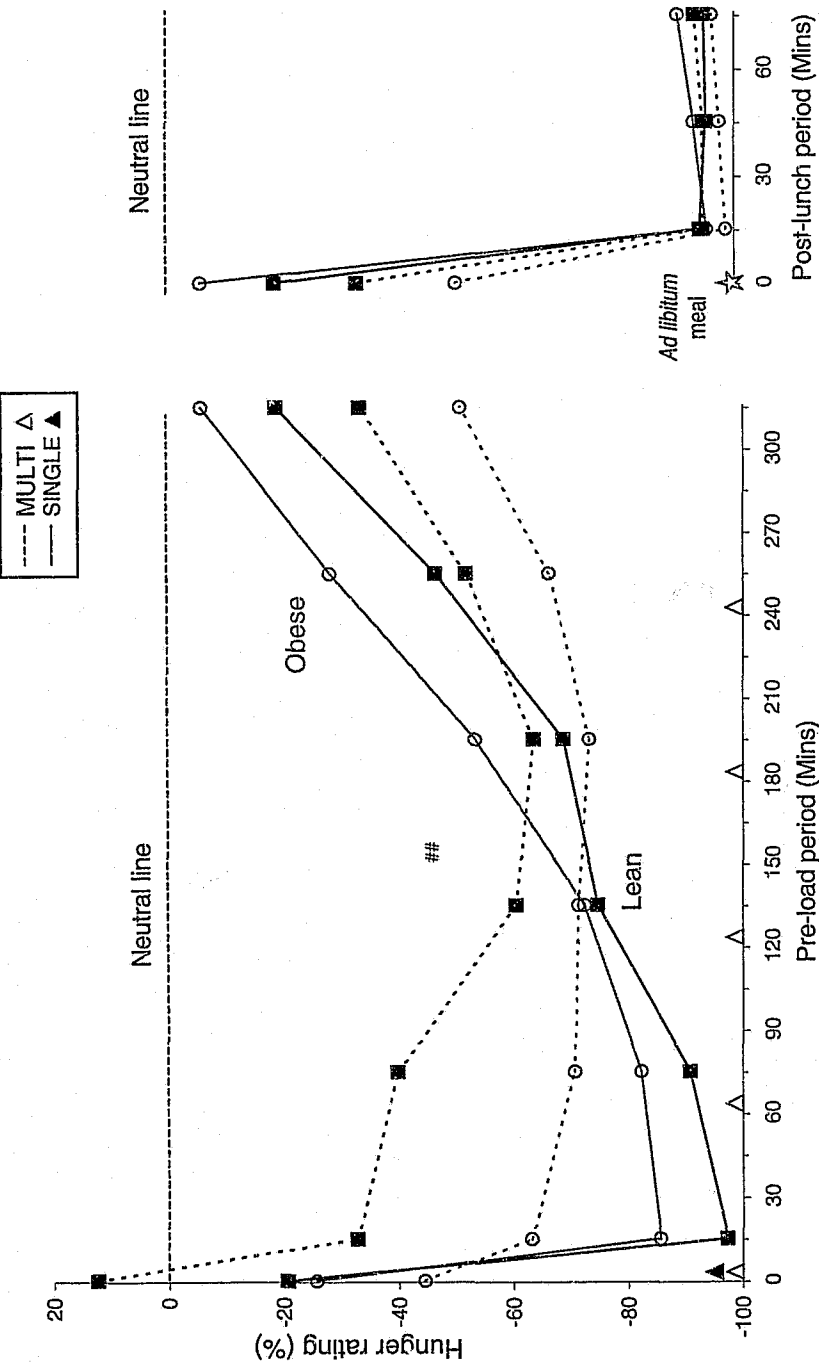
When the effect of meal frequency on insulin secretory rates (area under the insulin curves) was compared, more insulin was secreted over the 315-minute SINGLE pre-load treatment [$F_{1,20} = 7.39$, $P < 0.05$] compared to that secreted over the 315-minute MULTI pre-load treatment (FIGURE 6-4). Furthermore, when this parameter was compared between the lean and obese groups, it was found that the obese group secreted more insulin [$F_{1,20} = 42.6$, $P < 0.01$] for the same amount (and macronutrient value) of food intake over the 315-minute period than the lean group did.



Visual Analogue Scales for subjective satiety

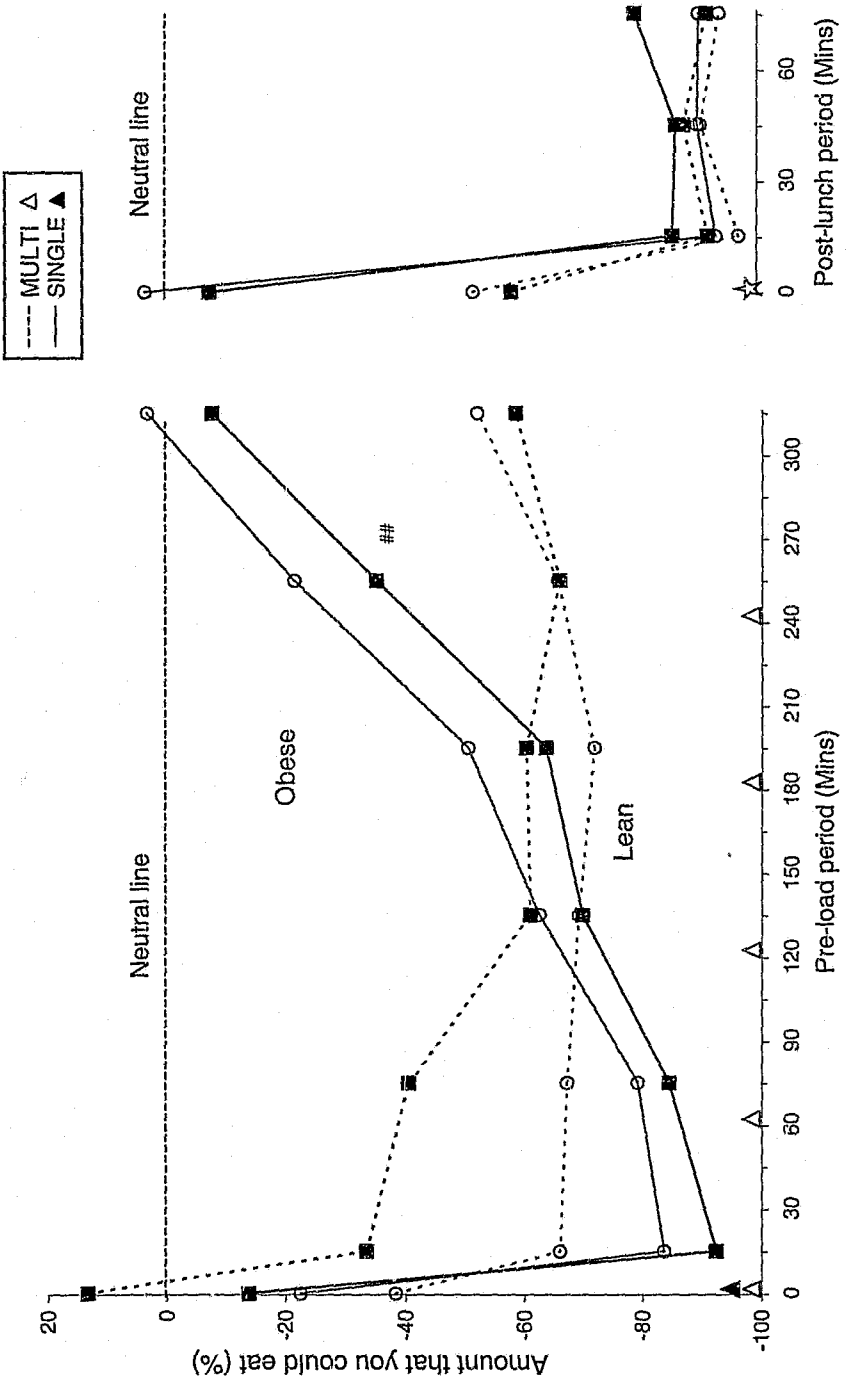
FIGURES 6-5, 6-6, and 6-7 depict the changes in VAS scores for the three sub-factors over the entire experimental period. In both the subjective *hunger ratings* (FIGURE 6-5) and the *amount each subject felt they could consume* (FIGURE 6-6) there were significant interactions between the lean and obese groups over time [$F_{6,120} = 2.8$, $P < 0.05$], and in all three subjective VAS ratings, there were differences between the patterns of eating over the pre-load period: the MULTI meal pattern eliciting a reduced response in subjective hunger scores.

FIGURE 6.5 Changes in subjective hunger ratings between lean (■) and obese (○) males in the SINGLE (——) and MULTI (-----) treatments in the OVERFEEDING trial.



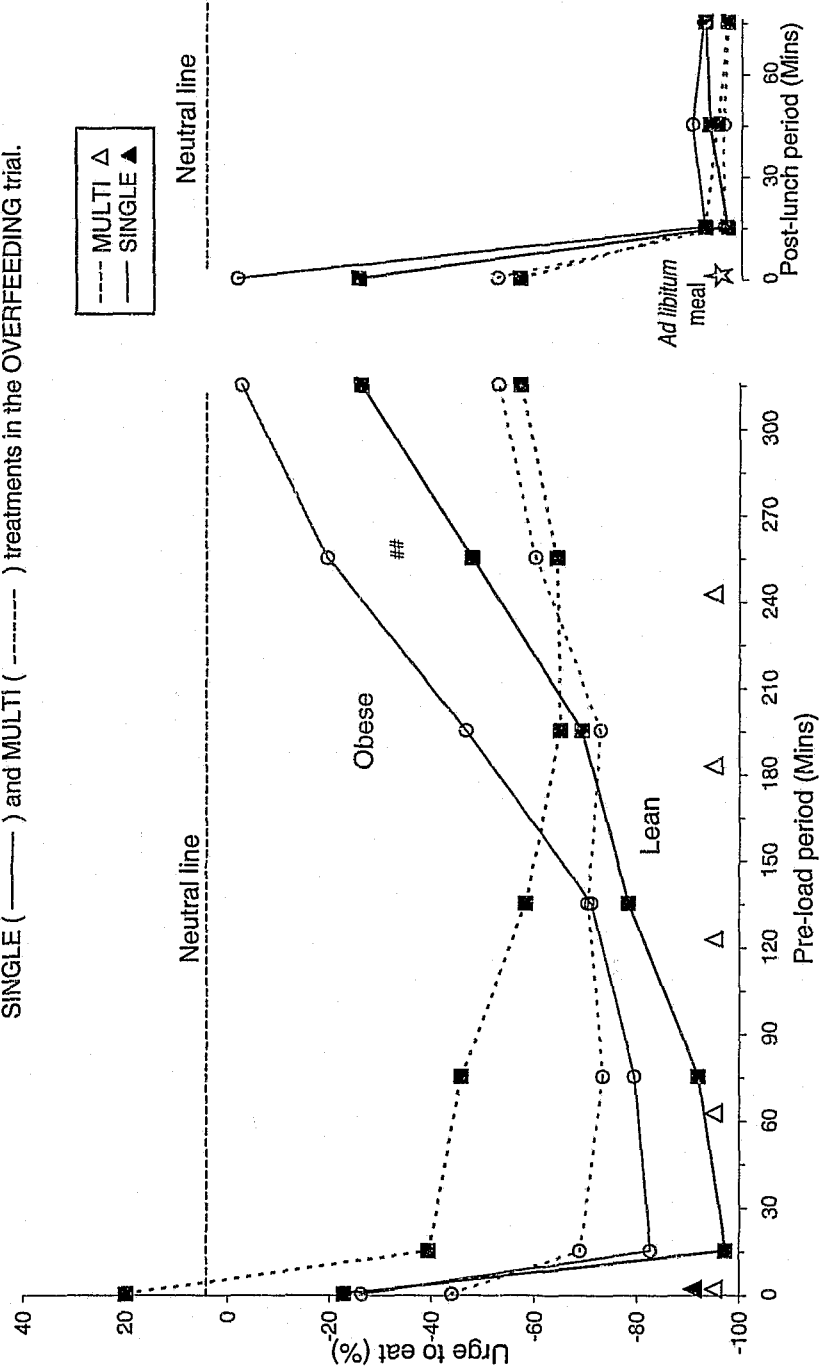
Where ## indicates significant difference ($P < 0.01$) between feeding patterns

FIGURE 6-6 Changes in the amount each subject perceived they could eat in lean (■) and obese (○) males in the SINGLE (——) and MULTI (-----) treatments in the OVERFEEDING trial.



Where ## indicates significant differences ($P < 0.01$) between feeding patterns

FIGURE 6.7 Changes in subjective urge to eat ratings between lean (■) and obese (○) males in the SINGLE (——) and MULTI (-----) treatments in the OVERFEEDING trial.



Where ## indicates significant difference ($P < 0.01$) between feeding patterns

The post lunch period

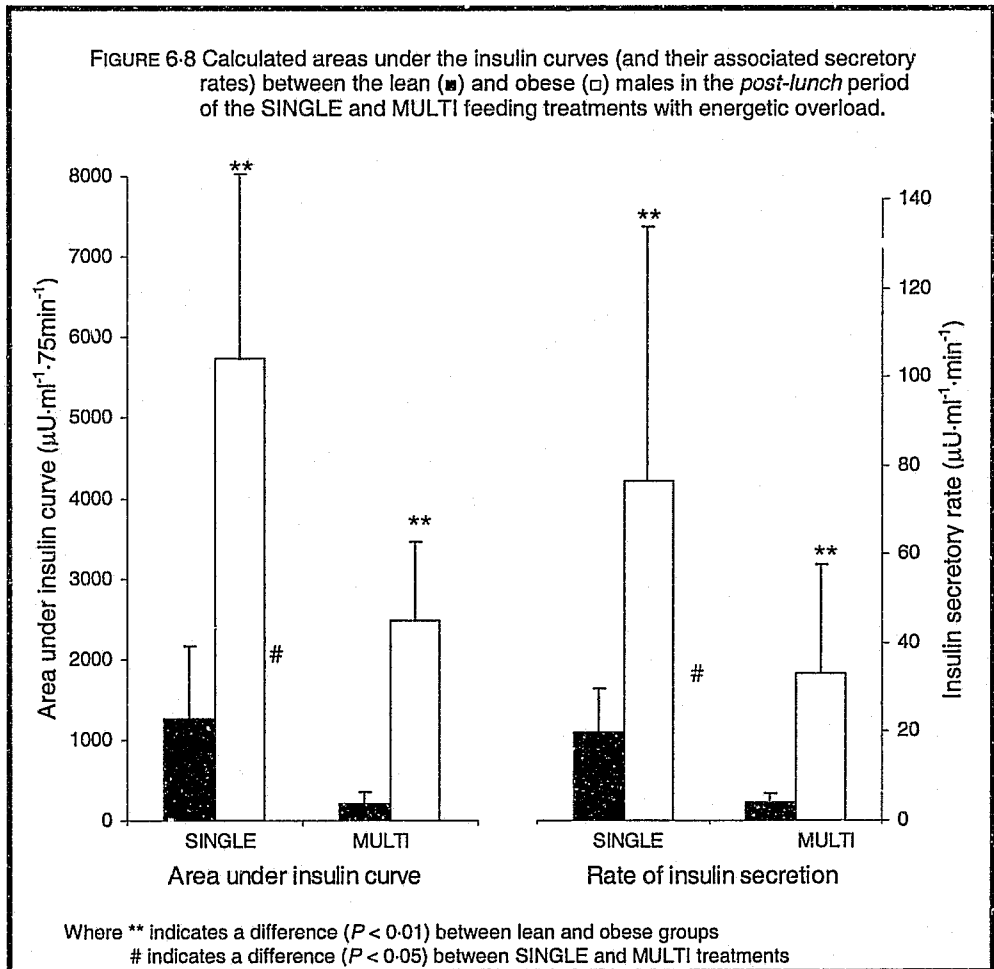
Plasma glucose responses

Immediately before lunch was served, plasma glucose concentrations were similar. However, consumption of the *ad libitum* test meal caused plasma glucose responses in the obese group to be higher [$F_{1,20} = 3.39$, $P < 0.01$] in the 75-minute post-lunch period compared to those responses observed in the lean group in the same period (FIGURE 6-2). Furthermore, the plasma glucose responses observed in both groups were different [$F_{3,60} = 3.09$, $P < 0.05$] as a result of the two pre-load feeding patterns in the 75-minute post-lunch period.

Serum insulin responses

The effect of consuming the test meal caused the insulin to rise higher [$F_{1,20} = 41.94$, $P < 0.01$] in the obese group compared to the lean group. Consequently, the insulin responses in the obese group in the 75-minute post-lunch period were *steeper*¹¹ [$F_{3,60} = 7.36$, $P < 0.01$] than those observed in the lean group (FIGURE 6-3). Finally, the insulin responses following the different patterns of eating in the 75-minute post-lunch period were significantly different [$F_{3,60} = 18.66$, $P < 0.01$].

Accordingly, the areas under the insulin curves in the post-lunch period were greater [$F_{1,20} = 18.66$, $P < 0.01$] in the obese responses (FIGURE 6-8) compared to the lean; in addition to the differences in the areas under the curves (and correspondingly insulin secretory rates) between the feeding patterns [$F_{1,20} = 7.67$, $P < 0.05$].

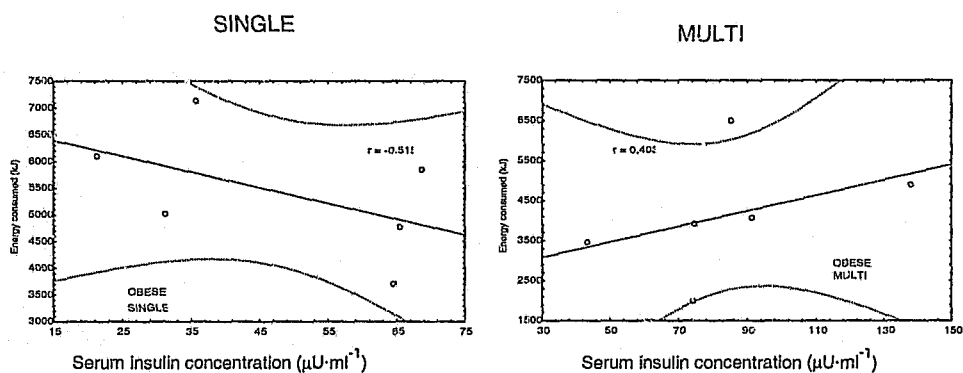


Visual Analogue Scales for subjective satiety

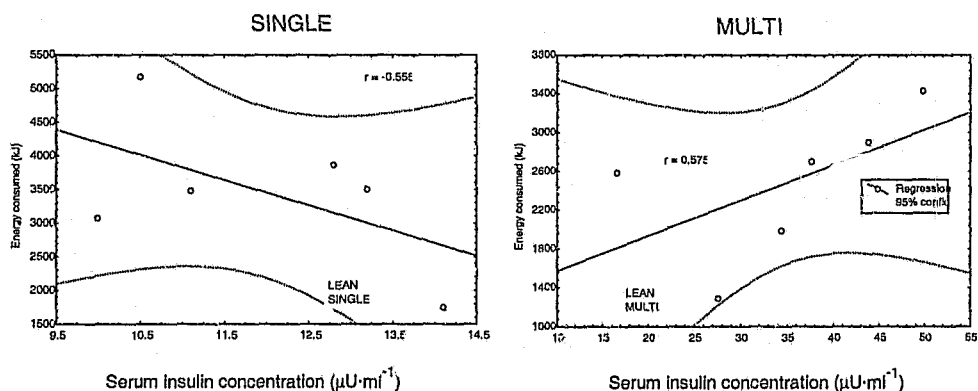
Even after the test meal, the VAS ratings differed as a function of the patterns in which the pre-load meal was consumed. FIGURE 6-9 depicts the scatterplots relating serum insulin concentrations immediately before lunch and the amount of energy consumed at the *ad libitum* lunch meal in the two different eating patterns in the lean and obese subjects. There were no significant relationships ($P > 0.05$), with the correlation values observed in the obese group were $r = -0.515$ and $r = 0.403$ in the SINGLE and MULTI respectively. The correlation values noted in the lean group were $r = -0.555$ and $r = 0.575$ in the SINGLE and MULTI respectively. Regardless of (a), whether the subjects were lean or obese; or (b), the frequency of eating the pre-load, there was no significant correlation between serum insulin concentration immediately before the test meal and the meal outcome under these testing conditions.

FIGURE 6.9 Scatterplots depicting the relationship between serum insulin concentration immediately before lunch and the amount of energy consumed at the *ad libitum* lunch in the two different eating plans on the OVERFEEDING pre-load trial.

OBESE SUBJECTS



LEAN SUBJECTS



DISCUSSION

The main finding in this experiment was that even when fed a high-fat, more palatable diet, increased frequency of smaller meals enhances appetite control in both lean and obese males. Increasing the frequency of eating a high-fat pre-load resulted in a substantial reduction in energy intake in both the obese (23.7%) and lean (28.5%) group, compared to when they consumed the same pre-load in a SINGLE sitting. The obese group consumed more ($P < 0.01$) energy than the lean group did, which is in line with all the other trials conducted in this study. Accordingly, the null hypotheses (that an increased frequency of feeding a high-fat preload had no effect on consequent energy intake in both lean and obese males), was rejected.

When the areas under the curves were compared, it was noted that the pattern in which the HF meal was consumed affected the amount of insulin secreted (FIGURE 6.4). Insulin secretion in the MULTI HF trial had a cumulative effect on the average rate of secretion over the 315-minute pre-load period. This was not seen in either of the lean, nor in the obese group at 33% ADER (Chapters 3, and 4). A plausible mechanism explaining this observation could be the reduced rate of gastric emptying that occurs when nutrients are high in fat. This would sustain stimulation of insulin secretion from the pancreatic β cells. The increased amount of insulin secreted in the MULTI treatment may have had a *positive effect* on satiety in both lean and obese males. This would give credence to the hypothesis that serum insulin exerts a positive effect on appetite-satiety mechanisms (Le Magnen, 1956; Woods, 1997).

Smaller, more frequent pre-load meals maintained subjective appetite ratings (in all three sub-factors) in a more stable manner compared to the greater ($P < 0.01$) fluctuations observed in the SINGLE treatment. The VAS responses of the obese group (*hunger ratings* (FIGURE 6.5) and *urge to eat* (FIGURE 6.7)) over the course of the pre-load period were different to that of the lean group ($P < 0.05$). These results suggest that the high-fat overloaded pre-load was less satisfying to the obese group compared to the lean group, given the same amount of food. As such both the high energy density and the low satiating effects of a high-fat diet contribute to the greater energy consumption by obese people (Kissileff *et al.*, 1984; Blundell & Macdiarmid, 1997). This is exacerbated by heightened palatability of high-fat foods (Rolls *et al.*, 1988; Drewnowski *et al.*, 1991; Drewnowski *et al.*, 1992). These individuals tend to prefer the tastes for those energy dense foods (Rolls *et al.*, 1988; Drewnowski *et al.*, 1992), and so the cycle will move into a self-potentiating and self-sustaining spiral to obesity.

These findings concur with previous reports that feeding high-fat diets to both laboratory rats and humans results overeating and weight gain (Friedman, 1998; Drewnowski *et al.*, 1991). Furthermore, a high-fat intake is linked to low lipid oxidation via reduced β oxidation, whereas high carbohydrate intake is linked to increased carbohydrate oxidation (Frayn & Kingman, 1995; Horton *et al.*, 1995; Stubbs, 1995; Stubbs *et al.*, 1995). This would further exacerbate the problem of weight gain on a high-fat diet.

If high-fat foods are consumed in a more frequent pattern, an element of control may come into the equation. This *periodicity of feeding* effect has been shown to exist in both lean (Speechly & Buffenstein, 1999) and obese males (Speechly *et al.*, 1999) when fed a prudent macronutrient diet. This study has shown that the frequency with which an obese individual consumes food (irrespective of its macronutrient value) the greater the probability that there will be enhanced control over appetite-satiety mechanisms. Moreover, the intake of dietary fat does not affect the rate of β -oxidation: the net result is the relative ease with which excessive energetic ingestion can occur in the absence of lipolysis, and net lipogenesis (and consequently obesity) follows.

Although I have found that eating smaller meals in a more frequent temporal pattern (nibbling) may exert short term benefits on metabolic and energy dynamics than eating fewer, larger meals, this study has been performed under highly controlled settings. The findings thereof may not be the case in the reality of free-living individuals, and this needs to be addressed in more long-term studies and under free-living conditions.

Visual Analogue Scales and Restraint

Throughout the pre-load period, the only differences that were noted in appetite ratings were a function of the varied meal frequency. Given the differences detected in the Dutch Restraint Questionnaire profiles between the lean and obese groups (TABLE 6.1), this inter-group similarity

was not the expected outcome (Westerterp-Plantenga & Ten Hoor, 1991; Federoff *et al.*, 1997). Dietary restraint is defined as the "the deliberate effort to combat the physiologically-based urge to eat in order to lose weight, or maintain a reduced weight, through caloric restriction" (Federoff *et al.*, 1997). Schachter (1971) proposed that obese individuals are relatively insensitive to internal physiological events. He further postulated that instead, obese behaviour is governed by external stimuli in the environment. Herman & Polivy (1975) extended this observation by proposing that dietary restraint, rather than body weight per se is the differentiating factor that contributes to the obese pattern of eating. Such a difference was not apparent in the current study, with both groups showing inter-treatment differences only.

CONCLUSION

In summary, this study showed that lean individuals show accurate compensation after pre-loads that are energetically overloaded (with fat). Although this compensation was not detected through the technique of visual analogue scales, the consequent intake of food was significantly less than that noted in the obese group. The obese group showed less precise energy compensation and appeared to have insensitivity to the satiety value of fat. Despite fat exerting a relatively lower satiating effect in the obese group compared to that observed in the lean group, eating the high-fat pre-load in a more frequent manner resulted in similar appetite control in both lean and obese males. In considering the strategies employed at reversing weight loss, the improved satiety achieved through the increased frequency of feeding, even with high-fat foods, should be investigated more thoroughly.

Chapter Seven

Enhanced appetite control in both lean and
obese males with increased frequency
of feeding on a restrictive diet.

INTRODUCTION

The treatment of obesity has turned into a lucrative multi-billion-dollar industry (Investors Chronicle, 2000), with many of the large pharmaceutical companies searching for the panacea to the obesity epidemic. Despite countless billions of dollars being poured into obesity research every year, success rates in obesity management have been remarkably poor (Hyman *et al.*, 1993; Glenney *et al.*, 1997; Carmichael, 1999; Miller, 1999). For the average person attempting weight-loss, the most common method of *self-treatment* is through energetic restriction (Miller, 1999). Accordingly, the obese individual embarks on a restrictive diet on a pre-determined day for which he/she has prepared psychologically. More often than not, such motivation follows with periods of excessive eating and feelings of physical and psychological discomfort (Smith *et al.*, 1999). Excessive hunger and reduced motivation with prolonged dietary restriction often lead to subsequent increased food intake and, in particular, the overconsumption of high-fat foods (Spitzer *et al.*, 1991; Marcus *et al.*, 1995; Costanzo *et al.*, 1999). Weight-loss success rates are so alarmingly poor, that the "dieting cycle" has no long-term effect on weight management in the obese.

The major obstacle to reversing obesity appears to hinge on a lack of understanding in the aetiology of the syndrome (Prentice & Jebb, 1996, Jebb 1999). However, there is increasing evidence that links the chronic state of positive energy balance with appetite dysfunction (Cooling & Blundell, 1997; Rolls *et al.*, 1992; Drewnowski *et al.*, 1991).

The chronic ingestion of low-fat foods may present a functional opportunity in the therapeutic management of obesity (Brehm *et al.*, 1999; Harvey, 1999), provided this diet does not negatively impact upon satiety and hunger. Previously, I have shown that when a prudent diet is administered as smaller, more frequent meals, appetite is enhanced and subsequent intake is lower in both lean (Speechly & Buffenstein, 1999) and obese males (Speechly *et al.*, 1999).

Based on the two premises that a) spreading an isoenergetic load enhances appetite control, and b) low-fat diets minimise *de novo* lipogenesis (and are associated with the reduced prevalence of obesity), I questioned whether the increased frequency in which a hypoenergetic low-fat meal is consumed (as a pre-load) would enhance appetite control in obese males, and reduce their energy intake at a consequential *ad libitum* test meal.

MATERIALS AND METHODS

Subjects

The same twelve (six lean men with BMI < 23 kg·m⁻², and six obese men with BMI > 32 kg·m⁻²) healthy, non-diabetic obese men that were used previously (in chapter five; Speechly and Buffenstein, 1999) were also used in this experiment. The details of the subject characteristics are contained in Chapters 5 and 6, but are tabulated below for convenience.

TABLE 7-1.

Characteristics of study participants.

	Lean	Obese	Significance
N	6	6	
Age (years)	26.67 ± 5.47	39.83 ± 19.03	NS
Weight (kg)	77.13 ± 5.60	125.88 ± 43.82	* t = 2.76
Height (m)	1.85 ± 0.61	1.79 ± 0.075	NS
BMI (kg·m ⁻²)	22.50 ± 1.08	39.05 ± 11.63	** t = 3.45
WHR	1.01 ± 0.04	1.17 ± 0.09	** t = 3.91
ADER (kJ)	12,098 ± 1,103	12,225 ± 3,543	NS
20% ADER (kJ)	2,419 ± 220	2,445 ± 709	NS
Relative energy reqs (kJ·kg ⁻¹)	156.8 ± 6.38	98.1 ± 7.66	** t = 14.4
DRQ			
External	2.65 ± 0.88	3.18 ± 1.05	** t = 3.08
Emotional	1.62 ± 0.77	2.65 ± 1.53	** t = 5.29
Restraint	2.30 ± 1.29	3.08 ± 0.96	** t = 3.77

Where BMI = body mass index; WHR = waist:hip ratio
and DRQ = Dutch Restrained Questionnaire.

And * $P < 0.05$, and ** $P < 0.01$ between lean and obese groups

It must be pointed out that the SINGLE treatment data contained in this experiment have been presented in Chapter 5, and are used in this chapter to highlight the effects that increasing frequency of feeding may have on appetite – satiety mechanisms in obese males.

Protocol

In this study, I investigated the effects of meal frequency on appetite control and serum insulin concentration when the pre-load meal was energetically restricted (20% ADER) and low in fat. The effect of meal frequency on appetite, perception of hunger, glucose and insulin concentrations was assessed by randomly assigning subjects, on their first visit, to one of two feeding regimes. Each subject took each eating pattern in random order with 50% of the study cohort taking the *nibbling* diet first. 20% of their average daily energy requirement (ADER) (as determined by the Harris-Benedict equation (Van Way, 1992)) was either given as a single meal (SINGLE) or divided into five equal portions given at hourly intervals (MULTI).

Testing Considerations

All subjects reported to the Sleep and Metabolic Laboratory at the University of the Witwatersrand Medical School, Johannesburg, at 07h00 in the morning. The laboratory is at an altitude of 1,800m (625 torr) and regulated at a room temperature of 20-22°C. The subjects were monitored throughout the testing period, and they were not allowed to leave the laboratory for any reason. Subjects were asked to refrain from eating and drinking anything other than that prescribed in the testing protocol. The first pre-load meal was given at ~07h30. Irrespective of whether a single meal or one of the multi-meals was given, the men were subjected to identical treatments and identical nutrient intake (TABLE 7-2): visual

analogue scales (VAS) and blood measurements were collected hourly for 5 hours. After five-and-a-half hours (~13h00), subjects were given an *ad libitum* lunch (of pre-prepared cottage pie and orange juice, TABLE 7.2). This was eaten in isolation. Subjects were told to eat as much as they wanted and for as long as they desired. They were unaware that the quantity of food ingested was being monitored in any way. The amount of food (and energy) consumed at the *ad libitum* lunch was measured by weighing the cottage pie (in its container) and the orange juice carton, immediately before presenting it to the subjects, and then re-weighing these after the completion of the test lunch meal. Although baked low-fat chicken & broccoli, "Slimmer's" ice-cream and diabetic chocolate are not a traditional breakfast in a South African context, it was decided to use this food combination as the pre-load meal as it allowed for a convenient covert manipulation of macronutrient sources in the pre-load.

TABLE 7.2.
The nutritional information of foods (per 100g) served in the underfeeding pre-load and test meal.

	Carbohydrate (grams)	Protein (grams)	Fat (grams)	Energy (kJ)
Underfeeding trial				
Chicken & broccoli	5.2	9.0	2.5	327
Orange juice	12.1	0.3	0.1	209
Ice-cream (Slim)	16.4	4.5	2.5	431
Chocolate snack	6.2	6.1	0.4	204
Test meal				
Orange juice	12.1	0.3	0.1	209
Cottage Pie	5.1	9.2	8.8	572

Timing of the time taken to consume the *ad libitum* lunch meal began immediately as the meal was presented to the subject. Subjects were instructed to start eating when they were presented with the food, and then to leave the table upon completion. Accordingly, each subject was monitored covertly throughout the eating process.

Food considerations

Every effort was made to ensure that the food presented to all subjects on all feeding treatments was identical (relative to each specific subject) in relative weight and energetic value, texture, temperature, and appearance. Although the temperature of the food was not measured, the preparation in all foods was sufficiently identical (storage, and cooking time) to satisfy us that the foods were similar when they were presented to the subjects.

The *ad libitum* meal (cottage pie) was considerably different in macronutrient value and energetic value to the pre-load meal(s) (Chicken & Broccoli bake) (TABLE 7.2) to ensure that the results obtained in this study were not the affects of sensory specific-satiety rather than the frequency of feeding.

Body mass (accurate to 0.05kg, Mettler TE/J, Zurich, Switzerland) and height (accurate to 0.1cm, SECA, Germany) were measured at the start of the experiment.

Scores for satiety

Subjective satiety and hunger were assessed by three VAS scales that accounted for the degree of changes in the following factors: hunger; thoughts of the amount of food that could be eaten; and the urge to eat (Hill *et al.*, 1984). The VAS scales were all 100mm in length, which were

anchored at either extremity with terms indicating extremes as depicted below.

An example of the visual analogue scale (VAS) used to assess hunger.

The diagram shows a horizontal line representing a visual analogue scale. Above the left end of the line is a box containing the text "How hungry do you feel?". Below the left end of the line is the text "Not at all hungry". Below the right end of the line is the text "As hungry as I have ever felt".

In analysis of the VAS data, the mid-point of the 100mm line (ie 50mm) was arbitrarily assigned the neutral line (Jenkins *et al.*, 1992). The value determined in this manner was then converted into a percentage of the extreme being measured, and subsequently analysed as such.

Plasma glucose and serum insulin analyses

Venous blood was drawn into prepared tubes and centrifuged at 5,000 rpm for 15 minutes for plasma and serum collection. These samples were stored at -70°C until assayed. The plasma samples were assayed for blood glucose levels using the GLUCOSE (Glu-cinet®) Technicon method ID-2G46-E94 (Sclavo SpA, Siena, Italy). The serum samples were assayed for insulin levels using the Coat-A-Count® method 91145 via the

¹²⁵I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The precise techniques and test principles are given in Appendix A.

Statistical methods

Changes in blood glucose and insulin over time, and changes in VAS ratings were statistically tested using the multiple analysis of variance (MANOVA) for repeated measures, and the Scheffé Test was used as an *ad hoc* test for differences between means. The data on time taken to consume the *ad-libitum* meal, the weights of food consumed, and the amounts of energy consumed at that meal were compared using the paired *t*-test. Multiple regression (FIGURE 7-9) analyses were used to determine the correlation between VAS scores completed immediately before lunch and energy intake during lunch (VAS ratings and treatment were the independent variables). All statistical tests were conducted using the software package STATISTICA, developed by Statsoft, Tulsa, OK, USA. In the text, tables, and graphs, data are presented as means $\pm$ standard deviation (sd) of the mean. Data were considered statistically significant at $P < 0.05$.

RESULTS

When the obese group consumed the isoenergetic pre-load as 5 smaller meals, they consumed 17.0% less energy [$F_{1,20} = 5.74$, $P < 0.05$] at the test meal compared to the single pre-load treatment ($4,736 \pm 1,308\text{kJ}$ vs $5,709 \pm 923\text{kJ}$ MULTI vs SINGLE respectively). When the lean group consumed the isoenergetic pre-load as 5 smaller meals, they consumed 11.7% less energy at the test meal compared to that consumed on the single pre-load treatment ($4,067 \pm 1,243\text{kJ}$ vs $4,604 \pm 1,183\text{kJ}$ MULTI vs SINGLE respectively).

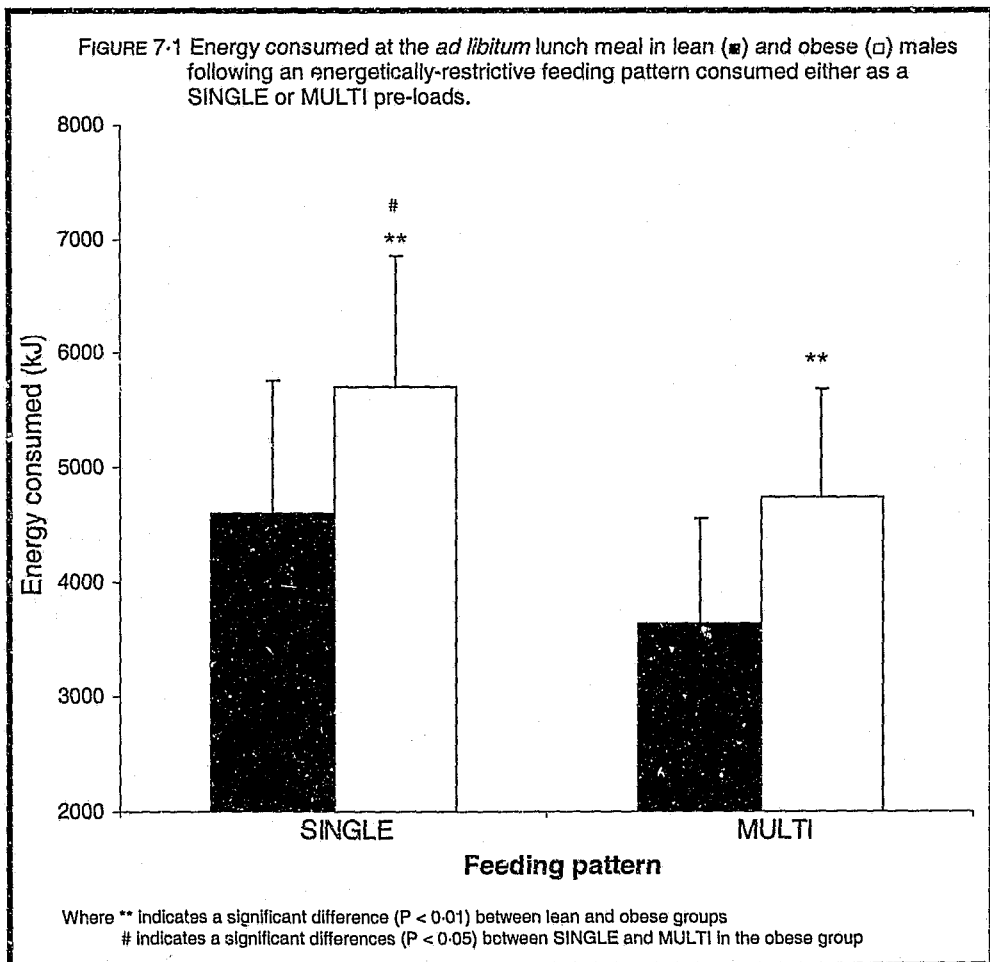


Table 7-3
Energy intake between lean and obese males consuming an energetically-restricted pre-load either as a SINGLE or as MULTI meals

	SINGLE		MULTI	
	Lean	Obese	Lean	Obese
Cottage Pie (g)	624 ± 177	# 757 ± 192 *	519 ± 206	638 ± 202 *
Juice (ml)	495 ± 249	661 ± 244	525 ± 228	518 ± 140
Cottage Pie (kJ)	3,569 ± 1,011	# 4,328 ± 1,238 *	2,968 ± 1,176	3,654 ± 1,153 *
Juice (kJ)	1,035 ± 520	1,381 ± 510	1,098 ± 476	1,084 ± 294
Total energy (kJ)	4,604 ± 1,183	# 5,709 ± 923 **	4,067 ± 1,243	4,736 ± 1,308 **
Time of lunch (min)	16.5 ± 6.1	19.7 ± 5.5	12.7 ± 3.1	18.3 ± 6.3
Rate of consumption (kJ·min ⁻¹)	299 ± 95.6	306 ± 81.1	299 ± 95.6	270 ± 62.0

Where *, ** $P < 0.05$ and $P < 0.01$ between lean and obese groups within SINGLE or MULTI feeding treatments;

$P < 0.05$ between SINGLE or MULTI feeding treatments within lean and obese groups;

Table 7-4
Relative comparison highlighting the effects of increasing feeding frequency between lean and obese males on energy intake at an ad libitum meal

		SINGLE		MULTI		DIFFERENCE
Trial		Lean	Obese	Lean	Obese	
33% ADER	Pre-load	3,404	4,100	3,404	4,100	
	ad lib	5,111	5,261	3,753	3,763	$S_L = S_O \quad P > 0.05$
			S_O/S_L		M_O/M_L	$M_L = M_O \quad P > 0.05$
			2.90%		- 2.66%	
	Total	8,515	9,361	7,157	7,863	
	Total ADER	10,212	12,300	10,212	12,300	
20% ADER	Pre-load	2,419	2,445	2,419	2,445	
	ad lib	4,604	5,709	4,067	4,736	$S_O \quad 24.0\% > L_O \quad P < 0.01$
			S_O/S_L		M_O/M_L	$M_O \quad 16.4\% > M_L \quad P < 0.05$
			24.0%		16.44%	
	Total	7,023	8,154	6,486	7,181	
	Total ADER	12,098	12,225	12,098	12,225	
	% consume	58.35	66.70	53.61	58.74	
	Degree of Compensation (%)	-30.38%	-12.35%	-23.50%	-8.10%	
Difference			S_O/S_L		M_O/M_L	$S_O \quad 59.3\% > L_O \quad P < 0.01$
			59.3%		65.5%	$M_O \quad 65.5\% > M_L \quad P < 0.01$

S_L is SINGLE (treatment) Lean (group)

S_O is SINGLE (treatment) Obese (group)

M_L is MULTI (treatment) Lean (group)

M_O is MULTI (treatment) Obese (group)

% consume is the proportion of ADER that was consumed in total (Pre-load + ad libitum lunch)

Degree of compensation = %consume [50% ADER - 20% ADER] - 20% ADER

When the energetically-restricted pre-load was consumed as a SINGLE meal, the obese group consumed 24% more energy [$F_{1,20} = 14.39$, $P < 0.01$] at the test meal compared to the lean group ($5,709 \pm 923\text{kJ}$ vs $4,604 \pm 1,183\text{kJ}$, obese vs lean respectively). Furthermore, the obese group consumed 16.4% more energy than the lean group at the test meal when the energetically-restricted pre-load was consumed as 5 smaller meals (MULTI treatment) (TABLE 7-3).

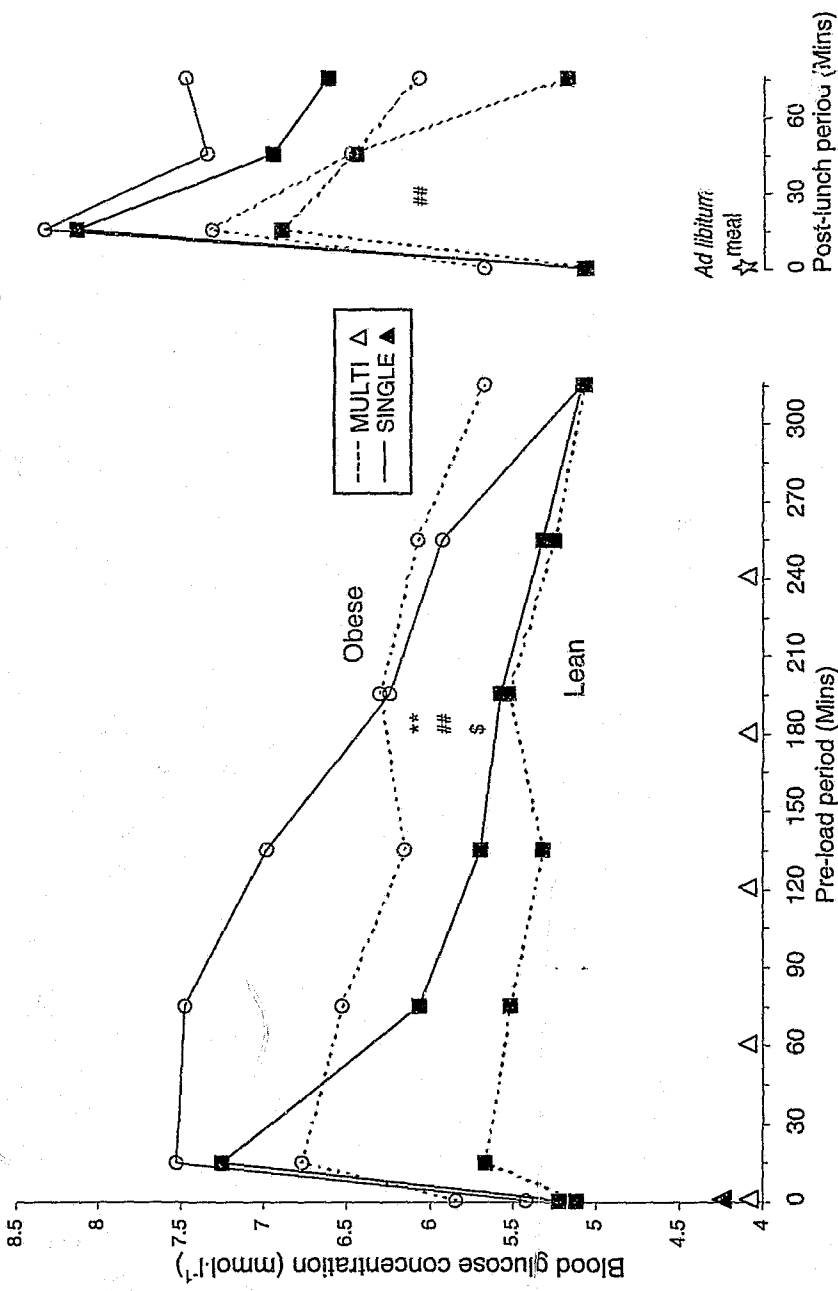
TABLE 7-4 above shows that when confronted with an energy restriction in the pre-load, neither group compensated at the ad libitum meal to make up the energy that was ingested at a prudent (33% ADER) described previously. In these terms, the obese group showed that their intake was ~60% that of the lean group in these trials.

The pre-load period

Plasma glucose responses

Although the plasma glucose responses on the MULTI treatment were lower [$F_{6,120} = 6.8$, $P < 0.01$] than those elicited on the SINGLE meal treatment over the course of the 315-minute pre-load period (FIGURE 7-2), there were no specific differences ($P > 0.05$) in glucose responses between feeding frequency within the specific groups (lean and obese).

FIGURE 7-2 Changes in plasma glucose concentrations between lean (■) and obese (○) males in the SINGLE (——) or MULTI (-----) treatments.



Where ** Indicates significant difference ($P < 0.01$) between Lean and Obese groups
Indicates significant difference ($P < 0.01$) between feeding patterns
\$ Indicates significant difference ($P < 0.05$) between groups and feeding treatments as a function of time

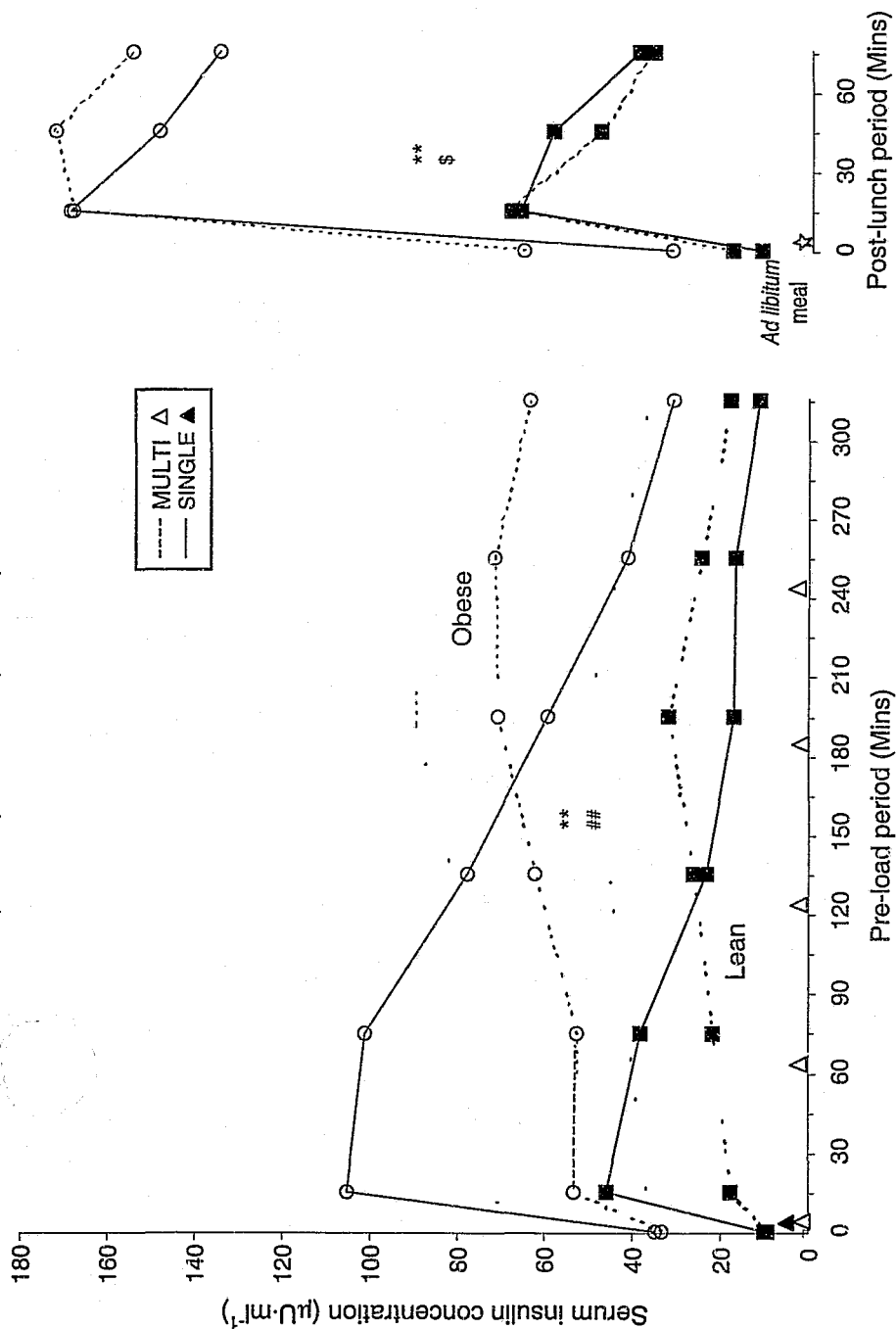
Serum insulin responses

Fasting serum insulin concentration of the obese group on both experimental days was more ($P < 0.01$) than 3x that of the lean group (FIGURE 7-3; Obese: $34.9 \pm 12.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $33.3 \pm 16.5 \mu\text{U}\cdot\text{ml}^{-1}$, SINGLE and MULTI respectively; vs Lean: $9.9 \pm 2.9 \mu\text{U}\cdot\text{ml}^{-1}$ and $9.3 \pm 3.0 \mu\text{U}\cdot\text{ml}^{-1}$, SINGLE and MULTI respectively).

The obese group's response in insulin concentration was higher [$F_{1,20} = 18.5$, $P < 0.01$] throughout the pre-load period on both SINGLE and MULTI treatments compared to the insulin responses elicited by the lean group on both treatments.

The two feeding patterns elicited different [$F_{6,120} = 10.5$, $P < 0.01$] insulin responses over the 315-minutes pre-load period, and FIGURE 7-3 depicts the different insulin dynamics invoked by the two feeding treatments. In the SINGLE treatment, the insulin concentration rose sharply to its zenith at 15 minutes after completion of the pre-load meal ($105.3 \pm 25.7 \mu\text{U}\cdot\text{ml}^{-1}$ in the obese, and $46.0 \pm 8.3 \mu\text{U}\cdot\text{ml}^{-1}$ in the lean) and then slowly declined back to baseline values throughout the 315-minutes pre-load period. In contrast, the MULTI treatment resulted in attenuation in insulin levels in both lean and obese groups, where insulin concentrations reached their zenith ~190 minutes (or after the fourth smaller pre-load meal) after the first pre-load meal.

FIGURE 7.3 Changes in serum insulin concentrations between lean (■) and obese (○) males in the SINGLE (——) and MULTI (-----) treatments in the UNDERFEEDING trial.



Where ** indicates significant difference ($P < 0.01$) between Lean and Obese groups
indicates significant difference ($P < 0.01$) between feeding patterns
\$ indicates significant difference ($P < 0.05$) between groups and feeding treatments as a function of time

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