INVESTIGATION OF THE PHYSIOLOGICAL MECHANISMS REGULATING APPETITE AND FOOD INTAKE IN HUMANS

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Summary

It is generally accepted that human appetite is under multifactoral control, and that investigation of each facet implicated in appetite regulation is required to develop understanding in this area. The current research aimed to investigate subjective appetite ratings and *ad libitum* food intake in response to manipulated preloads, while simultaneously measuring the hormone and metabolite response and gastric emptying rate of those preloads. This tripartite approach was then used to explore the relationship between the gastrointestinal hormones GLP-1 and CCK, and appetite. Manipulated preloads differing in their carbohydrate and fat content were used to investigate the post-prandial response. Increasing the carbohydrate and energy content of a preload was shown to decrease *ad libitum* food intake, although there were no obvious effects on subjective appetite ratings. In addition the secretion of GLP-1, GIP, insulin and glucose were significantly increased as preload carbohydrate and energy content increased. The gastric emptying of the preload was slower as carbohydrate and energy content increased. The manipulation of preload fat and energy content showed a similar pattern of response, with decreased *ad libitum* energy intake and delayed gastric emptying as preload fat and energy content increased. Hormone and metabolite responses showed significant increases in CCK and GLP-1 with increasing preload fat and energy content.

To investigate further the role of GLP-1 in the regulation of human appetite, appetite responses during a GLP-1 infusion were assessed against a saline control. The infusion of GLP-1 did not have a significant effect on self rated appetite or *ad libitum* energy intake, and was not supportive of a major role for GLP-1 as a satiety hormone in man. However GLP-1 infusion significantly delayed gastric emptying of a water load, supporting the role of GLP-1 as a regulator of gastric emptying.

The role of CCK in human appetite was similarly investigated, using an infusion of the CCKA receptor antagonist loxiglumide against a saline control. Loxiglumide infusion significantly increased *ad libitum* energy intake, and significantly increased the rate of gastric emptying of a high energy liquid preload. The infusion was also found to significantly increase GLP-1 and insulin responses, and the increased *ad libitum* energy intake was observed in spite of high GLP-1 levels with loxiglumide infusion.
The *ad libitum* test meal intake data from the manipulated preload studies showed that a small number of subjects did not adjust their intake according to preload energy intake. Thus the role of habitual exercise levels on food intake response was assessed to determine if this poor response was related to lifestyle differences. Individuals who participated in regular recreational exercise were found to better adjust *ad libitum* energy intake in response to manipulated preloads than those who undertook no regular recreational exercise. These data provide support a role for exercise in the regulation of food intake.

Taken together these data provide evidence that the differing hormonal responses to manipulated preloads are involved in the regulation of appetite response. Investigations of the roles of GLP-1 and CCK in appetite suggest that CCK is a more potent regulator of appetite than GLP-1. The role of exercise in determining the appetite response to manipulated preloads suggests that the investigation of lifestyle differences is also of importance in the understanding of human appetite.
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<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CPM</td>
<td>Counts per minute</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>DEBQ</td>
<td>Dutch eating behaviour questionnaire</td>
</tr>
<tr>
<td>EIE</td>
<td>Epigastric impedance epigastrography</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
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<tr>
<td>GE</td>
<td>Gastric emptying</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose-dependant insulinotropic polypeptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1 (7-36) amide</td>
</tr>
<tr>
<td>GRP</td>
<td>Gastrin releasing peptide</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>ICV</td>
<td>Intracerebroventricular</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>kJ</td>
<td>Kilojoule</td>
</tr>
<tr>
<td>LH</td>
<td>Lateral Hypothalamus</td>
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<tr>
<td>MJ</td>
<td>Megajoule</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non esterified fatty acids</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
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<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
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<tr>
<td>PZ</td>
<td>Pancreozymin</td>
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<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>T50</td>
<td>Gastric half emptying time</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue rating scales</td>
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<tr>
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<td>Ventromedial hypothalamus</td>
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Chapter One
1. Introduction

1.1. Background

1.1.1. Hunger, satiety and appetite

Human appetite is a complex mixture of physiological and psychological phenomena including the feelings of hunger and satiety, and is influenced by the motivational behaviour of an individual (Blundell et al., 1995b). Hunger can be described as the sensation associated with a need for food (Mattes & Friedman, 1993) which arises through internal signals that stimulate food consumption (Castonguay & Stern, 1990). Satiety is generally agreed to be the reciprocal of hunger, and the feelings of fullness experienced during eating (satiation) will lead to the cessation of food intake (satiety) (Blundell & Tremblay, 1995). The term appetite tends to be used interchangeably to describe both the eating behaviour comprising hunger and satiety, and with regard to the desire to eat, sometimes directed toward a particular food item, without necessarily feeling hungry (Castonguay & Stern, 1990). Although hunger and satiety are interactive states they may not work through the same mechanisms, and for the purpose of appetite research are treated separately.

The degree to which a food item or meal satisfies the appetite and leads to satiety has been termed the satiating efficiency (Kissileff, 1984), and this is defined as the extent to which each megajoule (MJ) of positive nutrient balance suppresses subsequent energy intake (Stubbs, 1998). Food intake inhibits or delays the onset of further eating through a number of mechanisms classified as sensory, cognitive, post-ingestive and post-absorptive, and these processes interact to influence eating behaviour at different times before, during or after feeding. The interaction of these mechanisms has been termed the ‘satiety cascade’ (Blundell & Tremblay, 1995) (figure 1), forming a framework through which many of the factors influencing eating may be interpreted. The cascade suggests that sensory cues such as the sight and taste
of food are extremely important at the onset of a meal in determining the amount that will be consumed, closely followed by cognitive cues e.g. self restraint and externality (section 1.8). Post-ingestive (or pre-absorptive) and post-absorptive cues exert their effect following ingestion of part or all of the meal, generating the neural and hormonal signals that control appetite (Blundell & Tremblay, 1995).

**Figure 1.1 A diagram of the ‘Satiety Cascade’ illustrating the interaction of psychological and physiological cues at different stages during food intake.** From Blundell & Tremblay, (1995).

It is important that while investigating the physiological control of food intake appetite researchers acknowledge the role of psychological cues in human eating behaviour, as external events often interfere with the eating pattern and can lead to confounding factors during laboratory investigations. Employment of certain methodologies minimise this (section 1.2), but the influence of behavioural and physiological factors acting at each stage of the satiety cascade should be borne in mind during data interpretation.

While it is acknowledged that psychological influences play an important role in shaping human eating behaviour, the studies presented in this thesis are primarily designed to investigate the physiological regulation of appetite. Consequently this
review focuses on the physiological aspects of food intake control, with a brief discussion of psychological influences in section 1.8.

1.1.2. The importance of understanding appetite
The interaction of behavioural and physiological influences on appetite has led to a wide variety of research into food intake control, involving many disciplines and providing greater understanding of a number of biological processes. Such research is of great importance due to the increasing incidence of overweight and obesity in western countries, and the ability to successfully tackle this problem relies heavily on an understanding of the processes controlling food intake and energy balance. As costs to the health service and social pressures due to obesity increase, it becomes more important to elucidate the underlying mechanisms. In addition to the problems of obesity, appetite research can help improve the understanding and eventual treatment of eating disorders such as anorexia nervosa (Phillipp et al., 1991), as well as disease states which include overt and unusual effects on appetite, e.g. Prader Willi syndrome (Holland et al., 1992). Thus appetite research not only improves understanding of the physiological control of food intake and energy balance, but will ultimately be of benefit to a number of population groups.

1.2. Experimental approaches to appetite investigation

1.2.1. The preloading paradigm
Research methods in appetite have been adapted to minimise the effects of individual variation and differences in the cognitive control of eating between subjects. A within-subjects experimental design allows for differences in habitual energy intakes and subjective appetite ratings as each subject is used as their own control (Hill et al., 1995). In terms of experimental design the preloading paradigm is often used for comparison of manipulations in energy or macronutrient content of a meal (Stubbs et al., 1998). The manipulations to be investigated are given to subjects in the form of a ‘preload’, using a randomised repeated measures design (Hill et al., 1995). Subjective ratings of hunger, satiety, desire to eat, etc. are then made for a set period of time.
following preload consumption. The time period investigated is dependant upon the expected time course of the development of satiety, with cognitive and pre-absorptive effects occurring up to 60 minutes after consumption. The time course of post-absorptive effects may vary depending upon the meal consumed, with simple glucose meals having the potential to influence appetite 15 to 30 minutes after consumption. In general short term post-absorptive studies investigate appetite responses over periods of 1 - 4 hours, the normal inter-meal interval, while longer term studies may investigate appetite effects into the next day.

1.2.2. Assessment of ad libitum food intake

To investigate the energy intake of a subject following different preload manipulations ad libitum food intake may also be assessed. A common approach is to present subjects with a set meal, commonly referred to as the ‘test meal’, from which energy and macronutrient intakes are calculated (Rogers, 1993). Alternatively food may be continuously weighed during consumption to determine the amount eaten and the rate of food intake (Kissileff et al., 1980). As food intake at this meal is responsive to the preload manipulation, energy intake provides a measure of the relative satiating capacities of the preload. The test meal often consists of a range of familiar foods appropriate to the time of day, and during consumption of the meal subjects are segregated from one another to minimise the effects of social interaction on food intake, which has been shown to influence the amount consumed (de Castro & Brewer, 1992). The variety and palatability of foods in the test meal are also an important determinant of the amount eaten, as highly palatable foods can increase hunger ratings (Hill et al., 1984), and may lead to increased intake unrelated to the preload manipulation. This can be minimised by ensuring subjects are not provided with their favourite foods in a test meal. Increased variety also elevates intake (Spiegel & Stellar, 1990), while consumption of water with the meal should be controlled to prevent any effect on intake (Lappalainen et al., 1993). Thus test meal content must be kept constant for the duration of a study to ensure comparability of data.
1.2.3. **Subjective appetite ratings**

Subjective ratings of hunger and satiety are frequently applied in appetite investigations, with 10 cm visual analogue scales (VAS) used for subjects to rate their feelings appropriately. Differences may arise in the use of these scales between subjects (Hill *et al.*, 1995), and the effects of this are minimised by using a repeated measures experimental design and pertinent statistical techniques. These scales have been reported to have both good (Porrini *et al.*, 1995) and poor (Raben *et al.*, 1995) reproducibility which may depend upon the experience of the subject using ratings scales and on day to day variations in self rating. However as self rating is one of the few available methods for assessing appetite these scales continue to be used and still provide valuable data. Similar to the VAS, food preference checklists may be used as an additional method of hunger assessment (Hill *et al.*, 1995) which utilise a menu choice system to determine how many calories the subject would like to eat. This has been shown to correlate well with subsequent food intake (Hill *et al.*, 1995).

The relationship between changes in hunger and satiety ratings and test meal energy intake can be used to calculate a satiety quotient, which assesses the satiating effect of the eating episode in relation to the energy consumed (Green *et al.*, 1997). If VAS ratings are continued for some time after test meal consumption, this calculation allows comparison of the effects of foods on satiety over a period of time. If a single measure of appetite is made following test meal consumption the method provides a measure of change in appetite in relation to the amount consumed, and may help compensate for problems arising due to over- or under-consumption at the test meal. Similarly Kissileff (1984) reported on a method to calculate the satiating efficiency of a food or macronutrient, where the relationship between preload energy intake and test meal energy intake is calculated, and the extent to which preload energy suppresses subsequent energy intake can be determined. In order to benefit from this calculation the nutrient under investigation must be preloaded at different levels to determine the extent to which test meal intake responds to the manipulation. These data can then be compared to that for other nutrients and the relative satiating efficiencies determined.
1.2.4. Assessment of salivary output

In addition to the subjective measures of food intake and appetite, salivation has been investigated in relation to hunger (Wooley & Wooley, 1973). It has been shown that salivation in fasted subjects increased with the presentation of food, and the increase in saliva correlates positively with body weight (Sahakian et al., 1981), and as a function of prior food deprivation and hunger ratings (Wooley & Wooley, 1973). Although a measure of salivation provides an objective measure of appetite, research is conflicting and has shown that anticipatory salivation is affected by a large number of variables not directly related to food intake (Hill et al., 1995). Consequently the majority of appetite studies rely on subjective methods of assessment for interpretation of the actions of physiological hunger and satiety signals.

1.3. Theories of short term appetite regulation

Perhaps the earliest published appetite research dates from the beginning of the 19th century, when William Beaumont reported on his investigations into digestion using a human subject with an open gastric fistula caused by a bullet wound to the stomach (Beaumont, 1846). This unusual condition enabled study of the progress and requirements of digestion, and Beaumont noted that food items which took the longest to digest had the greatest effect on appetite. Since this time the investigation of appetite has been pursued in order to determine the mechanisms through which food intake and hunger are regulated.

Early appetite research into the ‘hunger contractions’, so called as hunger was attributed to contractions of the digestive canal (Cannon, 1911), suggested roles for blood glucose (Bulatao & Carlson, 1924), insulin (Maclagan, 1937) intestinal loads and contractions (Cannon, 1929) and energy balance (Maclagan, 1937). These possible mechanisms have since been revisited and shown to have some role in appetite regulation (discussed later in this chapter), however much early research investigated the role of the brain as the centre of feeding control. In animal studies lesions of the diencephalon, the region including the thalamus and hypothalamus, were found to cause weight loss or weight gain through variation in food intake.
Chapter 1

(Hetherington & Ranson, 1939 cited in De Groot, 1998). Such data led to the suggestion that the hypothalamus was primarily responsible for both the stimulation of food intake, and the induction of satiety signals causing cessation of a meal. However as Adolf proposed; appetite is unlikely to be under single factor control, particularly in the well fed state when hunger and satiety would respond to multiple stimuli (Adolf, 1947). Later theories have recognised this multifactorial approach, and encompass peripheral responses to food intake in relation to central nervous system (CNS) stimulation. The CNS is likely to be responsive to changes in circulating hormone and metabolites following food intake, and the glucostatic (section 1.3.2), lipostatic (section 1.3.3) and aminostatic (section 1.3.4) theories of food intake regulation encompass a role for CNS response and regulation in eating behaviour. The time frame in which each theory of regulation may work also varies, with glucostatic and aminostatic mechanisms most likely to regulate short term intake. The lipostatic hypothesis may represent a longer term regulation of feeding behaviour, over periods of longer than one day. However hormonal responses to fat ingestion and the subsequent effects upon appetite point to a short term mechanism of fat mediated satiety operating in addition to any long term regulation.

Although it is probable, and generally accepted, that a relationship between central and peripheral factors is responsible for the regulation of appetite, much work remains to be completed before the relative roles of the central and peripheral satiety system, and all the factors within, can be sufficiently understood to enable more successful treatment of appetite related disorders.

1.3.1. Role of the hypothalamus in feeding behaviour

Data from early animal experimentation suggested that the hypothalamus was primarily responsible for the control of weight loss and gain, although the precise mechanisms through which these events occurred were, and remain, unknown. Further research suggested that observed changes in body weight following experimentally induced hypothalamic lesions were brought about through differences in food intake and appetite rather than changes in metabolism (cited in De Groot, 1998). Studies directed at different regions of the brain subsequently showed there to
be a dual nature to the observed hypothalamic regulation, with damage to the ventromedial hypothalamus (VMH) resulting in increased food intake and subsequent weight gain (e.g. Hetherington & Ranson, 1942; Brobeck et al., 1943; Hervey, 1959), and lateral hypothalamic (LH) lesions found to cause refusal of food and water intake (e.g. Anand & Brobeck, 1951; Williams & Teitelbaum, 1959). Furthermore bilateral VMH lesions were found to have a more profound effect on food intake than unilateral lesions (Mayer & Barmett, 1955b; Wagner & De Groot, 1962), suggesting the hypothalamus may be capable of grading appetite responses, rather than ‘switching’ food intake on or off. These data led to the VMH being termed the ‘satiety centre’, while the LH was termed the ‘feeding centre’. Further evidence for the LH as a feeding centre came from data showing an inhibition in electrical activity of this area during food intake (Anokhin & Sudakov, 1966), possibly leading to the cessation of food intake, and data is suggestive of an interaction between the VMH and LH in the regulation of feeding.

It has been suggested that the VMH acts through laterally and caudally projecting axons to inhibit the feeding centre and initiate satiety (Anand, 1961), although the exact localisation of the feeding area in the LH remains in doubt (De Groot, 1998). To investigate the role of neurotransmitters in this process studies utilising drugs that either stimulate or inhibit neurotransmitter action implied the involvement of a range of CNS transmitters in appetite (Grossman, 1998). Although there are many peptide based neurotransmitters linked to appetite regulation, discussed later in this chapter (section 1.5.2), some non-peptide central transmitters have also been shown to have an effect upon food intake. For example serotonin has been shown to have an inhibitory effect on food intake (Blundell, 1984), which is thought to be due to its action in the paraventricular nucleus (PVN) (Morley, 1987). Traditionally serotonergic effects upon appetite have been linked to the role of carbohydrate cravings in depression (Mela & Rogers, 1998), with the hypothesis that the depleted brain serotonin (5-hydroxytryptamine (5-HT)) seen in depressed patients leads to the stimulation of carbohydrate intake, which in turn promotes the entry of tryptophan (the 5-HT precursor) into the brain. Due to the action of insulin, uptake into peripheral tissues of many amino acids, except tryptophan, is facilitated. As
tryptophan competes with other similar (large neutral) amino acids for uptake into the brain, carbohydrate intake has the ultimate effect of increasing brain tryptophan levels (Mela & Rogers, 1998). Consequently increased 5-HT levels following carbohydrate intake induce feelings of relative calmness and help relieve some symptoms of depression (Møller, 1992; Maes & Meltzer, 1995). This in turn results in satiation of the appetite for carbohydrate rich foods, and would help explain the finding that serotonin reduces meal size and duration (Leibowitz & Shor-Posner, 1986).

However, serotinergic food intake response has been shown to reduce fat as well as carbohydrate consumption (Orthen-Gambill & Kanarek, 1982) suggesting that serotonin may play a more complex role in appetite. This theory is supported by studies using the 5-HT agonist 8-hydroxy-2(di-n-propylamino) tetralin, which causes a non-nutrient specific hyperphagia following administration in rats (Bendotti & Samanin, 1986; Dourish et al., 1986). Conversely peripheral administration of the serotonin antagonist metergoline has been shown to increase carbohydrate intake, while PVN administration of 5-HT itself decreased carbohydrate intake (Leibowitz et al., 1993), and 5-HT has been shown to decrease overall food intake in rats (cited in Blundell et al., 1995a). Furthermore 5-HT can cause reduction of fat intake (Blundell et al., 1995a), and lead to weight loss in obese patients (Cangiano et al., 1992).

Additional evidence of a role for serotonin in appetite comes from the satiety hormone cholecystokinin (CCK) (section 1.6), which has been shown to increase hypothalamic 5-HT during food intake and during systemic administration of the hormone in fasted rats.

In addition to serotonin, norepinephrine has been shown to enhance food intake when injected into the PVN through action on the α₂ adrenergic receptors (Goldman et al., 1985). This may explain the increased appetite associated with the tricyclic group of antidepressant drugs which modulate the affinity of these receptors (Morley, 1987). Dopamine has also been implicated in appetite, with hypothalamic administration of the neurotransmitter shown to cause hyperphagia (Friedman et al., 1973), and increased turnover of brain dopamine observed following a high fat diet (Levin et al., 1986). GABA (γ-amino butyric acid) has also been shown to decrease food intake, and consequently growth, when included in the diets of rats (Tews et al., 1980),
providing evidence that a large number of CNS neurotransmitters are likely to feature in appetite regulation to some extent.

With many studies focusing on the CNS it is difficult to separate pharmacological effects from physiological effects, particularly due to the trauma induced by experimental methods, and for obvious reasons this research is rarely, if ever, undertaken in the human subject. Although hypothalamic systems clearly play a role in the regulation of appetite and are a target in the search for appetite suppressant drugs, in the healthy human peripheral physiological signals are likely to exert a greater control over food intake and provide stimuli from which the hypothalamus receives primary appetite information.

1.3.2. The glucostatic theory of appetite regulation

In 1953 Mayer reported on a proposed glucostatic theory of appetite regulation following data showing there to be relationships between insulin and food intake (Morgan & Morgan, 1940) and glucose and food intake (Mayer & Bates, 1951; Mayer, 1953; Van Itallie et al., 1953) in both rat and man. The glucostatic theory proposed that hypothalamic feeding centres would consist of glucoreceptors, and thus circulating insulin and glucose levels would have a direct effect on the CNS and food intake response. The close homeostatic regulation of glucose levels was thought to be good evidence for a glucose based appetite control system (Mayer, 1955a), however Mayer also recognised that peripheral signals were likely to be part of this mechanism, and conflicting reports of the effect of insulin upon feeding behaviour (e.g. Grossman, 1955; Steffens, 1970) led to the suggestion that insulin and glucose may be involved both in the central control of appetite, through brain sensitivity to changing glucose concentration, with a more immediate effect on a peripheral system sensitive to circulating glucose levels (Woods & Porte, 1978).

Since this time much research has centred upon the relationship between insulin, glucose and appetite. The intravenous (IV) administration of insulin was found to increase food intake in normal weight rats, but decrease intake in obese animals (Orosco et al., 1994). Thus responses to insulin may either be dependant upon body
weight or occurrence of obesity, or else there is no direct effect on insulin upon food intake in these animals. The hepatic-portal administration of insulin during a meal was shown to decrease food intake in normal weight rats (Vanderweele, 1994). The authors suggest this may be due to increased glucose uptake into peripheral tissue, suggesting an indirect action of insulin upon appetite through its regulation of glucose. A small reduction in circulating glucose was shown to occur 5 to 6 minutes before onset of the next meal in free feeding rats (Louis-Sylvestre & Le Magnen, 1980a), and a lowering of circulating glucose following IV insulin may explain the increase in food intake observed in normal weight rats (Orosco et al., 1994). As the liver is involved in nutrient regulation it has been suggested that the hepatic system may regulate food intake by responding to alterations in blood glucose (Russek, 1981). Russek speculated that following glucose absorption from the intestine an increased liver pyruvate hyperpolarises the hepatocyte membrane, and inhibits hunger. However the mechanism through which this process would ultimately inhibit hunger was not suggested, and other authors have presented data arguing that hepatic glucoreceptors have no significant control over food intake. Liver denervation has not been shown to elicit any changes in ad libitum feeding behaviour in animals, suggesting either that the liver has no direct effect upon food intake or that its influence upon feeding behaviour is relatively small in comparison to other central or peripheral factors (Bellinger et al., 1976; Louis-Sylvestre et al., 1980b; Louis-Sylvestre, 1981). In addition to the above findings Vanderweele et al. (1974) showed no effect of hepatic-portal infusions of a 30% glucose solution upon food intake in rabbits, whilst a duodenal perfusion of glucose solution resulted in reduced food intake. Such data highlights the importance of gastrointestinal contact with nutrients for the successful regulation of appetite, and suggests that while specific organ systems may have a role in the regulation of appetite, they act secondary to pre-absorptive and early post-absorptive factors. As Friedman pointed out, although the liver may play a role in appetite regulation, there are a number of important areas in the control of appetite, and care should be taken not to focus too closely upon a single area of control (Friedman, 1981).
Human studies investigating the role of insulin and glucose levels in appetite have provided conflicting results. Exogenous insulin administration has been shown to lead to overeating (Grossman & Stein, 1948; Lovett & Booth, 1970), and hyperinsulinaemia may result in increased food intake independent of glucose status (Rodin et al., 1985). In addition Holt and Miller (1995) obtained results from a food intake study suggesting higher endogenous insulin responses to be associated with lessened satiety. However higher satiety ratings have also been shown following a meal which elicited a higher insulin response than a second test meal (Raben et al., 1994). This study showed differing levels for a range of hormones including glucose-dependant insulino tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), and conclusions as to the action of insulin cannot be drawn directly. Similarly Raben et al. (1996) showed strong correlations between change in satiety ratings and changes in glucose, insulin, lactate and GIP, but due to intercorrelations between these hormones causality could not be established.

Although the above investigations have shown an effect of insulin upon appetite, this effect is not uniform across studies and it therefore seems unlikely that insulin acts directly on food intake. There is no evidence of a direct correlation between satiety ratings and either glucose or insulin response (Holt et al., 1996), and infusion of an insulin-glucose mixture during food intake against a saline control resulted in elevated plasma insulin levels with similar circulating glucose concentrations, but did not affect meal size or duration in normal weight subjects (Woo et al., 1984). It is thus possible that the influence of insulin on appetite seen in previous studies is attributable to its action upon glucose status. Although an IV infusion of glucose has been shown to have no effect upon food intake (Lavin et al., 1996), intraduodenal infusion of glucose was shown to increase satiety, suppress hunger and reduce subsequent food intake in humans (Lavin et al., 1996). This provides evidence for an interaction between nutrient induced secretions and gastrointestinal loads in the induction of satiety. In addition intestinal absorption of glucose in this study may have promoted an interaction between glucose and insulin levels which itself influenced satiety. Consequently the relationship between glucose and insulin status
may be more important than absolute circulating levels of these parameters in appetite
regulation.

Taken together animal and human data do provide some evidence of a role for insulin
and glucose in feeding behaviour. However there is little support for a direct effect of
either insulin or glucose upon appetite and it is probable that these factors would work
in combination with other post-prandial responses to regulate appetite. With infusion
and feeding studies it is extremely difficult to quantify the effects of hormonal
differences in response to nutrient administration due to the increase of a number of
hormones and metabolites following food intake. Furthermore carbohydrate ingestion
not only increases glucose and insulin levels, but also stimulates GLP-1 secretion, and
as GLP-1 is a recent candidate as a satiety hormone (section 1.7) a potential role for
this hormone within the glucostatic hypothesis is possible. Consequently the
probability that factors other than glucose and insulin are influencing appetite in the
above studies must be considered, and it is possible that a glucostat model operates
within a multifactor post-prandial response which acts to regulate appetite.

1.3.3. The lipostatic theory of appetite regulation
The lipostatic hypothesis suggests that an appetite centre is primarily influenced by
levels of circulating fat or fat metabolites. First proposed by Kennedy (1953) with
additional work involving Mayer (Bates et al., 1955) the theory postulates that
animals spontaneously mobilise fat in amounts proportional to their total fat reserves,
and this is also dependent on fat distribution, ad libitum diet and exercise levels.
Receptors in the CNS would then monitor and regulate these levels of fat or fat
metabolites, and stimulate appetite accordingly (Geiselman, 1996). It was initially
proposed that this mechanism could account for the long term regulation of food
intake and body weight, whilst the glucostat was more likely to control short term
regulation of food intake (Mayer, 1955a).

The majority of evidence for a lipostatic mechanism of appetite regulation comes
from animal studies, and changes in the size of fat stores have been reported to result
in hyper or hypophagia accordingly (Cohn & Joseph, 1962; Harris et al., 1986;
Scharrer & Langhans, 1988; Plata-Salaman, 1991). Thus it is possible that changes in circulating fat metabolites attributable to changes in fat stores cause an alteration in food intake to enable the animal to maintain fat balance. The food hoarding behaviour of rats has also been shown to correlate with adipose tissue stores (Cabanac & Gosselin, 1996), suggesting that the loss of fat mass provides a signal to initiate feeding or gathering of food. However, surgical removal of body fat in rats was not found to cause any changes in food intake for 12 weeks post-operatively (Kral, 1976), leading to the suggestion that the size rather than the number of adipocytes may be important in the lipostatic regulation of appetite.

There is speculation as to how changes in adipose tissue may regulate appetite. Reputed fat based signals include free fatty acids (FFA), glycerol, apolipoprotein A-IV and 3-hydroxybutyrate (Tso et al., 1995; Geiselman, 1996), all of which are shown to be elevated during hypophagia (Geary et al., 1982). Carpenter and Grossman (1983) tested the relationships between fat metabolites and food intake using an infusion of glycerol, ketone bodies, FFA and Intralipid; and showed ketone bodies and FFA to cause the greatest reduction in food intake, with glycerol having the smallest affect on food consumption. It has also been suggested that the VMH and LH may be sites of action for fat based signals (Kasser et al., 1989) with FFA shown to have a direct effect upon the hypothalamic sites involved in the control of appetite (Oomura, 1989), and FFA oxidation in the liver a possible initiator of signals used to control feeding behaviour (Campfield, 1997).

1.3.3.1. Leptin

The discovery of leptin, the protein product of the obesity (ob) gene (Zhang et al., 1994) led to a resurgence of interest in the lipostatic theory of appetite regulation. Leptin is released by adipose tissue, possibly regulated by insulin (Utrianen et al., 1996), and provides an ideal candidate for a fat based satiety signal which could regulate adipose tissue stores. Central administration of leptin has been shown to depress food intake in rodents (Campfield et al., 1995; Van Dijk et al., 1996; Schwartz et al., 1996), and it has been suggested that peripheral release of the hormone may operate through a feedback system to act as a central satiety signal.
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(Rohner-Jeanrenaud & Jeanrenaud, 1996). There is a strong relationship between adipose tissue mass and circulating leptin levels in humans (Considine et al., 1996). However although a relationship between leptin and appetite has been demonstrated in animals (Friedman & Halaas, 1998) the same does not appear to be true in humans, particularly in obese subjects who have poor regulation of energy intake and weight gain despite having a large adipose tissue mass and high circulating leptin levels. Thus there may be resistance to the action of leptin in some obese individuals (Rohner-Jeanrenaud & Jeanrenaud, 1996). In terms of human appetite regulation the role of leptin remains poorly understood, although it seems likely that the hormone acts on a long term basis, over periods of 2 - 3 days, to regulate energy balance (Friedman & Halaas, 1998). This is consistent with the early interpretation of the lipostatic hypothesis as a mechanism for the long term regulation of food intake (Mayer, 1955a). Leptin levels have not been shown to increase during a meal and there is no relationship between meal termination and circulating leptin (Friedman & Halaas, 1998), providing further evidence of a role for the hormone in long term rather than short term appetite regulation.

1.3.3.2. Short term regulation of appetite through fat ingestion

Although the discovery of leptin provides much support for a lipostatic mechanism, there is poor evidence that dietary hyperphagia is generally self limiting and overweight per se does not successfully suppress appetite (Ramirez, 1990). In comparative short term human feeding studies fat has been shown to induce least satiety (section 1.5.1), suggesting that a fat based mechanism of appetite regulation may be relatively weak. However infusion of fat into the small intestine has been shown to result in increased feelings of fullness and reduced food intake (Welch et al., 1985; Read, 1992), suggesting that dietary fat is important in the generation of short term intestinal satiety signals (Blundell & Tremblay, 1995). The satiety hormone CCK (section 1.6) is released primarily in response to fat ingestion, and provides a probable mechanism for short term fat mediated satiety. Additional data has also suggested that fat type influences satiety, with medium chain triglycerides found to be more satiating than long chain triglycerides in animals and man (Furuse et al., 1992; Furuse et al., 1993; Stubbs & Harbron, 1996). Thus it appears that the carbon chain
length of fat is important in determining subsequent food intake, although the mechanism through which this action occurs remains unknown.

High fat foods often lead to passive overconsumption in man (Green et al., 1994; Blundell & Tremblay, 1995). Thus the elucidation of these mechanisms is of particular importance for the understanding and treatment of obesity, and further investigation of fat mediated long and short term satiety is warranted.

1.3.4. The aminostatic theory of appetite regulation

Although not of direct relevance to the work presented in this thesis, the aminostatic hypothesis must be mentioned as a strong candidate in appetite regulation. A protein based mechanism of food intake was proposed following observations that a rise in serum amino acid concentrations was concurrent with a reduction in appetite (Mellinkoff et al., 1956). Although the authors noted that amino acid concentrations did not predict the degree of hunger, it is probable that higher amino acid levels were responsible for initiating a satiety signal, and as protein has been shown to be the most satiating of the macronutrients (section 1.5.1) it seems likely that this macronutrient plays an important role in appetite regulation under normal physiological conditions.

A number of animal studies have provided evidence for a protein driven appetite (e.g. Kyriazakis & Emmans, 1991; Webster, 1993; Yahya & Millward, 1994); showing animals to select food intake to meet protein needs, and human studies have shown a marked stimulation of appetite during catch up growth until body protein stores have been repleted (Ashworth, 1974; Kabir et al., 1993). Millward (1995) proposed a ‘protein-stat’ mechanism by which body protein is regulated in relation to differing habitual intakes, and included in this model a method by which dietary protein may be involved in appetite regulation and the induction of satiety. Ultimately the model suggests that differences in habitual protein intakes may lead to differences in the satiating power of a protein load due to variance in amino acid oxidation and clearance from the circulation. If this were found to be true such data would provide strong evidence of a role for protein in the appetite of the well nourished individual. To date a single study investigating the role of habitual protein intake in protein
induced satiety has supported this hypothesis (Long et al., 2000), and this area is worthy of further investigation.

Although the precise mechanism through which protein may act on appetite is unknown, it is probable that the proposed hypothalamic appetite centres are responsive to changes in amino acid concentrations. It has been shown that dietary proteins cause changes in circulating amino acid concentrations (Fernstrom et al., 1979), and direct administration of amino acids can decrease food consumption in the obese and normal weight subject (Butler et al., 1981; Silverstone & Goodall, 1984; Rogers & Blundell, 1992). Furthermore different protein sources have been shown to result in different ratings of satiety (Uhe et al., 1992). These data implied a correlation between the tryptophan:LNAA ratio (Harper & Peters, 1989) and satiety, suggesting a serotonergic route of appetite control may be involved in protein mediated satiety.

The direct administration of phenylalanine in particular has been associated with a reduction in food intake (Ballinger & Clark, 1994; Rogers & Blundell, 1994), and the consumption of aspartame sweetened soft drinks is associated with a reduced energy intake during periods of consumption (Tordoff & Alleva, 1990). While the lower energy intake observed in the latter study may have been attributable to the consumption of the low energy soft drink itself, no subsequent compensation for this lower energy intake was seen during the investigation period, and it is possible that the phenylalanine constituent of the aspartame sweetener had some effect on appetite (Rogers et al., 1990). As this amino acid also stimulates secretion of CCK (Rogers & Blundell, 1992) it is probable that in addition to acting through central, amino acid sensitive mechanisms, dietary protein is able to act upon appetite through peripheral hormonal factors.

1.4. Gastrointestinal factors in appetite regulation

In addition to nutritive factors, food intake is influenced by gastrointestinal mechanisms that trigger both the initiation and termination of eating behaviours (Read
et al., 1994). As food intake is limited by the capacity of the gastrointestinal tract short term food intake must be regulated not only in terms of nutrient intake but also physical size of a meal, and the intervals between meals often seen in human eating behaviour corresponds to the time taken to process a meal in the GI tract (Read et al., 1994).

1.4.1. **Gastric distension and gastric emptying**

Gastric emptying is influenced by both the size and macronutrient composition of a meal, with meals of high energy density exhibiting a slower gastric emptying rate than those with a lower energy density (Wisen et al., 1993). Although energy dense loads take longer to empty completely from the stomach, the energy emptied into the duodenum for the first hour after ingestion is actually greater with these meals (Wisen et al., 1993). These data provide evidence for the role of small intestinal signals, initiated after movement of nutrients into the duodenum, in the regulation of gastric emptying. Rates of emptying have been shown to be faster in the initial 30 minutes after ingestion regardless of preload energy content (Hunt et al., 1985), and as this may represent the time taken for the stimulation and action of small intestinal factors, these data provide further evidence of a role for small intestinal factors in the regulation of gastric emptying.

It has been shown that the macronutrients have different emptying rates, with carbohydrate loads generally emptying faster than protein loads, which in turn empty faster than fat loads. The rate of emptying of each nutrient is related both to its energy density (Hunt & Stubbs, 1975), and to the level at which it stimulates secretion of hormones which regulate gastric emptying (sections 1.6 and 1.7). In terms of energy delivered to the small intestine per unit of time (kJ/min), the regulation of gastric emptying aims to maintain a similar rate of nutrient delivery to the duodenum after food intake (Hunt & Stubbs, 1975). However large increases in the volume or energy density of a preload may lead to an increased rate of nutrient delivery into the duodenum, as the stomach is unable to closely regulate emptying of a large load (Hunt et al., 1985).
In addition to the influence of energy content of a meal, the rate of gastric emptying is also influenced by acidity, osmolarity, conductivity and temperature (Hunt, 1956; Hunt & Knox, 1972), and it has been suggested that the effect of energy alone is not sufficient to determine gastric emptying (Shafer et al., 1985). Investigations into the relationship between gastric emptying and hormonal responses have provided much evidence for hormonal control of gastric emptying (e.g. Wettergren et al., 1993; Cote et al., 1995). Thus it is probable that the small intestinal signals required to regulate gastric emptying after the initial emptying phase are hormonal in origin.

As gastric emptying rate is responsive to the intake of different nutrients, and gastric distension is regulated through gastric emptying, it is probable that these factors may in turn influence post-prandial satiety. Correlations have been reported between gastric emptying rate and appetite ratings (Sepple & Read, 1989; Bergmann et al., 1992; Carbonnel et al., 1994), and there is evidence of a relationship between hormonal responses to a meal, gastric emptying and satiety in man (section 1.6.4 and section 1.6.5). The primary neural signalling pathway between the stomach and the CNS is the vagus nerve, and it has been proposed that the vagus mediates gastric satiety signals by increasing afferent impulses in the presence of a gastric load (Schwartz & Moran, 1996). Animal data has shown reduced food intake with increased gastric distension (Durrans et al., 1991; Phillipps & Powley, 1996), and food intake of obese subjects can be reduced by gastric balloon inflation or stomach stapling procedures (Pasquali et al., 1990), suggesting stomach size and gastric load to be important in the cessation of eating. In human studies gastric distension produced through inflation of a balloon results in increased feelings of pressure in the stomach (Khan & Read, 1992). However post-meal feelings of satiety were only produced with concurrent infusion of lipid into the duodenum during this study, suggesting the importance of nutrient contact with the gastrointestinal tract rather than gastric distension alone in the mediation of satiety.

There is evidence that effects of overt gastric distension, such as those produced by a gastric balloon, can disappear with time as the stomach adapts to the increased level of distension (Read et al., 1994). Although unlikely that the stomach expands or
shrinks over short periods of time it is possible that stretch receptors and nutrient receptors adapt to different levels of food intake. This may help explain habituation to large meal sizes in overweight individuals, continued overeating following periods of excess food intake, and persistent hunger at the beginning of a weight reduction diet (French et al., 1995). Changes in the rate of gastric emptying may also help explain this phenomenon, as it has been demonstrated that gastric emptying of a test meal was accelerated following consumption of a high fat diet (Cunningham et al., 1991) providing evidence of a role for this mechanism in the passive overconsumption observed with a high fat diet.

1.4.2. Nutrients in the small intestine

The rate of flow of nutrients into the small intestine may also influence satiety signals, explaining why the feelings of stomach pressure recorded with a gastric balloon became more pleasant feelings of satiety when nutrient was additionally infused into the intestine (Khan & Read, 1992). It has also been shown that lipid infusion in the small intestine induces feelings of satiety and a reduction in food intake, whereas IV infusion of the same lipid emulsion caused no appetite effects (Welch et al., 1985; Greenberg et al., 1989). Thus data suggest that pre-absorptive contact with the small intestine is necessary for a nutrient to potentiate satiety. Increasing the exposure time of the small intestine to nutrients has also been shown to influence satiety, and guar gum, which slows intestinal transit time and delays absorption of nutrients from the small intestine, has been reported to increase and prolong satiety (French & Read, 1994; Lavin & Read, 1995), as have other fibre sources (Haber et al., 1977; Raben et al., 1994). Gastric emptying can be delayed by dietary fibre in foods (Benini et al., 1995), and indirect appetite effects of high fibre foods may help explain the observation that lean subjects have an habitually higher fibre intake than obese subjects, with an inverse correlation between BMI and fibre intake (Alfieri et al., 1995). An observed reduction in lunch meal intake following a high fibre breakfast provides additional evidence of a role for fibre in the induction of satiety, and for the promotion of a high fibre diet to aid appetite control and weight loss (Turconi et al., 1993).
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The infusion of nutrients into the small intestine has been shown to have a strong effect upon appetite, and may elicit a greater satiety response than that seen with a gastric load (McHugh & Moran, 1985). Duodenal receptors which are able to detect the energy and macronutrient content of a gastric load have been proposed which could modulate satiety and regulate gastric emptying (Hunt, 1968; Houpt, 1980; Lin et al., 1989; Lavin & Read, 1995) and small intestinal factors such as gastrointestinal hormones are probably also required to potentiate satiety signals following nutrient ingestion. The existence of such factors provides some explanation of the need for nutrient contact with the intestine to initiate a satiety response, and prolonged contact with the small intestine which can occur with a higher fibre intake (French & Read, 1994), may therefore increase feelings of satiety. The portion of the small intestine infused differentially influences transit time, for example lipid infusion into the ileum slows intestinal transit time, but no effects are seen with jejunal or colonic infusion (Read & Kinsman, 1984). In addition, increasing the length of time the ileum is exposed to nutrients delays small intestinal transit and suppresses appetite (Barry et al., 1977). This effect of ileal fat perfusion has been termed the ‘ileal brake’, and lipid infusion into the ileum inhibits jejunal motility independent of jejunal contents and delays jejunal transit time (Spiller et al., 1984). This slower intestinal transit in turn influences appetite. A delayed gastric emptying has also been attributed to this mechanism (MacFarlane et al., 1983), and this is a potential mechanism through which fat, and possibly other nutrients, may induce satiety. More recently further evidence of a role for the ileal brake in appetite regulation is a proposed hormonal control of the brake by GLP-1 (Giralt & Vergara, 1999), a putative satiety hormone.

1.5. Role of the macronutrients in the appetite response

1.5.1. Relative satiating efficiencies of protein, carbohydrate and fat

Energy intake alone is generally a poor regulator of appetite, with diets of high energy density leading to increased energy intake (Duncan et al., 1983; Lissner et al., 1987; Thomas et al., 1992), and subjects finding it difficult to distinguish between high and low energy meals (Wooley et al., 1972). Although some short term studies have shown high energy preloads to be more satiating than low energy preloads (Pliner,
1973), due to the body’s aim to maintain protein and energy stores it is probable that appetite is also responsive to changes in macronutrient ingestion (Danforth, 1985), and post-prandial hormone responses and differences in the metabolic oxidation of each macronutrient are likely to interact in the control of food intake (Stubbs, 1998). However it is important to determine the physiological relevance of each macronutrient in food intake control, and comparative feeding studies have often been used to assess appetite responses to preloads of different macronutrient content.

The majority of human feeding studies have shown protein preloads to be more satiating and result in smaller subsequent food intake than both carbohydrate and fat (Hill & Blundell, 1986; de Castro, 1987; de Castro & Elmore, 1988; Barkeling et al., 1990; Johnson & Vickers, 1993; Johnstone et al., 1996; Poppitt et al., 1998); while fat has also been found to as less satiating than carbohydrate (e.g. Blundell et al., 1993; Green et al., 1994). This has led to a suggested hierarchy in the satiating efficiency of the macronutrients (Stubbs et al., 1996), and while some researchers have found no difference between the satiating efficiency of each macronutrient (Geliebter, 1979; Driver, 1988; de Graaf et al., 1992) or no significant effect of protein on appetite (Sunkin & Garrow, 1982), these negative studies have commonly fed a relatively low level of energy in each preload, probably at levels insufficient to induce a suitable degree of fullness in the individuals studied. In addition differences in the physical size of the preload and previous dietary status of subjects may have confounded results. Thus it seems likely that such a hierarchy does exist under some conditions.

As mentioned previously, energy intake has been shown to depend upon the energy density of a diet, and a greater energy intake occurs whilst consuming diets of high energy density (Duncan et al., 1983). Furthermore distinction between preloads of different energy content has been shown to be relatively poor (de Graaf & Hulshof, 1996; Himaya et al., 1997), and it has been suggested that food consumption throughout the entire day is ‘elastic’, with little physiological regulation of energy intake (de Castro, 1993). Thus the consumption of high fat (the most energy dense macronutrient) snacks tends to lead to a greater overall energy intake throughout the day (Green et al., 1994), as calorie for calorie high fat foods are less effective at
suppressing subsequent food intake (Green & Blundell, 1996) and excess intake more easily occurs with a high fat diet (Lawton et al., 1993). Whilst this poor ability to distinguish between different energy levels helps to account for passive overconsumption on high fat diets, the mechanism behind this phenomenon remains poorly understood (Warwick, 1996). There is some evidence to suggest that satiety with a high fat diet occurs when carbohydrate intake corresponds to expected daily carbohydrate oxidation (Tremblay et al., 1991), and it is possible that with consumption of a high fat diet carbohydrate intake is important for appetite control.

The presence of fat in the intestine has been shown to generate potent satiety signals (Welch et al., 1985; Lieverse et al., 1994c) probably through a CCK mediated mechanism (section 1.6.4). Conversely passive overconsumption during high fat diets suggests fat has a weak effect on satiety and this contradictory evidence on the role of fat in the control of energy intake has been termed the fat paradox (Blundell et al., 1996). Explanations for the paradox include the oral stimulation from fatty foods, which promote food intake by influencing sensory mechanisms early in the satiety cascade, and the energy density of high fats foods, such that a large amount of energy is consumed before fat is delivered to the small intestine and is able to induce satiety signals (Blundell et al., 1996). Studies with sucrose polyester (SPE) fat replacers (which include the product “Olestra” now marketed in the U.S.) may help explain the paradox, as their sensory qualities are similar to natural fat while delivering no absorbable calories. Data suggest that SPE can reduce subsequent food intake in lean male and female subjects (Hulshof et al., 1995) and has the same effect on satiety as the fat containing preload following a lunchtime or evening test meal (Cotton et al., 1996a). This would imply that either sensory qualities of fat or size and bulk of the preload are contributing to feelings of satiation. The hormonal responses to SPE has not been compared to a similar size fat load, and this needs to be explored further before it can be ruled out as a mechanism of action. It should also be noted that a similar study using a larger increase in SPE as fat replacement was found to result in 74% compensation and increased feelings of hunger (Cotton et al., 1996b), suggesting that if dietary manipulation is too severe in terms of calorie reduction, compensatory response will increase and adherence to such a diet would be more difficult.
There appears to be a biological drive to maintain energy intake which leads humans to overeat, and this is illustrated by data from the free living situation. Subjects have been found to completely compensate for a covert reduction in energy of available foods by increasing their food intake, but when foods were returned to the usual higher energy level no compensation was apparent, leading to over consumption (Foltin et al., 1988). There is also some evidence that high fat preloads suppress subsequent intake less than high carbohydrate preloads in obese and restrained eaters, whereas normal weight males were able to compensate for the preload in both instances (Rolls et al., 1994; Rolls & Hammer, 1995). Such data suggest that certain groups of individuals may compensate for caloric differences to a lesser extent, be it due to a predisposition towards high fat foods, physiological differences or behavioural differences. Due to the fat paradox, and a possible difference in response between obese and lean individuals, further research into the comparable hormone and metabolite responses to high and low energy dense foods, and their relation to appetite and food intake, would be beneficial in increasing the understanding of fat mediated satiety.

1.5.2. Hormonal response as a potential mediator of satiety

It is probable that differences in satiety following preloads of varying size and composition are attributable to a differing peripheral physiological responses which in turn stimulates central feeding centres. A number of the peptides released during or after a meal have been implicated in providing signals for satiety, and of these CCK is probably the most investigated (section 1.6.4).

Bombesin was initially isolated from frog skin (Anastasi et al., 1971) and found to be widely distributed in mammalian systems as gastrin releasing peptide (GRP) (Morley, 1987). Due to the possibility that it may promote CCK secretion (Ersperner et al., 1974) the effect of bombesin on food intake has been investigated and both bombesin (Gibbs et al., 1979) and GRP (Stein & Woods, 1982) were shown to decrease food intake in rats, although to a lesser extent than CCK. Human studies have also shown bombesin to reduce food intake in lean (Lieverse et al., 1993b; Lieverse et al., 1994a) but not obese (Lieverse et al., 1994a) subjects. There is also some question as to
whether aversive signals from bombesin cause this appetite reduction (Morley, 1987), although in humans reduced food intake has been shown at doses below the threshold for nausea (Muurahainen et al., 1993). Bombesin has since been shown to operate through different mechanisms than CCK to reduce food intake (Moran et al., 1996), and animal data suggest bombesin alone is unlikely to be a primary physiological mediator of satiety (Morley, 1987).

Somatostatin, found throughout the gastric and duodenal mucosa and in the pancreas (Johnson, 1985) has also been shown to inhibit food intake in animals (Lotter et al., 1981) and man (Lieverse et al., 1995a; Aarts et al., 1996). It is probable that somatostatin acts through vagal afferents (Levine & Morley, 1982), similar to the action of CCK (section 1.6), and possible interaction between these peptides may act to modulate satiety. A number of other peptide hormones have also been shown to have effects on food intake. Motilin, a stimulator of GI tract contractions and motility, is stimulated by the ingestion of fat (Christofides et al., 1979) but inhibited by carbohydrate and amino acid ingestion, and the peptide has been shown to increase food intake in fasted rats (Garthwaite, 1984) and in mice (Asakawa et al., 1998). The role of pancreatic hormones in the control of food intake has been extensively reviewed (e.g. Grossman, 1986; Morley, 1987). In general glucagon has been shown to decrease food intake in rat (Geary et al., 1993; Geary et al., 1997) and man (Geary et al., 1992), while insulin and glucose have been shown to have differential effects on food intake (section 1.3.2). In addition pancreatic polypeptide (PP) has been associated with decreased food intake in mice (Ueno et al., 1999), and as PP levels increase during a meal it has been suggested this may be a candidate hormone for meal termination (Morley, 1987).

While a number of hormones are implicated in appetite regulation, and it is likely that all hormonal signals interact to signal the cessation of eating, much research has been undertaken to determine primary regulators of food intake in man. Traditionally CCK has been studied as a satiety hormone, with a more recent focus upon the role of GLP-1 in satiety, and the actions of these hormones are discussed below.
1.6. Cholecystokinin

1.6.1. Background

Cholecystokinin (CCK) was isolated during the late 1920's as the substance responsible for control over gallbladder contractions (Ivy & Oldberg, 1928). Subsequently pancreozymin (PZ) was identified (Harper & Raper, 1943) and shown to stimulate the secretion of pancreatic hormones. Some time later in 1966 Jorpes and Mutt published work describing the purification of CCK from the small intestinal cells of pigs, and demonstrated that the 33 amino acid peptide caused both gallbladder contraction and release of pancreatic hormones (Jorpes & Mutt, 1966). Consequently it became apparent that CCK and PZ were the same substance, and that the hormone (CCK-PZ) played a primary role in the process of digestion. The hormone is now referred to as CCK, and subsequent research has demonstrated the presence of other molecular forms of CCK in both the CNS and circulation, ranging from short peptides (CCK-4, -5 and -8) to longer amino acid chains (CCK-39 and -58) (Calam et al., 1982; Walsh et al., 1982; Eberlein et al., 1988). Synthesis of the different forms of CCK is from preprocholecystokinin (Deschenes et al., 1984); the long chain (115 amino acid) precursor from which CCK is synthesised by post-translational processing (Gubler et al., 1984). The biological activity of each form of CCK appears to be contained in the C-terminal octapeptide with an O-sulphated tyrosine residue (Ondetti et al., 1970), and the peptide appears to be well conserved across mammalian species. However cleavage of the hormone to inactive fragments has been shown to occur through a number of peptidases (Deschodt-Lanckman et al., 1981; Zuzel et al., 1985), and the peptide has a relatively short half-life of less than 3 minutes in the circulation.

1.6.2. Distribution and localisation of CCK

Immunohistochemical techniques have shown the peptide to be primarily distributed in the upper small intestine of the gastrointestinal tract, localised within the I cells of the duodenum and jejunum (Sjolund et al., 1983). The type of CCK found in the small intestine appears to be species dependant, with CCK-58, CCK-39, CCK-33 and CCK-8 being the most abundant forms in human, dog and cat. CCK-LI (cholecystokininin-
like immunoreactivity) had also been shown in enteric nerves, smooth muscle and mucosa of the lower GI tract (Larsson & Rehfeld, 1979) and pancreatic and vagal afferent nerve fibres (Dockray et al., 1981). Investigation of blood plasma has shown the predominant circulating form of CCK to be the short chain sulphated CCK-8 molecule, although other forms are present (CCK-33, -39 and -58) (Calam et al., 1982), and the majority of plasma CCK is thought to be secreted from the intestinal mucosa (Rehfeld et al., 1982). In addition to CCK peripherally distributed throughout the small intestine, CCK-LI was detected in the CNS (Vanderhaeghen et al., 1975), the main molecular form of which is CCK-8S (Dockray, 1978). Further research has provided evidence for the release of CCK from nerve terminals suggesting action as a neurotransmitter (Schick et al., 1994), and it is probable that its action as a neurotransmitter is important in the role CCK plays in appetite.

1.6.3. CCK receptors

Animal studies have shown CCK to be widely distributed throughout the CNS in both brain and spinal cord regions (e.g. Hokfelt et al., 1988), and radioligand binding studies using different fragments of CCK from the brain and pancreas have shown two types of CCK receptor: peripheral receptors and brain receptors (Innis & Snyder, 1980). Additional work by Moran et al. (1986) demonstrated the presence of both receptors in the brain, leading to the classification of the different receptors as the subtypes CCKA: those receptors which are found mainly in the periphery but also in some areas of the CNS, and CCKB: receptors found mainly in the CNS. While CCKA receptors have a high affinity for the sulphated CCK octapeptide (CCK-8S), CCKB receptors have a high affinity not only for CCK-8S, but also the unsulphated octapeptide (CCK-8US), CCK-5 and CCK-4. However both peripheral and CNS CCKA and CCKB receptors have been shown to be identical in structure, and CCKB receptors show similarities to gastrin receptors (Wank et al., 1994). Peripheral CCKA receptors have been found in areas of known CCK action; including the pancreatic acinar cells, gall bladder and smooth muscle of the pylorus (Baldwin et al., 1998). Centrally these receptors have been shown in areas of the brain such as the hypothalamus and area postrema, although pharmacological studies suggest their presence in a number of other brain sites (Crawley, 1992). Similarly CCKB receptors
are found in a large number of regions in the brain, including the hypothalamus and cerebral cortex (Hill et al., 1992). Of the peripheral nervous system the vagus nerve has been shown to carry both CCKA and CCKb receptors on its afferent fibres (Corp et al., 1993) and as the vagus transmits information from the abdomen to the CNS it is likely that vagal signals are a primary mechanism in the role of CCK in appetite.

1.6.4. The role of CCK in appetite

The vagus nerve is the primary link between intestinal sites exposed to the breakdown products of ingested nutrients and the CNS, which ultimately mediates the control of food intake. Consequently vagal afferents have been a major focus in animal studies investigating the role of feedback signals in the control of food intake (Schwartz & Moran, 1996), and vagotomy studies have demonstrated significant changes in food intake and gastrointestinal function (Kraly et al., 1986; Schwartz et al., 1993). Due to the localisation of both CCKA and CCKb receptors on vagal afferent fibres it is likely that any control over food intake mediated through changes in circulating CCK would act, at least in part, though vagal signals.

A large number of investigations have centred upon the role of CCK in the control of food intake, and as long ago as 1937 duodenal extracts, which are likely to have contained some CCK, were shown to reduce food intake in animals (Maclagan, 1937). Much later Koopmans and colleagues (Koopmans et al., 1972) showed that intraperitoneal (IP) administration of porcine CCK inhibited food intake in fasted mice, while Sjodin (1972) reported the suppressing of food intake in dogs after administration of extracts containing CCK. Following further work investigating the appetite effects of CCK in rats (Leibling et al., 1975) and rhesus monkeys (Gibbs et al., 1976) it was suggested that CCK was mediating intestinal satiety, and a new role for the hormone as a putative satiety signal was proposed (Smith & Gibbs, 1975). Since this time the majority of animal studies investigating the role of CCK in the suppression of food intake have supported a role for peripheral CCK in satiety (table 1.1).
### Table 1.1  Summary of a selection of animal studies investigating the role of peripheral CCK in the regulation of appetite and food intake.

<table>
<thead>
<tr>
<th>Author &amp; year</th>
<th>Animal</th>
<th>Nature of study</th>
<th>Support for CCK?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koopmans et al., 1972</td>
<td>Mouse</td>
<td>IP injection of CCK against saline control in fasted animals showed effects on food intake.</td>
<td>Yes</td>
</tr>
<tr>
<td>Gibbs et al., 1976</td>
<td>Monkey</td>
<td>IV infusion of CCK and gastric preload of L-phenylalanine both produced dose related suppression of feeding.</td>
<td>Yes</td>
</tr>
<tr>
<td>Lorenz &amp; Goldman, 1982</td>
<td>Rat</td>
<td>IP injection of CCK-8 decreased meal size. The same dose had no effect on feeding behaviour when injected centrally.</td>
<td>Yes</td>
</tr>
<tr>
<td>Smith et al., 1984</td>
<td>Rat</td>
<td>IP administration of CCK-8 and the agonist ceruletide inhibited food intake. This effect was markedly reduced by bilateral abdominal vagotomy.</td>
<td>Yes</td>
</tr>
<tr>
<td>Willis et al., 1986</td>
<td>Rat</td>
<td>Peripheral and central injections of the CCK antagonist proglumide abolished the central satiety effect of CCK.</td>
<td>Yes</td>
</tr>
<tr>
<td>Bado et al., 1988</td>
<td>Cat</td>
<td>IV infusion of CCK-8 and CCK-7 inhibited sham feeding and feeding in fasted cats.</td>
<td>Yes</td>
</tr>
<tr>
<td>Moran et al., 1988</td>
<td>Rat</td>
<td>Removal of area of pyloric sphincter containing CCK receptors attenuated CCK satiety 2-3 weeks after surgery.</td>
<td>Yes</td>
</tr>
<tr>
<td>Moran &amp; McHugh, 1988</td>
<td>Rat</td>
<td>Investigation of relationship between inhibition of gastric emptying and reduction in food intake induced by CCK. GE accounts for some but not all of the variability in satiety action of CCK.</td>
<td>Yes</td>
</tr>
<tr>
<td>Canova &amp; Geary, 1991</td>
<td>Rat</td>
<td>IP injection of CCK-8 elicited a dose related decrease in meal size.</td>
<td>Yes</td>
</tr>
<tr>
<td>Schwartz et al., 1991</td>
<td>Rat</td>
<td>Effect of CCK analogue U-67827E on glucose intake with and without a gastric load. Load required to potentiate effects on food intake.</td>
<td>Yes</td>
</tr>
<tr>
<td>Reidelberger, 1992</td>
<td>Rat</td>
<td>IP administration of CCK-8 inhibited food intake. This effect was blocked by vagotomy and the CCKA receptor antagonist MK-329.</td>
<td>Yes</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Treatment</td>
<td>Effect</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>---------</td>
<td>-----------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ebenezer &amp; Parrot, 1993</td>
<td>Pig</td>
<td>The CCKA receptor antagonist A70104 dose dependently increased food intake in pigs.</td>
<td></td>
</tr>
<tr>
<td>Weatherford et al., 1993</td>
<td>Rat &amp; mouse</td>
<td>Effect of the CCK agonist JMV-180 investigated in rats and mice. Dose dependent effect on food intake through binding to low affinity receptors.</td>
<td></td>
</tr>
<tr>
<td>Cabanac &amp; Zhao, 1994</td>
<td>Rat</td>
<td>IP injection of CCK promoted negative alliesthesia in normal weight rats. Following weight loss negative alliesthesia was not observed.</td>
<td></td>
</tr>
<tr>
<td>Covasa &amp; Forbes, 1994</td>
<td>Chicken</td>
<td>IP injection of CCK-8 into free feeding animals reduced food intake. No colour aversion found.</td>
<td></td>
</tr>
<tr>
<td>Cox, 1994</td>
<td>Rat</td>
<td>Infusion of sucrose and a saline control with IP injection of CCK-8. CCK-8 promoted decreased food intake with sucrose infusion.</td>
<td></td>
</tr>
<tr>
<td>Eckel &amp; Ossenkopp, 1994</td>
<td>Rat</td>
<td>Administration of CCK decreased ingestive response to sucrose. No taste aversion was observed.</td>
<td></td>
</tr>
<tr>
<td>Ebenezer &amp; Baldwin, 1995</td>
<td>Rat</td>
<td>Infusion of antagonist (2-NAP) which is unable to cross blood brain barrier had no effect on food intake in rats.</td>
<td></td>
</tr>
<tr>
<td>Ebenezer, 1996</td>
<td>Rat</td>
<td>IP administration of CCK-8S produced a dose related suppression of water intake. Thus CCK may not have specific effects upon feeding.</td>
<td></td>
</tr>
<tr>
<td>Strohmayer &amp; Greenberg, 1996</td>
<td>Rat</td>
<td>Administration of the CCKA receptor antagonist devazepide increased food intake in male rats. No effect was seen in female rats.</td>
<td></td>
</tr>
<tr>
<td>Voits et al., 1996</td>
<td>Rat</td>
<td>IP administration of CCK-8S reduced food intake in normal but not obese rats. Higher doses reduced intake in obese rats.</td>
<td></td>
</tr>
<tr>
<td>Niederau et al., 1997</td>
<td>Rat</td>
<td>Subcutaneous injection of the CCK agonist caerulein dose dependently decreased food intake in lean &amp; obese rats. This effect was abolished in lean animals with vagal afferent blockade.</td>
<td></td>
</tr>
</tbody>
</table>
Human investigations also support a role for CCK in satiety, with the administration of exogenous CCK reported to decrease ratings of hunger and subsequent food intake whilst increasing feelings of satiety. Infusion of varying forms of the peptide have been shown to affect appetite, with CCK-33 shown to significantly decrease hunger and increase fullness in fasted lean and obese subjects (Lieverse et al., 1994b), with no differences in response between lean and obese groups. Lieverse and colleagues showed a similar response in fed lean and obese subjects (Lieverse et al., 1995b) suggesting the appetite responses to CCK can occur in both the pre and post-prandial state, and does not require a gastric load to potentiate the effect. Under similar conditions infusion of CCK-8 has also been shown to reduce appetite (Kissileff et al., 1981). Conversely while CCK-9 was shown to reduce food intake in normal weight subjects against a saline control (Schick et al., 1991), Schick and colleagues suggested a pharmacological rather than physiological effect of CCK in appetite due to concurrent reduction in water intake during the experimental period coupled with the high dose of CCK-9 required to elicit an appetite responses (500 pmol/kg/h⁻¹).

More recent work addressing the drawbacks of infusing at supraphysiological levels has shown reduced food intake with CCK-8 infusion at physiological post-prandial levels, and lends much support to a physiological role for CCK in satiety (Ballinger et al., 1995).

It has been suggested that the vagal signals mediating CCK induced satiety require some degree of gastric distension for increased fullness to be expressed, and vagal afferent signals following stimulation with CCK have been shown to be increased in the presence of a gastric load (Schwartz & Moran, 1996). Ratings of fullness were also reported to be higher during CCK-8 infusion when a gastric balloon was inflated to the highest tolerated levels, compared to CCK-8 infusion alone (Melton et al., 1992), leading to the suggestion that CCK-8 may sensitise subjects to gastric pressure through its actions on the stomach. This finding was supported by investigations showing lower food intake with CCK-8 infusion following ingestion of a gastric load (Muurahainen et al., 1991; Ballinger et al., 1995). A similar approach using IV infusion of CCK-33 without a gastric load saw no difference in food intake between the treatment and control infusions (Lieverse et al., 1993a). As no difference in dose-
related inhibition of food intake was seen between CCK-8 and CCK-33 in rats
(Melville et al., 1993), and other studies have shown an effect of CCK-33 upon
satiety (Lieverse et al., 1994b; Lieverse et al., 1995b), administration of either CCK-8
or CCK-33 would be expected to have effects upon appetite. Thus it is probable that
CCK requires a gastric load to modulate its effects upon appetite.

The majority of human investigations therefore support a role for exogenous CCK in
satiety, and animal studies have helped in elucidating the probable role of gastric
distension and vagal signals in this process. Furthermore studies with populations
who often report abnormal levels of satiety in relation to their food intake provide
further evidence of a role for CCK in satiety. For example post-prandial CCK has
been shown to peak earlier and to a greater extent in anorexic patients than normal
weight controls (Harty et al., 1991), and to be greater in anorexic than bulimic
patients and normal weight subjects (Phillipp et al., 1991). Such data provide a
physiological rationale for the increased satiety reported by patients with eating
disorders, and similarly malnourished elderly patients who often report a suppressed
appetite and consequently have poorer food intakes, were shown to have significantly
higher post-prandial CCK than both healthy age matched controls and young adult
subjects (Berthelemy et al., 1992). This provides a possible link between suppressed
appetite and early cessation of eating with CCK in this group.

To determine the physiological significance of CCK upon feeding behaviour, appetite
responses to the endogenous hormone have been investigated. L-phenylalanine (but
not D-phenylalanine) stimulates endogenous CCK release, and following the
administration of 10g L-phenylalanine test meal intake was significantly lower than
with D-phenylalanine and placebo (Ballinger & Clark, 1994), supporting a
physiological role for endogenous CCK in satiety. However, circulating CCK has
been shown to correlate poorly with ratings of hunger and satiety following nutrient
ingestion (French et al., 1993; Lieverse et al., 1993c), implying no direct relationship
between CCK and feelings of hunger or fullness. Additional studies have utilised
CCK receptor antagonists to block the action of the hormone following the
stimulation of its release with a high energy premeal. Loxiglumide, a CCKA receptor
antagonist (Makovec et al., 1985) has been shown to block the activity of CCK on
gallbladder contraction (Lieverse et al., 1995a), and simultaneous measurement of
plasma CCK using radioimmunoassay showed an increase in CCK following an
intraduodenal gastric load, providing evidence that loxiglumide was blocking the
CCKA receptors in man as opposed to preventing the release of CCK.

Although there are relatively few human appetite studies using CCK receptor
antagonists, data shows conflicting results as to the extent of the effect of endogenous
CCK on food intake, with a similar number of studies showing appetite effects
(Lieverse et al., 1994c; Matzinger et al., 1999) as those which did not (Drewe et al.,
1992; French et al., 1994; Lieverse et al., 1995c). The mechanisms through which
peripheral CCK may act are probably modulated by gastric distension and vagal
signals. Vagal afferent impulses following stimulation with CCK have been shown to
be higher with a gastric load (Schwartz & Moran, 1996), and CCK has been shown to
have a greater effect on appetite with a gastric load (Muurahainen et al., 1991; Melton
et al., 1992). As CCK is also known to regulate the rate of gastric emptying, the
relationship between CCK induced satiety and gastric emptying is also likely be of
importance. However the extent to which the gastric emptying rate modulates satiety
induced by CCK is not clear, and although it is highly probable that CCK is a satiety
hormone, additional research to elucidate mechanisms of action and the extent to
which CCK is important in day to day feeding are required.

1.6.5. CCK and gastric emptying

In addition to its probable role in the regulation of appetite, CCK has been shown to
influence the rate of gastric emptying, providing one mechanism through which CCK
may act to regulate food intake. Animal studies have shown both exogenous and
endogenous CCK to delay gastric emptying of a lipid load (Moran et al., 1993; Jin et
al., 1994; Scarpignato et al., 1996). Thus it appears that CCK is involved in the
inhibition of gastric emptying following ingestion of a fatty meal. Human studies
have also provided evidence for the regulation of gastric emptying by endogenous
CCK, with the CCKA receptor antagonist loxiglumide shown to accelerate fat induced
inhibition of gastric emptying in healthy humans (Meyer et al., 1989; Konturek et al.,
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Infusion of CCK was also shown to delay gastric emptying in healthy subjects and those with functional dyspepsia to the same extent (Cote et al., 1995). As functional dyspepsia is associated with increased post-prandial satiety the authors concluded that this appetite response was unlikely to be mediated through overt effects of CCK upon gastric emptying in these patients.

Investigation of differential gastric emptying rates between the solid and liquid phase of a mixed meal showed loxiglumide to accelerate gastric emptying of both phases of the test meal (Borovicka et al., 1996). However recent investigation of the new CCKA receptor antagonist lintitript showed different effects of this antagonist upon the emptying of solid and liquid meals, with accelerated emptying of a solid but not a liquid load (Kreiss et al., 1998). It is therefore possible that CCK may act primarily through regulation of emptying of the solid phase of a meal, and that this part of a meal has the greatest influence on satiety.

Although the mechanisms through which CCK regulates gastric motor mechanisms remain unclear, the investigation of gastric emptying by magnetic resonance imaging suggested that changes in contractile activity of the antrum may be responsible for the regulation of gastric emptying by CCK (Schwizer et al., 1997). In addition a role for antral signals in the mediation of satiety is supported by a correlation between subjective satiety ratings and the antral area as assessed by ultrasonographic measurement (Santangelo et al., 1998). Since it is also likely that vagally mediated signals are necessary for CCK to regulate gastric emptying (Schwartz et al., 1993), and vagal signals have been shown to relate to food intake (section 1.6.4), it is possible that CCK may act to regulate appetite through its effects upon gastric emptying.

1.7. Glucagon-like peptide-1

1.7.1. Identification of glucagon-like peptide-1

More recently GLP-1 has been considered as a putative satiety hormone due its central action on food intake in rodents (Schick et al., 1992). The hormone was first
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reported when gut glucagon-like immunoreactants (GLI’s) were found in intestinal extracts by Unger et al. (1961) as gut peptides of unknown structure and function which demonstrated cross reactivity with antiglucagon antisera. Following this discovery the tissue distribution of these peptides in man was assessed (Unger et al., 1966), describing high concentrations of GLI’s in L-cells of the intestinal mucosa, the greatest concentrations of which are in the lower small intestine and colon (Eissele et al., 1992). Structural identification of these glucagon-like peptides was later made by Lund et al. (1982) who detected two peptides with a similar amino acid structure to glucagon. These findings were confirmed by Bell et al. (1983b), who named the peptides glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), and found their amino acid sequence to be conserved and identical across a range of species (Bell et al., 1983a).

The presence of GLP-1 and GLP-2 in a number of mammalian species suggested the peptides should have biological significance, and expression of the proglucagon gene, from which both glucagon and the glucagon-like peptides are derived (Lopez et al., 1983; Mojsov et al., 1986; Ørskov et al., 1986) in the pancreas and intestine (Novak et al., 1987), was further suggestion of a probable physiological role. The peptides resulting from proglucagon differ in the pancreas and intestine, and while glucagon is the primary peptide produced in the pancreas, the L-cells of the small intestine produce glicentin, GLP-1 and GLP-2 (Mojsov et al., 1986; Ørskov et al., 1987; Holst et al., 1987).

The major circulating form of the peptides produced in the L-cell is the truncated form of glucagon-like peptide-1, GLP-1(7-36)amide, and this has been shown to be the major molecular form in human intestinal tissue (Kreymann et al., 1987; Ørskov et al., 1989). Glucagon-like peptide-2 was originally thought to have little or no biological significance, however recent data suggest it has trophic actions on the intestine (Drucker, 1999). A physiological role for the GLP-1(7-36)amide (GLP-1) hormone is well documented, and the peptide is a potent incretin (Zunz & La Barre, 1929), acting to potentiate the release of insulin following a meal in the presence of glucose (Kreymann et al., 1987; Nauck et al., 1993; Wang et al., 1995). Secretion of
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the peptide is seen in response to mixed meals (Ørskov & Holst, 1987; Elliott et al., 1993; Herrmann et al., 1995; Herrmann et al., 1995) and oral glucose loads (Ørskov et al., 1991). However while both oral and duodenal administration of GLP-1 result in a typical post-prandial response of raised circulating GLP-1 concentrations, intravenous glucose loads have not been shown to result in an increase of the hormone (Herrmann et al., 1995), suggesting that GLP-1 is released in response to nutrient stimulation of the intestinal mucosa rather than post-absorptive factors.

Post-prandial studies of GLP-1 have all reported an increase in circulating GLP-1 within 30 minutes of ingestion of the meal. However, when taking into consideration the position of intestinal L-cells in the lower intestine, and the probable necessity for nutrient stimuli to promote secretion of GLP-1, the speed of the post-prandial response suggests there may be an additional mechanism promoting the release of the hormone. It is also possible that neural signalling from the upper to lower small intestine following a meal promotes the release of GLP-1 prior to direct nutrient stimulation in the lower small intestine.

1.7.2. Glucagon-like peptide-1 and gastric emptying

GLP-1 has also been shown to play an active role in the regulation of gastric emptying, with an increase in circulating GLP-1 corresponding to a decrease in gastric emptying rate. Following research showing a delay in gastric emptying induced by the administration of glucagon Schjoldager et al. (1988), Wettergren et al. (1993), Young et al. (1996) and Willms et al. (1996) demonstrated a significantly decreased gastric emptying rate in rat and man respectively with the administration of intravenous GLP-1 to physiological post-prandial levels, although the mechanism of action of the hormone was unknown. This finding was supported by Schirra et al. (1997), who used a subcutaneous route of administration for GLP-1, and showed a dose-dependant delay in gastric emptying with increasing GLP-1 concentration. Schirra and colleagues noted that although GLP-1 prolonged the lag phase (the time taken to reach maximal emptying velocity) and half time of gastric emptying, the overall time to complete gastric emptying was not significantly different between treatments and the control. Consequently they suggested that GLP-1 initially inhibits
and then transiently stimulates gastric emptying rate (Houghton et al., 1988). The suppression of gastric acid secretion has also been seen with GLP-1 administration (Schjoldager et al., 1989), which could be involved in the delay of gastric emptying induced by the peptide.

Obese subjects have been shown to have lower post-prandial levels of GLP-1 than their lean counterparts following an oral carbohydrate load, suggesting that in obese subjects the release of GLP-1 is attenuated post-prandially (Ranganath et al., 1996). Recently Näslund et al. (1997) have shown that the lower post-prandial GLP-1 values seen in obese subjects following a high carbohydrate meal relate to a faster gastric emptying rate in these subjects. This faster rate of gastric emptying seen in obese persons has implications in the appetite control mechanisms of these individuals, as faster movement of food out of the stomach may result in a reduced period of satiety and a shorter delay until the onset of the next meal than in individuals with slower gastric emptying (section 1.4.1). Indeed, it has been shown that the administration of GLP-1 to obese men can slow gastric emptying and increase the period of post-prandial satiety (Naslund et al., 1998). However, GLP-1 has been shown in animal studies to have central actions upon appetite, suggesting that the hormone may have a role in eating behaviour which is not directly related to gastric emptying.

1.7.3. Glucagon-like peptide-1 and appetite

Following the identification of GLP-1-like activity in the rat brain by a number of research groups (Shimazu et al., 1987; Seifert et al., 1990; Schick et al., 1993; Göke et al., 1995; Calvo et al., 1995), and the detection of proglucagon mRNA in rat brain (Han et al., 1986), the localisation and identification of the central peptide as GLP-1(7-36)amide was made (Kreymann et al., 1989). Taken together these data suggested that there is central synthesis of the hormone as well as synthesis in the small intestine. Kreymann et al. (1989) found the highest brain concentrations of GLP-1 to be in the hypothalamus, and showed that the proglucagon processing of the hormone was similar in both the hypothalamus and rat ileum, suggesting that the proglucagon, and thus the GLP-1 product, is the same in the brain and intestines. This and additional work by the same group (Kanse et al., 1988), supported a role for GLP-
1 as a probable neurotransmitter, suggesting that the hormone has a physiological role in the mammalian central nervous system. As the hypothalamus has been shown as an area of central regulation of appetite, the possibility of a role for GLP-1, a hormone present in both the hypothalamus and the intestine, in food intake regulation was investigated. Schick et al. (1992) reported that an intracerebroventricular (ICV) injection of GLP-1 significantly attenuated feeding behaviour in fasted rats. This finding was later supported by Lambert et al. (1994), who found that ICV injection in rats dose-dependently reduced food intake in these animals, and the administration of a GLP-1 antibody produced an increase in food intake compared to controls. The implication of these data, that GLP-1 may have a central role in the development of satiety after a meal, possibly mediated by other neurotransmitters, was further investigated by Turton et al. (1996) and Tang-Christensen et al. (1996) who both found ICV administration of the hormone to suppress food intake in fasted rats. In both cases exendin (9-39), a GLP-1 receptor antagonist, blocked the inhibitory effect of GLP-1 on food intake. While both these studies showed exendin (9-39) alone to have no effect on fast-induced feeding, Turton et al. (1996) administered the antagonist to satiated rats, and found that subsequent food intake was more than doubled, suggesting that GLP-1 may have a role as a mediator of satiety in the fed rather than fasted state. Work demonstrating the GLP-1 induced inhibition of food intake in rats was replicated in chickens (Furuse et al., 1997) by comparison of the effects of ICV administration of both mammalian GLP-1 and chicken GLP-1 upon food intake in neonatal chicks. Food intake was shown to be significantly reduced following the administration of both peptides, suggesting that the conserved structure of GLP-1 seen in mammalian species is similar to other species, and consequently may be interchangeable between species. Early work had shown that the amino acid sequence of chicken GLP-1 differs from mammalian GLP-1 at only four positions (Hasegawa et al., 1990), and as both peptides exerted a suppressive effect on food intake in chickens, data suggest a conserved sequence of GLP-1 across species which is biologically active in the control of appetite. This in itself lends weight to the probability that GLP-1 is involved in appetite regulation, and led Furuse et al. (1997) to suggest that it is the N-terminus of the GLP-1 molecule, histidine, which is important in the feeding behaviour of rats and probably chickens. If this is indeed the
case, further work on GLP-1 receptor structure between species could clarify the action of GLP-1 with regards to food intake, and could lead to further areas of research for synthetic appetite suppressing peptides.

1.7.4. *Potential mechanisms through which GLP-1 may regulate appetite*

It has now been shown that peripheral GLP-1 is able to access the area postrema and subfornical organ of the brain through highly dense permeable capillaries in these regions (Ørskov et al., 1996). Although in this study peripheral GLP-1 was not shown to be able to cross the blood brain barrier and directly access the hypothalamus, the area postrema receives afferent input from both the hypothalamus and the periphery via vagal fibres, and the efferent neurons from the area postrema to hypothalamic neurosecretory neurons and vagal motor neurons could in turn be stimulated by peripheral GLP-1. More recently a similar study has shown GLP-1 to be transported across monolayers of porcine brain capillary endothelial cells (Drewe et al., 1998), at a higher rate than CCK-8, suggesting that the hormone may be able to cross the blood-brain barrier. As it has been shown that GLP-1 receptors in the brain are identical in structure to peripheral β-cell GLP-1 receptors (Wei & Mojsov, 1995) these data together suggest that peripheral post-prandial GLP-1 could act to control food intake through cerebral actions. However it is unclear whether GLP-1 would act through a combination of peripheral and central actions, or central actions alone.

These data also imply that a central response to GLP-1 is necessary for its physiological functions, and it is therefore possible that appetite effects of this hormone would need to be centrally mediated. This hypothesis is supported by Tang-Christensen *et al.* (1996), who administered GLP-1 to rats by IP injection during a study into the effect of ICV administration of the hormone. No effect on eating behaviour was found following IP administration, although a significant suppression of water intake was observed. These data suggest that peripheral administration of GLP-1 was unable to activate the hypothalamic neurons which may be necessary for the induction of satiety. However it is equally possible that concentration of the hormone given was too low to produce either a peripheral effect, or a concentration which was able to access the brain. Additionally, the reduction in water intake seen
may be translated as a weak effect on the hormone on ingestive behaviour, which at higher concentrations could affect food intake.

1.7.5. The role of GLP-1 in human appetite regulation

A natural progression from the findings of a suppressive effect of GLP-1 on food intake in animals was to investigate the effect of the hormone upon human appetite, and such a study forms part of this thesis. At the time this research was undertaken there was no published work investigating the role of GLP-1 in human appetite. However during the course of our investigations similar studies were published which showed GLP-1 to have a role in the mechanism of satiety in humans. Gutzwiller et al. (1997) intravenously infused three doses of GLP-1 in glucose against a glucose control, and found a dose dependent reduction in food intake following infusion in normal weight males. As GLP-1 and glucose act together to promote the release of insulin, it is possible that GLP-1 could exert an appetite effect through a glucostat related mechanism. Furthermore this mechanism of action may allow another incretin hormone, GIP, to act upon appetite. As GIP is released in response to nutrient absorption (Morgan, 1996), the hormone could be involved in post-ingestive mechanisms of appetite regulation. Thus the relationship between GLP-1, GIP and a glucostat requires further investigation with regard to appetite. Additional evidence of a role for GLP-1 in appetite was provided by Flint and colleagues (1998), who found increased satiety with GLP-1 infusion. These ratings were made following consumption of a breakfast meal, and it is possible that delayed gastric emptying of the meal may be responsible for this outcome. A slower rate of gastric emptying was also accompanied by a prolonged period of satiety following consumption of a test meal study in obese subjects during GLP-1 infusion (Näslund et al., 1998). Thus it is probable that any satiety action of GLP-1 operates to some extent through the effect of the hormone upon gastric emptying, although the degree to which this is the case, and the extent to which central response to GLP-1 directly influences satiety remains unknown.

Although both animal and human data do suggest an effect of GLP-1 upon appetite, a conditioned taste aversion produced by ICV GLP-1 has been reported (Thiele et al.,
1997; Van Dijk et al., 1997), implying that animals may be responding to this aversion when reducing their food intake, and not to a direct satiety effect of GLP-1. However, in these studies the taste aversion was in response to re-feeding following a second dose of GLP-1, and in randomised appetite studies this effect should be controlled for. Also it is not apparent if this taste aversion is seen following peripheral infusion of GLP-1, as used in human studies. Consequently, further evidence for a conditioned taste aversion is required before this can be said to have an influence on GLP-1 induced satiety. In addition further human appetite studies with GLP-1, and using the GLP-1 receptor antagonist exendin (9-39) are necessary before firm conclusions on the role of GLP-1 in appetite can be drawn.

1.8. Additional factors which may influence hunger and satiety in man

As mentioned previously there are a large number of psychological as well as physiological factors that may affect food intake. Although beyond the scope of this thesis it is important to recognise the range of influences in human eating behaviour, and give consideration to these influences during interpretation of appetite data.

It has been shown that the amount of food eaten in adult life could be influenced by learned eating habits during early life (Oscali & McGarr, 1978), and while this may be due to physiological adaptation to different levels of food intake, there is likely to be much involvement of learned feeding behaviour in this phenomenon. In addition the habitual diet of an individual influenced hunger ratings following a lunchtime test meal (Craig & Richardson, 1989), thus it could be said that eating behaviour in early life sets food intake levels in adult life which in turn perpetuates eating habits, as appetite is dependant to some extent on habitual food intake. Sensory specific satiety is also likely to be attributable to a combination of psychological and physiological factors, as appetite has been satisfied while an individual also tires of a particular taste (Johnson & Vickers, 1992; Vandewater & Vickers, 1996), and there is evidence that some individuals are more sensitive than others to learned flavour cues, such that their
intake reflects expected energy content of a meal rather than actual energy content (Booth et al., 1976; Tepper et al., 1991). It is possible that this mechanism is important in the aetiology of obesity.

It is also important that cognitive processes are taken into account in appetite research, as subjects who exhibit a high level of dietary restraint, emotionality or externality may react differently to preload manipulations. It has been shown that restrained eaters, those who usually exhibit a large degree of self control over food intake, eat more when presented with an identified high energy preload, and less when presented with a low energy preload (Herman & Mack, 1975). This is attributed to a breakdown in imposed self restraint, often occurring in ‘dieters’, and results in overeating due to the feeling that because restraint has been broken, the individual may as well continue to eat (Mela & Rogers, 1998). The externality theory suggests individuals to be more sensitive to external cues e.g. palatability, than internal hunger cues, and is often associated with obesity, while emotionality describes overeating in response to emotional stimuli such as fear, loneliness or rage, and the act of eating then modifies the emotional state. Thus food intake becomes a coping response rather than due to physiological needs (Mela & Rogers, 1998). As individuals who overtly exhibit these tendencies show different appetite responses than people who do not, large variation in the appetite responses of a group of randomly selected individuals may occur. When investigating the physiological controls of appetite subjects who exhibit a smaller response to external and emotional cues are required to enable any appetite effects seen to be attributed to physiological rather than psychological differences. The use of questionnaires such as the Dutch Eating Behaviour Questionnaire (Van Strein et al., 1986) can determine the extent to which a volunteer displays emotionality, externality and restraint in their eating habits. Thus the influence of these factors can be minimised during volunteer recruitment.

Additional factors without a psychological basis have also been identified as influencing satiety in man. The physical composition of a meal has been investigated with regard to its satiating properties, and data is inconclusive as to whether solid or liquid preloads are more satiating (chapter 3, section 3.1). Furthermore the lifestyle of
a subject is increasingly being recognised as a factor which may influence appetite responses, both in terms of habitual macronutrient intake (Long et al., 2000), and due to the possible influence of exercise levels upon the regulation of food intake (chapter 8, section 8.1).

The major factors which influence food intake are summarised in figure 1.2. It includes a number of physiological research areas which remain worthy of further investigation for the elucidation of appetite control mechanisms.
Figure 1.2 Diagram showing a summary of the relationships between psychological, peripheral and central factors important in the regulation of appetite. Adapted from Blundell & Tremblay (1995) and Castonguay & Stern (1990).
1.9. Aims of the current research

In order to elucidate further some of the physiological mechanisms of food intake regulation, the current research aims to investigate the roles of gastrointestinal hormones and nutrient metabolites in the regulation of satiety following nutrient ingestion in man. The role of two of the primary satiety hormones, CCK and GLP-1, will be specifically addressed. As these hormones have also been shown to regulate gastric emptying, their effect upon gastric emptying rate and the relationship between gastric emptying and satiety will also be assessed.

To achieve these aims the research has the following specific objectives:

a) To validate the self-rating scale and test meal intake methods frequently used in the investigation of appetite. The appetite responses using different types of preload and test meals used will also be specifically investigated to determine how differences in methodology may influence the appetite data obtained.

b) To investigate the role of circulating hormones and metabolites, and gastric emptying on the appetite and food intake responses following preloads of increasing fat and carbohydrate content. These factors will be simultaneously assessed to elucidate the degree to which appetite responses following manipulated preloads may be attributable to differences in hormone and metabolite responses and gastric emptying rate.

c) To determine the effects of an infusion of exogenous GLP-1 at physiological post-prandial concentrations in the regulation of short term satiety and food intake in man. As GLP-1 has previously been shown to regulate the rate of gastric emptying following a meal, gastric emptying rate will be assessed simultaneously, and any relationship between GLP-1, appetite and gastric emptying will be explored. Hormone and metabolite responses will also be measured to elucidate any relationships between GLP-1 and other hormones and metabolites in the satiety response, and to investigate the influence of GLP-1 in the post-prandial response.
d) To investigate the role of endogenous CCK in appetite and food intake regulation in humans. Using an infusion of the CCKA receptor antagonist loxiglumide, appetite and gastric emptying responses to a high fat meal will be explored. Simultaneous assessment of hormone and metabolite responses will help elucidate the mechanisms through which CCK may act upon satiety, and enable investigation of the role of CCK in the post-prandial response.
Chapter Two
# 2. Materials and methods

## 2.1. Materials

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<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
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</thead>
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<tr>
<td>Autoanalyser cups</td>
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<td>Cannulae (Y-Can 19g &amp; 21g)</td>
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<td>Fluoride Oxalate Tubes (1ml)</td>
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<td>LIP Ltd., Shipley</td>
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<td>Lithium heparin tubes (5ml, 10ml)</td>
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<td>LIP Ltd., Shipley</td>
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<td>LP4 tubes</td>
<td>LIP Ltd., Shipley</td>
</tr>
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<td>Whatman No.1 filter paper</td>
<td>Whatman International, Maidstone, Kent</td>
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<td>Aprotinin</td>
<td>Sigma Chemicals, product code A-1153</td>
</tr>
<tr>
<td>BSA Fraction V, RIA grade</td>
<td>Sigma Chemicals, product code A-7888</td>
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<tr>
<td>CCK-8 (sulphated) (26-33)</td>
<td>Bachem Ltd., product code H2080</td>
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<td>$^{125}$I CCK-8 (sulphated) radiolabel</td>
<td>Amersham Pharmacia Biotech, product code IM159</td>
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<td>Charcoal - Norit PN.5</td>
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<td>Citric acid</td>
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<td>Dextran grade C</td>
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<td>EDTA di-sodium salt</td>
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<td>Supplier Details</td>
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<td>Fisher Chemicals, product code S/2120/50</td>
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<td>Sodium azide</td>
<td>BDH Chemicals, product code 30111</td>
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<tr>
<td>Sodium hydrogen carbonate</td>
<td>Fisher Chemicals, product code S/4240/53</td>
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<td>Sodium carbonate</td>
<td>Fisher Chemicals, product code S/2920/53</td>
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<td>Sodium chloride</td>
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<td>Sodium dihydrogen orthophosphate</td>
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<td>Sodium Iodide</td>
<td>ICN Chemicals, product code 63034</td>
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<td>Sodium Metabisulphite</td>
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<td>Synthetic GLP-1 (7-36) amide</td>
<td>Peninsula Laboratories, product code 7168</td>
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<tr>
<td>Synthetic human GIP</td>
<td>Sigma Chemicals, product code G-2269</td>
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**Cobas-Mira Test Kits**

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<thead>
<tr>
<th>Test Kit</th>
<th>Supplier Details</th>
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<tbody>
<tr>
<td>Unimate 5 Glucose HK test kit</td>
<td>Roche Diagnostic Products Ltd., Welwyn Garden City, Hertfordshire</td>
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<tr>
<td>Unimate 5 Trig test kit</td>
<td>Roche Diagnostic Products Ltd., Welwyn Garden City, Hertfordshire</td>
</tr>
<tr>
<td>WAKO NEFA C ACS-ACOD test kit</td>
<td>Alpha Laboratories, Eastleigh, Hampshire</td>
</tr>
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</table>
Paracetamol Assay Kit
(Acetaminophen)

Antisera
Anti-rabbit Sac-Cel
Donkey anti-guinea pig serum
Donkey anti-rabbit serum
Guinea pig anti-insulin antisera
Normal guinea pig serum
Normal rabbit serum
Rabbit anti-CCK antisera
Rabbit anti-GIP antisera
Rabbit anti-GLP-1 antisera

Buffers
Carbonate/bicarbonate pH9.8
0.04M Phosphate pH7.4
0.4M Phosphate pH6.5
0.1M Sodium acetate pH5.0
CCK assay diluent pH 7.4
Sodium acetate (CCK) pH 3.6

Cambridge Life Sciences plc, Ely, Cambridgeshire
IDS, Tyne & Wear, product code AA-SAC1
Guildhay Antisera Ltd., Guildford
Gift from Sheila Hampton, University of Surrey
Gift from Sheila Hampton, University of Surrey
Gift from Sheila Hampton, University of Surrey
Sigma Chemicals, product code R-9133
From John Calam, RPMS, Hammersmith
Gift from Linda Morgan, University of Surrey
Gift from Linda Morgan, University of Surrey

10.6g sodium carbonate + 8.5g sodium hydrogen carbonate in 1 litre distilled water.
23g disodium hydrogen orthophosphate anhydrous + 5.97g sodium dihydrogen orthophosphate in 5 litres distilled water.
54.4g potassium dihydrogen orthophosphate + 56.8g disodium hydrogen orthophosphate anhydrous in 1 litre distilled water.
8.2g sodium acetate anhydrous in 1 litre distilled water
6.89g sodium dihydrogen orthophosphate 1-hydrate + 3.72g EDTA + 2.5g gelatin + 200mg sodium azide into 1 litre distilled water. Warm and mix.
1.02g sodium chloride + 3ml glacial acetic acid + 6.3ml 1M sodium hydroxide in 100ml distilled water.
2.2. Methods

2.2.1. Measurement of Satiety

2.2.1.1. Recruitment criteria

All volunteers for studies were asked to complete the Dutch Eating Behaviour Questionnaire (DEBQ, appendix I) during recruitment (Van Strein et al., 1986). The DEBQ determines the level of restrained, emotional and external eating for each individual (chapter 1, section 1.8), and scores of less than 3.5 are considered to indicate acceptable levels of eating behaviour. Volunteers with scores of more than 3.5 in the DEBQ were not accepted for participation in the study. In addition to the DEBQ, all participants were non dieters, and had not experienced any periods of rapid weight loss during the 2 years prior to the study.

2.2.1.2. Visual analogue scales

The assessment of hunger and satiety was made using self-rating visual analogue scales (VAS, appendix II). These scales are commonly used to rate subjective feelings in the sociological and psychological sciences, and were first described in 1921 by Hayes & Patterson. Early studies into appetite successfully implemented the use of VAS ratings for the assessment of appetite (Silverstone & Stunkard, 1968), and their use has been previously validated for subjective measures (Bond & Lader, 1974).

Throughout each study period subjects rated their feelings of hunger and satiety at regular intervals specified in the experimental protocol. Each scale consisted of a 10 cm line anchored at either end with extreme statements; e.g. the scale ‘how hungry do you feel?’ ranges from ‘not at all hungry’ to ‘as hungry as I have ever felt’ (figure 2.1). Subjects were instructed to rate themselves by marking the scale at the point which was most appropriate to their feeling at that time, and during each study individuals were reminded of the importance of using the scales in a consistent manner to improve comparability of results. Ratings on the scales were converted to a score in centimetres for statistical analysis of the appetite responses to each treatment.
Figure 2.1 Example of visual analogue scales (VAS) used to assess self-rated hunger and satiety in appetite studies. The full VAS is included as appendix II.

<table>
<thead>
<tr>
<th>How hungry are you?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
</tr>
<tr>
<td>hungry</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How full do you feel?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
</tr>
<tr>
<td>full</td>
</tr>
</tbody>
</table>

2.2.1.2.1. Adjustment for differences in the use of rating scales

As different subjects use rating scales to different extents, i.e. a small range of movement around the centre of the scale or a large range of movement using the extremes of the scale; the use of mathematical and statistical adjustment of the raw data can be implemented to reduce variation in subjective data. In this thesis adjustment for differences in baseline ratings and movement along the scale (multiplicative adjustment for baseline), and the transformation of data to the number of standard deviations from the mean each rating is (z score) have been used when the raw data shows large variation and no clear post-prandial differences.

Multiplicative adjustment for baseline

Using the following formula an adjusted score is calculated for each subjective rating, and analysed using the same technique as that used for the raw data (section 2.2.4).

\[
\text{adjusted rating} = \frac{(\text{rating} - \text{baseline rating})}{\text{baseline rating}}
\]

Transformation to z scores

The z score transforms each rating to the number of standard deviations that the value is from the mean using the formula:

\[
\text{z score} = \frac{(\text{rating} - \text{mean of subject ratings})}{\text{SD of subject ratings}}
\]

Z scores are analysed using the same technique as that used for the raw data (section 2.3).
2.2.1.3. Food preference checklist

Food preference checklists were also given to subjects as a measure of appetite, and required the subject to judge how many food items they would like to eat at any given time (Hill et al., 1995)(appendix III). This menu-choice procedure used was first reported by Fuller in 1970 who gave subjects a list of 15 items on which to rate both pleasantness and their desire to eat each item. The ratings used in these studies are those devised by Hill et al. (1986), and subjects were instructed to select from a list of 30 foods those which they would like to eat at a particular time (figure 2.2). Subjects were instructed to consider each food item individually rather than build a meal, and data was analysed in terms of the number of items and total energy selected. This provides an additional hunger assessment, and the method has proved sensitive to experimental manipulation of caloric density, as both the number of items selected and total energy selected correlate significantly with ratings of hunger and satiety (Hill et al., 1995).

Figure 2.2 Example of items included on the food preference checklist (FPC) used to assess hunger in appetite studies. The full FPC is included as appendix III.

Please indicate which of the food items below you would like to eat at this moment. Consider each item in turn and independently from the other items - you are not being asked to construct a menu from these foods.

- A medium sized roast chicken breast
- A small baked potato
- A medium portion of boiled rice
- A slice of melon
- A plain omelette (2 eggs)
- A large portion of tuna (canned in brine)
- A medium sized bowl of cornflakes with semi-skim milk & sugar
- Two slices of salami
- A large banana
During each investigation subjects were asked to complete both the visual analogue scale and the food preference checklist at the same time and at regular intervals. From herein these assessments together will be termed the “VAS ratings”.

2.2.1.4. *Ad libitum* buffet test meal

A widely used method of investigating appetite response is to provide subjects with a test meal from which energy and macronutrient intakes are recorded by weighing food items before the meal is offered to the subject, and re-weighing them when the subject has finished eating (Rogers, 1993). Test meal intake in the investigations described in chapters 3-6 and 8 of this thesis was measured by offering subjects a buffet style meal consisting of a range of familiar foods. Subjects were instructed to eat as little or as much as they liked from this selection until feeling comfortably full, and intake from the buffet meal was monitored. During consumption of the test meal subjects were separated from one another to minimise the effects of social interaction on food intake, which has been shown to influence the amount eaten (de Castro & Brewer, 1992).

Before participation in a study subjects were provided with a list of food choices, and asked to rate foods in order of preference. They were given their second or third choice of food in the buffet test meal in order to minimise over consumption due to the free availability of palatable food. The first investigation carried out for this thesis (described in chapter 4) used a highly palatable buffet with a large amount of available energy (table 2.1), and it was observed that subjects ate significantly more than their usual lunchtime intake with this buffet test meal. Consequently the second investigation (described in chapter 6) used a smaller number of the same food types as those used in chapter 4. As high levels of intake were also observed during this investigation the palatability of the buffet test meal was reduced for subsequent investigations (described in chapters 3,5 and 8), and use of a single food choice test meal compared to the buffet style test meal was investigated (chapter 3, section 3.4). Following this study we adopted use of a single food choice test meal (described in
chapter 3, section 3.4.3.2.2) for the final investigation in this series of experiments (chapter 7).

Table 2.1  Foods included in the buffet style test meal given to subjects following a preload.

<table>
<thead>
<tr>
<th>Food Offered</th>
<th>Amount Offered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chapter 4</td>
</tr>
<tr>
<td>Sandwiches: choose 2 fillings*</td>
<td></td>
</tr>
<tr>
<td>turkey slice</td>
<td>12 x ¼ of each filling</td>
</tr>
<tr>
<td>grated cheese</td>
<td>selected</td>
</tr>
<tr>
<td>ham</td>
<td></td>
</tr>
<tr>
<td>tuna</td>
<td></td>
</tr>
<tr>
<td>Biscuits: choose 1 from</td>
<td></td>
</tr>
<tr>
<td>Chocolate digestives, Hobnobs</td>
<td>8 biscuits</td>
</tr>
<tr>
<td>Lincoln, Rich tea, Fruit shortie, All butter, Almond shortie</td>
<td>-</td>
</tr>
<tr>
<td>Cakes: choose 1 from</td>
<td></td>
</tr>
<tr>
<td>Chocolate mini rolls, Lemon slices, Almond slices</td>
<td>8 x ½ cake</td>
</tr>
<tr>
<td>Crisps: choice of flavour (25g packet)</td>
<td>2 packets</td>
</tr>
<tr>
<td>Pot dessert: choice of flavour</td>
<td></td>
</tr>
<tr>
<td>Fruit fool (150g pot)</td>
<td>1 pot</td>
</tr>
<tr>
<td>Fruit yoghurt (200g pot)</td>
<td>-</td>
</tr>
<tr>
<td>Fruit: choose from</td>
<td></td>
</tr>
<tr>
<td>Orange, grapes</td>
<td>1 orange or 200g grapes</td>
</tr>
<tr>
<td>Average available energy (kJ)*</td>
<td>16720</td>
</tr>
</tbody>
</table>

* Subjects were also provided with salad garnish and pots of mayonnaise and mustard dressing from which they could eat ad libitum. These items were weighed before and after consumption and included in the calculation of energy intake.

†Average available energy calculated from the mean energy available from each food selection. Actual available energy varies with subject selection, but identical food items are offered throughout the study once subjects have made their food choice.

All food items were weighed and counted before and after consumption of the buffet test meal to allow the calculation of energy and macronutrient intake. In addition to
total food intake data, quotients were calculated to assess the change in hunger satiety ratings during consumption of the *ad libitum* test meal in relation to energy intake from the meal (Green *et al.*, 1997). The satiety quotient (SQ) is determined by the following equation:

\[
\text{SQ} = \frac{\text{post meal rating} - \text{baseline rating}}{\text{test meal intake}}
\]

and the hunger quotient (HQ) as:

\[
\text{HQ} = \frac{\text{baseline rating} - \text{post meal rating}}{\text{test meal intake}}
\]

Therefore SQ and HQ increase either as food intake decreases in relation to the change in ratings before and after consumption, or as the change in ratings increases in relation to the amount of food consumed during the test meal.

### 2.2.2. Measurement of gastric emptying

Gastric emptying was measured by electrical impedance epigastrography (EIE) using methods established by McClelland & Sutton (1985). EIE utilises a small alternating current applied to the abdominal area, and changes in the conductivity of the stomach as a meal is ingested and emptied are assessed by changes in impedance of the electrical current. As the conductivity of the abdominal tissue remains constant, changes in conductivity are therefore attributable to changes in the stomach contents. Consequently the impedance (total resistance) measured is proportional to the conductivity of stomach contents plus surrounding tissue at a given time, and as tissue conductivity is constant the rate of change in impedance following a meal can be used to assess rate of stomach emptying.

In order to measure epigastric impedance four pairs of voltage sensing electrodes and two pairs of current injecting electrodes were attached to the abdominal area of each subject (over the stomach and lower back). This provided impedance data from 4 sets of electrodes (termed channels), from which a gastric emptying curve was obtained for comparison and analysis of the emptying rates of different test meals. An average of the data from each channel was used for analysis. The percentage of each meal remaining in the stomach was determined post-prandially at regular time intervals, and the half time of gastric emptying (T50), defined as the time taken to return to 50%
of the peak signal produced in response to ingestion of a test meal, was determined from this data.

Due to the nature of the EIE measurement it can only be used to assess emptying of homogenous liquid test meals, and the method assumes that the meal is emptying at a constant rate. Subjects were required to remain in a semi-supine position, making as little movement as possible, for the duration of the measurement period. This prevented alterations in conductivity measurements which may result from abdominal muscle contraction and a change in the path of electrical current.

Although this method has been reported to compare well with other methods of assessing gastric emptying (Sutton et al., 1985; Sutton, 1987) more recent data suggests that although gastric emptying times from EIE are comparable within individuals, the gastric half emptying times assessed by this method are not quantitatively similar to those obtained by the recognised standard of gastric emptying measurement, scintigraphy (Giouvanoudi et al., 2000). In the current investigations the T50 data is therefore used only to rank the rate of gastric emptying of test meals, and not to provide quantifiable data on true gastric emptying times.

All gastric emptying measurements, formulation of emptying curves and calculation of T50’s in these investigations were performed by A. Giouvanoudi and W.B. Amaee of the Physics Department at the University of Surrey.

2.2.3. Laboratory analysis

2.2.3.1. Collection of blood samples

Blood samples were drawn at regular intervals during the investigation period (see study protocols for further details on blood sampling times). Samples were decanted into 10 ml lithium heparin tubes for the analysis of TAG, NEFA, insulin, and GIP, 5 ml lithium heparin tubes with additional aprotinin as a preservative (200 KIU/ml blood) for the analysis of GLP-1, and 1 ml fluoride oxalate tubes for the analysis of glucose. For CCK analysis 4 ml of blood was collected into 5 ml glass tubes
containing 1 ml sodium acetate buffer. Following collection tubes were kept in an ice bath to reduce degradation. Whole blood was then spun in a refrigerated centrifuge at 897g (3000 rpm) for 10 min. After centrifugation the plasma was separated from red cells using a plastic pasteur pipette, and stored in LP3 tubes, frozen at -20°C for subsequent analysis.

2.2.3.2. Preparation of quality control samples for use in plasma assays
Plasma glucose, triacylglycerol and non-esterified fatty acids were analysed using automated enzymatic methods (see below). Quality control (QC) samples for these analyses were obtained from Randox Laboratories Ltd, County Antrim, UK, and control serum was supplied with mean, minimum and maximum levels for acceptance. These QC’s were used in all automated analysis.

Quality control samples for use in the radioimmunoassays were prepared at the University of Surrey. Fasted and post-prandial blood samples were taken from human volunteers and spun in a refrigerated centrifuge at 897g (3000 rpm) for 10 min. Plasma from these samples was then decanted and pooled to provide QC’s in the lower and upper range of the standard curve. Ten samples from each batch of QC’s were run against a standard curve to determine the range of acceptable values for the batch.

2.2.3.3. Plasma glucose analysis
Plasma glucose was analysed by an automated enzymatic colorimetric method on the Cobas Mira Biochemical Analyser (Roche Products Ltd., Welwyn Garden City, Hertfordshire). The Unimate 5 Glucose HK test kit was used (Roche Products Ltd.) which utilises the hexokinase and glucose-6-phosphate dehydrogenase catalysed reactions of glucose and glucose-6-phosphate to produce NADH. The concentration of NADH formed is directly proportional to the amount of glucose in the sample, which is determined by measuring the absorbance of NADH at 340 nm.
Chapter 2

hexokinase

\[
\text{D-glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{D-glucose-6-P} + \text{ADP}
\]

\[
\text{D-glucose-6-P} + \text{NAD}^{+} \xrightarrow{\text{G-6-P DH}} \text{D-gluconate-6-P} + \text{NADH} + \text{H}^{+}
\]

Quality control samples were included at the beginning and end of each assay. Using QC values, the inter-assay coefficient of variation was calculated at 1.3%, and the intra-assay variation was 2.0%.

2.2.3.4. Plasma triacylglycerol analysis

Plasma triacylglycerol (TAG) concentrations were also analysed by an automated enzymatic method on the Cobas Mira Biochemical Analyser using the Unimate 5 Trig test kit (Roche Products Ltd.). This method is a colorimetric test which ultimately measures the oxidative coupling of 4-chlorophenol and 4-aminophenazone to a red coloured quinoneimine derivative in the presence of hydrogen peroxide. The hydrogen peroxide is proportional both to the amount of colour produced and to the concentration of triacylglycerol in the sample, and is produced by the reactions outlined below.

\[
\text{LPL} \quad \text{Triacylglycerol} \xrightarrow{\text{LPL}} \text{glycerol} + \text{fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-P} + \text{ADP}
\]

\[
\text{Glycerol-3-P} + \text{O}_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + \text{H}_2\text{O}_2
\]

LPL = Lipoprotein lipase
GPO = Glycerol-3-phosphate oxidase

The colour intensity of the quinoneimine derivative is therefore proportional to triacylglycerol concentration, which is determined by measuring absorbance at 500 nm.
Chapter 2

Quality control samples were included at the beginning and end of each assay, and both the inter and intra assay CV were 2.0%.

2.2.3.5. Plasma non-esterified fatty acid analysis

Plasma non-esterified fatty acid (NEFA) was measured on the Cobas Mira Biochemical Analyser using the WAKO NEFA C ACS-ACOD method test (WAKO Chemicals GmbH, Neuss, Germany). Also a colorimetric reaction this method utilises the enzymatically catalysed production of hydrogen peroxide from NEFA in the reaction below.

\[
\text{Acyl-CoA synthetase} : \quad \text{NEFA} + \text{ATP} + \text{CoA} \rightarrow \text{acyl-CoA} + \text{AMP} + \text{PPi} \\
\text{Acyl-CoA oxidase} : \quad \text{Acyl-CoA} + \text{O}_2 \rightarrow 2,3\text{-trans-enoyl-CoA} + \text{H}_2\text{O}_2
\]

The hydrogen peroxide produced then reacts with 3-methyl-N-ethyl-(β-hydroxyethyl)-aniline (MEHA) to form a purple adduct, and the concentration of this adduct is proportional to the concentration of NEFA in the sample. Its absorbance is measured at 550 nm.

\[
\text{Peroxidase} : \quad \text{H}_2\text{O}_2 + \text{MEHA} + \text{4-aminoantipyrine} \rightarrow \text{Purple adduct}
\]

Quality control samples at the beginning and end of each assay were used to calculate the inter and intra assay CV. Coefficients of variation were 4.9% and 5.6% respectively.

2.2.3.6. Plasma paracetamol analysis

Plasma paracetamol was measured on the Cobas Mira Biochemical Analyser using the Acetaminophen Assay Kit (Cambridge Life Sciences plc, Ely, Cambridgeshire). The enzymatic reaction cleaves the paracetamol molecule to yield p-aminophenol. This compound then reacts with o-cresol in ammoniacal copper solution to produce a blue colour. The absorbance of this blue colour is measured at 615 nm. Standards for
the assay were supplied with the assay kit, and lay within the specified range in all assays.

2.2.3.7. Plasma Insulin analysis

Plasma insulin concentrations were measured by radioimmunoassay established at the University of Surrey (Hampton & Withey, 1993). The assay uses antisera raised in guinea pig against porcine insulin conjugated to ovalbumen. This cross reacts 100% with human insulin, 61% with des 31-32 biosynthetic proinsulin, and less than 0.01% with either plasma C-peptide or proinsulin. Insulin radiolabelled with $^{125}$I was produced at the University of Surrey using the method described in section 2.2.3.9, and was used as a tracer in this assay. The separation of the bound and free insulin is by the addition of a double antibody plus polyethylene glycol method. Natural insulin extracted from human pancreas was used as the standard, and was obtained from The National Institute for Biological Standards and Controls.

Analysis was performed at 4°C and all samples were assayed in duplicate. Plasma samples were defrosted at the time of assay and centrifuged at 897g (3000 rpm) for 10 minutes at 4°C to spin down any fibrin which may otherwise interfere with accurate pipetting. An assay diluent of 0.04M phosphate buffer (pH 7.4) containing 0.5% w/v BSA was used throughout the assay, and was added to all tubes (table 2.2). Standards ranging from 1500 pmol/l to 23 pmol/l were prepared by double dilution from previously made human standards, and 50μl added to standard curve tubes using positive displacement pipettes. Charcoal stripped serum (CSS, section 2.2.3.10) (50μl) was then added to the standard curve tubes, and 50μl of sample plasma was added to sample tubes and one non specific binding (NSB) tube. An NSB tube was set up in duplicate for each subject and treatment condition within the assay, and determines any binding of the label to the assay tubes used. Positive displacement pipetting was used for all samples. Anti-insulin antiserum was diluted 1:15000 (from stock) in assay diluent and 100μl added to each tube except NSB tubes. Tubes were then vortexed and incubated overnight at 4°C.
The $^{125}\text{i}$ iodine label was prepared by Sheila Hampton at the University of Surrey using the chloramine-T method of iodination (section 2.2.3.9). The label was diluted to 10,000 counts per minute (cpm) per 100µl and added to all assay tubes. Total tubes containing only 100µl of label were also set up. Tubes were vortexed and incubated overnight at 4°C.

On the last day of the assay normal guinea pig serum (NGPS) was diluted 1:200 with assay diluent, and donkey anti-guinea pig serum (DAGP) was diluted 1:16 with assay diluent. One hundred microlitres of each antibody and 700µl of 4% w/v polyethylene glycol (PEG) were added to each tube to facilitate separation of the bound and free antibody. All tubes were vortexed and incubated at 4°C for 2 hours before centrifugation at 623g (2500 rpm) for 30 minutes. The supernatant was aspirated under vacuum, and the remaining pellet containing the bound insulin counted on a gamma counter for 2 minutes per sample (Wizard 1470, Wallac International, Finland). Each sample was read against the standard curve by an automated program on the Wizard 1470 to determine the insulin concentration of each sample.

Low (fasting) and high (post-prandial) value quality control samples were included at the beginning and end of each assay. The high QC’s showed an inter-assay CV of 10.7% and an intra-assay CV of 5.7%. CV calculated for the lower end of the sample range from the low QC’s were similar at 8.4% and 4.2% respectively.
Table 2.2 Summary of insulin radioimmunoassay protocol

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Tubes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Totals</td>
<td>NSB</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay</td>
<td>-</td>
<td>350μl</td>
</tr>
<tr>
<td>Diluent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CSS</td>
<td>-</td>
<td>50μl</td>
</tr>
<tr>
<td>QC Plasma</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample Plasma</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plasma Antisera</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Vortex all tubes and incubate for 24h at 4°C*

| Day 2          |       |    |     |    |    |    |     |        |
| Label          | 100μl| 100μl| 100μl| 100μl| 100μl| 100μl| 100μl| 100μl   |

*Vortex all tubes and incubate for 24h at 4°C*

| Day 3          |       |    |     |    |    |    |     |        |
| NGPS           | -     | 100μl| 100μl| 100μl| 100μl| 100μl| 100μl| 100μl   |
| DAGP           | -     | 100μl| 100μl| 100μl| 100μl| 100μl| 100μl| 100μl   |
| 4% PEG         | -     | 700μl| 700μl| 700μl| 700μl| 700μl| 700μl| 700μl   |

*Vortex all tubes and incubate for 2h at 4°C. Centrifuge at 2500 rpm for 30min.*

2.2.3.8. Plasma GLP-1 analysis

Plasma glucagon-like peptide-1 (GLP-1) concentrations were measured by a radioimmunoassay established at the University of Surrey, first described by (Elliott et al., 1993), and adapted by (Norris, 1997). The assay uses antisera raised in rabbit against synthetic human GLP-1(7-36) amide conjugated to bovine serum albumin. This is specific for the C-terminal amidated form of glucagon-like peptide-1, and cross reacts 100% with GLP-1 (7-36) amide but less than 0.2% with either GLP-1 (7-37) and GLP-1 (1-37). Cleavage of the GLP-1 molecule by the enzyme DPP IV yields the biologically inactive (9-36) peptide. Due to its C-terminal this peptide also cross reacts with the assay antisera, thus both inactive and active GLP-1 are measured with this assay. GLP-1 radiolabelled with $^{125}$I was produced at the University of Surrey.
using the method described in section 2.2.3.9 and was used as the tracer in this assay. The separation of the bound and free GLP-1 is by addition of Sac-Cel, a donkey antirabbit solid phase second antibody in cellulose suspension. Standards used were prepared from synthetic human GLP-1(7-36) amide (Peninsula Laboratories), freeze dried and stored at -20°C until used in the assay. The standards were reconstituted and double diluted in charcoal stripped serum for each assay.

As with the insulin assay, analysis was performed at 4°C throughout and samples were assayed in duplicate. Plasma samples were defrosted at the time of assay and centrifuged at 897g (3000 rpm) for 10 minutes at 4°C. An assay diluent of 0.04M phosphate buffer (pH 6.5) containing 0.5% w/v HSA and aprotinin at 50,000 KIU/100 ml was used throughout the assay, and was added to all tubes (table 2.3). Standards ranging from 160 pmol/l to 5 pmol/l were prepared by double dilution with CSS from previously made standards, and 200µl was added to standard curve tubes. Plasma samples (200µl) were added to sample tubes and one NSB tube for each treatment condition. Anti-GLP-1 antiserum was diluted 1:6000 (from stock) in assay diluent, and 100µl added to each tube except NSB tubes. All tubes were then vortexed and incubated overnight at 4°C.

The 125iodine label was prepared at the University of Surrey (section 2.2.3.9). The label was diluted to 5,000 cpm per 100µl and added to all assay tubes. Total tubes containing only 100µl of label were also set up. All tubes were then vortexed and incubated for 48 hours at 4°C.

On the last day of the assay 100µl of anti-rabbit Sac-Cel was added to each tube. All tubes were vortexed at 15min intervals for one hour, and kept at room temperature for that hour. After 60 minutes 1 ml of distilled water was added to each tube, and all tubes were centrifuged at 897g (3000 rpm) for 30 minutes. The supernatant was aspirated under vacuum, and the remaining pellet containing the bound GLP-1 counted on a gamma counter for 2 minutes per sample (Wizard 1470, Wallac International, Finland).
## Table 2.3 Summary of GLP-1 radioimmunoassay protocol

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSB Std</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Zero Std</td>
<td>100µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Std</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>QC NSB</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Sample</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Tubes</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NSB Sample</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
<tr>
<td>Sample</td>
<td>200µl</td>
<td>100µl</td>
<td>100µl</td>
</tr>
</tbody>
</table>

**Assay**

Day 1:
- 200µl lOOµl lOOµl 200µl lOOµl 200µl 100µl
- Vortex all tubes and incubate for 24h at 4°C

Day 2:
- 100µl 100µl 100µl 100µl 100µl 100µl 100µl 100µl
- Vortex all tubes and incubate for 48h at 4°C

Day 4:
- 100µl 100µl 100µl 100µl 100µl 100µl 100µl 100µl

Vortex every 15min for 1h at room temp. Add 1ml of distilled water to each tube & centrifuge at 3000rpm for 30min.

High and low quality control samples were included at the beginning and end of each assay. Inter and intra assay CV calculated from the high QC values were 13.2% and 8.0% respectively. CV from the low QC's were 6.3% and 2.9% respectively.

### 2.2.3.9. Plasma GIP analysis

Glucose dependant insulinoertropic polypeptide (GIP) concentrations were measured by radioimmunoassay established at the University of Surrey first described by Morgan et al. (1978). The assay uses antisera raised in rabbit against purified natural porcine GIP conjugated to ovalbumen. This antisera cross reacts 100% with human GIP, and has negligible cross reactivity with other hormones (Morgan et al., 1978). Radiolabelled GIP produced at the University of Surrey using the method described in
section 2.2.3.9 was used as the tracer, and the separation of the bound and free peptide was by addition of a double antibody plus polyethylene glycol method. Synthetic human GIP (Sigma Chemicals) was used to make pre-prepared GIP standards which had been freeze dried and stored at -20°C. The standards were reconstituted and double diluted with assay diluent for use in each assay as described below.

Plasma samples were assayed in duplicate at 4°C, and defrosted at the time of assay. Centrifugation at 897g (3000 rpm) for 10 minutes spun down any fibrinogen in the samples which may have interfered with accurate pipetting. An assay diluent of 0.04M phosphate buffer (pH 6.5) containing 0.5% w/v HSA and aprotinin at 50,000 KIU/100 ml was used throughout the assay, and diluent was added to all tubes (table 2.4). Standards ranging from 800 pmol/l to 25 pmol/l were prepared by double dilution in assay diluent from previously made standards, and 100μl was added to the appropriate standard curve tubes. CSS (100μl) was then added to the standard curve tubes, and 100μl sample plasma was added to sample tubes and one non specific binding (NSB) tube. Anti-GIP antiserum was diluted 1:9000 (from stock) in assay diluent, and 100μl added to each tube except NSB tubes. All tubes were then vortexed and incubated overnight at 4°C.

The 125iodine label was prepared at the University of Surrey (section 2.2.3.9) and was diluted to 5,000 cpm per 100μl for addition to the assay tubes. Total tubes containing only 100μl of label were also set up, and all tubes were vortex mixed and incubated for 48 hours at 4°C.

On the last day of the assay normal rabbit serum (NRS) was diluted 1:135 with assay diluent, and donkey anti-rabbit serum (DAR) was diluted 1:16 with assay diluent. Fifty microlitres of each antibody and 100μl 14% w/v polyethylene glycol (PEG) was added to each tube to facilitate separation of the bound and free antibody. All tubes were vortexed and incubated at 4°C for 2 hours before centrifugation at 623g (2500 rpm) for 30 minutes. The supernatant was aspirated under vacuum, and the remaining pellet containing the bound GIP counted on a gamma counter for 2 minutes per sample (Wizard 1470, Wallac International, Finland).
High and low QC samples were included at the beginning and end of each assay, and the CV's calculated from the high QC's were 2.1% and 4.1% respectively for inter and intra assay variation. Low QC values gave slightly higher CV's of 5.6 and 5.9% for inter and intra assay variation at the lower end of the standard curve.

| Table 2.4 Summary of GIP radioimmunoassay protocol |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Reagents** | **Tubes** |
| | Totals | NSB Std | Zero Std | Std | NSB QC | QC | NSB Sample | Sample |
| **Day 1** | | | | | | | | |
| Assay | - | 300µl | 200µl | 100µl | 300µl | 200µl | 300µl | 200µl |
| Diluent | | | | | | | | |
| Standard | - | - | - | 100µl | - | - | - | - |
| CSS | - | 100µl | 100µl | 100µl | - | - | - | - |
| QC Plasma | - | - | - | - | 100µl | 100µl | - | - |
| Sample | - | - | - | - | - | 100µl | 100µl | |
| Plasma | - | - | 100µl | 100µl | - | 100µl | - | 100µl |
| Antisera | - | - | - | - | - | - | - | - |
| **Day 2** | | | | | | | | |
| Label | | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl |
| **Day 4** | | | | | | | | |
| NRS | - | 50µl | 50µl | 50µl | 50µl | 50µl | 50µl | 50µl |
| DAR | - | 50µl | 50µl | 50µl | 50µl | 50µl | 50µl | 50µl |
| 14% PEG | - | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl | 100µl |

*Vortex all tubes and incubate for 24h at 4°C*

2.2.3.10. Iodination of GIP and GLP-1

The peptides GIP and GLP-1 were iodinated with $^{125}$iodide using the chloramine-T method of iodination described by Kwasowski (1986) and adapted from Greenwood & Hunter (1963). Approximately 24 hours prior to iodination 3g of Sephadex G-15 beads were mixed with 15 ml of 0.1M sodium acetate buffer (pH 5.0) to swell.
overnight. On the day of the iodination the Sephadex/sodium acetate mixture was degassed with a suction pump to remove any excess air. An elution column was set up using a plastic disposable column to which approximately 30 cm of 1 mm PVC tubing was attached at the bottom tip, and filled with swollen Sephadex taking care to avoid any banding or air pockets. Excess sodium acetate buffer was allowed to drain from the column via the PVC tubing. Protein elution buffer was prepared using 50 ml of 0.1M sodium acetate pH 5.0 to which 0.5% w/v HSA and 5000 KIU/ml aprotinin were added. Fifteen millilitres of this buffer was then passed through the column, and the column stopped before it ran dry. Sodium metabisulphite (10 mg) and chloramine-T (7.5 mg) were each dissolved in 5 ml of 0.4M phosphate buffer (pH 7.4) and either 3 μg of GIP or 5 μg of GLP-1(7-36) amide dissolved in 10 μl of 0.4M phosphate buffer (pH 7.4) immediately prior to the iodination. The dissolved peptide was placed in an autoanalyser cup for iodination.

The iodination procedure was carried out in a radiochemical laboratory behind lead shielding. The elution column was clamped in place, and the autoanalyser cup positioned behind the lead shielding. One millicurie (10 μl) of $^{125}$sodium iodide ($^{125}$NaI) was added into the bottom of the autoanalyser cup. Chloramine-T (10 μl) was then added to start the reaction between the peptide and $^{125}$NaI, and all reagents were mixed with a pipette for 15 - 20 seconds. The reaction time was reduced if ambient temperature exceeded 20°C. At the end of the reaction time 20 μl of sodium metabisulphite was added to the autoanalyser cup and mixed with the reagent to stop the reaction. Two hundred microlitres of protein elution buffer was also added and the contents of the autoanalyser cup layered onto the elution column using a disposable pipette, taking care to avoid air bubbles. A fraction collector was used to collect approximately 20 fractions of eluent into LP4 tubes, at 10 drops per fraction, giving a total volume of approximately 380 μl per fraction. Protein elution buffer was layered onto the column to allow elution of both peptide-bound and free $^{125}$iodide into LP4's, and the column was not allowed to run dry.

A 10 μl sample of each fraction was taken into LP3 tubes and counted for 10 seconds on a gamma counter (Wallac 1260 Multigamma II, Wallac International) to determine
which fractions contained the $^{125}$iodine labelled peptide. Figure 2.3 illustrates a successful iodination, with 2 visible peaks. The first peak of radioactivity is the iodinated peptide, while the second is the unbound $^{125}$iodide. The fraction containing the iodinated peptide and those either side of it were retained for use in the radioimmunoassays, and the remaining fractions were discarded in accordance with government legislation on disposal of radioactivity. The chosen fraction were stored as 20μl aliquots in 100μl 10% w/v HSA, and frozen at -20°C until required. At the time of assay the label was affinity purified and diluted with assay diluent to give 5000 cpm/100μl.

Figure 2.3 Example of a successful GLP-1 iodination. The first peak to be eluted contains the iodinated peptide, the second peak is free iodide.

Iodination of insulin was by a similar method and performed at the University of Surrey by Dr. Sheila Hampton.

2.2.3.11. Affinity purification of GIP and GLP-1(7-36) amide radiolabel

The affinity purification of each label was carried out using a plastic disposable column containing small pore glass beads covalently bound to the low avidity antibody for each hormone. The storage buffer (0.1M carbonate / bicarbonate buffer pH 9.8) was drained from the column, and the column washed with 10 ml of 0.3% v/v
HC1, which was passed through under pressure from a syringe, and 20 ml of assay diluent which was allowed to drain through at its own pace. The column was stoppered just before it ran dry, and aliquots of the radioactive label were thawed and diluted with 3 ml of assay diluent. The label was then layered onto the column, and the column was capped and inserted into a boiling tube to roll mix for 30 minutes. During this time a 5 ml glass vial used for collecting the purified label was filled with assay diluent and also roll mixed, coating the vial with diluent to reduce binding of the label to the vessel.

After 30 minutes the column was re-clamped and the beads allowed to settle before washing with 20 ml of distilled water. The diluent was washed from the glass vial, and the purified label eluted into the vial with 5 ml 0.3% HCl. The column was then washed with 10 ml each of distilled water and carbonate / bicarbonate buffer, and stored in carbonate/bicarbonate buffer at 4°C.

One hundred microlitres of the purified label was counted on a gamma counter to calculate the dilution factor required to give final counts of 5000cpm/100μl, and the label was diluted appropriately with assay diluent for addition to the assay.

2.2.3.12. Preparation of charcoal stripped serum

Charcoal stripped serum (CSS) was used in the standard curve of the insulin, GLP-1 and GIP RIAs. Plasma proteins cause depression of the binding of the radiolabel to the antiserum in unknown samples and use of CSS in the standard curve matches for this effect. The hormone free CSS was prepared in large batch quantities prior to the assay by taking approximately 300 ml of fasting blood from a number of volunteers and leaving it to clot overnight at 4°C. The serum was then decanted from each clot, all serum pooled, and the total volume measured.

Agarose coated charcoal was previously prepared by the addition of 100g charcoal to 25g agarose dissolved in 500 ml of distilled water and heated to 70°C in a water bath. After the addition of charcoal, the solution was mixed thoroughly, cooled to 50°C, and poured into 1L of acetone. This mixture was stirred and then filtered through
Whatman No.1 filter paper to remove excess liquid. The resultant charcoal residue was left to dry overnight. The agarose coated charcoal was then 'de-fined' to remove any fine dust-like particles of charcoal which may interfere with the assay. The defining process involves washing the charcoal 2 or 3 times in distilled water to remove suspended particles, and drying overnight at 37°C. Preparation of the charcoal should be completed at least 24h prior to charcoal stripping of serum to allow the charcoal time to dry fully.

The prepared agarose coated charcoal was added to the pooled serum at 25g per 200ml, and stirred overnight at room temperature. The charcoal/serum solution was then centrifuged at 11400g (10000 rpm) for 1h and the supernatant removed and re-spun under the same conditions. The final supernatant was filtered with Whatman No.1 filter paper to remove any fine pieces of charcoal. A small sample (1.5 ml) of the CSS was spun at 11400g (10000 rpm) for 10 minutes in a bench top microcentrifuge to check for the presence of fine particles, and if necessary the serum was re-filtered. The CSS was then divided into aliquots and stored at -20°C.

2.2.3.13. Plasma CCK analysis

Plasma cholecystokinin (CCK) was analysed by radioimmunoassay adapted from the method developed at the Royal Postgraduate Medical School (RPMS), Hammersmith (Beardshall et al., 1992). The assay uses Dino-7 antisera raised in rabbits and supplied by John Calam & Mark Jordinson, RPMS, Hammersmith which cross reacts 100% with the human sulphated CCK-8 fragment (Jordinson et al., 1996). This antisera has some cross reactivity with CCK-33 and the peptide gastrin, and ethanol extraction of the plasma prior to analysis aims to minimise this cross reactivity. Radiolabelled sulphated CCK-8 was used as the tracer in this assay (Amersham Pharmacia Biotech), and separation of the bound and free CCK was facilitated with the use of dextran coated charcoal (DCC) in the original assay protocol.
2.2.3.13.1. Adaptation of CCK radioimmunoassay

The procedure for the original assay was obtained from the RPMS, Hammersmith (appendix IV) along with 2 freeze dried stock standards and dino-7 antisera. Two standard curves were set up according to the protocol in table 2.5, however the first curve used the addition of 100μl DCC to facilitate separation of the bound and free hormone, while 100μl anti-rabbit Sac-Cel was used in the second standard curve. Both methods produced comparable results, with slightly higher binding of the label to the hormone and greater precision with the use of Sac-Cel (figure 2.4).

Figure 2.4 Comparison of standard curves for CCK radioimmunoassay using dextran coated charcoal or anti-rabbit Sac-Cel for separation of the bound and free hormone. Standards used were obtained from the RPMS, Hammersmith. Duplicate sample are shown.

In order to run assays at the University of Surrey appropriate standards were prepared to provide the same range of values as those obtained from the RPMS (0.2 pmol/l to 100 pmol/l by logarithmic dilution). Synthetic CCK-8(sulphated) was purchased from Bachem Ltd. (code H2080), and to obtain a concentration of 1.6 pmol per freeze dried standard, a stock solution of CCK-8S was made up to 160 pmol/ml in a stable freeze drying solution. For 100 ml of freeze dry solution the following recipe was used (obtained from M. Ghatei, RPMS):
Chapter 2

5g lactose, 2.5g BSA, 200mg citric acid, 100mg L-cysteine HCL, 50,000 KIU aprotinin, 100ml 0.1M formic acid and to this solution 18.3μg of CCK-8S (MW 1143.3g) was added. This stock solution was then diluted 1:100 to give a standard solution of 1.6 pmol/ml, and 1 ml aliquots of the solution were freeze dried and stored at -20°C until use.

To assess these standards against those supplied by the RPMS, standard curves were set up using the Sac-Cel and DCC methods, with both the RPMS and new standards. The new standards showed the same range of values as those from the RPMS standards with both Sac-Cel and DCC (figure 2.5), and as a result all assays were run using the Sac-Cel method with the standards prepared at the University of Surrey.

Figure 2.5 Comparison of standard curves for CCK radioimmunoassay using standards obtained from the RPMS, Hammersmith, and standards developed at the University of Surrey. Separation of the bound and free hormone was facilitated with anti-rabbit Sac-Cel. Duplicate samples are shown.

Standards from RPMS

Standards from Surrey

2.2.3.13.2. CCK sample extraction and assay procedure

Plasma samples were extracted in duplicate with the ethanol extraction method to precipitate protein of high molecular weight. One millilitre of ethanol was added to
0.5 ml plasma and vortexed for 10 seconds. The solution was then centrifuged at 897g (3000 rpm) for 15 minutes, and the resulting supernatant collected into LP4 tubes. The supernatant was stored at -80°C and dried overnight in a centrifugal evaporator (Savant Speedvac SC210A, Lifesciences International, Basingstoke, Hampshire) for analysis.

The assay procedure was performed at 4°C throughout and CCK assay diluent (pH 7.4) was added to each dried sample and standard curve tube (table 2.5). Freeze dried standards were reconstituted with 1.6 ml assay diluent, dilutions of 1:10 and 1:100 were made and either 100μl, 50μl or 20μl of each standard dilution was added to standard curve tubes as specified in table 2.5. Antisera was diluted 1:100 with assay diluent and 100μl added to each tube except NSB tubes. The CCK-8S radiolabel purchased from Amersham was diluted to 1000cpm/400μl and added to each assay tube plus total tubes containing only label. All tubes were then vortexed and incubated for 72 hours at 4°C.

Following the three day incubation, 100μl of anti-rabbit Sac-Cel was added to each tube (except total tubes), all tubes were vortexed at 15 minute intervals for 30 minutes and kept at room temperature for that time. Tubes were then centrifuged at 897g (3000 rpm) for 15 minutes, and the supernatant from each sample collected into a separate LP4 tube. Both pellet (bound label) and supernatant (free label) were counted on a gamma counter (Wizard 1470, Wallac International) for 60 seconds, and the ratio of bound and free label was calculated (bound ÷ free). The B/F ratio of the standards was plotted logarithmically for the standard curve and the mean B/F from duplicate samples was read manually from this curve. As 0.5 ml sample plasma was assayed in each tube, and standard values were per ml of sample, values obtained from the standard curve were multiplied by 2 to get a final CCK-8S concentration of each sample in pmol/l.
Table 2.5 Summary of CCK radioimmunoassay protocol

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Reagents</th>
<th>Diluent</th>
<th>Standard</th>
<th>Antisera</th>
<th>Label</th>
<th>Sac-Cel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>400μl</td>
<td>100μl</td>
</tr>
<tr>
<td>NSB</td>
<td>500μl</td>
<td>100μl</td>
<td>-</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
<tr>
<td>Zero</td>
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<td>-</td>
<td>100μl</td>
<td>400μl</td>
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</tr>
<tr>
<td>1:100 dil^n</td>
<td>480μl</td>
<td>20μl</td>
<td>100μl</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
<tr>
<td>1:10 dil^n</td>
<td>450μl</td>
<td>50μl</td>
<td>100μl</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
<tr>
<td>Stock sol^n</td>
<td>400μl</td>
<td>100μl</td>
<td>100μl</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
<tr>
<td>Samples</td>
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<td>-</td>
<td>100μl</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
<tr>
<td>QC</td>
<td>500μl</td>
<td>-</td>
<td>100μl</td>
<td>400μl</td>
<td>100μl</td>
<td></td>
</tr>
</tbody>
</table>

Quality control samples of post-prandial (high) and fasted (low) plasma were included at the beginning and end of each assay. The CV calculated from high QC’s showed the inter assay variation to be 18.2% and intra assay variation to be 10.5%. Inter assay variation calculated from the low QC’s was lower (10.6%), with a similar intra assay variation of 10.9%.

2.3. Statistical analyses

Distribution of data was assessed using the Shapiro-Wilks W test. If the W value in this test was not significant then data was assumed to be normally distributed. Parametric statistics have been used in this thesis unless otherwise stated in the relevant text.

The statistical analysis for each chapter has been described in the methods section of the relevant chapter. In general plasma hormone and metabolite responses to different conditions were evaluated using suitable statistical tests.
preloads were compared using 2 factor repeated measures analysis of variance (ANOVA), using preload and time as the repeated measures (within subjects) factors. Where appropriate individual time points have been analysed using one way repeated measured ANOVA, with preload as the repeated measures factor.

Ratings from the visual analogue scales, and transformed rating scores were compared using 2 factor repeated measures analysis of covariance (ANCOVA), using preload and time as the repeated measures factors and the baseline VAS reading as the covariate. Food preference checklist data was also analysed in this way. Buffet meal intake data and further testing of individual time points was carried out using one way repeated measures ANOVA, with preload as the repeated measures factor.

The hunger and satiety quotient scores for each subject were analysed using students t-test (paired, 2 tailed), to compare two treatment conditions, and repeated measures ANOVA, with preload as the within subjects factor for 3 or more treatment conditions.

The level of significance in all tests was taken at p < 0.05, and where ANOVA or ANCOVA showed significant differences, the Duncans multiple range post hoc test was used to identify where those differences lay. Correlations were analysed using the Pearson Product Moment Correlation test. Where additional statistical techniques have been applied they are described in the relevant text.

The ‘Statistica for windows’ software package, release 5.1 (Statsoft Inc, 2300 East 14th Street, Tulsa, OK 74104, USA) was used for statistical analyses.
Chapter Three
Chapter 3

3. Investigation of methods used in the study and assessment of appetite.

3.1. General introduction

Investigations into the effects of varying energy levels and macronutrient content upon appetite often employ the preload paradigm (Stubbs et al., 1998) to directly compare satiety response and subsequent food intake between different preloads. Preloads used may be either liquid (e.g. de Graaf et al., 1992; Poppitt et al., 1998) or solid (e.g. Hill & Blundell, 1986; Lawton et al., 1993; Green & Blundell, 1996). However while both preload designs have yielded data on comparative satiating efficiencies of different macronutrients, there is still speculation as to whether the physical form of a preload has effects on the satiety response (Kissileff, 1985). As the investigations in this thesis have adopted liquid test meals, primarily due to their necessity for the EIE assessment of gastric emptying and the ease of their manipulation, a comparison of liquid and solid preloads has been undertaken to determine the relative benefits of using either method (section 3.3).

The visual analogue scales applied in this thesis are used in the vast majority of appetite investigations. Although these scales have been validated for use as subjective measurements (Bond & Lader, 1974), relatively few studies have validated this technique in relation to appetite, and this has been addressed as part of the current research (section 3.2). In addition the test meal used to assess food intake following a preload manipulation has been explored further to determine if there are any benefits of using a single choice as opposed to a mixed choice meal (section 3.4). Other factors which may have influenced appetite ratings during our research are explored in section 3.5, and this investigation was primarily undertaken to determine whether cannulation and venipuncture of subjects could have adverse effects upon appetite.

The research presented in the present chapter was undertaken towards the end of the experimental work reported in this thesis to elucidate any factors which may have
unduly influenced our data. Due to the general nature of this chapter and its examination of some methodological issues in appetite research, it was felt that although not in chronological order, it provided an introductory overview and was better presented at the beginning of this work. Consequently recommendations which have come from the current research have not been adopted in the majority of studies reported in chapters four to eight due to the time scale of investigations.

3.2. Evaluation of the use of visual analogue scales and the *ad libitum* test meal in the assessment of appetite response

3.2.1. Introduction

Although visual analogue scales have been validated for use in rating subjective feelings (Bond & Lader, 1974), there is relatively little data on their reliability and reproducibility in appetite research. Conflicting reports of good (Porrini *et al*., 1995) and poor (Raben *et al*., 1995) reproducibility for appetite ratings represent the literature in this area, but these scales remain in common use for ratings of hunger, satiety and desire to eat (e.g. Hill & Blundell, 1986; Barkeling *et al*., 1990; Rogers *et al*., 1990; Uhe *et al*., 1992) and can illustrate significant differences in appetite response between preload or other physiological manipulations (e.g. Lieverse *et al*., 1994b; Flint *et al*., 1998). These data suggest that self-rating is an acceptable and reproducible method to apply in appetite research. Conversely a number of our studies (reported in this thesis) have shown there to be a large variation in appetite ratings throughout the post prandial study period, and while a mean difference in ratings is apparent, significant differences in appetite response between preload manipulations were not obtained.

3.2.2. Aim

Therefore the present study aimed to investigate the use of self rated appetite scores and test meal energy intake in appetite research. Post-prandial appetite responses
following identical preloads were assessed on 3 separate occasions to assess reproducibility and reliability of these methods.

3.2.3. Study design

3.2.3.1. Subjects

Eighteen healthy subjects (10 male, 8 female) of normal weight for height (BMI 20 - 25 kg/m²) were recruited from staff and postgraduates at the University of Surrey. All volunteers were non dieters and were asked to complete the DEBQ during recruitment. Only individuals with scores of < 3.5 in restrained, emotional and external eating were accepted for participation in the study.

Prior to commencement of the study all volunteers were informed that they must refrain from strenuous exercise and alcohol consumption the day before and on the day of each investigation. Subjects were also asked to choose a standard lunch time meal (see appendix V for meal choices). This meal was served on each test occasion to standardise food intake during the study day.

3.2.3.2. Test meals

One preload was developed for use on each study occasion and provided 1756 kJ of energy per 450 ml serving (table 3.2.1). Each preload was served as a flavoured milkshake, and subjects were able to choose either strawberry or banana flavouring for the preload (Nesquick milkshake powder, Nestlé, PO box 207, York, Y091 1XY). The flavour of the preload remained constant for each subject throughout the study.

Table 3.2.1 Macronutrient composition of 1756 kJ liquid preload

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ)</td>
<td>915 (70)</td>
</tr>
<tr>
<td>Fat (kJ)</td>
<td>828 (22)</td>
</tr>
<tr>
<td>Protein (kJ)</td>
<td>13 (3.2)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1756</td>
</tr>
</tbody>
</table>

Ingredients: 46g double cream, 50g maltodextrin, 20g nesquick
3.2.3.3. Study protocol

Using a single blind crossover design appetite response to the 1756 kJ preload was investigated on three separate occasions at least 7 days apart. Subjects were unaware of the intention of the study and no indication of preload composition was given at any stage during the investigation.

On the morning of each study subjects were asked to consume their usual breakfast, avoiding fried breakfasts or extremely fatty foods, and were instructed to avoid drinks containing caffeine throughout the day. Following the consumption of breakfast subjects were able to continue with their normal activities, excepting sport and recreational exercise, until lunchtime. At 1230 hours individuals were provided with a standard lunch meal served in the investigation unit, after which subjects were instructed to consume nothing except water until returning to the investigation unit for the post-prandial study.

Subjects returned to the investigation unit at 1715 hours and following a 15 minute rest period completed baseline VAS ratings. Subjects consumed the preload and completed a second rating. VAS ratings were then completed at 20 minute intervals for the next 60 minutes, after which time subjects were offered an ad libitum buffet test meal from which energy and macronutrient intake were calculated. Subjects were instructed to eat as little or as much as preferred from this meal until feeling comfortably full. To prevent over consumption due to freely available food, subjects were informed they could take home anything they did not want to eat at that time. Following consumption of the buffet meal subjects were free to leave the investigation unit and resume their normal activities.

3.2.3.4. Statistical analyses

In addition to the test described in chapter 2, section 2.3, the coefficient of variation was calculated to determine between subject and within subject variation in appetite ratings and food intake following repeated consumption of an identical preload.
3.2.4. Results

3.2.4.1. Hunger ratings

The coefficient of variation (CV%) was calculated for each subject at each time point using VAS ratings following preload consumption on the three test occasions (appendix VI). Within subject CV’s were found to range between 0.8% and 96.7% throughout the post-prandial period, with a mean within subject variation across all time points of 23.5% (SD 16.8%).

Between subject variation was found to be greater than within subject variation (mean CV 38.6% SD 18.1%). It was noted that variation in hunger ratings was greatest following buffet meal consumption (within subject mean 42.6% SD 29.0%, between subject mean 69.1% SD 24.8%).

To determine whether there were any significant differences in hunger ratings following three identical preloads a repeated measures ANCOVA was applied to compare hunger responses on each test occasion. This test also illustrated whether any order effect was apparent in hunger ratings. Mean hunger values were found to be similar on each test occasion (figure 3.2.1) with no significant difference in hunger ratings at any time during the post-prandial period (p > 0.9).
Figure 3.2.1 Hunger ratings following consumption of a 1756 kJ preload on three separate test occasions

Hunger ratings (mean ± SD, n = 18) after consumption of a 1756 kJ preload on three separate test occasions (▲ = first, △ = second, ▼ = third occasion). There were no significant differences in hunger ratings between test occasions (p > 0.9).

3.2.4.2. Satiety ratings

The within subject variation in satiety ratings was found to be greater than those for hunger ratings, with a range of 0% to 150% (mean 36.1% ± 25.5%) (see appendix VII for individual values). Between subject variation was also greater than those for hunger ratings (mean 45.2% ± 18.9%), and greater than the within subject CV for satiety. Variation in ratings was greatest at baseline (mean 57.4% ± 25.8%) and immediately before consumption of the buffet test meal (mean 58.7% ± 34.8%), while the smallest CVs were seen following the buffet meal (mean 16.5% ± 4.8%).

ANCOVA of satiety data showed no significant differences in satiety ratings between study occasions (p > 0.5), and mean values at each time point during the post-prandial period were similar (figure 3.2.2). No differences were observed between male and female subjects in hunger or satiety ratings.
Figure 3.2.2 Satiety ratings following consumption of a 1756 kJ preload on three separate test occasions

Satiety ratings (mean ± SD, n = 18) following consumption of a 1756 kJ preload on three separate test occasions (▲ = first, ▲ = second, ▲ = third occasion). There were no significant differences in satiety ratings between test occasions (p > 0.5).

3.2.4.3. *Ad libitum* buffet meal intake

Mean buffet energy intakes were found to decrease with each repetition of the study (figure 3.2.3), and analysis with one way ANOVA showed a significant difference in buffet energy intake between test occasions (p = 0.006). Post hoc analysis showed there to be a significant difference in energy intake between the 1st and 3rd (p = 0.004) and 2nd and 3rd (p = 0.01) test occasions, with no significant difference between the 1st and 2nd study days (p = 0.5). Further analysis with order as a random effect elucidated no other differences.
Figure 3.2.3 Buffet meal energy intake 60 minutes after consumption of a 1756 kJ preload on three separate test occasions

Buffet energy intakes (mean ± SD, n = 18) following consumption of a 1756 kJ preload on three separate occasions (第一, 第二, 第三 occasion). Subjects had a significantly lower buffet intake on the third test occasion compared to the first (p = 0.004) and second (p = 0.01) test occasions. There was no significant difference in intakes between the first and second test occasions (p = 0.5).

The difference in energy intake was reflected in protein (p = 0.002), carbohydrate (p = 0.006) and fat (p = 0.03) intakes. Post hoc analysis of macronutrient intakes again showed significant differences between the 1st and 3rd study occasion (p < 0.02 all nutrients), and the 2nd and 3rd study occasion (p < 0.03 all nutrients). There were no significant differences between the 1st and 2nd study occasion (p > 0.3 all nutrients).

Analysis of buffet energy intakes of male and female subjects showed female subjects to decrease their intake in a stepwise manner on each consecutive test occasion (p = 0.02), with the decrease significant between the 1st and 3rd test day (569 kJ, p = 0.007). Male subjects showed as slightly smaller decrease in intake of 781 kJ between the 1st and 3rd test occasion which approached significance (p = 0.06). There was no difference in energy intakes on the 2nd and 3rd test occasion in male subjects (figure 3.2.4).
Buffet energy intakes (mean ± SD, n = 18) of male (dotted line) and female (solid line) subjects following consumption of a 1756 kJ preload on three separate occasions (■ = first, ■ = second, ■ = third occasion).

Between subject CV in energy intake was also calculated, with an average of 27.5% (SD 3.1%) across the three study days. This was an average variation of 299 kJ. Within subject CVs were found to be much smaller, ranging from 7.2% to 25.8% (mean 13.9% SD 5.6%, appendix VII for individual values). An average variation of 327 kJ to 1171 kJ. Although there was no significant difference in CVs of male and female subjects (unpaired t-test $p = 0.2$), males did exhibit a slightly larger variation in intake than females (male mean 15.3% SD 6.6%; female mean 12.1% SD 3.7%).

3.2.5. Discussion

Data from this investigation shows similar variation to previous work (Raben et al., 1995), and shows VAS ratings to have low reproducibility when used in the assessment of hunger and satiety. Although no significant difference was seen between hunger and satiety ratings, suggesting that appetite response to the preload was the same on each test occasion, the large within subject variation seen in the present study could effectively mask differences in response to manipulated test preloads in similar studies. While this could be overcome to some extent by
implementing statistical techniques to minimise or standardise variation in data, e.g. the z-score (section 2.2.1.1.1), it is arguable whether data transformation is beneficial in instances where CVs range between 0 - 150%, as seen in within subject ratings of satiety in the current study. It is possible that the familiarity of subjects to the rating scales and their experience of self-assessment is a primary determinant of the size of variation seen in VAS ratings. In the present study the majority of subjects recruited had not used VAS ratings previously, and received only a verbal instruction of how to use the scales. Although subjects were asked if they understood what was required, and were reminded on each test occasion, it is probable that inexperience was an important determinant both of their confidence in making subjective ratings, and in the accuracy of their ratings. Nevertheless those subjects who had used VAS ratings in previous appetite studies (n = 6) showed similar variation at each time point to those who had not, although these subjects had generally been involved in only 1 previous study. It was also noted that the largest variation occurred at times when the parameter being assessed was likely to be at its lowest, i.e. in hunger following the buffet test meal, and in satiety at baseline and immediately before consumption of the buffet test meal. This suggests subjects to have more difficulty in rating themselves at the extreme ends of the VAS when there is little stimulus for hunger or satiety, and this may account for the over consumption which can occur following periods of low intake or at times of great hunger.

While VAS ratings showed appetite response was not influenced by any order effect, ad libitum test meal intakes clearly show a decrease in energy intake with each consecutive test occasion. There are a number of possible explanations for this response, primarily relating to psychological rather than physiological processes. Due to the nature of the buffet; a relatively palatable and attractive meal comprising a variety of foods, subjects may have overeaten on the first study day. As the buffet meal had been altered previously to make it more bland (chapter 2, section 2.2.1.3), the novelty of the study situation and food presented may also have acted to increase their intake at the first sitting. A novelty effect could be eliminated by implementing a ‘practice session’ in which subjects were introduced to the foods used and the study environment. However this data shows no significant difference in intake between
the first and second test occasion, and does not support a novelty effect in this case. In addition to possible over consumption on the first study day subjects were shown to significantly reduce energy intake on the third test occasion compared to both the second and first test occasions. Thus it is probable that boredom and familiarity with the test meal and study environment acted to reduce food intake on the last test occasion, and if any novelty effect was acting to increase intake, that this effect was also reduced on the third test occasion. It is also likely that the boredom and familiarity would be greater than any novelty effect seen at the beginning of the study. Additional external factors should also be considered for their role in food intake response. This study was undertaken during the summer over a period of approximately 6 weeks, and during this time the ambient temperature fluctuated considerably. Unfortunately temperature readings were not made on each test day, but it is possible and relatively likely that on the hottest days food intake would have been reduced. It was also interesting to observe a difference in buffet intake response between male and female subjects, with females showing a decline in food intake across each study day. No control for the menstrual cycle was made in this investigation, and it is possible that stage of the menstrual cycle had some effect on food intake. However, as the variation in intake was smaller in female than male subjects it is possible that menstrual cycle would not have had a significant effect upon food intake. The number of male and female subjects were relatively small (n = 10 and n = 8 respectively), and further investigations into gender differences observed in appetite ought to aim for 15 - 20 subjects to improve statistical power. Although male and female subjects showed a different pattern of response, in both cases intake was lowest on the third test occasion, supporting a role for familiarity and boredom. And if the observed reduction in food intake is attributable to familiarity with the buffet meal these data suggest that female subjects may be more readily influenced than male subjects.

Order effects can be minimised by using randomised repeated measures analysis (Hill et al., 1995), which improves the power of the experimental design by using each subject as their own control and allowing for differences in use of the rating scales between subjects. However in instances where the randomised design becomes
imbanced, e.g. subjects withdraw or are excluded due to illness, differences in intake may be seen which should be attributed to external factors rather than the preload manipulation. It has been suggested (Peter Rogers, unpublished observations) that such variation could be minimised by using a between subjects rather than within subjects experimental design. While this approach would require a much larger number of subjects it would effectively eliminate the occurrence of any order effects. In order to compare appetite the response to different preloads between subject groups baseline VAS and food intake data in response to a standardised preload would be required. Test data could then be adjusted as a function of baseline data, to allow for the difference in response between subjects. As this would double the workload required, validation of the technique in relation to the present data would be required to determine any benefits over the current design before use in an experimental situation. However the data generated from this investigation can be used in the power calculations needed to determine the number of subjects which would be required in order to detect a significant difference in satiety ratings. For example with CVs in the range of 30% to 40% for hunger and satiety ratings, and aiming to detect differences in ratings between treatments in the region of 2 cm on the appetite scale, power calculations suggest using upwards of 50 subjects in order to determine a statistically significant difference at the 5% level in appetite ratings between manipulated preloads. However the majority of investigators recruit less than 50 subjects and are often able to detect difference between preloads, again suggesting that subject familiarisation with self-rating scales may be important.

Although these data suggest that use of rating scales is open to large variation, the absence of any reliable non subjective techniques for the assessment of appetite means that the data generated is still extremely valuable. In terms of the ad libitum buffet test meal, it may be beneficial to implement a practice session for subjects to become used to the meal and test procedure, further reduce the palatability of the test meal or use a single food choice test meal. This would then minimise any influence of novelty in causing over consumption.
This work also provides further data on variation in appetite ratings which may be implemented in power calculations. Thus the number of subjects recruited could be adjusted to account for the variation seen and improve results from such studies. In addition trained subjects should be used to improve VAS data who are both familiar with self rating techniques and the scales to be used. This may explain why some studies have shown statistically significant difference in appetite ratings (e.g. Lieverse et al., 1994b) when others have not (Drewe et al., 1992). The use of a between subjects rather than within subjects design is also worthy of further investigation, as this approach, although not preferred in the past, may provide a useful tool in eliminating many of these confounding factors.

3.3. Does the physical state of a preload influence appetite response?

3.3.1. Introduction

The preloading method is commonly used in appetite studies to assess satiety response and food intake in response to various manipulations of energy and macronutrient content. Previously researchers have reported on the effects of manipulating both liquid (e.g. Poppitt et al., 1998) and solid preloads (e.g. Lawton et al., 1993). While the energy and macronutrient content of these preloads may be similar, difference in their physical characteristics may result in different rates of digestion and differences in appetite response. Consequently when comparing data between different studies the physical form of the preload is a factor which must be considered as influencing appetite responses. However there are relatively little data which directly compare the satiating efficiency of preloads with different physical states, or investigates differences in the level of compensation or ability to detect energy differences in manipulated preloads which may also be influenced by the physical state of a meal. Although it has previously been shown that compensation to energy manipulations of liquid and solid preloads is similar (Pliner, 1973) results were shown to differ in obese subjects, with poorer compensation following solid preloads, thus differences may occur in compensatory responses when preload energy is delivered in differing forms.
As the content of liquid meals is much easier to manipulate than that of solid meals, and the EIE assessment of gastric emptying requires liquid meals, this approach has been adopted in our studies. The use of liquid preloads is also supported by previous findings showing them to be more satiating and reduce subsequent food intake to a greater extent than solid preloads (Kissileff et al., 1984; Kissileff, 1985; Rolls et al., 1999), particularly if liquefied soup is used as the liquid preload. Rolls and colleagues speculated that decreasing the energy density and increasing the volume of a preload acts to increase fullness and reduce subsequent energy intake, and this provides a potential mechanism for the action of liquid preloads on appetite. However this hypothesis does not explain findings where chunky soup has been shown to be more satiating than an identical liquefied soup preload (Himaya & Louis-Sylvestre, 1998), or those studies which have found no difference in food intake between chunky and homogenised preloads (Kissileff et al., 1980). Previous investigations have often used soup as the liquid preload, and although use of a hot food liquid meal may have different appetite effects to that of a cold liquid drink it is likely that similar comparative appetite responses would be seen between varying energy levels of a soup preload and varying energy levels of a cold liquid preload. However in two of our preloading studies (chapters 4 and 5) a distinct difference in appetite response was not observed, particularly in terms of the VAS ratings. While the degree of variation seen when using VAS ratings may to some extent explain this data (section 3.2 above), the preloads used in these investigations were given in cold liquid form (as a milkshake). It is possible that subjects found the meal unsatisfying, particularly as a breakfast meal following an overnight fast, which in turn led to more overt feelings of hunger or a lesser degree of satiety than would have been experienced with a solid meal. Thus the psychological aspects of consuming solid food, considered ‘a proper meal’, may act to improve subjective appetite data over use of a liquid meal. Two studies reporting a direct comparison of liquid and solid meals have shown subjects to rate themselves as more satiated following a solid preload compared to a liquid preload of identical energy content (Hulshof et al., 1993), and have shown 24 hour food intake to be lower with solid than liquid meals (Tournier & Louis-Sylvestre, 1991). However, in relation to the data presented in this thesis, and
to add to the small number of studies in this area, a comparison of cold liquid preloads with nutritionally comparable solid meals would be beneficial.

3.3.2. Aim

Therefore the current study aimed to investigate appetite responses following liquid and solid preloads of similar energy and macronutrient content using VAS ratings and an *ad libitum* test meal. The compensation for manipulations of liquid and solid preloads was also investigated. In order to analyse data with respect to our other studies the liquid preloads were based on those used in previous preloading studies.

3.3.3. Study design

3.3.3.1. Subjects

Fourteen healthy subjects (7 male, 7 female) were recruited from the undergraduate student population at the University of Surrey. All subjects were of normal weight for height (mean BMI 23.0 ± 1.8 kg/m²), with an age range of 19 - 30 years. All volunteers selected were non dieters, and were asked to complete the Dutch Eating Behaviour Questionnaire prior to inclusion in the study. Only those subjects scoring less than 3.5 as restrained, emotional and external eaters were accepted onto the study.

Prior to commencement of the study all volunteers were instructed that they must refrain from alcohol and strenuous exercise the day before each test occasion. A liking of the preloads to be used was also established, and only those subjects expressing a liking for the food used in the study were recruited.

3.3.3.2. Test meals

Four test meals (2 liquid and 2 solid) were developed to provide preloads of high and low energy content. The liquid preloads were given as a 450 ml milkshake consisting of sucrose, maltodextrin (Cerestar Pur 01915, Cerestar UK Ltd., Trafford Park, Manchester; M17 1PA) double cream and ‘Nesquick’ flavouring (Nestlé, PO box 207,
York, YO91 1XY), and were made up to volume with water. The low energy liquid preload (LL) was designed to provide 1346 kJ, and the high energy liquid preload (HL) was designed to provide 2755 kJ. Solid preloads were developed to match the total macronutrient and energy content of the liquid preloads as closely as was possible with the use of 'real food' items, and were based on an apple crumble recipe. Ingredients were manipulated to provide a similar level of energy and macronutrient per serving (245g) as the liquid preloads with a similar energy difference between high and low energy preloads (liquid 1409 kJ, solid 1392 kJ). The low energy solid (LS) preload provided 1393 kJ, and the high energy solid (HS) preload provided 2785 kJ. The energy density of the solid preloads was higher than that of the liquid preloads when assessed in terms of energy per ml or gram of the preload. The LL preload provided 3.0 kJ/ml and the LS preload provided 5.7 kJ/g. The HL preload had an energy density of 6.1 kJ/ml, while the HS preload had an energy density of 11.4 kJ/g.

The sucrose content of each solid preload was the same, and no differences in sweetness were detectable between the liquid and solid preloads due to the sweetness provided by the ‘Nesquick’ flavouring in the liquid preloads. Preload compositions are outlined in table 3.3.1 below.

Table 3.3.1 Composition and formulation of liquid and solid preloads. Amounts given are per portion.

<table>
<thead>
<tr>
<th></th>
<th>Liquid preloads</th>
<th>Solid preloads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low energy†</td>
<td>High energy‡</td>
</tr>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>1108 (66)</td>
<td>1108 (66)</td>
</tr>
<tr>
<td>Fat (kJ (g))</td>
<td>234 (6)</td>
<td>1622 (43)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>4 (0.25)</td>
<td>25 (1.5)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1346</td>
<td>2755</td>
</tr>
</tbody>
</table>

† LL : 13g double cream, 48g maltodextrin, 19g nesquick.
‡ HL : 90g double cream, 48g maltodextrin, 19g nesquick.
§ LS : 140g flour, 40g low fat spread, 425g apples, 40g sugar, 5g maltodextrin, 80g skim milk; weigh 245g serving from this when cooked.
* HS : 180g flour, 125g butter, 426g apples, 40g sugar, 40g maltodextrin, 105g double cream; weigh 245g serving from this when cooked.
3.3.3.3. Study Protocol

Using a randomised cross over design the appetite responses to each preload were investigated. Although subjects were aware of the obvious difference between liquid and solid preloads, they were blind to the energy manipulations of each preload.

On the morning of each study subjects were asked to consume their normal breakfast before 0930 hours, and to consume the same breakfast on each test occasion. Subjects were free to undertake their usual activities until arrival at the investigation unit at 1100 hours, although they were instructed not to participate in any sport or recreational exercise.

Following a rest period in the investigation unit subjects completed baseline VAS ratings, and were offered one of the four preloads. The liquid preloads were consumed at room temperature, while the solid preloads were warmed slightly in a microwave to improve palatability. Subjects were instructed to eat or drink at a steady pace until all the preload was consumed, and completed a second VAS rating after consumption of the preload. VAS ratings were then completed at 20 minute intervals for the following 60 minutes, after which time subjects were offered an ad libitum buffet style test meal from which they could eat until feeling comfortably full. To avoid over consumption of buffet food because it was free and available subjects were advised they could take with them anything they did not want to eat at that time. From this meal energy and macronutrient intakes were calculated, and following the test meal subjects were free to leave the investigation unit and resume their normal activities.

3.3.3.4. Statistical analyses

Comparison of hunger and satiety response to the high and low energy preloads within the liquid and solid preload manipulation was analysed using repeated measures ANCOVA with preload (high vs. low) and time as the repeated measures factors. Buffet test meal energy intake from these treatment conditions was compared using a paired, 2-tailed t-test (high vs. low).
To analyse differences in hunger and satiety ratings between the liquid and solid preloads, repeated measures ANCOVA was used with each preload condition as a repeated measures factor (LL, HL, LS, HS), and time as a repeated measure. Buffet test meal intake between these conditions was similarly analysed using repeated measures ANOVA.

For analysis of appetite ratings the baseline rating (-5 min) was used as the covariate, and time points +5 to 60 minutes were the repeated measures. Ratings following consumption of the buffet test meal were analysed separately as they we no longer directly responsive to preload energy manipulations.

3.3.4. Results

3.3.4.1. Hunger ratings

There was no significant difference in hunger ratings between the low and high energy solid preloads (figure 3.3.1, \( p = 0.3 \)). However ANCOVA between the high and low energy liquid preloads showed subjects to rate themselves more hungry following the LL than HL preload, and this difference approached significance \( (p = 0.09) \).

Analysis of hunger ratings between the high energy preloads and low energy preloads showed no significant difference in hunger ratings between the HS and HL preloads \((p = 0.5)\). However subjects rated themselves significantly more hungry following the LL compared to the LS preload \((p = 0.003)\). In addition subjects experienced significantly greater hunger following the LL preload compared to the HS preload \((p = 0.03)\). There was no significant difference in hunger ratings between the HL and LS preloads \((p = 0.1)\).

There were no significant differences in hunger ratings following the buffet test meal \((p > 0.1 \text{ between all preloads})\).
Figure 3.3.1 Hunger ratings following consumption of liquid preloads containing 1346 kJ (LL) and 2755 kJ (HL), and solid preloads containing 1393 kJ (LS) and 2785 kJ (HS)

Hunger ratings (mean ± SEM, n = 14) following consumption of HL (▲), LL (▲), HS (▲) and LS (▲) preloads. Subjects tended to be more hungry after the LL compared to the HL preload (p = 0.09), and significantly more hungry after the LL compared to the LS preload (p = 0.003).

3.3.4.2. Satiety ratings

Satiety ratings following preload consumption showed a similar order of response to hunger ratings (figure 3.3.2). Analysis of HS and LS data showed there to be no significant difference in satiety ratings between these preloads (p = 0.1). However, with the liquid preloads subjects rated themselves significantly more satiated after consumption of the HL compared to the LL preload (p = 0.03).

Comparison of satiety ratings between the high energy and low energy preloads was undertaken as above. Post hoc testing of the ANCOVA showed no significant difference in satiety ratings following consumption of the HL and HS preloads (p = 0.3). However following the low energy preloads subjects rated themselves significantly more satiated after LS than LL (p = 0.005). There were no significant differences in satiety between the HS and LL preloads (p = 0.15) or the HL and LS preloads (p = 0.5), and no significant difference in satiety ratings following the buffet test meal (p > 0.1 between all preloads).
Satiety ratings (mean ± SEM, n = 14) following consumption of HL (▲), LL (▲), HS (▲) and LS (▲) preloads. Subjects were significantly more satiated after the HL compared to the LL preload ($p = 0.03$), and after the LS compared to the LL preload ($p = 0.005$).

### 3.3.4.3. *Ad libitum* buffet meal intake

Analysis of buffet meal intakes showed no significant difference in energy intakes between the solid preloads ($p = 0.5$). However, energy intake was significantly greater following the LL compared to the HL preload ($p = 0.007$, figure 3.3.3).

Analysis of macronutrient intakes reflected the energy data and showed no significant differences between the solid preloads ($p > 0.2$ all nutrients). Intakes following the LL preloads were significantly greater than following the HL preload for all nutrients (protein: $p = 0.02$, fat: $p = 0.006$, carbohydrate: $p = 0.002$, weight: $p = 0.01$). There were no significant differences in energy or macronutrient intakes between HL and HS, and LL and LS preloads ($p > 0.1$ in all cases).
Buffet energy intakes (mean ± SEM, n = 14) following consumption of HL (■), LL (■), HS (■) and LS (■) preloads. Intake was significantly higher following the LL preload compared to the HL preload \( (p = 0.007) \). There was no difference in intakes between LS and HS preloads.

The difference in buffet meal intakes between HS and LS was 143 kJ, showing subjects to make a 10% compensation for the 1392 kJ difference in solid preload energies. Compensation was better with the liquid preloads, with subjects eating 647 kJ less with HL, and making a 46% compensation for the 1409 kJ difference in preload energy.

Quotients were also calculated for hunger and satiety ratings. There were no significant differences in hunger quotients \( (p > 0.3) \) or satiety quotients \( (p > 0.4) \) following any of the preloads (figure 3.3.4).
Figure 3.3.4 Hunger and satiety quotients calculated from buffet meal energy intake and change in hunger and satiety ratings 60 minutes after consumption of liquid preloads containing 1346 kJ (LL) and 2755 kJ (HL), and solid preloads containing 1393 kJ (LS) and 2785 kJ (HS)

Hunger and satiety quotients (mean ± SEM, n = 14) following consumption of HL (■), LL (■), HS (■) and LS (■) preloads. There were no significant differences in quotients between any of the preloads.

3.3.5. Discussion

One objective of this investigation was to determine whether compensation for differences in preload energy occurred more readily in response to solid or liquid manipulations. Data shows that subjects were more able to adjust for energy differences in liquid rather than solid form, although compensation was poor in both cases (< 50%). Better compensation with liquid preloads was an unexpected finding, as a previous study has shown similar compensation following liquid and solid preloads in lean subjects (Pliner, 1973), and data from our additional work (chapters 4 and 5) has shown poor compensation following milkshake style liquid preloads. The difference in appetite response between HL and LL was also apparent in VAS ratings, where subjects rated themselves as more hungry ($p = 0.09$) and less satiated ($p = 0.03$) following the LL preload. Interestingly there were no significant differences in
appetite response to the HS and LS preloads either in hunger \( (p = 0.3) \), satiety \( (p = 0.1) \) or energy intake \( (p = 0.5) \), and it is possible that the weight of the preload had an overriding effect on appetite response. Also due to the use of food items rather than basic macronutrients in preparation of the solid preload, the fibre and protein content of the solid meals may have had additional effects on appetite which would not occur with the liquid meals. However, in terms of a comparison between the two solid preloads distribution of protein and fibre was similar, and a comparative effect of appetite could still be assessed.

While data suggests liquid preloads to be preferable to solid preloads in assessing appetite response to energy manipulations, it does not show liquid preloads to be more satiating than solid preloads per se. Although the HL preload was found to be more satiating than the HS preload it was not more satiating than the LS preload, implying that the LS preload was acting to induce satiety through factors which were not dependant on energy intake alone. In addition subjects rated themselves as less hungry following the LS preload, while ratings between the HS and HL preloads were similar, supporting the finding that the high energy liquid preload was not consistently more satiating than both of the solid preloads. It was noted that the LL preload was consistently less satiating than the LS preload, implying that at lower energy levels a solid preload is more satiating than a liquid preload. This data is converse to previous work showing liquid preloads as generally more satiating (Kissileff et al., 1984; Rolls et al., 1999), as at higher energy levels there was little difference in response to solid and liquid preloads although at lower levels the liquid preload was more satiating. The different effects of pre- and post-absorptive factors may go some way to explaining this data. As Rolls suggested, decreasing the energy and density and increasing the volume of a preload acts to increase satiety (Rolls et al., 1999), probably through an increased rate of gastric emptying and subsequent increased stimulation of small intestinal receptors responsible for the induction of satiety signals (Welch et al., 1985; Greenberg et al., 1989). Coupled with this may be hormonal response to nutrients in the small intestine which itself can promote satiety (section 1.5.2, 1.6 & 1.7). It is likely that the liquid preload is emptied from the stomach more quickly than the solid preload, and thus acts through small intestinal mechanisms to generate satiety soon
after ingestion. The solid preload however is likely to exert a lesser degree of small intestinal response, coupled with a greater or prolonged satiety due to gastric distension (Khan & Read, 1992). Therefore although the high energy liquid and solid preloads may be acting through different mechanisms over the time scale of this study they would both result in a similar degree of satiety. If this were the case, a similar explanation could be applied at lower energy levels. In this instance the LL preload would empty quickly from the stomach, and due to its lower energy level than the HL preload would act to a comparably lesser extent to small intestinal satiety factors. In addition there would be very little gastric load remaining 60 minutes into the study - at the time the buffet test meal was given to subjects, resulting in least satiety following this meal. The LS preload however would provide a greater stomach distension, and due to its solid nature would require more digestive action before emptying was initiated and would consequently be emptied at a slower rate (Malagelada et al., 1979). Thus satiety signals following the LS preload may arise from gastric distension, and this may account for the greater satiety following the LS preload as opposed to the LL preload in the 60 minutes following ingestion during this study. The rate of gastric emptying may also explain differences in the slope of hunger and satiety ratings following preload consumption as a sharper decline in satiety is seen following both the LL and HL preloads.

While the extent to which pre and post absorptive factors of appetite control are involved in the responses observed cannot be determined from this study, the above speculation goes some way to suggesting why these results may have occurred. In retrospect it was unfortunate that hormonal and gastric emptying data was not obtained during the investigation, and in future studies of a similar nature this could add valuable information to the relative roles of gastric, small intestinal and hormonal factors in appetite control. However this investigation set out to explore the extent of the differences between use of liquid and solid preloads, and has been successful in illustrating that compensation appears to occur more readily with a liquid preload. In terms of appetite research this data supports the use of a liquid preload against a solid preload when discrimination between two or more dietary manipulations is investigated. The data also provide evidence that the preload meals used elsewhere in
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this thesis are capable of eliciting differences in appetite response, and that the meals themselves are unlikely to be a confounding factor in data interpretation.

3.4. Use of a mixed food buffet test meal compared to a single food pasta meal for the assessment of ad libitum food intake

3.4.1. Introduction

An important factor in the preloading design is the use of an ad libitum test meal from which subsequent food intake is assessed. Different studies have adopted a variety of different approaches to this test meal, ranging from use of single food items such as banana slices (Lieverse et al., 1995c) to offering mixed food items including a savoury hot meal plus cakes, chocolate and crisps (Ballinger et al., 1995) and a buffet comprising sandwiches, cakes, biscuits, crisps, yoghurt and fruit (Rogers et al., 1990). The latter buffet style test meal has been used in a number of studies in this thesis and allows subjects to select as little or as much as preferred from the range of foods to satisfy their hunger. By offering small discrete portions of each item (e.g. quarter of a sandwich, half slices of cake) it is hoped that subjects will eat until comfortably full rather than feeling they should finish an entire portion of food. However the buffet test meal is both palatable and novel for the subjects at the beginning of a study, and this may lead to over consumption. There is a well documented relationship between the variety and palatability of food available and increased food intake (Rolls, 1979; Hill et al., 1984; Johnstone et al., 1998), and one of our studies (chapter 4) has shown very high energy intakes, in the region of 7500 kJ, from a buffet style test meal. Although over consumption may occur with the buffet test meal, the primary objective of most appetite studies is to determine the relative satiating effects of preload manipulations, and if the same buffet meal with the same food choices is used throughout the study food intake should still be directly comparable. However as a range of food items of different energy and macronutrient composition are available, variations in test meal intake could arise through chance differences in selection rather than due to differences in appetite. Thus a ‘grazing’ effect is more likely to occur
with a variety of foods, and this may mask differences in test meal intake in response to preload manipulations. An additional concern with a buffet style test meal is that raised in section 3.2; that food intake may reduce as the study progresses because the perceived novelty of the buffet meal declines. This could be overcome to some extent by presenting a less palatable test meal which holds little novelty for the subjects, and use of such a meal may also act to prevent over consumption. It is thus possible that a subject’s ability to detect differences in preload energy manipulations would become more apparent with use of a single food test meal, showing improved compensation for such manipulations due to a reduction of the influences of palatability and variety on food intake. Furthermore a direct comparison of different test meals would provide good evidence to advocate the use of such test meals in future studies.

3.4.2. Aim

The present study therefore aimed to assess food intake following preloads of high and low energy content using a mixed food buffet style test meal and a single food test meal. Subjects’ compensatory response to preloads of differing energy content was also investigated in relation to the type of test meal used, and hunger and satiety ratings were assessed during the post-prandial period.

3.4.3. Study design

3.4.3.1. Subjects

Fourteen healthy subjects (7 male, 7 female) were recruited from the undergraduate population at the University of Surrey. Volunteers selected were non obese (mean BMI 24.5 ± 3.4 kg/m²) with an age range of 18 - 24 years. All subjects were non dieters and completed the DEBQ during recruitment. Only those subjects with scores of < 3.5 in restrained, emotional and external eating were accepted onto the study.

Prior to commencement of the study subjects were advised that they must refrain from alcohol and strenuous exercise for the 24 hours before each study occasion. In
addition subjects were asked to make food choices for their buffet and pasta test meals as detailed in section 2.2.1.3 and section 3.2.3.2 respectively.

### 3.4.3.2. Test meals

#### 3.4.3.2.1. Preloads

Two preload meals were developed to provide a high (HE, 2513 kJ) and low (LE, 1008 kJ) energy density. Each preload was given as a 450 ml milkshake consisting of double cream, maltodextrin and sucrose, and was made up to volume with bottled water. Energy manipulation was achieved through the alteration of carbohydrate content, while the fat and protein content of each preload remained constant (see table 3.4.1 below). Each milkshake was flavoured with 1.5 ml vanilla essence (Langdales vanilla flavouring, E.F. Langdale Ltd., Chase Road, Northern Way, Bury St Edmunds, Suffolk, IP32 6NT) to improve palatability.

| Composition and formulation of high and low energy preloads per 450 ml serving. |
|---------------------------------|---------------------------------|
| Low energy preload | High energy preload |
| Carbohydrate (kJ (g)) | 167 (10) | 1672 (100) |
| Fat (kJ (g)) | 828 (22) | 828 (22) |
| Protein (kJ (g)) | 13 (0.8) | 13 (0.8) |
| Total energy (kJ) | 1008 | 2513 |

*LE : 46g double cream, 10g sucrose  
*HE : 46g double cream, 10g sucrose, 90g maltodextrin

#### 3.4.3.2.2. Ad libitum test meals

The *ad libitum* buffet test meal described in section 2.2.1.5 requires subjects to select from a number of items those which they would like to be offered for the test meal. Depending on which food items are selected there is a maximum total energy available of 12611 kJ and a minimum of 10751 kJ. Subject are then free to select food items from this total, and actual energy intake is calculated accordingly. This buffet selection was used as the mixed food test meal in the present study, and
subjects were asked to rank the food items available for the buffet in order of taste preference (section 2.2.1.3, table 2.1). Subjects were offered their second or third choice of each food item in the buffet in order to minimise overeating due to the free availability of highly palatable food, and were offered the same number of the same food items on each test occasion. The buffet was prepared prior to the study and presented to each subject on an individual tray. Subjects were instructed that they may select from any of the food items on the tray when eating, and to eat until feeling comfortably full. Water (500 ml) was available whilst consuming the buffet test meal.

A pasta based meal was developed for the single food ad libitum test meal. The meal was designed to provide approximately 9500 kJ with a macronutrient composition of 13% protein : 50% carbohydrate : 37% fat (see table 3.4.2 for composition). The pasta test meal provided less total energy than the buffet test meal as it was observed from previous studies that subjects rarely ate more than 8300 kJ from the buffet test meal. Two different meal flavours based on commercially available pasta sauces were developed to be identical in macronutrient composition and energy content, providing subjects with a choice of test meal. Subjects chose their preferred pasta flavour at the beginning of the study, and were offered the same weight of the same test meal on each test occasion.

**Table 3.4.2 Composition of pasta test meals, amounts given are per serving.**

<table>
<thead>
<tr>
<th></th>
<th>Pasta and tomato sauce</th>
<th>Pasta and carbonara sauce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>4682 (280)</td>
<td>4581 (274)</td>
</tr>
<tr>
<td>Fat (kJ (g))</td>
<td>3499 (93)</td>
<td>3536 (94)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>1371 (82)</td>
<td>1338 (80)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>9552</td>
<td>9455</td>
</tr>
</tbody>
</table>

**Ingredients of pasta meals (per serving):**
Pasta and tomato sauce: 1 x 300g jar ‘Tesco original recipe pasta sauce’ (Tesco Ltd, Cheshunt, EN8 9SL), 100g turkey mince, 65g cheddar cheese, 50 ml olive oil, 350g pasta.
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*Pasta and carbonara sauce:* 1 x 300g jar ‘Dolmio carbonara creamy ham pasta sauce’ (Master Foods UK, Kings Lynn, Norfolk, PE30 4JE), 100g unsmoked back bacon, 50g cheddar cheese, 100 ml skimmed milk, 15 ml olive oil, 350g pasta.

All pasta meals were prepared in a domestic kitchen prior to the study and re-heated in a microwave oven immediately before serving. The meal was offered to each subject individually in a covered casserole dish, and subjects were instructed to serve from the casserole into a side dish before eating. The subjects were told to eat until comfortably full, and were able to refill the side dish as often as they liked, although they were asked to keep the casserole covered between servings. Replacing the casserole lid aimed to prevent grazing of the meal by the subjects, although participants were informed that the food would cool too quickly if the lid was not replaced. Water (500 ml) was available whilst consuming the pasta test meal.

Subjects were segregated during consumption of both test meals to minimise the effects of social interaction on food intake, and conditions were kept constant during each test occasion.

### 3.4.3.3. Study protocol

Using a randomised crossover design appetite response to the high and low energy preloads was investigated. In order to compare *ad libitum* food intake between the buffet and pasta test meals each subject attended the investigation unit on four separate occasions, and was offered the high and low energy preload twice. Although aware of the obvious difference in test meals subjects were blind to the preload energy manipulations.

On the morning of each study subjects were instructed to consume their normal breakfast before 0930 hours, and to consume the same breakfast on each test occasion. Subjects were free to undertake their usual activities between breakfast and arrival at the investigation unit although they were instructed not to participate in any sport or recreational exercise during this time.
Following arrival at the investigation unit at 1100 hours, subjects had a 15 minute rest period and completed baseline VAS ratings. They were then offered either the high or low energy preload which they were instructed to consume at a steady pace. Following consumption of the preload subjects completed a second VAS rating, and completed subsequent VAS ratings at 20 minute intervals for the next 60 minutes. After this time subjects were offered either the buffet or pasta test meal from which they were instructed to eat as little or as much as they liked until feeling comfortably full. Energy and macronutrient intakes were calculated from each meal. Following consumption of the *ad libitum* test meal subjects were free to leave the investigation unit and resume their normal activities.

### 3.4.3.4 Statistical analyses

Although 2 preloads were used (HE and LE), with each subject consuming each preload twice, the repeated measures design of the investigation is such that this data should not be combined for valid statistical analysis. Comparison of combined HE and combined LE data would result in \( n = 28 \) rather than \( n = 14 \) and would therefore carry the assumption that 28 different subjects were investigated. Consequently VAS ratings during each test day were analysed with ANCOVA (using preload and time as the repeated measures), and post hoc testing (Duncans multiple range test) was used to compare response between HE and LE preloads.

As the study aimed to investigate the discriminatory power of the buffet and pasta test meals, analysis of test meal intake was undertaken using a paired, 2 tailed t-test (comparison of pasta meal intake following the HE and LE preloads, and buffet meal intake following the HE and LE preloads). To determine differences in intake between the pasta and buffet test meals, repeated measures ANOVA with post hoc testing was used.
3.4.4. Results

3.4.4.1. Hunger ratings

There was no significant difference in hunger ratings between the high and low energy preloads on any test occasion (figure 3.4.1, \( p > 0.3 \)), and no clear trend for subjects to rate themselves more hungry following the LE preload than the HE preload. Post hoc testing of the ANCOVA also showed there to be no significant differences in hunger ratings made following repeat consumption of the high \( (p = 0.9) \) and low \( (p = 0.1) \) energy preloads.

Figure 3.4.1 Hunger ratings following consumption of a low energy preload (1008 kJ) and a high energy preload (2513 kJ) consumed twice each on four separate occasions

![Hunger ratings graph](image)

Hunger ratings (mean ± SEM, \( n = 14 \)) following HE (■) and LE (■) preloads consumed twice on four separate occasions. There were no significant differences in hunger ratings between preloads \( (p > 0.3) \).

3.4.4.2. Satiety ratings

Mean satiety ratings showed subjects to rate themselves as more satiated following consumption of the HE preload compared to the LE preload on each test occasion (figure 3.4.2) although this difference was not significant \( (p > 0.1) \). Post hoc testing
of this analysis showed no significant differences between satiety ratings following repeat consumption of the HE preload \( (p = 0.3) \) and the LE preload \( (p = 0.9) \).

**Figure 3.4.2** Satiety ratings following consumption of a low energy preload (1008 kJ) and a high energy preload (2513 kJ) consumed twice each on four separate occasions

Satiety ratings (mean ± SEM, \( n = 14 \)) following HE (■) and LE (■) preloads consumed twice on four separate occasions. There were no significant differences in satiety ratings between preloads.

### 3.4.4.3. Ad libitum test meal intake

Mean energy intakes from the pasta and buffet test meals are illustrated in figure 3.4.3. Paired t-test analysis of pasta meal intakes showed subjects to have a significantly lower energy intake following the HE preload compared to the LE preload \( (p = 0.009) \). As the pasta meal was homogenous, difference in macronutrient intake reflected that of energy intake \( (p = 0.009 \text{ all nutrients}) \). Analysis of buffet meal intakes also showed subjects to consume less energy following the HE preload compared to the LE preload \( (p = 0.04) \). Differences in buffet protein intake were also significant \( (p = 0.04) \), although fat and carbohydrate intake were not significantly different between preloads \( (p = 0.09 \text{ and } p = 0.2 \text{ respectively}) \). Comparison of the difference in energy intake between the HE and LE preloads with the buffet and pasta
test meals showed there to be a larger difference in intake with the pasta meal (mean 957, \( \text{SD} \) 1162 kJ) than the buffet meal (mean 782, \( \text{SD} \) 1283 kJ), although this difference was not statistically significant \( (p = 0.7) \) due to the large variation in each case. The percentage compensation for difference in preload energy intake was calculated from this data, and showed 64% compensation with the pasta meal, and 52% compensation with the buffet meal.

Further analysis of differences in intake between the pasta and buffet test meals following the HE preload showed a significantly higher energy intake with the buffet test meal \( (p = 0.02) \). Following the LE preload test meal energy intake tended to be higher with the buffet meal \( (p = 0.07) \).

Figure 3.4.3 Test meal energy intakes 60 minutes after consumption of a low energy preload (1008 kJ) and a high energy preload (2513 kJ)

Test meal energy intakes (mean ± SEM, \( n = 14 \)) following HE (■) and LE (■) preloads from the pasta test meal (solid columns) and buffet test meal (hatched columns). Subjects consumed significantly less energy after the HE preload with the pasta meal \( (p = 0.009) \) and buffet meal \( (p = 0.04, \text{energy}) \). Intake was also significantly higher with the buffet meal \( (p = 0.02, \text{HE preload}, p = 0.07, \text{LE preload}) \).

Quotients were calculated for hunger and satiety ratings from test meal intakes. Hunger quotients were higher following the HE preload compared to the LE preload.
during both test meals (figure 3.4.4). This difference was significant during consumption of the pasta meal ($p = 0.009$), but did not achieve significance following the buffet meal ($p = 0.1$). Analysis of hunger quotients within the HE and LE preloads showed a significantly higher quotient during the pasta meal following the HE preload ($p = 0.03$), and a higher quotient following the LE preload during the pasta meal which approached significance ($p = 0.09$).

Figure 3.4.4 Quotients calculated from test meal energy intake and change in hunger ratings 60 minutes after consumption of a low energy preload (1008 kJ) and a high energy preload (2513 kJ)

Satiety quotients showed similar results, with quotients greater during the pasta meal than the buffet meal (figure 3.4.5). This difference was significant following the HE preload ($p = 0.03$), but did not reach significance following the LE preload ($p = 0.3$). Analysis of satiety quotients following the HE and LE preloads showed there to be a higher quotient following the HE preload during the pasta and buffet test meals, although these differences were not significant ($p = 0.1$ and $p = 0.7$ respectively).
Satiety quotients (mean ± SEM, n = 14) following HE (■) and LE (■) preloads from the pasta test meal (solid columns) and buffet test meal (hatched columns). Quotients following the HE and LE preloads were not significantly different with the pasta and buffet test meals. The satiety quotient following the HE preload was significantly lower with the buffet meal than with pasta meal (p = 0.03).

3.4.5. Discussion

The primary finding of this study was that a single food (pasta) test meal is a more sensitive measure of appetite than a mixed food (buffet) test meal. Intake from the pasta test meal was found to be lower than that from the buffet test meal following the high and low energy preloads, suggesting either that subjects tend to over consume when presented with the mixed food test meal, or that they under consume when presented with a single choice pasta test meal. This was supported by compensation calculations, which showed subjects to more successfully adjust pasta meal intake than buffet meal intake to compensate for preload energy differences. The lower intake seen with the pasta meal may be attributable to the bland nature of the meal in relation to the buffet meal, and as the pasta meal was homogenous reduced variety also provides an explanation for the lower intake observed. This data supports that of
previous studies showing food intake to relate to palatability and variety (e.g. Johnstone et al., 1998) and provides good evidence for use of a single food test meal in appetite research. The homogenous nature of the pasta test meal also eliminated differences in intake which may arise due to the selection of different food items from the buffet test meal. For example selection of a quarter of cheese sandwich would provide almost twice as much energy as selection of a biscuit. While it may be argued that differences in food selection are themselves a function of appetite response, presentation of a range of food items is likely to increase this 'grazing' from the buffet meal, and subjects may inadvertently select a high energy item which does not act to reduce intake within the test meal (de Castro, 1993), although subsequent appetite may be altered. On the other hand use of a mixed food meal does allow investigation of differences in macronutrient intake in response to preload manipulation, or differences in selection of sweet and savoury items. Whilst this may yield useful data if investigating differences in food selection, in previous work the author has found no differences in macronutrient intake which were not attributable to the differences in energy intake observed.

The absence of significant differences in appetite ratings between the high and low energy preloads was surprising, particularly as differences in test meal energy intake were apparent and do support an effect of preload manipulations on appetite response. The subjects had not used VAS ratings for self assessment prior to the study, which may have led to difficulties in appetite assessment and provide an explanation for the results seen. However the subjects used in section 3.3 (above) were not trained in use of VAS ratings either, and in that study differences in appetite ratings following preloads of similar energy content were seen. While the preloads used in the current investigation were manipulated through changes in carbohydrate content, those in section 3.3 were manipulated through changes in fat content. Although macronutrient differences may to some extent explain the results seen, it is unusual that subjects responded to manipulations of fat content, traditionally viewed as a poor inducer of satiety signals and a promoter of passive over consumption (Green & Blundell, 1996). It is also a possibility that the subjects used in this investigation were themselves poor at self assessment, regardless of their lack of familiarity with the VAS ratings.
However the discrepancy between VAS data and test meal intake data suggests that use of the *ad libitum* test meal is extremely beneficial in appetite research, and as differences in energy intake were observed with both the pasta and buffet test meal in this study, data suggests that differences in appetite response were occurring following the high and low energy preloads. Test meal intake also enables the calculation of hunger and satiety quotients, which relate the amount of food eaten at the test meal to relative changes in appetite ratings during consumption. Although this can control for over or under consumption to some extent by showing, for example, that subjects ate more and reported greater feelings of fullness, in this study quotients did not elucidate any differences that were not already apparent from test meal intakes. Therefore it seems that test meal intake data provides a strong method of appetite assessment, particularly if subjects are unfamiliar with the VAS technique.

In conclusion data from this study suggests that use of a mixed food test meal may mask compensatory responses to preload manipulations, and this effect can be reduced by using a single food test meal. It is likely that over consumption occurs with a mixed food test meal due to palatability, variety, and grazing which acts to reduce the sensitivity of this method. Consequently unless aiming to investigate differences in macronutrient or flavour choice, use of a single food test meal is advocated for appetite investigations.

### 3.5. The effect of unfamiliar experimental conditions upon subjective ratings of hunger and satiety

#### 3.5.1. Introduction

The biological and psychological approach to appetite often requires multidisciplinary studies involving subjective appetite ratings and physiological analysis. As hunger and satiety can be influenced by a variety of external cues including pain and anxiety (Mela & Rogers, 1998) it is conceivable that exposure of subjects to novel or unusual experimental conditions may adversely influence appetite ratings. Prior to the current
research, our studies had required the cannulation of subjects to obtain blood samples, and the use of a gastric monitor to assess rates of gastric emptying. The process of cannulation can be very unsettling, particularly if a subject has not experienced the procedure before. Although cannulation generally causes little pain, the accompanying anxiety and nervousness may have some affect upon appetite. In terms of gastric emptying measurements, the EIE technique (section 2.2.2) requires subjects to remain semi-supine for the duration of the post prandial period. Maintaining this position for the duration of the study can affect the positioning of a meal in the stomach (Read et al., 1994) which may in turn adversely affect satiety. In addition increased boredom in the subjects may arise through having to remain still, and this is also a factor which can influence appetite and food intake (Mela & Rogers, 1998). In our first multidisciplinary study (chapter 4) we observed little difference in appetite response to preload energy manipulations, and it was speculated that the above factors may have contributed to the findings. To investigate further a study investigating appetite response to manipulated preloads when subjects were continuing their normal daily routine may provide insight as to whether experimental conditions do have adverse effects upon appetite. Although such a design may in itself be open to confounding factors, such as poorly controlled physical activity levels and subject dietary compliance, a within subjects repeated measures design can be implemented to minimise these effects. Additional data on the difference in hunger and satiety ratings with and without cannulation would also provide insight into the effect of these techniques in appetite investigations.

3.5.2. Aim

Therefore the present study aimed to investigate appetite response to three liquid preloads identical in composition to those used in chapter 4. Assessments were made whilst subjects undertook their normal activities, and the data was interpreted in relation to that obtained previously to determine the effect of an unfamiliar study environment (i.e. cannula and EIE equipment) compared to a ‘normal’ environment upon appetite ratings. In addition to this study the effects of cannulation and venipuncture were investigated through the assessment of appetite ratings before and after the procedures.
3.5.3. Effect of unfamiliar study conditions on ratings of hunger and satiety

3.5.3.1. Study Design

3.5.3.1.1. Subjects

Seven healthy male subjects of normal weight for height (BMI 20 - 25 kg/m²) were recruited from staff and postgraduates at the University of Surrey. All volunteers were non dieters and were not taking any regular medication other than minor analgesics. Prior to commencement of the investigation all subjects were informed that they should avoid alcohol and strenuous exercise for 24 hours before each study day.

3.5.3.1.2. Test meals

Three test meals (preloads) were developed to provide low carbohydrate and energy density (LC: 1008 kJ), medium carbohydrate and energy density (MC: 1677 kJ), and high carbohydrate and energy density (HC: 2513 kJ). The composition of each preload is detailed in table 3.5.1, and preloads are identical to those used in chapter 4.

<table>
<thead>
<tr>
<th>Carbohydrate (kJ (g))</th>
<th>Low carbohydrate*</th>
<th>Medium carbohydrate*</th>
<th>High carbohydrate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>167 (10)</td>
<td>836 (50)</td>
<td>1672 (100)</td>
</tr>
<tr>
<td>Fat (kJ (g))</td>
<td>828 (22)</td>
<td>828 (22)</td>
<td>828 (22)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>13 (0.8)</td>
<td>13 (0.8)</td>
<td>13 (0.8)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1008</td>
<td>1677</td>
<td>2513</td>
</tr>
</tbody>
</table>

* LC preload: 46g double cream, 10g sucrose
* MC preload: 46g double cream, 10g sucrose, 40g maltodextrin
* HC preload: 46g double cream, 10g sucrose, 90g maltodextrin

3.5.3.1.3. Study Protocol

A single blind randomised crossover design was used to investigate the appetite response to each preload. On three occasions at least seven days apart subjects arrived at the investigation unit at 0900 hours following an overnight fast. After a 20
minute rest period subjects were asked to complete baseline VAS ratings of hunger and satiety, and were then offered one of three preloads. The preloads were consumed at room temperature, and subjects were instructed to drink at a steady pace. Straws were not used for preload consumption in this investigation.

Following consumption of the preload subjects completed a second set of VAS ratings. They were then free to leave the unit and resume their normal working activities, but were instructed to refrain from any sport or recreational exercise for the duration of the study as this may have considerable effects upon appetite. Subjects were required to complete VAS ratings hourly until lunchtime, and were instructed to eat or drink nothing except water until this time.

3.5.3.1.4. Statistical analyses

Hunger and satiety ratings were analysed with repeated measures ANCOVA as described in chapter 2, section 2.3. The baseline sample (-15 min) was used as the covariate, with preload and time (+5 to 180 min) used as repeated measure factors.

3.5.3.2. Results

3.5.3.2.1. Hunger ratings

Mean hunger ratings following consumption of each preload are shown in figure 3.5.1. Repeated measures ANCOVA showed there to be no significant difference in hunger ratings following each preload \((p = 0.5)\), although subjects were consistently more hungry during the study period following the LC preload compared to the MC and HC preloads.
Figure 3.5.1 Hunger ratings following consumption of 1008 kJ, 1677 kJ and 2513 kJ preloads on three separate occasions

Hunger ratings (mean ± SEM, n = 7) following consumption of the LC (■), MC (■) and HC (■) preloads on three separate occasions. There were no significant differences in hunger ratings between preloads (p = 0.5).

3.5.3.2. Satiety ratings

There were also no significant differences in subjective satiety ratings following each preload (figure 3.5.2; p = 0.5), and although subjects did rate themselves as slightly less satiated following the LC compared to the MC and HC preloads the difference was minimal. Multiplicative adjustment of the raw data, and conversion of ratings to z scores did not elucidate any further differences in satiety ratings.
Figure 3.5.2 Satiety ratings following consumption of 1008 kJ, 1677 kJ and 2513 kJ preloads on three separate occasions

Satiety ratings (mean ± SEM, n = 7) following consumption of the LC (■), MC (■) and HC (■) preloads on three separate occasions. There were no significant differences in satiety ratings between preloads ($p = 0.5$).

3.5.4. Effect of cannulation and venipuncture on ratings of hunger and satiety

3.5.4.1. Study design

Ten healthy subjects (6 male, 4 female) who were participating in other studies were recruited. Following an overnight fast, subjects were asked to complete VAS rating scales for hunger and satiety before and after the cannulation process. Following completion of the final VAS subjects continued participation in the study for which they had been cannulated (unrelated to these experiments). In addition a further 4 subjects (male) underwent venipuncture for blood sample extraction in the instance of a blocked cannula. These subjects were asked to complete VAS rating scales before and after the venipuncture procedure. Following completion of the final VAS these subjects were free to resume their normal activities.
3.5.4.2. Results

3.5.4.2.1. Hunger and satiety ratings

Ratings before and after each procedure were analysed using paired, 2 tailed t-tests. There were no significant differences in hunger \( (p = 0.4) \) or satiety \( (p = 0.2) \) ratings before and after cannulation (see table 3.5.1 for mean ratings).

<table>
<thead>
<tr>
<th>Table 3.5.2 Mean (SD) hunger and satiety ratings before and after cannulation of 10 subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cannulation</td>
</tr>
<tr>
<td>Hunger rating (cm)</td>
</tr>
<tr>
<td>Satiety rating (cm)</td>
</tr>
</tbody>
</table>

Similarly venipuncture had little effect on self rated hunger and satiety. As \( n = 4 \) a valid t-test could not be carried out on this data, however comparison of mean hunger and satiety ratings before and after venipuncture show little difference between ratings (table 3.5.2).

<table>
<thead>
<tr>
<th>Table 3.5.3 Mean (SD) hunger and satiety ratings before and after venipuncture of 4 subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before venipuncture</td>
</tr>
<tr>
<td>Hunger rating (cm)</td>
</tr>
<tr>
<td>Satiety rating (cm)</td>
</tr>
</tbody>
</table>

3.5.5. Discussion

The results from these short investigations suggest that experimental conditions during study days are unlikely to have a major confounding effect upon subjective appetite response. Data from investigation 3.5.3 found no significant differences in hunger and satiety responses between each preload. While this is disappointing in terms of ability to detect appetite differences in response to the manipulated preloads,
these data are similar to that obtained in the first multidisciplinary study (chapter 4), which also showed no differences in hunger and satiety ratings following identical preloads. Consequently it would appear that the study environment itself, including the semi-supine position adopted for assessment of gastric emptying and cannulation for blood sampling, was unlikely to have interfered with appetite ratings to the extent that data were confounded. Further evidence that the cannulation procedure has little effect on appetite ratings comes from investigation 3.5.4, which showed similar mean ratings before and after subjects were cannulated (table 3.5.2). Although very small increases in hunger and decreases in satiety were observed before and after cannulation and venipuncture in this study, this is most likely to be attributable to the passing of time. Any negative effects of the procedure would be expected to reduce hunger rather than increase it, particularly if changes were due to feelings of nervousness and apprehension.

In the current study additional factors must also be considered to explain the lack of significant differences between hunger and satiety ratings. Subjects did rate themselves as consistently more hungry and less satiated following the LC preload, and although differences in ratings between the MC and HC preload are similar, data suggests that differences in appetite between higher and lower energy levels were occurring. Variance in the use of the VAS rating scales may be a factor which contributed to this data, as although subjects were briefed on use of the scales at the beginning of the study they were not fully familiar with them. Due to the variance in subjective ratings a larger number of subjects would increase the power of this design for future studies. As subjects were continuing with their normal daily activities energy expenditure was not controlled during the study days, and may have had differential effects upon appetite. It is possible that resumption of normal activities in this case overrode differences in appetite, and as subjects were free to consume water this may also have had some effect on feelings of hunger, perhaps even used as a means of suppressing hunger during the study period. Thus it is possible that while an extremely controlled situation may have adverse effects upon appetite, a free living situation may also confound appetite data. Future investigations may benefit from a relaxed study environment with subjects asked to remain in the investigation unit but
be free to continue with their own work, watch television and move around the room. Energy expenditure would then be standardised while also maintaining a relatively ‘normal’ environment.

To conclude, data from these investigations suggest it is unlikely that the study environment and experimental procedure during our previous research unduly influence appetite response to such an extent that hunger and satiety ratings are confounded. It is more likely that familiarity with use of VAS rating scales, number of subjects and external factors such as boredom have a greater effect on appetite data, and this can be addressed in future studies.
Chapter Four
4. Effect of increasing the carbohydrate and energy content of a preload on post-prandial appetite responses, changes in circulating hormone and metabolite status and gastric emptying

4.1. Introduction

There have been a number of previous studies comparing the relative satiating efficiencies of protein, fat and carbohydrate (e.g. Geliebter, 1979; Hill & Blundell, 1986; de Graaf et al., 1992; Rolls et al., 1994), and the role that increasing the overall energy content of a preload may play in the control of appetite (e.g. Wooley et al., 1972; Duncan et al., 1983). However there is comparatively little work investigating appetite responses to different energy levels derived through the manipulation of a single macronutrient rather than manipulating levels of all preload constituents to increase total energy (e.g. Pliner, 1973; de Graaf et al., 1993). It is possible that preloading at different energy levels may explain discrepancies between studies which have shown there to be differences in the satiating efficiencies of the macronutrients (de Castro & Elmore, 1988; Johnstone et al., 1996; Poppitt et al., 1998) and those which have not (Geliebter, 1979; Driver, 1988; de Graaf et al., 1992). It is also possible that each macronutrient exerts a satiety effect at a specific energy level or range of energy levels, which could in part account for the different satiety responses to individual food items which have been reported (Rolls et al., 1990; Holt et al., 1995a).

The role of carbohydrate in appetite control is frequently debated, and studies have shown post-prandial insulin and glucose response to have some links to appetite (Holt & Miller, 1995b; Lavin et al., 1996; Raben et al., 1996). More recently the role of GLP-1 as a putative satiety hormone has been investigated (Schick et al., 1992;
Lambert et al., 1994; Tang-Christensen et al., 1996), and as a peptide released primarily in response to carbohydrate ingestion (Elliott et al., 1993) this provides an additional mechanism through which carbohydrate may act upon appetite. As GLP-1 acts as an incretin along with the gastrointestinal hormone GIP (Morgan, 1998), it is also possible that either of these hormones may act along with insulin and glucose in a carbohydrate mediated mechanism of appetite regulation. While it is probable that differences in post-prandial satiety following preloads of varying energy and macronutrient content are related to the post-prandial hormone and metabolite responses following these preloads, the role of other gastrointestinal factors can also influence satiety in man, and the rate of gastric emptying is known to be responsive to preloads of different energy content (Wisen et al., 1993). In turn gastric distension may be related to satiety, and it is therefore probable that gastric emptying rate is important in regulation of food intake (Sepple & Read, 1989; Carbonnel et al., 1994).

As the regulation of gastric emptying is responsive to hormonal signals such as GLP-1 (Schirra et al., 1997), assessment of the rate of gastric emptying in relation to hormonal responses following meals of varying carbohydrate and energy content would provide further elucidation of carbohydrate mediated post-prandial satiety mechanisms.

4.2. Aims

The present study therefore aimed to investigate post-prandial responses to meals of increasing energy content manipulated through the alteration of carbohydrate content. The appetite responses to each preload were assessed whilst measuring hormone and metabolite responses, and the gastric emptying rate of each meal was determined. This enabled the relationship between satiety and post-prandial changes in hormones and gastric emptying to be assessed.
4.3. Study design

4.3.1. Subjects

Nine healthy male subjects (age range 23 - 28 years) of normal weight for height (mean BMI 22.4 ± 1.7 kg/m²) were recruited from staff and postgraduates at the University of Surrey. All volunteers selected were non dieters, and were asked to complete the DEBQ prior to inclusion in the study. Only those volunteers who scored less than 3.5 on restrained, emotional or external eating were accepted onto the study. Subjects were also asked to complete a 7 day food diary to provide data on habitual dietary intakes.

Prior to commencement of the study all volunteers underwent standard haematological and biochemical screening conducted by the Royal Surrey County Hospital, and informed written consent was obtained from each individual. In addition screening for the subjects' suitability to the EIE method of gastric emptying was completed. The abdominal region of each subject was measured to locate the approximate position of the stomach, and electrodes were placed over the area located around the stomach. Subjects then consumed a 450 ml water load, and the gastric emptying rate of this water load was monitored to check the correct positioning of electrodes for each subject.

Preceding the study all subjects were instructed that they must refrain from strenuous exercise for the 24 hours before each study day, and avoid alcohol the evening before each study. Ethical approval for this research was obtained from the South West Surrey Local Research Ethics Committee at the Royal Surrey County Hospital.

4.3.2. Test meals

Three preload test meals were developed in collaboration with Dr. Peter Rogers (Dept. of Psychology, University of Bristol) to provide different energy densities through the manipulation of carbohydrate. Each preload was given as a 450 ml milkshake, consisting of double cream, maltodextrin and sucrose, and was made up to volume with water. The low carbohydrate preload (LC) was designed to provide 1008
kJ, the medium carbohydrate preload (MC) provided 1677 kJ and the high carbohydrate preload (HC) provided 2513 kJ. Fat and protein content remained constant in each preload (see table 4.1 for preload composition). Vanilla essence (Langdales vanilla flavouring, E.F. Langdale Ltd., Chase Rd, Northern Way, Bury St. Edmunds, Suffolk, IP32 6NT) improved palatability of the preload, and 1.5 ml was added to flavour each milkshake.

Table 4.1 Composition of preloads with different carbohydrate and energy content

<table>
<thead>
<tr>
<th></th>
<th>Low carbohydrate*</th>
<th>Medium carbohydrate*</th>
<th>High carbohydrate*</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1008</td>
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<td>2513</td>
</tr>
</tbody>
</table>

* LC preload : 46g double cream, 10g sucrose
† MC preload : 46g double cream, 10g sucrose, 40g maltodextrin
‡ HC preload : 46g double cream, 10g sucrose, 90g maltodextrin

Prior to the present study, appetite responses to each preload were tested in a pilot study at the Institute of Food Research, Reading, to determine whether the differences in preload energy and carbohydrate content produced detectable differences in hunger and satiety. These data are presented and discussed with results from the current study.

4.3.3. Study protocol

Using a single blind within subjects randomised crossover design appetite, gastric emptying and hormonal responses to each preload were investigated on three separate occasions seven days apart.

On the evening before each study subjects were asked to consume a standard evening meal (appendix VIII) before 2230 hours, and after this time to drink only water until arrival at the investigation unit. Following the overnight fast subjects arrived at the
investigation unit at 0830 hours, and after a 15 minute rest period were cannulated in an antecubital forearm vein. Electrodes were positioned over the abdominal area and lower back for the measurement of gastric emptying by epigastric impedance epigastrography (EIE) and subjects adopted the semi-supine position required for the measurement of gastric emptying (section 2.2.2). Two baseline blood samples were taken (-15 and 0 min), and subjects completed the first set of VAS ratings. A baseline reading was also obtained on the epigastrograph for approximately 20 minutes. Subjects were then instructed to consume the 450 ml liquid preload at a steady pace. The preload was consumed at room temperature, and drunk through a straw to minimise ingestion of air which could have interfered with the EIE.

Following consumption of the preload post-prandial VAS ratings were completed at 30 minute intervals, and venous blood samples were taken at regular intervals for 2.5 hours (15, 30, 45, 60, 75, 90, 120, 150 min). Gastric emptying of the preload was measured throughout. After the final blood sample (+150 min) the subjects’ cannula was removed and EIE measurements stopped. Subjects were then offered an ad libitum buffet style test meal and instructed to eat as little or as much as preferred, until feeling comfortably full. From this meal macronutrient and energy intake was calculated. Subjects were separated during eating to minimise any effects of social interaction upon food intake, and completed a final VAS when finished. Subjects were then free to leave and resume their normal behaviour, although a food diary was provided and they were asked to record their consumption for the remainder of the study day up to an including breakfast the next morning.

4.3.4. Statistical analyses

Hunger and satiety ratings were analysed using repeated measures ANCOVA. The baseline rating (-15 min) was used as the covariate, with preload and time (+5 to 150 min) as repeated measures factors. Ratings made following consumption of the buffet test meal were analysed separately as they were not primarily responsive to the manipulated preload. Buffet test meal intakes and satiety quotients were analysed using repeated measures ANOVA, with preload as the repeated measures factor.
Hormone and metabolite responses and gastric emptying data were analysed using repeated measures ANOVA, with preload and time (-15 to 150 min) as repeated measures factors.

Significant ANOVA / ANCOVA results were analysed further with post hoc testing, to determine between which preloads significant differences lay.

4.4. Results

4.4.1. Appetite responses

4.4.1.1. Hunger ratings

Mean hunger ratings following consumption of the LC, MC and HC preloads are illustrated in figure 4.1. There was no significant difference in hunger response to each preload for the 150 minutes following preload consumption \( (p = 0.2) \), and no difference in hunger ratings following consumption of the buffet test meal \( (p = 0.1) \).

It was noted that the greatest difference in hunger response occurred 30 minutes after consumption of the preload, and repeated measures ANOVA at +30 min showed there to be a significant difference in hunger ratings at this time \( (p = 0.05) \). Post hoc testing showed this to be due to significantly lower hunger following the HC preload compared to the LC preload \( (p = 0.02) \). It was at this time following preload consumption (30 minutes) that the greatest difference in gastric emptying and volume of the preload remaining in the stomach occurred (section 4.4.3), and this relationship is discussed in section 4.5. Transformation of hunger data to z scores and multiplicative adjustment for baseline did not elucidate any further differences in hunger responses.
Figure 4.1 Hunger ratings following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

![Hunger ratings following consumption of preloads](image.png)

Hunger ratings (mean ± SEM, n = 9) following consumption of LC (■), MC (■) and HC (■) preloads. There were no significant differences in hunger response to each preload. At +30 minutes hunger ratings were significantly lower following the LC compared to the HC preload ($p = 0.02$).

As a pilot study had been run prior to this investigation to determine appetite responses to the LC, MC and HC preloads, the hunger data from this study was combined with that of the pilot study to increase the power of the statistical analysis. The pilot study involved only appetite ratings, and the time points used for VAS ratings were the same as in the current investigation. Hunger ratings of the combined data were found to be different between preloads at a level approaching significance ($p = 0.06$, figure 4.2). Post hoc analysis of the ANCOVA showed hunger ratings to be significantly higher following the LC preload than the HC preload ($p = 0.03$). There was no significant difference in hunger ratings between the LC and MC ($p = 0.2$) and MC and HC preloads ($p = 0.3$).
Figure 4.2 Hunger ratings following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Combined data from the current investigation and the pilot study.

Hunger ratings (mean ± SEM, n = 18) following consumption of LC (■), MC (■) and HC (■) preloads. Ratings were significantly higher following the LC compared to the HC preload ($p = 0.03$). There was no significant difference in hunger ratings between the LC and MC ($p = 0.2$) and MC and HC ($p = 0.3$) preloads.

4.4.1.2. Satiety ratings

Satiety ratings showed a similar (inverted) pattern to hunger ratings, and there were no significant differences in satiety response following preload consumption (figure 4.3, $p = 0.2$). However, it was observed that subjects rated themselves consistently more satiated following consumption of the HC preload than either the MC or LC preloads. Following consumption of the buffet test meal there was no significant difference in satiety ($p = 0.4$), and no significant difference in ratings was observed between preloads 30 minutes after preload consumption ($p = 0.3$).
Figure 4.3 Satiety ratings following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Satiety ratings (mean ± SEM, n = 9) following consumption of the LC (■), MC (■) and HC (■) preloads. There were no significant differences between preloads ($p = 0.2$).

Satiety data was transformed to z scores for additional analysis, and repeated measures ANCOVA on this data showed a significant main effect of preload on satiety ratings ($p = 0.02$, figure 4.4). Post hoc testing of the sample showed subjects to be significantly less satiated following the MC than either the LC ($p = 0.02$) or the HC ($p = 0.03$) preload. There was no significant difference in z scores between the LC and HC preloads ($p = 0.6$).
Figure 4.4 Z scores calculated from satiety ratings following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC).

Satiety z scores (mean ± SEM, n = 9) following consumption of the LC (■), MC (■) and HC (■) preloads. There were significant differences in adjusted satiety ratings between the MC and LC preloads ($p = 0.02$) and the MC and HC preloads ($p = 0.03$).

Combined data from the current investigation and the pilot study showed there to be a significant difference in satiety ratings between preloads ($p = 0.04$, figure 4.5). Post hoc analysis of data showed satiety ratings to be significantly higher following the HC preload compared to the LC ($p = 0.03$) and MC ($p = 0.04$) preloads. There was no significant difference in satiety ratings between the LC and MC preloads ($p = 0.8$).
4.4.1.3. Food preference checklist data

The number of items and total energy selected were calculated from the FPC’s (figures 4.6 and 4.7 respectively). Subjects selected a greater amount of energy and larger number of items from the FPC as preload energy content decreased, although this did not achieve statistical significance ($p = 0.4$ in both cases). Transformation of data to $z$ scores and multiplicative adjustment for baseline did not highlight any additional differences between preloads.

Macronutrient choice from the FPC was also analysed by dividing the food items selected into high protein, high carbohydrate, high fat, low energy or mixed macronutrient foods. The distribution of foods selected was similar following each preload. It was noted that the number of high carbohydrate food items selected was not different between preloads at any time during the study period.
Figure 4.6 Number of items selected from the food preference checklist following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Number of items (mean ± SEM, n = 9) selected from the FPC following consumption of the LC (■), MC (■) and HC (■) preloads. There were no significant differences in the number of items selected following each preload (p = 0.4).

Figure 4.7 Total energy selected from the food preference checklist following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Total energy (mean ± SEM, n = 9) selected from the FPC after consumption of the LC (■), MC (■) and HC (■) preloads. There were no significant differences (p = 0.4).
4.4.1.4. Ad libitum buffet meal intake

Ad libitum intakes from the buffet test meal showed a trend to decrease as energy content of the preload increased, although this difference did not achieve statistical significance ($p = 0.3$, figure 4.8). Macronutrient intakes and the weight of food eaten reflected the changes in energy intake in each instance, and were not significantly different ($p > 0.1$ in all cases, figure 4.9). Analysis of intakes in relation to subject bodyweight (intake in kJ/kgbw or gram/kgbw) did not show any further differences in buffet energy intake.

Figure 4.8 Buffet meal energy intakes 150 minutes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Buffet meal energy intake (mean ± SEM, n = 9) following consumption of the LC (■), MC (■) and HC (■) preloads. Intakes were not significantly different between preloads ($p = 0.3$).
Figure 4.9 Buffet meal macronutrient intakes and weight of food eaten 150 minutes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Buffet meal macronutrient intakes and weight of food eaten (mean ± SEM, n = 9) following consumption of the LC (■), MC (■) and HC (■) preloads. Intakes were not significantly different between preloads (p > 0.1).

Hunger quotients were calculated from energy intake as described in section 2.2.1.2. and showed little difference in response following the LC and HC preloads (figure 4.10). However repeated measures ANOVA showed a difference in hunger quotients approaching significance (p = 0.07) due to a significant difference in the hunger quotient between the MC and HC preloads (p = 0.05).

Satiety quotients were also calculated and showed there to be a difference approaching significance between each preload (p = 0.06, figure 4.11). Post hoc testing showed this difference to be due to a significantly lower satiety quotient following the HC compared to the MC preload (p = 0.03), with a greater decrease in satiety during the buffet meal following the MC preload.
Figure 4.10 Hunger quotients calculated from buffet meal energy intake 150 minutes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Hunger quotients (mean ± SEM, n = 9) after consumption of the LC (■), MC (■) and HC (■) preloads. Difference between preload approached significance ($p = 0.07$).

Figure 4.11 Satiety quotients calculated from buffet meal energy intake 150 minutes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Satiety quotients (mean ± SEM, n = 9) after consumption of the LC (■), MC (■) and HC (■) preloads. Difference between preloads approached significance ($p = 0.06$).
Buffet meal energy intake data from the current study was combined with that of the pilot study (figure 4.12). There was a clear stepwise decrease in energy intake as preload carbohydrate and energy content increased, with a significant difference in energy intakes between preloads ($p = 0.05$). Post hoc analysis showed energy intake to be significantly greater following the LC preload compared to the HC preload ($p = 0.02$). There was no significant difference in intakes between the LC and MC preloads ($p = 0.1$), or the MC and HC preloads ($p = 0.4$).

**Figure 4.12** Buffet meal energy intakes 150 minutes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Combined data from the current investigation and the pilot study.

![Energy Intake Chart](image)

Buffet meal energy intake (mean ± SEM, n = 18) following consumption of the LC (■), MC (■) and HC (■) preloads. Intake was significantly greater after the HC preload compared to the LC preload ($p = 0.02$). There was no significant difference in intakes between the LC and MC ($p = 0.1$), or MC and HC ($p = 0.4$) preloads.

To determine the extent to which compensation occurred for differences in preload energy during consumption of the buffet meal, the total intake during the study period (preload + buffet meal energy) was calculated and compared between preloads (figure 4.13). There was no significant difference in total intake between the LC (mean 9008 SD 2997 kJ) and MC (mean 9050 SD 2374 kJ) preloads ($p = 0.9$), although total energy
intake following the HC preload (mean 10049 ± 2136 kJ) was significantly higher than both the LC ($p = 0.03$) and MC ($p = 0.03$) preloads.

**Figure 4.13** Total energy intake throughout the study period with preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Energy intake (mean ± SEM, n = 9) throughout study period from preload energy (■) plus buffet intake following consumption of the LC (■), MC (■) and HC (■) preloads. Total energy intake was significantly higher with the HC preload compared to the MC and LC preloads ($p = 0.03$ in both cases).

Percentage compensation in buffet meal energy intake for preload energy differences was calculated, with subjects showing 93% compensation between the LC and MC preloads for the 669 kJ preload energy difference. No compensation was seen for the difference in preload energy (836 kJ) between the MC and HC preload, with subjects eating more following the HC preload. There was a 31% compensation in energy intake between the LC and HC preload.

Further analysis of buffet meal energy intake showed there to be distinct differences in individual response to preload manipulations, and it was observed that only 3 out of 9 subjects decreased their energy intake with increasing preload energy content.
(figure 4.14). Of the remaining subjects 4 out of 9 had similar intakes following each preload, while 2 out of 9 subjects actually increased their energy intake as preload energy content increased. Repeated measures ANOVA with test occasion as the repeated measures factor was used to determine any significant order effect in this data. There was no significant difference in buffet test meal energy intake between test occasions ($p > 0.1$).

**Figure 4.14** Buffet meal energy intake of individual subjects following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Buffet meal energy intakes of each subject following consumption of the LC (■), MC (■) and HC (■) preloads.

The relationship between self rated appetite and food intake was assessed, with correlations between buffet meal energy intake and the VAS and FPC ratings made immediately before buffet meal consumption (time +150 min). There was no relationship between energy intake and self-rated hunger ($r = -0.31, p > 0.1$) or satiety ($r = -0.02, p > 0.1$), and no relationship between energy intake and number of items ($r = 0.07, p > 0.1$) or total calories ($r = 0.1, p > 0.1$) selected from the food preference checklist.
4.4.1.5. Food intake during the remainder of the study day

After the study period subjects were asked to complete a food diary for the remainder of the study day, up to and including breakfast the following morning. In order to determine whether compensation for differences in preload energy occurred at any time during the 24 hours after each preload mean intakes for the rest of the study were calculated (table 4.3, see also figure 4.16).

Subjects had a significantly greater energy intake during the rest of the study day following the LC preload compared to the MC and HC preloads ($p = 0.04$ in both cases). Consequently total energy intake during the study day was higher after the LC than either the MC or HC preloads, although this differences was not significant ($p > 0.1$). Differences in carbohydrate intake throughout the rest of the day were similar to those for energy, and following the LC preload subjects had a greater mean carbohydrate intake than after the MC or HC preloads ($p > 0.1$).

Fat intake during the rest of the study day followed the same pattern as energy intake ($p > 0.1$). Total fat intake during the study day was significantly higher following the LC than the MC preload ($p = 0.039$), and tended to be higher than following the HC preload ($p > 0.1$). Protein intakes throughout the study day were similar after the MC and HC preloads, and slightly elevated following the LC preload ($p > 0.1$).
Table 4.2 Mean (SD) energy and macronutrient intakes throughout the study day

<table>
<thead>
<tr>
<th></th>
<th>LC</th>
<th>MC</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preload intake</td>
<td>241 (0)</td>
<td>401 (0)</td>
<td>601 (0)</td>
</tr>
<tr>
<td>Buffet meal intake</td>
<td>1923 (720)</td>
<td>1766 (568)</td>
<td>1802 (511)</td>
</tr>
<tr>
<td>Rest of day intake</td>
<td>1577 (404)</td>
<td>1171 (480)</td>
<td>1159 (371)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3741 (862)</td>
<td>3338 (917)</td>
<td>3562 (791)</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preload intake</td>
<td>10 (0)</td>
<td>50 (0)</td>
<td>100 (0)</td>
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<tr>
<td>Buffet meal intake</td>
<td>232 (89.4)</td>
<td>215 (72)</td>
<td>218 (69)</td>
</tr>
<tr>
<td>Rest of day intake</td>
<td>170 (38)</td>
<td>148 (84)</td>
<td>149 (65)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>412 (85)</td>
<td>413 (132)</td>
<td>467 (122)</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preload intake</td>
<td>22 (0)</td>
<td>22 (0)</td>
<td>22 (0)</td>
</tr>
<tr>
<td>Buffet meal intake</td>
<td>86 (33)</td>
<td>77 (25)</td>
<td>80 (23)</td>
</tr>
<tr>
<td>Rest of day intake</td>
<td>61 (31)</td>
<td>40 (29)</td>
<td>47 (18)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>169 (39)</td>
<td>139 (42)</td>
<td>149 (35)</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preload intake</td>
<td>0.2 (0)</td>
<td>0.2 (0)</td>
<td>0.2 (0)</td>
</tr>
<tr>
<td>Buffet meal intake</td>
<td>51 (18)</td>
<td>50 (18)</td>
<td>49 (13)</td>
</tr>
<tr>
<td>Rest of day intake</td>
<td>54 (34)</td>
<td>42 (14)</td>
<td>45 (8)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>105.2 (40)</td>
<td>92.2 (17)</td>
<td>94.2 (14)</td>
</tr>
</tbody>
</table>

4.4.1.6. Habitual dietary intake and appetite responses

All subjects were instructed to complete a 7 day food diary before commencement of the study to provide data on habitual dietary intake (appendix IX).

To determine the extent to which habitual intake influenced the food intake response of subjects during the study, the mean buffet energy intake of each subject was correlated with habitual daily energy intake. Although there was no correlation between habitual daily energy intake and mean energy consumed during the buffet ($r = 0.35 \ p > 0.1$), the individuals with the highest habitual energy intakes tended to consume most during the buffet test meal. It was noted that subjects consistently overate during each study day such that both their buffet intake was significantly higher than their habitual lunchtime intake ($p < 0.001$ following each preload, figure 4.15), and their total energy intake during the study day was significantly higher than
their habitual daily intake ($p < 0.03$ following each preload, figure 4.16). To determine whether the ability to detect differences in preload energy content was influenced by the habitual intake of a subject the difference in buffet meal intake between the LC and HC preloads was correlated with habitual energy intake. There was no relationship between difference in buffet energy intake and habitual dietary intake ($r = -0.2, p = 0.5$).

**Figure 4.15  Mean buffet energy intakes after consumption of manipulated preloads compared to habitual lunchtime energy intakes of individual subjects.**

Mean buffet meal energy intakes (■) after the LC, MC and HC preloads compared to habitual lunchtime energy intakes (■) of each subject. Subjects consistently ate more from the buffet test meal than their usual lunchtime intake.
Figure 4.16 Mean buffet energy intakes after consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC) compared to habitual energy intakes of individual subjects

Comparison of total study day energy intakes (mean ± SEM, n = 9) from the preload (■), buffet meal (LC (■), MC (■) and HC (■) preloads) and rest of day (hatched area) and habitual daily energy intake (■). Mean study day energy intake was significantly higher than habitual daily energy intake (p < 0.03).

The habitual energy intakes of the subject group varied by approximately 4180 kJ /day (range 9137 to 12423 kJ/day), and the hunger and satiety responses of individuals to each preload was investigated with respect to their habitual energy intakes. Subjects were retrospectively divided into 2 groups of higher (mean 11579 SD 506 kJ/day, n = 5) and lower (mean 9727 SD 548 kJ/day, n = 4) energy intakes, and their appetite responses compared using repeated measures ANOVA with habitual intake as a between subjects factor, and time as the repeated measures factor. There was shown to be no significant difference in the appetite responses of subjects with higher and lower habitual energy intakes (hunger p > 0.4 all preloads, satiety p > 0.3 all preloads). Furthermore there were no significant differences in hunger and satiety responses immediately following consumption of the preload (time 0, p > 0.1), and before or after consumption of the buffet test meal (time +150 and +165, p > 0.1).

The change in hunger during the study period was not correlated with habitual energy.
intake, and there was no significant relationship between changing hunger and habitual energy intake following either preload (LC: $r = -0.27, p = 0.4$, MC: $r = -0.1, p = 0.8$, HC: $r = 0.2, p = 0.6$).

Data were also analysed to compare habitual carbohydrate intakes with appetite responses following the LC, MC and HC preloads. Subjects were retrospectively divided into groups of lower (mean 282 SD 23.6 g/day, $n = 5$) and higher (mean 354 SD 34.2 g/day, $n = 4$) habitual carbohydrate intakes, and hunger and satiety responses following each preload analysed as described above. It should be noted that the high carbohydrate intake group differed from the high energy intake group by one subject who had a large energy intake from fat. Hunger and satiety data showed a similar pattern of response to the results above, with no significant differences in appetite responses between the high and low intake groups ($p > 0.3$, all preloads), and no significant correlation between buffet intake and habitual carbohydrate intake ($p > 0.3$, all preloads).

4.4.2. Blood metabolite and hormone responses

4.4.2.1. Plasma glucose

Post-prandial plasma glucose levels were significantly different between preloads ($p < 0.0001$, figure 4.17) and circulating glucose concentration increased as energy and carbohydrate content of the preload increased. Post hoc testing of this analysis showed glucose to be significantly higher following the HC preload compared to the LC ($p = 0.0001$) and MC ($p = 0.009$) preloads. Glucose levels were also significantly higher following the MC compared to the LC preload ($p = 0.01$). A significant preload x time interaction was observed between preloads ($p < 0.0001$), due to the faster return to baseline following the MC preload.

As carbohydrate and energy content of the preload increased, the mean time for which glucose remained elevated also increased (LC: 60 min, MC: 95 min, HC: 150 min). The time taken to return to baseline was analysed by ANOVA, and was found to be significantly longer following the HC than both the MC ($p = 0.0002$) and LC
(\(p < 0.0001\)) preloads. Time to return to baseline was also significantly longer after consumption of the MC compared to the LC preload (\(p = 0.0008\)).

Figure 4.17 Plasma glucose levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Post-prandial glucose concentrations (mean ± SEM, \(n = 9\)) following consumption of the LC (■), MC (■) and HC (■) preloads. Glucose levels were significantly different between the LC and MC (\(p = 0.01\)), LC and HC (\(p = 0.0001\)), and MC and HC preloads (\(p = 0.009\)). A significant preload \(\times\) time interaction was also observed (\(p < 0.0001\)).

4.4.2.2. Plasma insulin

Circulating insulin concentrations were also seen to increase as carbohydrate and energy content of the preload increased (figure 4.18). Prolonged elevated levels of insulin were observed following the HC preload; these had not returned to basal levels by the end of the study session (150 min). Following the LC preload insulin had returned to baseline approximately 60 min after the preload, and approached basal levels approximately 150 min following the MC preload. ANOVA showed there to be a significant difference in insulin response between preloads (\(p = 0.0003\)), with post-prandial levels significantly higher following the HC than both the MC
(\(p = 0.005\)) and LC (\(p = 0.0002\)) preloads. There was also a significantly increased insulin response following the MC compared to the LC preload (\(p < 0.0007\)). A significant preload \(x\) time interaction was also observed (\(p < 0.0001\)), attributable to the differences in rate of return to baseline between the preloads.

Figure 4.18 Plasma insulin levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

![Graph showing plasma insulin levels](image)

Post-prandial insulin concentrations (mean ± SEM, \(n = 9\)) following consumption of the LC (■), MC (■) and HC (■) preloads. Significant differences were seen between the LC and HC preloads (\(p = 0.0002\)) and the MC and HC preload (\(p = 0.005\)). Difference between the LC and MC preloads approached significance (\(p < 0.0007\)).

4.4.2.3 Plasma GIP

Post-prandial plasma GIP concentrations following each preload are illustrated in figure 4.19. Consumption of each preload resulted in an immediate rise in circulating GIP levels which remained elevated throughout the study period. Increasing the carbohydrate and energy content of the preload significantly increased post-prandial GIP levels (ANOVA, \(p = 0.003\)). Post hoc testing showed significant differences in post-prandial GIP concentrations between the LC and HC preloads (\(p = 0.001\)), and the MC and HC preloads (\(p = 0.04\)). Differences on overall post-prandial GIP
concentrations between the LC and MC preloads approached significance ($p = 0.08$). For the first 45 minutes after preload consumption plasma GIP levels were significantly greater with the MC compared to the LC preload ($p = 0.04$), and peak GIP concentration was significantly higher following the MC preload than the LC preload ($p = 0.0004$). A significant preload x time effect was seen between the preloads ($p = 0.001$), due to differences in GIP response during the first 45 minutes after preload consumption.

**Figure 4.19** Plasma GIP levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

![Graph showing plasma GIP levels](image)

Post-prandial GIP concentrations (mean ± SEM, $n = 9$) after consumption of the LC (■), MC (■) and HC (■) preloads. GIP was significantly higher following the HC preload than the LC ($p = 0.001$) and MC ($p = 0.04$) preloads. The difference between LC and MC preload approached significance ($p = 0.08$), although peak GIP concentrations were higher with the MC preload ($p = 0.0004$).

### 4.4.2.4. Plasma GLP-1

Post-prandial GLP-1 exhibited a large rise immediately after consumption of the HC preload compared to both the MC and LC preloads (figure 4.20), with GLP-1 concentrations significantly higher as carbohydrate and energy content of the preloads
increased ($p = 0.001$). Post hoc testing showed the HC preload to produce a significantly greater GLP-1 response than the LC and MC preloads ($p = 0.002$ in both cases). Although there was a slightly higher peak GLP-1 following the MC preload compared to the LC preload, overall post-prandial response was similar and not significantly different between these preloads ($p = 0.9$). It was noted that peak GLP-1 concentration was reached earlier with higher preload carbohydrate and energy content, and after the HC preload peak concentration was reached within 15 minutes. Following the MC and LC preloads peak levels were reached after 30 and 45 minutes respectively. A significant preload x time interaction was observed ($p = 0.0002$), attributable to the difference in rate of change of GLP-1 concentrations for the first 60 minutes after preload consumption.

Figure 4.20 Plasma GLP-1 levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Post-prandial GLP-1 concentrations (mean ± SEM, n = 9) after consumption of the LC (■), MC (■) and HC (■) preloads. GLP-1 was significantly greater following the HC preload than the LC and MC preloads ($p = 0.002$). There was not a significant difference between the LC and MC preloads ($p = 0.9$).
4.4.2.5. Plasma TAG

Circulating TAG showed little difference in response for 90 minutes following consumption of the preloads (figure 4.21). There was no significant difference in TAG response between preloads ($p = 0.5$), and circulating levels showed no observable increase during the first 90 min post-prandially. A significant preload x time interaction was observed ($p < 0.0001$), due to the difference in TAG concentrations apparent at the end of the study period (90 to 150 min). At 150 min after preload consumption TAG levels were significantly higher following the LC than the MC preload ($p = 0.02$), and higher following the LC compared to the HC preload ($p = 0.003$). There was no significant difference in TAG between the MC and HC preloads ($p > 0.05$).

Figure 4.21 Plasma TAG levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Post-prandial TAG concentrations (mean ± SEM, $n = 9$) after consumption of the LC (■), MC (■) and HC (■) preloads. There was no significant difference between preloads until +150 min. At this time TAG was significantly higher following the LC than the MC ($p = 0.02$) and HC ($p = 0.003$) preloads.
4.4.2.6. Plasma NEFA

A reduction in plasma NEFA concentration was seen following consumption of the preloads, with a greater reduction following the HC preload (figure 4.22). ANOVA showed there to be significant difference in NEFA concentrations \((p = 0.001)\), with the LC preload resulting in less suppression of NEFA than either the MC \((p = 0.004)\) or HC \((p = 0.0006)\) preloads. Although NEFA levels were higher following the MC than the HC preload, this difference was not statistically significant \((p = 0.3)\). A significant preload \(\times\) time interaction was apparent \((p > 0.0001)\) due to differences in NEFA levels from 45 minutes after preload consumption. ANOVA of this time period \((45\) to \(150\) min) showed NEFA to be significantly higher with the LC preload compared to the MC \((p = 0.0003)\) and HC \((p < 0.0001)\) preloads during this time.

Figure 4.22 Plasma NEFA levels following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Post-prandial NEFA concentrations (mean ± SEM, \(n = 9\)) following consumption of the LC (■), MC (■) and HC (■) preloads. NEFA was significantly higher after the LC preload than either the MC \((p = 0.004)\) and HC \((p = 0.0006)\) preloads.
4.4.3. *Gastric emptying response*

The percentage of the preload remaining in the stomach was calculated at 15 minute intervals following consumption, and is shown in figure 4.23. Analysis by repeated measures ANOVA showed the percentage of the meal remaining in the stomach to be significantly higher as the carbohydrate and energy content of the preload increased \( (p < 0.0001) \), thus the rate of emptying was slower as carbohydrate and energy content of the preload increased. The LC preload emptied more quickly than the MC \( (p = 0.0002) \) and HC \( (p = 0.00008) \) preloads, with a smaller difference between the MC and HC preloads \( (p = 0.004) \). The lag period at the beginning of the emptying curve was shortened with lower carbohydrate and energy content of the preload.

**Figure 4.23** Percentage of the meal remaining in the stomach for 120 minutes after ingestion of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

Mean ± SEM \( (n = 9) \) percentage of meal remaining in the stomach after consumption of the LC (■), MC (■) and HC (■) preloads. Gastric emptying rate was significantly slower as carbohydrate and energy content of the preload increased \( (p < 0.001 \) between all preloads).

Half emptying rates \( (T_{50}) \) were calculated from the gastric emptying curves generated by EIE (figure 4.24). Preload composition was shown to have a significant effect on
half emptying rate \((p < 0.0001)\), and post hoc testing showed a significant increase in half emptying time with increasing carbohydrate and energy content of the preload. The HC preload exhibited a significantly longer T50 than both the MC \((p = 0.0007)\) and LC \((p < 0.0001)\) preloads, and the MC preload had a significantly longer T50 than the LC preload \((p = 0.0006)\).

Figure 4.24 Time taken for half of the meal to empty from the stomach (T50) following consumption of preloads containing 1008 kJ (LC), 1677 kJ (MC) and 2513 kJ (HC). Differences in energy content of preload were achieved through the manipulation of carbohydrate.

![Graph showing T50 times for LC, MC, and HC preloads.](image)

Half emptying time (T50) \((\text{mean } \pm \text{ SEM, } n = 9)\) following consumption of the LC (■), MC (■) and HC (■) preloads. Half emptying rate was significantly shorter as fat and energy content of the preload increased \((p < 0.001 \text{ between all preloads})\).

The T50 of gastric emptying was correlated with total area under the curve for GLP-1, and these factors were found to significantly correlate \((r = 0.4, p = 0.04, \text{ all data})\). Plasma GLP-1 was also found to correlate with the percentage of the preload remaining in the stomach \((r = 0.36, p < 0.01, \text{ all data})\).
4.5. Discussion

While it has been previously shown both that subjects find it difficult to (Wooley et al., 1972) or are able to (Pliner, 1973) distinguish between preloads of different energy content in their appetite responses, data from the present study suggest that increased satiety and decreased food intake are associated with increased energy and carbohydrate content of a preload. Furthermore this study suggests that the number of subjects used in appetite investigations is a critical factor in determining the extent of the differences seen.

Hunger and satiety ratings following each preload showed small difference as carbohydrate and energy content increased, with subjects tending to rate themselves as less hungry and more satiated following the HC preload. Similar responses were seen in data from the food preference checklists, with subjects tending to select a greater number of items with decreasing preload energy and carbohydrate content. Transformation of satiety data to z scores elucidated larger difference in appetite responses, and although this was not in the direction expected - subjects were found to be least satiated following the MC preload, this suggests that the large variance in sample means has the effect of reducing differences in ratings between preloads. The large variation seen in subjective appetite ratings causes problems in the interpretation of VAS data. Often mean differences are in the direction expected and following the pattern expected, but significant differences are not apparent due to between subject and within subject variation. As the variation in a sample is a primary factor in determining the number of subjects required and the power of the statistical analyses, use of a larger subject group is likely to help minimise the problems induced by large between and within subject variation. A pilot study had been undertaken prior to this investigation to assess the appetite responses following preload carbohydrate and energy manipulations in nine subjects. The pilot study did find appetite differences between the LC, MC and HC preloads (Day et al., 1998), and to assess the effect of increasing the number of subjects, data from the pilot study and the current study were combined for analysis. It should be noted that during the pilot study gastric emptying measurements and blood samples were not taken, but timing of VAS ratings and the buffet test meal was identical to that of the present investigation. Doubling
the number of subjects investigated increased the mean difference in appetite responses between preloads, and resulted in a stepwise increase in hunger as preload carbohydrate and energy content decreased \((p = 0.06)\), with significantly greater hunger following the LC compared to the HC preload \((p = 0.03)\). Similarly the difference between satiety ratings was more apparent \((p = 0.04)\), with significantly greater satiety following the HC preload compared to both the LC \((p = 0.03)\) and MC \((p = 0.04)\) preloads.

Buffet intake data support the above observation, as data from the current study showed a trend for subjects to decrease buffet energy intake as preload energy content increased, but with no significant differences. Combined analysis of these data with pilot study data again increased the differences seen between preloads, and resulted in a significant effect of preload upon energy intake \((p = 0.05)\). There was a stepwise decrease in energy intake with increasing energy content of the preload, and this difference was significant between the LC and HC preloads \((p = 0.02)\). Taken together the appetite responses and food intake data from the pilot and current studies support an effect of the preload manipulations upon appetite, with the difference between the LC and HC preload being the most pronounced. The distinction between energy levels would be expected to be better when there is a larger difference between the energy content of preloads, and this would explain why the largest differences in appetite responses in this study tend to be seen between the LC and HC preload in each parameter. This may also help explain why the literature in this area shows conflicting data, with Pliner (1973) using a similar difference in preload energy manipulations to this study (1672 kJ (400 kcal)) and showing compensation for this difference in subsequent food intake, while investigations using smaller energy manipulations, for example 369 kJ (95 kcal) (Rolls et al., 1994) and 819 kJ (196 kcal) (Wooley et al., 1972) found little difference in appetite responses and food intake between preloads. The design of the present investigation does not allow the responses seen to be attributed to either energy or carbohydrate differences, and to determine whether macronutrient or energy content was the primary influence the relative effects of increasing fat or protein content of a preload would also need to be
assessed at the same energy levels. The appetite responses to increases in energy through the variation in fat content will be discussed in the next chapter.

Food intake responses from the current study were examined further to determine whether individual differences in response could account for the variation observed. It was found that the subjects had differences in their food intake responses which could be grouped as either a similar intake between each preload, increased intake as preload energy and macronutrient content increased, or decreased intake as preload energy and macronutrient content increased. Although reasons for this can only be speculated, it was noted that those subjects who responded as would be expected, by decreasing their test meal energy intake as preload energy and macronutrient content increased, were those who undertook regular recreational exercise, and the effect of exercise upon responses to manipulated preloads was investigated further and is discussed in chapter 8. It is possible that individuals who tended to eat the same following each preload manipulation ate in response to their expectations of the energy content of the preload rather than in response to actual preload manipulations (Tepper et al., 1991), or that they ate what would be considered a 'usual lunchtime intake' regardless of preload energy levels. In addition individuals who ate more as preload energy and carbohydrate content increased may have been those individuals who exhibited higher levels of dietary restraint, as restrained eaters have been shown to eat more following a greater known preload energy intake (Herman & Mack, 1975). However in this investigation the subjects recruited exhibited low levels of restraint (1.0 - 2.5), and would not be considered as restrained eaters by the DEBQ. Furthermore the subject with the highest score for restraint (2.5) was one of those who responded by reducing energy intake as preload energy and carbohydrate content increased. Thus it seems that other factors are likely to be responsible for food intake in these individuals, and poor regulation of intake could be attributed to a number of determinants, e.g. study conditions, poor ability of the subjects themselves to judge energy manipulations, and prior nutritional status. As all subjects showed a grossly elevated intake at the buffet test meal compared to their normal lunchtime intake, and throughout the entire test day compared to their usual daily intake it seems likely that a novel situation and the presentation of a large amount of freely available highly
palatable food promoted over consumption. This is likely to have contributed considerably to the food intake data seen, and the variety, palatability and amount of test meal food used should be addressed as an area of possible error in future investigations.

It was noted that macronutrient intake at the test meal and throughout the rest of the study day differed little between preloads, suggesting that short term food intake is unlikely to be adjusted to account for differences in macronutrient intake. If the oxidation of substrates is responsible for some degree of appetite control (Stubbs, 1998) then any adjustments in macronutrient ratio is likely to occur over longer periods of time. In light of recent work by the author suggesting that habitual protein intake influences the satiating efficiency of a protein load (Long et al., 2000) the influence of habitual diet on appetite responses was retrospectively investigated in the current study. There was found to be no relationship between habitual energy or carbohydrate intake of the group and appetite responses to each preload, suggesting that an appetite mechanism similar to the protein-stat (Millward, 1995) is unlikely to be operating in the regulation of food intake through carbohydrate, and supporting a primary role of oxidation for this action (Stubbs, 1998). However the number of subjects in this investigation does not allow adequate comparison of different levels of habitual food intake with appetite responses, and more work is needed in this area to elucidate the role of habitual diet. It was noted that subjects with a higher habitual energy intake tended to consume more during the buffet test meal. Although this was unlikely to have adversely affected the food intake data due to the within subjects experimental design, it does provide some suggestion of an influence of habitual intake on food intake control.

As mentioned above it has been speculated that oxidation rates of macronutrients are responsible for part of their control over food intake, with carbohydrate exerting a stronger appetite effect than fat due to its smaller storage capacity, which through feedback mechanisms may regulate energy intake (Flatt, 1987). While such a mechanism may act to regulate appetite over periods of longer than a few hours, in this study we aimed to examine differential hormone and metabolite responses to
Chapter 4

Preloads of increasing carbohydrate content to determine whether these factors are responsible for the short term regulation of food intake (i.e. periods of 1 - 4 hours, the usual inter-meal intervals). Glucose and insulin responses seen were as expected with preloads of increasing carbohydrate content, with insulin levels significantly greater as preload carbohydrate content increased. Overall plasma glucose response was also significantly greater with higher carbohydrate intake. Peak glucose levels were actually similar between the MC and HC preloads, but plasma levels remained elevated for longer with the HC preload. Although insulin and glucose responses were increased with increasing carbohydrate and energy content of the preload, with appetite ratings and food intake showing a similar relationship with changes in preload content, data from this investigation could not be used to investigate any direct relationships between these parameters due to the small differences seen in appetite responses with the 9 subjects used. However as the combined pilot study / current study data showed lower hunger and greater satiety when levels of insulin (and other hormones) were greater, and these data are consistent with some effect of hormonal responses upon appetite ratings.

Plasma GIP and GLP-1 responses were also in line with those seen previously after meals of differing carbohydrate content (Hampton et al., 1986; Morgan, 1998), and showed a significantly increased response as preload carbohydrate and energy content increased. For GLP-1 peak concentrations were slightly elevated following the MC compared to the LC preload, although overall response was similar, while after the HC preload levels were more than doubled compared to the other two preloads. This relates to some extent to appetite data, as ratings with both 9 and 18 subjects showed the greatest satiety following the HC preload, with little difference in satiety between the MC and LC preloads. As the buffet test meal was given to subjects 150 minutes following the preload food intake would not have been responsive to GLP-1 levels; as by this time post-prandial GLP-1 concentrations had nearly returned to baseline and there was no difference in plasma values between each preload. Thus if circulating GLP-1 were to have any effect on food intake it would not be apparent from this study. However it is interesting to note some similarity between GLP-1 and appetite responses, particularly as GLP-1 has been proposed as a satiety hormone (Turton et
al., 1996), and it is possible that GLP-1 could interact with a glucostatic mechanism of food intake control due to its release in response to carbohydrate, and similar pattern of response to insulin (Lavin et al., 1998). Non-esterified fatty acids were found to decrease as carbohydrate and energy content of the preload increased, which may be attributable to the insulin response following these preloads suppressing hormone sensitive lipase with consequent attenuation of NEFA release from adipose tissue. Plasma TAG levels showed little response to preload manipulations for the first 90 minutes after consumption, with no difference between preloads. However after 90 minutes a differential response was seen. As insulin suppresses hepatic TAG release the lower insulin levels seen with the LC preload could enable greater hepatic TAG output and therefore greater circulating TAG levels. It was interesting to note that immediately before the buffet test meal NEFA and TAG levels were higher and food intake greater following the LC preload, although no relationship between these circulating fat related factors and appetite can be inferred from these data.

The gastric emptying rate of each preload was determined, and showed some relationship with appetite responses and other post-prandial changes. The T50 of gastric emptying was found to increase as preload energy and carbohydrate content increased, suggesting that emptying rate was slower at higher energy and carbohydrate levels and supporting previous work suggesting gastric emptying to respond to energy density (Shafer et al., 1985; Velchik & et al, 1989; Wisen et al., 1993). The percentage of the meal remaining in the stomach at 15 minute intervals following preload ingestion supports this view, and shows the LC preload to empty significantly faster than the MC and HC preloads. Although the EIE method of gastric emptying cannot provide quantitative data on actual emptying times (chapter 2, section 2.2.2), the emptying rates obtained are relative to one another, and data can be used to rank emptying rates of the preloads. As the MC preload was found to empty significantly faster than the HC preload, and more slowly than the LC preload, a dose response relationship between nutrient intake and gastric emptying is implied. Although this response could be due to either the carbohydrate content or total energy content of the preload, gastric emptying rate was found to correspond to hormone response following preload consumption. It has previously been shown that gastric
emptying rate is responsive to GLP-1 administration (Willms et al., 1996; Schirra et al., 1997), and in the present study T50 was shown to positively correlate with total GLP-1. Although cause and effect cannot be inferred from these data in light of the previous research it seems likely that gastric emptying rate is responsive to GLP-1.

Due to the large variability in appetite data little inference can be made on the relationship between gastric emptying and appetite. It was notable that a significant difference in hunger ratings was observed between the LC and HC preloads 30 minutes after consumption, and this corresponded to a time at which the biggest difference in gastric fullness was likely to have occurred. Although satiety data did not show a significant difference at this time subjects did rate themselves as more satiated at 30 minutes with the HC preload. As the mean T50 of the LC preload was shorter than that for the HC preload, it is possible that at 30 minutes post-prandially the LC preload had largely emptied from the stomach while a greater proportion of the HC preload remained in the stomach. As mentioned previously data produced by the EIE is not quantitative, and while these data provide some support for a role of differences in gastric fullness in the appetite process (Khan & Read, 1992), a direct relationship cannot be assessed in the current investigation. Additional investigation of this phenomenon by administering a test meal at the 30 minute interval may provide further evidence for the role of gastric fullness in food intake control.

Taken together these data support a short term appetite regulation which is responsive to differences in energy and carbohydrate intake. This effect may be due in part to differences in the gastric emptying rate of manipulated meals, and the influence of differences in gastric distension in appetite regulation. Although no direct relationship could be established between hormone and metabolite responses and appetite, differences in response to preloads of increasing carbohydrate and energy content follow a similar, stepwise increase to that observed with appetite and food intake data. Thus data are suggestive of a role for gastric emptying and hormone and metabolite responses in the regulation of appetite. Results of this investigation cannot elucidate whether it was the manipulation of carbohydrate or total energy ingested which were responsible for the differences in appetite responses seen. Consequently
similar investigation of different energy levels manipulated through fat content will be used to investigate appetite responses further.
Chapter Five
Chapter 5

5. The effect of increasing fat and energy content of a preload upon post-prandial appetite responses, changes in circulating hormone and metabolite status and gastric emptying

5.1. Introduction

A high fat intake is reported to result in passive overconsumption ((Blundell & Tremblay, 1995), and fat has often been shown as the least satiating of the macronutrients (de Castro, 1987; Blundell et al., 1993; Green et al., 1994; Johnstone et al., 1996). However studies infusing fat into the small intestine have found effects of these infusions on satiety, suggesting that fat does play some part in the induction of short term satiety signals (Welch et al., 1985; Lieverse et al., 1994c). The precise mechanisms through which fat may exert this satiety effect remain unknown, although the role of CCK, a hormone released in response to fat ingestion, is well documented, and this hormone is arguably the primary short term route through which fat acts to control food intake (Schick et al., 1991; Lieverse et al., 1994b; Lieverse et al., 1995b; Matzinger et al., 1999). In addition to the role of CCK other gastrointestinal hormones could be important in some circumstances. For example GLP-1, also a putative satiety hormone, has been shown to respond to fat intake (Elliott et al., 1993) and may be important in satiety signalling following nutrient ingestion.

As described in the previous chapter, there are relatively few studies which have investigated appetite responses to different energy levels derived through manipulation of a single macronutrient. However such an approach would help elucidate the relative role of the macronutrient based satiety signals in the control of appetite, and could be used to provide further evidence for the relative roles of fat mediated satiety signals. While hormonal responses may account for much of the
satiety response seen with high fat preloads, gastric emptying rates may also account for differences in the satiety response as gastric emptying has been shown to vary with size and nutrient content of a meal (Wisen et al., 1993). In addition gastric emptying is under hormonal control by both CCK (Liddle et al., 1986) and GLP-1 (Willms et al., 1996), and it is possible that fatty meals may act through both gastric emptying and hormonal mechanisms to potentiate satiety.

5.2. Aims

The present study therefore aimed to investigate post-prandial responses to meals of increasing energy density manipulated through fat content. Appetite responses to each preload were assessed whilst simultaneously measuring hormone and metabolite status and gastric emptying rate. This enabled the relationship between satiety and post-prandial physiological changes to be assessed.

5.3. Study Design

5.3.1. Subjects

Ten healthy subjects (five male, five female) were recruited from staff and students at the University of Surrey. All subjects were of normal weight for height (mean BMI 23.3 ± 1.9 kg/m²), with an age range of 23 - 29 years. Individuals completed the DEBQ prior to the study, and only those subjects with scores of less than 3.5 in restrained, emotional and external eating were accepted onto the study. All volunteers underwent standard haematological and biochemical screening during recruitment, and completed a medical health questionnaire to ensure they were not taking regular medication other than oral contraceptives and minor analgesics. Informed written consent was obtained from each individual, and the gastric emptying of a water load by EIE was used to determine the correct positioning of electrodes for each subject prior to the study.
Preceding the study all subjects were informed they must refrain from strenuous exercise during the 24 hours before each study day, and avoid alcohol on the evening before the study. They were also asked to make food selections for their *ad libitum* buffet test meal (section 2.2.1.3). Ethical approval for this research was obtained from the South West Surrey Local Research Ethics Committee at the Royal Surrey County Hospital.

### 5.3.2. Test meals

Three preloads were developed to contain different energy densities through the manipulation of fat content. Each meal consisted of double cream and maltodextrin, and was made up to a constant volume of 450 ml with bottled water. The low fat preload (LF) was designed to provide 1067 kJ, the medium fat preload (MF) provided 1756 kJ, and the high fat preload (HF) provided 2596 kJ. Carbohydrate content of each preload remained similar, and although protein content varied due to the use of more double cream in the higher fat preloads, total protein levels were minimal (table 5.1).

**Table 5.1** Composition of preloads with different fat content. Amounts given are per 450ml serving.

<table>
<thead>
<tr>
<th></th>
<th>Low fat</th>
<th>Medium fat</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>915 (70)</td>
<td>915 (70)</td>
<td>915 (70)</td>
</tr>
<tr>
<td>Fat (kJ (g))</td>
<td>150 (4)</td>
<td>828 (22)</td>
<td>1655 (44)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>2 (0.5)</td>
<td>13 (3.2)</td>
<td>26 (6.4)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1067</td>
<td>1756</td>
<td>2596</td>
</tr>
</tbody>
</table>

† LF preload: 8g double cream, 50g maltodextrin, 20g nesquick
‡ MF preload: 46g double cream, 50g maltodextrin, 20g nesquick
* HF preload: 92g double cream, 50g maltodextrin, 20g nesquick

Each preload was given as a milkshake, flavoured with 20g ‘Nesquick’ milkshake power to improve palatability. Subjects were able to choose their preferred milkshake flavour from either strawberry or banana, which remained constant throughout the study.
5.3.3. **Study protocol**

Post-prandial responses to each preload were investigated on three separate occasions at least one week apart using a single blind randomised within subjects crossover design. On the day before each study subjects were asked not to undertake any strenuous exercise and to avoid alcohol consumption. Individuals were also instructed to consume their normal evening meal before 2200 hours the evening before the study, and to consume nothing except water after this time until arrival at the investigation unit the following morning. Following the overnight fast subjects arrived at the investigation unit at 0800 hours and were allowed a 15-30 minutes rest period to adjust to their surroundings. Subjects were then cannulated in an antecubital forearm vein, and electrodes were placed over the abdominal area and lower back for the assessment of gastric emptying with EIE (section 2.2.2). Subjects adopted a semi-supine position for the remainder of the study to facilitate the measurement of gastric emptying. Two fasting blood samples were taken and subjects completed basal VAS ratings prior to consumption of the preload. A baseline reading was also obtained on the epigastrograph for approximately 20 minutes, after which time subjects consumed one of the 3 preloads through a drinking straw at a steady pace.

Following preload consumption gastric emptying rate was monitored for 90 minutes, and post-prandial blood samples were taken at regular intervals (15, 30, 45, 60, 75, 90, 120 minutes). VAS ratings were completed just after preload consumption and every 30 minutes for the remainder of the post-prandial test period. After 90 minutes EIE measurements were stopped and subjects were offered an *ad libitum* buffet style test meal from which macronutrient and energy intake was calculated. They were instructed to eat as little or as much as preferred until feeling comfortably full. To minimise over consumption due to the availability of free food, subjects were aware they could take away any food they didn’t eat at that time. During consumption of the test meal subjects were separated from one another to minimise the effects of social interaction on food intake. Following the test meal a final blood sample was taken and subjects completed another VAS. They were then free to leave the investigation unit and resume their usual activities.
5.3.4. Statistical analyses

Hunger and satiety ratings were analysed using repeated measures ANCOVA. The baseline rating (-5 min) was used as the covariate, with preload and time (+5 to 90 min) as repeated measures factors. Ratings made following consumption of the buffet test meal (105 min) were analysed separately as they were not primarily responsive to the manipulated preload. Buffet test meal intakes and satiety quotients were analysed using repeated measures ANOVA, with preload as the repeated measures factor.

Hormone and metabolite responses and gastric emptying data were analysed using repeated measures ANOVA, with preload and time (-15 to 90 min) as repeated measures factors.

Significant ANOVA / ANCOVA results were analysed further with post hoc testing, to determine between which preloads significant differences lay.

5.4. Results

5.4.1. Appetite responses

5.4.1.1. Hunger ratings

Hunger ratings following consumption of each preload are illustrated in figure 5.1. There were no significant differences in hunger response following consumption of each preload (ANCOVA, \( p = 0.9 \)), and no significant difference in hunger ratings between preloads following the buffet test meal (\( p = 0.6 \)).
Figure 5.1 Hunger ratings following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Hunger ratings (mean ± SEM, n = 10) following consumption of LF (▲), MF (▲) and HF (▲) preloads. There was no significant difference between preloads ($p = 0.9$).

Transformation of the hunger data to $z$ scores did not elucidate any further differences between preloads. However multiplicative adjustment for differences in baseline showed subjects to rate themselves as more hungry following the LF preload than either the MF or HF preloads, although this difference was not significant (ANOVA, $p > 0.2$) (figure 5.2).
Figure 5.2 Hunger ratings adjusted for differences in baseline rating following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Hunger ratings (mean ± SEM, n = 10) which have been multiplicatively adjusted for differences in baseline following consumption of LF (▲), MF (▲) and HF (▲) preloads. There were no significant differences between preloads (p > 0.2) although subjects rated themselves to be consistently more hungry following the LF preload.

5.4.1.2. Satiety ratings

Satiety ratings following consumption of each preload are illustrated in figure 5.3. There were no significant differences in satiety ratings following each preload (ANCOVA, p = 0.7), and no significant difference in satiety ratings following the buffet test meal (p = 0.9).

Transformation of the satiety data into z scores, and multiplicative adjustment for baseline did not highlight any further differences in satiety response between preloads.
Figure 5.3 Satiety ratings following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Satiety ratings (mean ± SEM, n = 10) following consumption of LF (▲), MF (▲) and HF (▲) preloads. There was no significant difference between preloads (p = 0.7).

5.4.1.3. Food preference checklist data

Food preference checklist data did not show any significant differences in subject response to each preload in either the number of items or total energy selected (figures 5.4 and 5.5, ANCOVA: p > 0.1 in both cases). Neither was there any significant difference in food preference checklist data following consumption of the buffet meal (p > 0.2 in both cases). Transformation of the raw data to z scores, and multiplicative adjustment for baseline did not show any further differences between preloads. In addition there were no differences in the number of high fat, protein or carbohydrate items selected after each preload from the food preference checklist.
Figure 5.4 Number of items selected from the food preference checklist following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

![Graph showing number of items selected from the FPC following consumption of LF, MF, and HF preloads.]

Number of items selected (mean ± SEM, n = 10) from the FPC following consumption of LF (▲), MF (▲) and HF (▲) preloads. There was no significant difference between preloads (p = 0.2).

Figure 5.5 Total energy selected from the food preference checklist following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF), and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

![Graph showing total energy selected from the FPC following consumption of LF, MF, and HF preloads.]

Number of items selected (mean ± SEM, n = 10) from the FPC following consumption of LF (▲), MF (▲) and HF (▲) preloads. There was no significant difference between preloads (p = 0.2).
5.4.1.4. Ad libitum buffet meal intake

Energy intakes decreased as preload fat and energy intake increased although this difference was not significant (figure 5.6, $p = 0.1$). Differences in macronutrient intake and the weight of food consumed reflected the difference in energy intake and were not significantly different between preloads ($p > 0.1$ all nutrients).

Figure 5.6 Buffet test meal energy intake 90 minutes after consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Buffet meal energy intakes (mean ± SEM, $n = 10$) 90 minutes after consumption of LF (■), MF (■) and HF (■) preloads. There were no significant differences between preloads ($p = 0.1$).

The hunger quotients were calculated and showed a difference between preloads which approached significance (figure 5.7, ANOVA: $p = 0.06$). Post hoc testing showed the hunger quotient to be significantly lower following the LF preload compared to both the MF ($p = 0.04$) and HF ($p = 0.04$) preloads, suggesting subjects ate a greater amount following the LF preload to achieve the change in hunger observed. There was no significant difference in hunger quotient between the MF and HF preloads.
Figure 5.7 Hunger quotients calculated from buffet meal energy intake 90 minutes after consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Hunger quotients (mean ± SEM, n = 10) following consumption of LF (■), MF (■) and HF (■) preloads. Quotients were significantly lower after LF compared to MF ($p = 0.04$) and HF ($p = 0.04$) preload. Quotients between MF and HF were not significantly different ($p > 0.2$).

The satiety quotient following each preload was also calculated and showed there to be no differences in the relationship between buffet meal intake and change in satiety between any of the preloads (figure 5.8, ANOVA: $p = 0.9$).
Figure 5.8 Satiety quotients calculated from buffet meal energy intake 90 minutes after consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Satiety quotients (mean ± SEM, n = 10) following consumption of LF (■), MF (■) and HF (■) preloads. There was no significant difference between preloads (p = 0.9).

To determine whether subjects overate during the study period the total energy intake during the study was calculated from preload energy + buffet meal energy (figure 5.9). Even though buffet meal energy intake tended to decrease as preload energy increased, total energy intake during the study was significantly increased as preload energy increased (ANOVA: p = 0.003), showing over consumption following preloads of higher fat and energy content. Post hoc testing showed total intake to be significantly greater following the HF preload compared to the MF (p = 0.04) and LF (p = 0.001) preloads. Intake following the MF preload was also higher compared to the LF preload (p = 0.08).
Figure 5.9 Total energy intake throughout study period with preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Energy intake throughout study period (mean ± SEM, n = 10) from the preload (■) and buffet test meal (90 minutes after LF (■), MF (■) and HF (■) preloads). Total energy intake during the study period was significantly increased with the HF preload compared to the MF (p = 0.04) and LF (p = 0.001) preloads. Intake was also higher with the MF compared to the LF preload, and this difference approached significance (p = 0.08).

Individual buffet energy intakes (figure 5.10) showed subject response to the preload manipulation to be very variable, with only 2 of the 10 subjects decreasing their buffet intake when fat and energy content of the preload increased. The remaining subjects showed no consistent pattern of response with 2 subjects increasing buffet meal energy intake as preload fat and energy increased, and 5 subjects showing the greatest food intake after the MF preload. One subject ate least energy on the MF and most on the HF preload. Repeated measures ANOVA with test occasion as the repeated measures factor was used to determine any significant order effect in these data. There was no significant difference in buffet test meal energy intake between test occasions (p > 0.1).
Figure 5.10 Buffet test meal energy intakes of individual subjects following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Buffet test meal energy intakes of each subject following consumption of LF (■), MF (■) and HF (■) preloads. Pattern of energy intakes varied between subjects.

5.4.2. Blood metabolite and hormone responses

5.4.2.1. Plasma glucose

There was a rise in circulating glucose observed following consumption of each preload (figure 5.11), with no significant difference in glucose response between preloads (ANOVA: $p = 0.1$). A significant preload $\times$ time interaction was observed following preload consumption ($p = 0.0001$), and is attributable to the increased time taken to reach peak glucose concentration as the fat and energy content of the preload increased. There was no significant difference in glucose concentrations following consumption of the buffet test meal ($p = 0.2$).
Figure 5.11 Plasma glucose levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma glucose concentrations (mean ± SEM, n = 10) following consumption of LF (▲), MF (▲) and HF (▲) preloads. A significant preload x time interaction was observed (p = 0.0001) as time to reach peak levels increased with fat and energy content of the preload.

5.4.2.2. Plasma insulin

Mean plasma insulin responses following consumption of each preload are illustrated in figure 5.12. As fat and energy content of the preload increased peak insulin response was seen to slightly decrease, with the time taken to reach peak insulin concentration marginally greater with the HF preload. ANOVA showed there to be no significant difference in insulin concentrations following either of the preloads (p > 0.7). A significant preload x time interaction was observed in insulin response between preloads (p = 0.04), due to the delayed time to peak following the HF preload compared to the MF and LF preloads. There was no significant difference in insulin levels following buffet test meal consumption (ANOVA: p = 0.6).
Figure 5.12 Plasma insulin levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma insulin concentrations (mean ± SEM, n = 10) following consumption of LF (△), MF (▲) and HF (▲) preloads. A significant preload x time interaction was observed (p = 0.04) as time to reach peak levels was delayed following the HF preload compared to the MF and LF preloads.

5.4.2.3. Plasma GIP

The largest increase in GIP concentrations were seen following consumption of the HF preload, and GIP levels following this preload continued to rise throughout the 90 minute post-prandial period (figure 5.13). Following the LF and MF preloads GIP concentrations were similar, although it was noted that peak GIP levels were greater with the MF than the LF preload. Repeated measures ANOVA did not show a significant difference between GIP levels following each preload (p = 0.2). A significant preload x time interaction was observed due to the prolonged elevated levels of GIP following the HF preload (p = 0.001). GIP levels following the buffet test meal were analysed using repeated measures ANOVA. GIP concentrations were significantly different between preloads at this time (p = 0.03). Post hoc testing showed GIP to be significantly higher with the HF preload compared to the LF.
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preload ($p = 0.01$). There was no significant difference in GIP levels between the LF and MF ($p = 0.2$) or MF and HF ($p = 0.1$) preloads.

Figure 5.13 Plasma GIP levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma GIP concentrations (mean ± SEM, $n = 10$) following consumption of LF (▲), MF (▲) and HF (▲) preloads. There was a significant preload x time interaction ($p = 0.001$) as GIP remained elevated for longer following the HF preload.

5.4.2.4. Plasma GLP-1

Plasma GLP-1 concentrations increased with increasing fat and energy content of the preload, and this difference approached statistical significance (figure 5.14, $p = 0.06$). Post hoc testing showed there to be significantly higher levels of GLP-1 following the HF compared to the LF preload ($p = 0.03$), with no significant difference between the HF and MF ($p = 0.1$) or the MF and LF ($p = 0.4$) preloads. It was observed that the HF preload elicited a large increase in GLP-1 of approximately 30 pmol/L, whilst consumption of the MF and LF preloads produced half this rise. Additionally the time taken for GLP-1 levels to return to baseline was longer with a higher fat and energy content of the preload. This difference is reflected in a significant preload x time interaction ($p < 0.0001$).
GLP-1 levels following consumption of the buffet test meal (+120 min) were compared using repeated measures ANOVA, and it was shown that GLP-1 concentrations after the test meal were significantly greater with higher fat and energy content of the preload \((p < 0.0001)\). Post hoc testing showed GLP-1 to be significantly increased between the LF and MF preloads \((p = 0.003)\), the LF and HF preloads \((p < 0.0001)\), and the MF and HF preloads \((p = 0.02)\).

**Figure 5.14** Plasma GLP-1 levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma GLP-1 concentrations \((\text{mean} \pm \text{SEM}, n = 10)\) following consumption of LF (▲), MF (▲) and HF (▲) preloads. Circulating GLP-1 increased as preload fat and energy content increased \((p = 0.06)\), and this difference approached significance. A significant preload \(\times\) time interaction was also observed \((p < 0.0001)\). Following the buffet test meal GLP-1 was significantly higher following the HF preload compared to the MF \((p = 0.02)\) and LF \((p < 0.0001)\) preloads, and significantly higher following the MF compared to the LF preload \((p = 0.003)\).

**5.4.2.5. Plasma TAG**

Mean post-prandial TAG concentrations are illustrated in figure 5.15. Following preload consumption there was a very small rise in circulating TAG levels, which was
sustained during the study period following the HF and MF preloads. TAG levels following the LF preload returned to baseline approximately 60 minutes after preload consumption. Repeated measures ANOVA showed there to be no significant effect of preload upon TAG response ($p = 0.3$), although there was a significant preload x time interaction ($p < 0.0001$) due to the sustained elevation of TAG following the HF and MF preload compared to the LF preload.

TAG concentrations following consumption of the buffet test meal (+120 min) were analysed by repeated measures ANOVA, and were found to significantly increase as the fat and energy content of the preload increased ($p = 0.008$). Post hoc testing of this effect showed TAG concentrations to be significantly lower following the LF preload compared to the HF preload ($p = 0.003$), and lower compared to the MF preload at a difference approaching significance ($p = 0.08$). There was no significant difference in post buffet meal TAG levels between the MF and HF preloads ($p = 0.1$).
Figure 5.15 Plasma TAG levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma TAG concentrations (mean ± SEM, n = 10) following consumption of LF (△), MF (▲) and HF (▲) preloads. There was a significant preload x time interaction between preloads (p < 0.0001) as circulating TAG lowered more quickly following the LF preload.

5.4.2.6. Plasma NEFA

Following preload consumption there was a reduction in circulating NEFA levels which was smaller as fat and energy content of the preload increased (ANOVA: p = 0.04, figure 5.16). Post hoc testing showed this to be due to significantly higher NEFA levels following the HF preload compared to the MF (p = 0.03) and LF (p = 0.03) preloads. There was no significant difference in NEFA concentrations between the MF and LF preloads (p = 0.9). The post-prandial differences in NEFA concentrations remained apparent following consumption of the buffet test meal (ANOVA: p = 0.0001). Post hoc testing of levels after buffet meal consumption showed significantly higher NEFA levels with the HF preload compared to the LF (p = 0.0001) and MF (p = 0.004) preloads. NEFA was also significantly greater with the MF compared to the LF preload at this time (p = 0.05).
Figure 5.16 Plasma NEFA levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma NEFA concentrations (mean ± SEM, n = 10) following consumption of LF (▲), MF (▲) and HF (▲) preloads. Circulating NEFA was higher as fat and energy content of the preload increased (p = 0.04). This difference remained significant following the buffet test meal (p = 0.0001).

5.4.2.7. Plasma CCK

Plasma CCK showed a significantly greater post-prandial response as preload fat and energy content increased (figure 5.17, p = 0.005). Post hoc testing of this difference showed plasma CCK levels to be significantly lower following the LF preload than after both the MF (p = 0.01) and HF (p = 0.003) preloads, with no significant difference in circulating CCK between the MF and HF preloads (p = 0.4). A significant preload x time interaction was also observed (p = 0.02). Following buffet meal intake there was no significant difference in CCK concentration (ANOVA: p = 0.1).
Figure 5.17 Plasma CCK levels following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Plasma CCK concentrations (mean ± SEM, n = 10) following consumption of LF (▲), MF (▲) and HF (▲) preloads. Circulating CCK was significantly lower following the LF preload compared to the MF (p = 0.01) and HF preloads (p = 0.003). There was no significant difference in CCK between the MF and HF preloads (p = 0.4).

5.4.3. Gastric emptying response

The percentage of the meal remaining in the stomach was calculated at 15 minute intervals during the 90 minute post-prandial period (figure 5.18). There was a significantly slower emptying rate for the preload as fat and energy content increased (p < 0.0002 between all preloads).
Figure 5.18 Percentage of the meal remaining in the stomach for 90 minutes after ingestion of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Percentage of meal remaining in stomach (mean ± SEM, n = 10) following consumption of LF (△), MF ( △) and HF ( △) preloads. The rate of gastric emptying significantly decreased as fat and energy content of the preload increased (p < 0.0001).

From percentage of meal remaining in the stomach the half emptying rate was determined (T50), and was shown to significantly increase as fat and energy content of the preload increased (figure 5.19, p < 0.0001). Post hoc testing showed the HF preload to have a significantly slower half emptying rate than both the MF and LF preloads (p = 0.0002 and p < 0.0001 respectively). The MF preload also had a significantly slower half emptying rate than the LF preload (p = 0.0002).
Figure 5.19 Time taken for half of the meal to empty from the stomach (T50) following consumption of preloads containing 1067 kJ (LF), 1756 kJ (MF) and 2596 kJ (HF). Differences in energy content of preload were achieved through the manipulation of fat.

Half emptying time (T50) (mean ± SEM, n = 10) following consumption of LF (■), MF (■) and HF (■) preloads. Half time of gastric emptying was significantly longer as fat and energy content of the preload increased (p < 0.0001).

The T50 from gastric emptying was found to correlate with the total area under the curve of GLP-1 (r = 0.4, p = 0.02, all data). In addition circulating CCK was found to correlate with the percentage of the preload remaining in the stomach (r = 0.36, p < 0.01, all data)
5.5. Discussion

Previous studies have reported that subjects find it difficult to adjust for different energy intakes (Wooley et al., 1972; de Graaf & Hulshof, 1996), and that fat consumed as part of an energy dense diet is likely to lead to passive overconsumption (Blundell & Tremblay, 1995) and increased food intake (Duncan et al., 1983). Data from the present study initially appear to support that of previous work, and shows subjects to respond poorly to increased fat and energy density both in terms of self rated appetite and subsequent food intake.

There were small differences in mean hunger ratings between each preload, with subjects generally rating themselves as more hungry immediately following the HF preload. As it has been shown previously that large variation may result from the use of VAS ratings, which could mask differences in appetite responses (chapter 3, section 3.2) hunger ratings were adjusted for differences in baseline values, and the adjusted data showed subjects to experience greater hunger following the LF preload compared to both the MF and HF preloads. Although this difference was not significant, an effect of preload fat and energy manipulation on appetite is also supported by elevated satiety ratings following the HF preload compared to the MF and LF preloads. Thus it appears that subjects were able to detect differences between the LF and HF preloads, with less ability to detect difference between the MF preload compared to either the LF or HF preloads. Food preference checklist data in the current study added little additional information, with a large variation in number of items and energy selected throughout the study period. The buffet meal energy intake however showed a stepwise decrease in food intake as preload fat and energy content increased, and although compensation for preload energy adjustments was poor a small mean energy intake response to preload manipulations was observed. It is noted that the current study used only 10 subjects due to difficulties during recruitment, and previous work suggested that a larger number of subjects can improve the statistical power in appetite studies (chapter 3 and chapter 4). It is therefore speculated that use of a larger subject group may better elucidated differences in appetite and food intake responses between preloads. Additional evidence for an effect of preload upon appetite response was provided by quotients
calculated from hunger and satiety data in relation to buffet energy intake. If again focusing upon the difference in response between the LF and HF preloads, the hunger quotient was significantly lower following the LF preload. These data imply that subjects needed a greater energy intake during the buffet meal to produce the relative change in hunger observed following the LF preload compared to the HF preload, and would therefore suggest that subjects were more hungry at the beginning of the test meal following the LF preload. Although a similar significant difference was not seen with satiety quotients calculated from this meal it is possible that hunger proved a more sensitive measure during food intake in this instance.

These findings are similar to those reported in our previous work, and although the buffet test meal and preload-test meal interval were slightly different between this and the previous study, these data suggest that detectable differences in appetite following preload manipulations require relatively large differences in energy intake. It is noted that previously reported studies showing differences in appetite responses to various energy intakes have used a similar difference in preload energy (e.g. Pliner, 1973) (energy difference of 1672 kJ), while those studies finding little or no differences in appetite have used smaller preload energy manipulations (Wooley et al., 1972) (energy difference of 819 kJ). As data from the current investigation was similar to that reported in chapter 4, appetite responses do not appear to differ in response to manipulation of carbohydrate and fat, but rather to be primarily responsive to differences in energy intake. This is illustrated well by the similar, stepwise decrease in buffet meal energy intake as preload energy content increased, regardless of whether the manipulation was produced by carbohydrate or fat. As different foods were included in the buffet test meals during this and the previous investigation these data cannot be directly compared. However they do question the finding that high fat foods are less effective at suppressing subsequent food intake than high carbohydrate foods (Green & Blundell, 1996).

As high fat diets are linked to passive overconsumption (Blundell & Tremblay, 1995) total food intake during the study period was investigated to determine whether overall energy intake was greater with the HF preload. Even though buffet test meal
intake was slightly reduced as preload energy increased, suggesting a small amount of compensation for preload energy manipulations, total energy intake throughout the test period was significantly higher as preload fat and energy increased. This supports previous observations of poor regulation of food intake with high fat consumption (Rolls et al., 1994), and suggests passive overconsumption is occurring. Although the current study cannot determine whether this difference is due to fat intake or energy intake, data from the previous study (chapter 4) showed a similar effect following a high energy preload, and taken together these data suggest that energy density rather than fat alone may promote overconsumption (Duncan et al., 1983). It is thus possible that fat is traditionally viewed as causing passive overconsumption as the majority of fatty foods are those which are energy dense.

While appetite responses provide some data to support differential responses between the LF and HF preloads, it was noted that the largest differences in hormone and metabolite responses also tended to occur after these preloads. Insulin and glucose showed a similar response between preloads over the 90 minute post prandial period due to the identical carbohydrate content of the preload. However differences in the time to reach peak glucose concentration were observed, and this may be attributable to the differences in gastric emptying rates of the preloads. The gastric emptying rate was shown to slow as fat and energy content of the preload increased, and as the rate of nutrient release into the small intestine would determine the rate of glucose appearance in the blood differences in gastric emptying could result in differences in plasma glucose. Differences in glucose appearance can also account for the slightly delayed insulin response following the MF and HF preload compared to the LF preload. TAG and NEFA levels also followed the pattern expected following ingestion of preloads with varying fat content, with circulating TAG returning to baseline more quickly following the LF preload. Following buffet test meal consumption circulating TAG was elevated with the HF preload, and it is likely that the fat content of the preload potentiated the release of enterocyte TAG following the buffet meal (Fielding et al., 1996).
Hormonal responses were found to differ as fat and energy content of the preload increased, with higher GLP-1, CCK and GIP levels following the HF preload. Hormone concentrations were also higher after the MF compared to the LF preload. While GLP-1 has been reported to respond to fat ingestion (Elliott et al., 1993), the stepwise increase observed here accompanied by high levels following the HF preload has not been previously reported. It was also interesting to note that GLP-1 secretion following the buffet test meal appeared to be related to preload fat and energy intake, with higher fat and energy content of the preload resulting in higher levels of GLP-1 following the buffet test meal. This occurred in spite of mean buffet energy intake being lower after the HF preload, and suggests that GLP-1 is sensitive to prior nutritional status, in much the same way as enterocyte TAG release may respond to prior nutritional intake. This has not been previously reported and the mechanism for such potentiation of GLP-1 release is unknown. It is possible that positioning of the GLP-1 releasing L-cells in the small intestine may be responsible for this response. Increased GLP-1 is generally reported within 15 - 30 minutes of meal ingestion, and nutrients could not have reached the lower small intestinal L-cells in time to stimulate release of GLP-1 so soon after food. As there are also small numbers of L-cells in the upper jejunum it has been suggested that GLP-1 is initially released from these cells after nutrient ingestion (Nauck, 1997). Neural signalling may then act to promote the release of more GLP-1 from the lower small intestinal L-cells, possibly through the action of GLP-1 released in the early stages of digestion. With the buffet test meal consumed 90 minutes after the preload, it is possible that the preload had by this time acted directly upon lower intestinal L-cells to promote GLP-1 secretion, with an additive effect from ingestion of the buffet meal leading to further stimulation of jejunal L-cells. GLP-1 release from these cells plus neural or hormonal stimulation to potentiate release of GLP-1 from the lower small intestine would act to release a greater amount of GLP-1. Thus a combined effect of preload nutrition and buffet meal intake may have determined the extent of GLP-1 response to the buffet meal. It is unlikely that differences in buffet meal intake were responsible for the differences in GLP-1 seen as buffet energy and macronutrient intakes were not significantly different between preloads. Although intakes did decrease slightly with increased fat and energy content of the preload this is the opposite of what would be expected to
promote increased GLP-1 release after the buffet meal with the HF preload. However a lower buffet meal intake accompanied by higher GLP-1 levels is consistent with the proposed satiety role for GLP-1 (Turton et al., 1996), and it is possible that a GLP-1 based mechanism could act to reduce energy intake within an eating episode. In the present study no relationship between GLP-1 levels and food intake could be determined due to the small differences in food intake between preloads. While GLP-1 may have no action on within meal satiety it is noted that the appetite and food intake data in this study showed little differences, and were unsuitable for use in assessing relationships between hormone and metabolite status and food intake control. However the role of GLP-1 in the control of human eating behaviour may be of much importance as a hormone responsive to both fat and carbohydrate ingestion, and a regulator of gastric emptying.

The role of GIP in appetite is not well documented, and it is unclear whether this hormone would have any direct influence on food intake. Due to its incretin properties, it is most likely that GIP would act together with glucose and insulin in a glucostatic mechanism to regulate appetite. It was observed that significantly higher levels of GIP occurred with a higher preload fat and energy content, and higher satiety ratings were made during this time with the HF preload. However at the time the buffet test meal was offered to subjects glucose levels had returned to baseline, and it is probable that any synergistic effects of GIP and glucose would not have been acting upon food intake in the current study. The role of CCK in fat mediated short term satiety has been frequently investigated (Kissileff et al., 1981; Lieverse et al., 1994b; Ballinger et al., 1995), and data from the current study provide evidence for differential CCK release following ingestion of varying fat loads. Although no relationship between CCK and satiety could be determined from these data it is possible that the lower hormone levels seen in response to ingestion of the LF preload contributed to the higher adjusted hunger ratings seen with this preload. However additional investigation of the effect of endogenous CCK upon appetite released in response to a high fat load would provide greater elucidation of the role of CCK in fat mediated satiety.
As described above the gastric emptying rate was shown to significantly decrease as fat and energy content of the preload increased. While this was not related to satiety and food intake in the present study, relationships between gastric emptying and hormonal responses were investigated further, and supported previous investigations showing a role for GLP-1 in the control of gastric emptying (Schirra et al., 1997). The half emptying time of all preloads was shown to positively correlate with total GLP-1 levels throughout the 90 minute period following preload consumption. While it is acknowledged either that GLP-1 could be regulating gastric emptying rate, or that rate of release of nutrient into the small intestine could be regulating GLP-1 secretion, in light of previous work showing administration of exogenous GLP-1 to delay gastric emptying it seems most likely that GLP-1 regulates gastric emptying. Furthermore it is probable that a feedback mechanism of regulation is active, such that rate of release of nutrient into the small intestine potentiates the release of GLP-1, which in turn regulates rate of nutrient release. While CCK has also been shown to control gastric emptying (Liddle et al., 1986) current data shows the strongest relationship between these parameters following the HF preload. Thus it is possible that endogenous CCK exerts an effect at higher circulating levels than GLP-1, although again a causal relationship cannot be inferred from these data.

Although the current data provide little elucidation of the role of hormone and metabolite responses in the regulation of food intake, it seems likely that differences in appetite responses do occur if the energy difference is large enough, and hormone and metabolite levels are likely to potentiate the response. Gastric emptying rate has been shown to relate to GLP-1 and CCK levels, providing further evidence for these hormones as regulators of gastric emptying. However no direct evidence was obtained for their role in satiety. As gastric emptying rate was shown to be slower with greater fat and energy content of the preload, and buffet test meal intake was lower at this time, there may be a relationship between rate of emptying or amount of the preload remaining in the stomach and food intake. However further investigation of the relationship between food intake and percentage of stomach fullness using a test meal much sooner after preload consumption would help elucidate the extent to which gastric fullness regulates appetite. To further investigate the role of GLP-1 and...
CCK in appetite, both of which are related to gastric emptying, direct infusion of the hormones or an antagonist would allow any direct effects of the hormone upon food intake and gastric emptying to be determined.
Chapter Six
6. Investigation into the effects of a GLP-1 infusion upon appetite, circulating hormones and metabolites, and gastric emptying.

6.1. Introduction

As explored by our previous studies, it is probable that the appetite responses to preloads of different macronutrient and energy content are controlled to some extent by the action of gastrointestinal hormones. As a hormone shown to be secreted in response to carbohydrate (Elliott et al., 1993) and fat (Herrmann et al., 1995), GLP-1 has recently been shown to have central actions in the control of food intake in rodents (Schick et al., 1992; Lambert et al., 1994; Turton et al., 1996; Tang-Christensen et al., 1996), with a reduced food intake following ICV administration of the hormone. Similar actions of GLP-1 have been shown in chickens (Furuse et al., 1997), and as the structure of this hormone is conserved across species (Bell et al., 1983) the question of a role for GLP-1 in the control of human appetite has been raised. GLP-1 receptors have been shown in both the hypothalamic area of the brain (Kanse et al., 1988) and the small intestine (Ørskov et al., 1987) and it is possible that the hormone may play a central or peripheral role in appetite regulation in man. Furthermore GLP-1 has been shown to inhibit gastric emptying (Schirra et al., 1997) and gastric acid secretion (Wettergren et al., 1993), both of which are likely to be important in the short term regulation of food intake, and have been implicated in CCK mediated satiety (Schwartz & Moran, 1996). At the time of this investigation there had been no work investigating the role of peripheral GLP-1 on appetite in man. During the course of this thesis additional investigations have been published and these will be discussed in relation to the data obtained in section 6.5.
6.2. Aim

The present study aimed to investigate the role of exogenous peripheral GLP-1 in the control of food intake and satiety. Appetite responses and changes in circulating hormone and metabolite levels were assessed during an infusion of GLP-1 against a saline control. The effect of this infusion upon gastric emptying was simultaneously investigated to determine the extent to which any satiety response may be attributable to changes in gastric emptying.

6.3. Study design

6.3.1. Subjects

Ten healthy male subjects (age range 22 - 29 years) of normal weight for height (mean BMI 23.2 ± 2.0 kg/m²) were recruited from staff and postgraduates at the University of Surrey. All volunteers selected were non dieters and were asked to complete the DEBQ during recruitment. Only those individuals with scores of less than 3.5 in restrained, emotional and external eating were accepted for participation in the study. All subjects underwent standard haematological and biochemical screening conducted by the Royal Surrey County Hospital to ensure they were of good health and able to participate in the investigation. The infusion procedure was fully explained to all volunteers, and GP approval was obtained for each subject before commencement of the study. Informed written consent was also obtained from each subject, and subjects were aware that they could withdraw from the study at any time. Ethical approval was obtained from the South West Surrey District and University of Surrey Ethical Committees.

Screening for EIE was undertaken using the water load test to locate the correct positioning for electrodes for each individual. Preceding the investigation all subjects were informed they must refrain from strenuous exercise and alcohol intake the day before and the day of each study occasion.
6.3.2. Infusions

The GLP-1 infusion aimed to increase circulating GLP-1 to physiological post-prandial levels. A delivery rate of 1.2 pmol GLP-1/kg per min has been shown by previously published work (Willms et al., 1996) to result in circulating plasma GLP-1 levels similar to those found after a meal.

Synthetic GLP-1 was supplied by Professor S. Bloom, Royal Postgraduate Medical School, Hammersmith Hospital, London. The material was >99% pure, with a peptide content of > 85% (mw 3295.3 determined by mass spectrometry). Infusions were prepared from 10 nmol aliquots of the sterile, freeze dried synthetic GLP-1; which were dissolved in 5 ml of aseptically prepared plasma obtained from the subject to be infused. The addition of plasma minimised adsorption of the peptide onto the infusion tubing apparatus. Following inversion mixing the plasma / GLP-1 solution was injected into 95 ml sterile saline (0.9% w/v sterile sodium chloride in 100 ml minibags for iv infusions, from which 5 ml saline had been removed), to give a concentration of 100 pmol GLP-1 per ml of saline. The prepared infusion bag was connected to a sterile infusion set (IVAC UK Ltd., Wade Road, Basingstoke, Hants, RG24 8NE), which was in turn connected to an IVAC 591 infusion pump for delivery.

The infusion rate set on the pump was calculated according to subject body weight using the following formula:

To deliver 1.2 pmol/kg per min

\[
1.2 \text{ pmol x body weight (kg)} = X \text{ pmol GLP-1 to be delivered per min}
\]

\[
X \text{ pmol } / 100 \text{ pmol (the concentration of GLP-1 in 1 ml saline)} = Y
\]

therefore need to infuse GLP-1 at rate of \( Y \) ml / min.

As the IVAC 591 infusion pumps set delivery rate in ml / hour; \( Y \times 60 \) gives a final infusion rate of \( Z \) ml / hour.

A control of saline was infused against GLP-1, and in this instance the pump was set to the same flow rate as that for the GLP-1 infusion. To record any adverse
physiological consequences of the infusions an additional VAS questionnaire was included to assess possible side effects (appendix X).

6.3.3. Study protocol

The two infusions were administered in a single blind randomised crossover design on separate occasions 7 days apart. On the morning of each study subjects were asked to consume their usual breakfast but to avoid fried and high fat foods. They were also instructed to avoid caffeinated drinks for the duration of the study day. Six hours before commencement of the study (1200 hours) subjects consumed a standard lunch selected from a range of oven ready meals chosen to be relatively low in fat and NSP content, both of which may have prolonged and variable effects on gastric emptying (see appendix V for meal choice). The same lunch meal was consumed on both occasions, and subjects were instructed to eat nothing further and drink only water after the lunch meal until arrival at the investigation unit.

Subjects arrived at the investigation unit at 1700 hours, and were cannulated in an antecubital vein in both forearms. As the GLP-1 in the infusion could interfere with hormone analysis, one cannula was used for administration of the infusion, and the second for the removal of venous blood samples. Three pairs of electrodes were positioned over the stomach and lower back for the EIE measurement of gastric emptying, and baseline impedance readings were taken for approximately 20 minutes. During this time 2 basal blood samples were taken, and baseline VAS ratings were completed for the assessment of appetite and adverse physiological consequences of the infusion.

GLP-1 or saline was then infused for 60 minutes, and venous blood samples were taken at regular intervals during this time (10, 20, 30, 40, 60 min). Subjects also completed VAS ratings at 20 minute intervals throughout the study period. Twenty minutes after the start of the infusion subjects were given a 400 ml water load to drink, and gastric emptying of this load was monitored for a further 20 minutes. At 40 minutes after the start of the infusion, and following completion of EIE assessment of the water load, electrodes were removed and subjects were offered an ad libitum...
buffet style meal from which energy and macronutrient intake were calculated. A further 200 ml of water was offered with the buffet meal, and subjects were instructed to eat as much as they preferred until feeling comfortably full. Each subject was segregated during buffet meal consumption to minimise the effects of social interaction on food intake, and to avoid over consumption due to the free availability of food subjects were informed they could take home any food they could not eat at that time. Following consumption of the buffet meal subjects were free to leave the investigation unit and resume their normal activities.

6.3.4. Statistical analyses

Hunger and satiety ratings were analysed using repeated measures ANCOVA. The baseline rating (-10 min) was used as the covariate, with infusion and time (+5 to 40 min) as repeated measures factors. Ratings made following consumption of the buffet test meal (60 min) were analysed separately. Buffet test meal intakes and satiety quotients were analysed using a paired, 2 tailed t-test.

Hormone and metabolite responses and gastric emptying data were analysed using repeated measures ANOVA, with preload and time (-10 to 40 min) as repeated measures factors. Data following consumption of the buffet test meal (60 min) was analysed separately using a paired, 2 tailed t-test.

6.4. Results

6.4.1. Occurrence of adverse physiological effects

Subjects completed rating scales to determine the occurrence of any side effects. Repeated measures ANCOVA of ratings during the saline and GLP-1 infusions showed no significant differences between ratings of sickness and nausea ($p = 0.1$), dizziness ($p = 0.4$), stomach ache ($p = 0.5$) and thirst ($p = 0.4$). Mean ratings of physiological effects are illustrated in figure 6.1. Subjects did not report any additional side effects during either the GLP-1 or saline infusion.
Figure 6.1 Ratings of adverse physiological events during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Ratings of any adverse physiological events (mean, n = 10) occurring during infusions of saline (■) or 1.2 pmol GLP-1/kg per min (■). There were no significant differences in ratings between infusions.

6.4.2. Appetite responses

6.4.2.1. Hunger ratings

There was no significant difference in hunger ratings between the GLP-1 and saline infusions (ANCOVA: p = 0.6, figure 6.2). Following consumption of the buffet test meal subjects rated themselves to be less hungry with the GLP-1 infusion, although this difference was not significant (p = 0.1). Transformation of hunger data into z scores, and multiplicative adjustment for baseline did not elucidate any further differences.
Hunger ratings (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference in hunger ratings between infusions (p = 0.6).

**6.4.2.2. Satiety ratings**

Mean satiety ratings during infusions of saline and GLP-1 are illustrated in figure 6.3. Repeated measures ANCOVA showed there to be no significant difference in satiety ratings between the infusions (p = 0.6). In addition there was no significant difference in satiety ratings following consumption of the buffet test meal (p = 0.7). Transformation of data to z scores did not elucidate any further differences, although multiplicative adjustment for baseline showed subjects to rate themselves as more satiated during the saline infusion than the GLP-1 infusion (p = 0.04, figure 6.4).
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Figure 6.3 Satiety ratings during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Satiety ratings (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1 /kg per min (■). There was no significant difference in satiety ratings between infusions (p = 0.6).

Figure 6.4 Satiety ratings adjusted for differences in baseline ratings during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Satiety ratings (mean ± SEM, n = 10) which have been adjusted for differences in baseline during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). Subjects were significantly more satiated during saline than GLP-1 infusion (p = 0.04).
6.4.2.3. Food preference checklist data

The number of items and total energy selected from the food preference checklists showed little difference between infusions (ANCOVA: $p = 0.7$, figures 6.5 and 6.6), and there was no significant difference in food preference checklist selections following the buffet test meal ($p > 0.9$ in both cases). In addition there were no differences in the type of food items selected from the FPC during each infusion.

Figure 6.5 Number of items selected from the food preference checklist during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Number of items (mean ± SEM, n = 10) selected from the food preference checklist during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference between infusions ($p = 0.7$).
Figure 6.6  Total energy selected from the food preference checklist during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Saline or GLP-1 infusion

Total energy (mean ± SEM, n = 10) selected from the food preference checklist during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference between infusions (p = 0.7).

6.4.2.4. Ad libitum buffet meal intake

Energy and macronutrient intakes were calculated from the buffet test meal and there was found to be no significant difference in buffet meal energy intakes between the saline and GLP-1 infusion (p = 0.2, figure 6.7). In addition there were no significant differences in macronutrient intake during the infusions (protein, p = 0.3; fat, p = 0.2, carbohydrate, p = 0.2; weight of food eaten, p = 0.3). Analysis of individual energy intakes showed 7 out of the 10 subjects to reduce their energy intake during the GLP-1 infusion compared to the saline infusion, while the remaining 3 subjects ate more during the GLP-1 infusion that the saline infusion (figure 6.8).
Figure 6.7 Buffet test meal energy intake consumed 40 minutes into an infusion of 1.2 pmol GLP-1/kg per min against a saline control

Buffet meal energy intakes (mean ± SEM, n = 10) consumed after 40 minutes of an infusion of saline or 1.2 pmol GLP-1/kg per min. There was no significant difference between energy intakes (p = 0.2).

Figure 6.8 Buffet test meal energy intake of each subject consumed 40 minutes into an infusion of 1.2 pmol GLP-1/kg per min against a saline control

Buffet meal energy intakes of individual subjects 40 minutes into an infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■).
Quotients were calculated for hunger and satiety from buffet meal energy intake. There was no significant difference in satiety \((p = 0.4)\) or hunger \((p = 0.2)\) quotients during either infusion, although the mean hunger quotient was higher during the GLP-1 infusion than the saline infusion (figure 6.9).

Figure 6.9 Hunger and satiety quotients calculated from test meal energy intake 40 minutes after the start of an infusion of saline or 1.2 pmol GLP-1/kg per min.

Satiety and hunger quotients (mean ± SEM, \(n = 10\)) calculated from buffet meal energy intake 40 minutes into an infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference in satiety \((p = 0.4)\) or hunger \((p = 0.2)\) quotient values between infusions.

6.4.3. Blood metabolite and hormone responses

6.4.3.1. Plasma GLP-1

Mean GLP-1 levels during the saline and GLP-1 infusion are illustrated in figure 6.10. During the GLP-1 infusion a large and sustained rise in circulating GLP-1 was observed, whilst during the saline infusion no change in circulating GLP-1 was seen for the first 40 minutes of infusion. GLP-1 reached approximately twice physiological post-prandial levels during the infusion, and repeated measures ANOVA showed the difference in GLP-1 levels between the infusions to be highly significant \((p < 0.0001)\).
Following consumption of the buffet test meal GLP-1 levels increased with the saline infusion, but the difference between infusions remained significant ($p < 0.0001$).

**Figure 6.10** Plasma GLP-1 concentrations during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Plasma GLP-1 concentrations (mean ± SEM, $n = 10$) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). Plasma GLP-1 was significantly higher with GLP-1 infusion ($p < 0.0001$). This difference remained significant following consumption of the buffet test meal ($p < 0.0001$).

### 6.4.3.2. Plasma glucose

Circulating glucose concentrations exhibited a small but sustained drop during the GLP-1 infusion from 4.9 (SD 0.35) mmol/l at baseline to 4.1 (SD 0.4) mmol/l by 40 minutes into the infusion (figure 6.11). This drop was apparent and significantly different from baseline after 10 minutes of the infusion (paired t-test, $p = 0.002$). Glucose was lowered further until 30 minutes into the infusion ($p < 0.001$) after which time no further drop was seen. No changes in circulating glucose levels were observed during the saline infusion, and throughout the infusion repeated measures ANOVA showed glucose to be significantly lower during the GLP-1 infusion than the
saline infusion ($p = 0.004$). Following consumption of the buffet test meal plasma glucose levels remained significantly lower with the GLP-1 infusion than the saline infusion ($p = 0.0003$).

Figure 6.11 Plasma glucose concentrations during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

![Figure 6.11 Plasma glucose concentrations during infusion of 1.2 pmol GLP-1/kg per minute against a saline control](image)

Plasma glucose concentrations (mean ± SEM, $n = 10$) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). Glucose was significantly lower during the GLP-1 infusion ($p = 0.004$). Following buffet meal consumption (+60) glucose was significantly lower with GLP-1 infusion ($p = 0.0003$).

6.4.3.3. Plasma insulin

Plasma insulin levels were unaffected by both the saline and GLP-1 infusions, and there was no significant difference in insulin levels between infusions (ANOVA $p = 0.2$, figure 6.12). Although a small transient rise in plasma insulin was observed 10 minutes into the GLP-1 infusion this was not significantly different from baseline ($p = 0.3$), and levels had returned to baseline after 20 minutes of the infusion. Following consumption of the buffet test meal plasma insulin levels were significantly lower with the GLP-1 infusion than the saline infusion ($p = 0.01$).
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Figure 6.12 Plasma insulin concentrations during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Plasma insulin concentrations (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference in insulin levels between infusions (p = 0.2). Following buffet meal consumption (+60) insulin was significantly lower with GLP-1 infusion (p = 0.01).

6.4.3.4. Plasma CCK

Cholecystokinin remained at basal levels throughout the first 40 minutes of the GLP-1 and saline infusions, and was not significantly different between infusions (ANOVA: p = 0.6, figure 6.13). Following consumption of the buffet test meal plasma CCK showed a trend towards being higher with the GLP-1 infusion than the saline infusion, (p = 0.07).
Plasma CCK concentrations (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference in CCK levels between infusions (p = 0.6). Following buffet meal consumption (+60) CCK was higher with GLP-1 infusion (p = 0.07).

6.4.3.5. Plasma GIP
Throughout the 40 minute pre-prandial infusion of saline and GLP-1, plasma GIP remained close to baseline levels (figure 6.14). Repeated measures ANOVA showed there to be no significant difference in GIP levels between the infusions (p = 0.08), although plasma GIP was marginally higher during the saline infusion than the GLP-1 infusion. Following consumption of the buffet test meal circulating GIP was significantly lower with the GLP-1 infusion than with the saline infusion (p = 0.02).
Plasma GIP concentrations (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference between infusions (p = 0.08). Following consumption of the buffet meal GIP was significantly lower with GLP-1 infusion (p = 0.02).

6.4.3.6. Plasma NEFA

Plasma NEFA concentrations during the saline and GLP-1 infusions are illustrated in figure 6.15. Differences in NEFA levels during the saline and GLP-1 infusions were not significantly different prior to the buffet test meal (p = 0.3). Following consumption of the buffet test meal plasma NEFA was significantly higher with the GLP-1 infusion than the saline infusion (p = 0.03).
Plasma NEFA concentrations (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no difference between infusions (p = 0.3) although NEFA was significantly higher following buffet meal consumption with GLP-1 infusion (p = 0.03).

6.4.3.7. Plasma TAG

Following the start of the GLP-1 and saline infusions a small and non significant drop in circulating TAG was observed during the first 20 minutes (figure 6.16). Throughout the pre-prandial study period there were no significant differences in plasma TAG between the saline and GLP-1 infusion (p = 0.7). In addition there was not a significant difference in plasma TAG following buffet meal consumption (p = 0.4).
Figure 6.16 Plasma TAG concentrations during infusion of 1.2 pmol GLP-1/kg per minute against a saline control

Saline or GLP-1 infusion

400 ml water  buffet meal

Plasma TAG concentrations (mean ± SEM, n = 10) during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). There was no significant difference between infusions (p = 0.7), and no significant difference in TAG levels following buffet meal consumption (p = 0.4).

6.4.4. Gastric emptying response

The gastric half emptying times for the water load to empty from the stomach during the saline and GLP-1 infusions are illustrated in figure 6.17. Analysis using a paired, 2 tailed t-test showed there to be a significantly greater T50 during the GLP-1 infusion compared to the saline infusion (p = 0.001).

Analysis of the percentage of the water load remaining in the stomach at 2 minute intervals following consumption showed a slower rate of gastric emptying with GLP-1 infusion. The GLP-1 infusion was seen to cause a more pronounced lag period at the beginning of the emptying curve, and emptying rate was significantly slower with GLP-1 infusion (p = 0.001, figure 6.18).
Figure 6.17  Half emptying time of a water load consumed 20 minutes after the start of an infusion of saline or 1.2 pmol GLP-1/kg per minute

Half emptying time (mean ± SEM, n = 10) of a water load during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). T50 was significantly longer with GLP-1 infusion ($p = 0.001$).

Figure 6.18  Percentage remaining in the stomach of water load consumed 20 minutes after the start of an infusion of saline or 1.2 pmol GLP-1/kg per minute

Percentage remaining (mean ± SEM, n = 10) of a water load during infusion of saline (■) or 1.2 pmol GLP-1/kg per min (■). Gastric emptying rate was significantly slower with GLP-1 infusion ($p = 0.001$).
6.5. Discussion

This study was successful in producing a rise in circulating GLP-1, reaching physiological levels seen at the higher end of the post-prandial range reported previously (chapter 4). No adverse effects of the infusion were reported, showing good toleration of the infusion by the subjects. Although physiological post-prandial levels of GLP-1 were reached, the infusion had little effect upon subjective ratings of hunger and satiety, or on food preference checklist data. Subjects rated themselves to be marginally less hungry during the first 20 minutes of GLP-1 infusion, and were slightly more satiated during this time. However the difference between ratings was rather small, and during the gastric emptying of the water load no differences in appetite ratings were seen. Thus it would appear that GLP-1 infusion has no effect on ratings of hunger and satiety in the fasted subject. Adjustment of satiety ratings to account for differences in baseline ratings and degree of movement along the visual analogue scale showed subjects to be more satiated with the saline infusion. This is contrary to what would be expected either from a direct satiety effect of GLP-1, or from differences in rates of gastric emptying as during GLP-1 infusion emptying was delayed in comparison to during saline infusion. Thus greater satiety would be expected with GLP-1 infusion due to greater gastric distension following the water load. Thus these data do not provide evidence of a direct effect for GLP-1 in the mediation of satiety.

Ad libitum buffet meal intake tended to be lower with GLP-1 infusion although this difference was not significant, and examination of individual buffet energy intakes showed that while a proportion of subjects (n = 4) exhibited a considerable reduction in buffet meal energy intake with GLP-1 infusion, 3 subjects showed a much smaller decrease in energy intake with GLP-1 infusion, and a further 3 subjects increased their food intake with GLP-1 infusion compared to the saline control. The absence of a significant effect of GLP-1 upon food intake is contrary to previous animal studies which have reported a reduction of food intake with central administration of the peptide (Schick et al., 1992; Lambert et al., 1994; Turton et al., 1996; Tang-Christensen et al., 1996). However a GLP-1 receptor -/- mouse has been shown not to
demonstrate any significant changes in body weight or food intake compared to control animals during short term feeding studies (Scrocchi et al., 1996), and it is possible that GLP-1 is not an essential regulator of feeding behaviour.

Greater evidence for some role of GLP-1 in appetite regulation was observed following buffet meal consumption, when there was a trend for subjects to experience less hunger after the buffet meal with GLP-1 infusion. It was noted that the reduced feelings of hunger occurred with a lower mean buffet meal intake, which provides some indirect evidence that GLP-1 infusion had the ability to enhance the satiating effect of the buffet meal. This observation is supported by differences in the mean hunger quotient calculated from buffet meal energy intakes, with a higher, but not significantly different, quotient during GLP-1 infusion. Thus during GLP-1 infusion subjects needed to eat less to achieve the reduction in hunger observed compared to the saline control. As there was no difference in satiety ratings and satiety quotients following the buffet test meal it is also suggested that in a post-prandial state GLP-1 may act to reduce hunger rather than induce satiety. This possible action of GLP-1 in post-prandial appetite control suggests that in common with the action of CCK (Ballinger et al., 1995), GLP-1 may mediate its effects through gastric emptying and gastric distension, and that some degree of stomach distension may be needed to amplify appetite responses to GLP-1. Data from the current study support a role for gastric emptying in GLP-1 induced satiety, as GLP-1 infusion was shown to delay the gastric emptying of a water load. This supports findings of previous work (Wettergren et al., 1993; Willms et al., 1996; Schirra et al., 1997) which have shown GLP-1 to delay gastric emptying, and our previous observations of a relationship between GLP-1 and gastric emptying (chapter 4, chapter 5). Differences in gastric emptying of the water load in the current study also demonstrate that this GLP-1 infusion was biologically active. While it has been observed that the lag period but not total emptying time of a mixed liquid meal is prolonged with GLP-1 infusion (Schirra et al., 1997) we observed prolongation of both the lag period and half emptying time, suggesting the infusion to act on total emptying time of the load. Thus it is probable that hunger effects seen following buffet test meal consumption
are due to the delayed emptying of the test meal with GLP-1 infusion, which mediated appetite effects through gastric distension (Read et al., 1994).

Plasma glucose levels were shown to decrease during the GLP-1 infusion which may be due to the facilitation of glucose uptake (Villanueva-Peñacarrillo et al., 1994) and glucagon suppression (Komatsu et al., 1994) by GLP-1. Insulin remained unaffected because glucose levels did not raise above fasting levels, and was lower after the buffet test meal with the GLP-1 infusion. This is likely to be due to the effect of GLP-1 infusion upon gastric emptying, with a delayed emptying leading to delayed nutrient absorption and lower glucose levels. Consequently lower plasma insulin was seen immediately following buffet meal consumption. CCK can be eliminated in the present study as exerting any effect upon appetite or gastric emptying following the water load, as CCK was unaffected by the GLP-1 infusion and remained at basal levels. Similarly there was no difference in plasma GIP, TAG and NEFA levels between the GLP-1 and saline infusions prior to buffet meal consumption. The lower levels of GIP and NEFA following buffet meal consumption may be attributable to delayed gastric emptying with GLP-1 infusion. Although there was no significant difference in CCK levels following the buffet test meal, higher levels of CCK were observed with GLP-1 infusion. This was unexpected as CCK is secreted by the I-cells of the duodenum, and the delayed gastric emptying during GLP-1 infusion should delay exposure of these cells to ingested nutrients. Although the CCK radioimmunoassay used has little cross reactivity with gastrin and extraction of plasma samples is used to reduce any gastric interference, cross reactivity of gastrin in the CCK assay cannot be ruled out. As gastrin is released by the antrum of the stomach delayed gastric emptying would increase exposure time of these endothelial cells to nutrient contact, thus promoting gastrin secretion. This would then increase the possibility of cross reactivity in the plasma assay. As CCK levels were increased following buffet meal intake with GLP-1 infusion a secondary effect of CCK upon gastric emptying rate of this meal is also possible. Thus delayed gastric emptying of the buffet test meal could be attributable to the actions of both hormones, and effects on hunger ratings could also be mediated by a combined post-prandial action of GLP-1 and CCK.
During the course of this investigation other reports were published which investigated the role of peripheral exogenous GLP-1 in human appetite, and showed an effect of the peptide on appetite ratings and food intake. Enhanced feelings of satiety and reduced feelings of hunger have been shown with GLP-1 infusion following a breakfast meal (Flint et al., 1998), and were accompanied by decreased energy intake from a test meal 4.5 hours into the infusion. Although no assessment of gastric emptying was made during this investigation, it is possible that effects on the emptying rate of the breakfast meal contributed to differences in appetite ratings after this meal. It was also noted that the GLP-1 infusion was continued for much longer by Flint and colleagues than in the present study, and the prolonged elevated levels of GLP-1 may have contributed to the significant reduction in test meal intake. The influence of a prolonged GLP-1 infusion is supported by (Näslund et al., 1998) who showed no effect of short term GLP-1 infusion on test meal food intake, but found effects on appetite ratings after a further 3.5 hours of infusion time. As the infusion was started at the same time that the test meal was offered in this study, it is possible this did not give enough time for raised GLP-1 levels to influence appetite and the amount of food consumed while the meal was eaten. Again it is speculated that delayed gastric emptying effects on food ingested during the test meal have influenced satiety after the test meal, although this study also provides evidence for a prolonged elevated GLP-1 level to induce satiety effects.

In a more short term experimental design similar to the current study (Gutzwiller et al., 1999) a reduction of food intake was seen with GLP-1 infusion levels of 0.375, 0.75 and 1.5 pmol/kg per min, with reduced hunger ratings at the highest dose. However this experimental design infused GLP-1 in a glucose vehicle, against a 5% glucose control while the current study used a saline based infusion solution. As we saw a drop in plasma glucose with GLP-1 infusion, and as glucose plays a role in the short term regulation of hunger and satiety (Campfield, 1997), it is possible that an interaction between glucose and GLP-1 levels is needed for the induction of short term satiety by the hormone. During this time Lavin et al. (1998) also conducted an investigation into the effects of insulin, GLP-1 and GIP upon appetite following
intraduodenal infusion of glucose, and obtained results suggesting appetite responses were more closely related to GLP-1 than other gastrointestinal hormones. The close post-prandial relationship of GLP-1, insulin and glucose also implies that GLP-1 could exert appetite effects not only directly and through alterations in gastric emptying rate, but also through a glucostat-related mechanism. To investigate further a short term investigation comparing the relative food intake and appetite responses to GLP-1 and GLP-1 with glucose against both glucose and saline controls is needed. Use of the glucose clamp technique could also help elucidate whether a critical level of glucose is necessary in this response.

In the present study the absence of clear effects of GLP-1 infusion on appetite ratings and food intake may in part be explained by the greater variability in these responses compared to physiological responses. As effects on gastric emptying and blood glucose were observed the hormone levels infused here did have biological activity, and lack of activity can therefore be eliminated as an explanation for the lack of difference in appetite responses. It is acknowledged that the number of subjects (n = 10) used in this investigation may in itself have reduced the power of the experimental design, and other studies have used twice this number (Flint et al., 1998; Gutzwiller et al., 1999). However this study does cast some doubt as to whether GLP-1 is a major satiety factor in man, and suggests that satiety actions of the hormone are likely to be mediated through the presence of gastric loads. Thus it would appear that a role for GLP-1 in appetite control may be of greater importance in the post-prandial rather than the fasted state. Additional work is now needed to investigate the role of endogenous post-prandial GLP-1 on appetite ratings, food intake and within meal termination of eating, and the receptor antagonist exendin (9-39) is an ideal candidate for this role (Schirra et al., 1998).
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7. The role of CCK in the control of appetite and gastric emptying: effect of an infusion of the CCKα receptor antagonist loxiglumide upon appetite, circulating hormones and metabolites, and gastric emptying

7.1. Introduction

Although fat is generally shown to be less satiating than other macronutrients (e.g. Johnstone et al., 1996) intestinal lipid infusions have been shown to have short term effects on satiety in man (Welch et al., 1985; Lieverse et al., 1994c). As a peptide released primarily in response to fat consumption, CCK has been implicated as the hormone which may mediate fat-induced satiety, and animal studies have shown reduced food intake with peripheral administration of CCK (Koopmans et al., 1972; Smith & Gibbs, 1975). In humans infusion of exogenous CCK has been shown to reduce food intake in normal weight and obese subjects (Kissileff et al., 1981; Schick et al., 1991; Lieverse et al., 1994b; Lieverse et al., 1995b), with positive effects of the hormone shown in both fed and fasting individuals. Although it was suggested that CCK may have a pharmacological rather than physiological effect on food intake (Schick et al., 1991), physiological levels of the exogenous hormone have also been shown to reduce food intake (Ballinger et al., 1995). A problem with ascribing physiological levels to circulating CCK remains the lack of a reliable and standardised assay (Rehfeld, 1998). Consequently use of a receptor antagonist of CCK is extremely important to investigate the influence of endogenous CCK. Studies using the peripheral CCKα receptor antagonist loxiglumide to investigate the role of endogenous CCK in human appetite have shown conflicting results. Authors have often adopted the approach of intra-intestinal administration of a fat load with and without loxiglumide infusion, and an intraduodenal fat load has been shown to elicit physiological concentrations of CCK and reduce food intake in lean subjects.
Infusion of loxiglumide for 2½ hours before a test meal was shown to increase food intake during the test meal, and subjects were shown to have higher circulating CCK during this time. Thus it appears that endogenous CCK does induce satiation and satiety in man. More recently a similar investigation has shown comparable results, with loxiglumide blocking the satiating effect of an intraduodenal fat load plus banana shake (Matzinger et al., 1999). Conversely Drewe and colleagues (Drewe et al., 1992) showed no effect of loxiglumide infusion on food intake during intrajejunal perfusion with fat even though circulating CCK was elevated. Also Lieverse et al. (Lieverse et al., 1995c) found no effect of loxiglumide on food intake and satiety in lean and obese subjects. However the methods used in these investigations may provide some explanation. While lean individuals were infused at 10mg/kg.h⁻¹ according to their actual body weight, the obese were infused at their ideal body weight, and it is possible that the dose of loxiglumide in these subjects was not sufficient to elicit a response. This is supported by the findings of the study which showed the lean group to have a slight increase in food intake with loxiglumide, but no effect in the obese group. Furthermore there was no initial loading dose of loxiglumide to ensure blockade of receptors, necessary as loxiglumide competes with CCK for CCKα receptor binding. This may have resulted in insufficient blocking of receptors and allowed CCK to maintain its biological action. It is also questionable whether the premeal used to promote the release of endogenous CCK was a sufficient stimulus. The meal consisted of 100g bananas blended with water to form a predominantly carbohydrate containing shake. As CCK is primarily released in response to fat it is unlikely that CCK response following this premeal would have any effect on appetite, thus the blockade of CCKα receptors would have no measurable effect on food intake in this instance. As the investigation made no measure of plasma CCK levels, these data should be interpreted with caution. An oral administration of the antagonist has also been shown to have no effect on food intake in the free living situation (French et al., 1994), although in this instance the dose and route of administration may not have been sufficient to block peripheral receptors.

At present data examining the role of endogenous CCK in satiety is incomplete, with similar numbers of studies showing positive and negative results. In those
investigations where inter-intestinal administration of nutrients are used it is probable that subject discomfort or unfamiliarity with procedures used may have negative effects on data, particularly with subjective appetite ratings. Delivery of nutrients through an oral fat load, with concurrent infusion of loxiglumide against a control would have the benefit of minimising subject discomfort and delivering nutrients to the intestine by the natural route. Also measurement of circulating CCK is needed to determine whether the concentration of loxiglumide administered is acting on receptors, and enable further assessment of the relationship between plasma CCK and appetite. Due to the relative lack of viable assays for this peptide few studies have measured circulating CCK. As gastric distension may be important for the CCK mediated satiety response the extent to which gastric emptying in humans relates to CCK mediated satiety and food intake is worthy of further investigation, particularly as few studies have examined the direct relationship between these parameters. Thus further research into the role of endogenous CCK in short term fat mediated satiety in relation to gastric emptying may help elucidate the physiological importance of this hormone in the control of human appetite.

7.2. Aim

The present study therefore aimed to investigate the role of peripheral endogenous CCK in the induction of satiety, and elucidate the role of gastric emptying in this mechanism. Appetite responses and post-prandial changes in circulating hormones and metabolites were assessed during an infusion of the CCKA receptor antagonist loxiglumide against a saline control. The effect of the infusions upon gastric emptying was simultaneously assessed.

7.3. Study design

7.3.1. Subjects

Eleven healthy, non dieting subjects (8 male, 2 female, age range 22 - 33 years) were infused with loxiglumide or saline in a single blind randomised crossover design. All subjects were recruited from staff and students at the University of Surrey and were of
normal weight for height (mean BMI 22.4 ± 1.8 kg/m²). During recruitment they were asked to complete the DEBQ and only those subjects scoring less than 3.5 in restrained, emotional and external eating were accepted for participation in the study. During the recruitment process all volunteers underwent standard haematological and biochemical screening conducted by the Royal Surrey County Hospital to ensure only those in good health were selected to participate. The infusion procedure was fully explained to subjects at that time, and GP approval was obtained. Written consent was also obtained from each volunteer, and subjects were aware that they could withdraw from the investigation at any time. Ethical approval for the study was obtained from the South West Surrey District and University of Surrey Ethical Committees.

Screening for EIE was undertaken to locate correct positioning of the electrodes for each subject. Preceding the study all subjects were informed that paracetamol would be used as an additional measure of gastric emptying, and only individuals without known adverse reactions to paracetamol were recruited. In addition all subjects were instructed to refrain from alcohol consumption and strenuous exercise the day before and the day of each test occasion, and to avoid paracetamol containing painkillers for the 24 hours prior to the study.

7.3.2. Infusions

The loxiglumide infusion aimed to give a loading dose of 30 mg/kg per hour for the first ten minutes, and thereafter a 10 mg/kg per hour maintenance dose for the duration of the infusion period. This delivery rate has been shown previously to sufficiently block CCK_A receptors and prevent peripheral CCK action (Lieverse et al., 1995a).

Loxiglumide was supplied as a sodium salt (code CR-1505, batch D/1150) by Dr. Massimo D'Amato, Rotta Research Laboratories, Milan, Italy. The material was determined by HPLC to be 99.8% pure, with a molecular weight of 483.4g/mol. The powder was made into a sterile infusion solution using the supplied instructions (appendix XI) by St Georges Hospital Pharmacy, Tooting, London, and supplied for
use in sterile infusion bags. Before infusing the loxiglumide a sample of the solution successfully completed sterility and pyrogenicity testing at St Georges. Each loxiglumide infusion was made the day before each study occasion, and stored overnight at 4°C. The solution was brought to room temperature on the morning of the study prior to commencement of the infusion. The prepared infusion bags were then connected to a sterile infusion set (IVAC UK Ltd., Wade Road, Basingstoke, Hants, RG24 8NE), which was in turn connected to an IVAC 591 infusion pump for delivery.

The infusion rate was set according to subject body weight using the following formula:

Each loxiglumide infusion bag (1 litre) contained 5g loxiglumide / 1 = 5mg / ml

To deliver a loading dose of 30 mg/kg per hour:

\[
30 \times \text{bodyweight (kg)} = X \text{mg loxiglumide / h}
\]

\[
X \text{mg} \div 5 \text{mg / ml} = Y \text{ml / hour infusion rate.}
\]

After first 10 minutes of infusion the dose is reduced to 10 mg/kg per hour, and the above infusion rate is divided by 3 to calculate the maintenance dose infusion rate.

A control of saline was infused against the loxiglumide, and in this instance the pump was set to the same flow rate as that calculated for loxiglumide infusion. All infusion bags were covered with an opaque, light restrictive shield to ensure subjects were unaware which treatment they were receiving. To record any adverse physiological consequences of the infusions an additional VAS questionnaire was included to assess possible side effects (appendix XII).

7.3.3. Test meals

7.3.3.1. Standard breakfast

All subjects were asked to consume a standard breakfast before 0815 hours on the morning of each study day. The breakfast consisted of rice krispies (50g) served with
skimmed milk (300 ml), and one cup of tea or coffee with skimmed milk or sugar as preferred (see table 7.1 for composition). Although subjects did not have to eat all the food available, they were asked to eat the same amount at the same time on each test occasion. By offering the subjects a breakfast meal it was hoped that feelings of extreme hunger at the start of the study would be avoided, as these may interfere with subsequent hunger assessment.

The breakfast meal was selected due to the relatively small hormone and metabolite responses it elicits, and a preliminary study (authors unpublished observations) established that the majority of the parameters measured in this investigation would return to basal or near basal levels by the start of the study period (1100 hours). In addition an investigation into the effect of the breakfast meal upon subsequent gastric emptying measurements was undertaken prior to the study, and there was shown to be no difference in gastric emptying of a water load taken 3 hours after consumption of the test breakfast against a water load taken following an overnight fast (A. Giouvanoudi, personal communication).

7.3.3.2. Preload

A high fat liquid preload was developed to provide 50g of fat per serving, and thus promote the release of post-prandial CCK. The preload was given as a 450 ml milkshake consisting of double cream (105g), maltodextrin (50g) and nesquick flavouring (20g), and was made up to volume with bottled water (see table 7.1 for composition). The nesquick flavouring improved palatability of the preload, and subjects were able to choose either banana or strawberry flavour. The same flavour preload was offered on each test occasion. As the blockade of CCKA receptors by loxiglumide may alter secretions into the stomach, and the conductivity of gastric secretions may influence the EIE assessment of gastric emptying, paracetamol (1.5g) was added to the preload as an additional method of assessing gastric emptying.
Table 7.1 Macronutrient composition of standard breakfast meal and preload. Amounts given are per serving.

<table>
<thead>
<tr>
<th></th>
<th>Standard breakfast</th>
<th>Preload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (kJ (g))</td>
<td>13 (0.8)</td>
<td>1881 (50)</td>
</tr>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>1003 (60)</td>
<td>1204 (72)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>217 (13)</td>
<td>30 (1.8)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1233</td>
<td>3115</td>
</tr>
</tbody>
</table>

7.3.3.3. Ad libitum test meal

The pasta test meal described in section 3.4. was used as the *ad libitum* test meal in the present study. Details of macronutrient composition and food choice have been described previously (section 3.4.3.2.2.).

7.3.4. Study protocol

The 2 infusions were administered using a single blind randomised crossover design on separate occasions 7 days apart. On the morning of each study subjects consumed the standard breakfast before 0815 hours, and were instructed to consume nothing except water after this time. Following arrival at the investigation unit at 1045 hours subjects were cannulated in an antecubital forearm vein, and a three way tap was fitted to the cannula to allow the administration of the infusion and taking of blood samples with one cannula. A three way tap was used as a number of subjects expressed discomfort and concern at the use of two cannula. Three pairs of electrodes were then positioned over the stomach and lower back for the EIE measurement of gastric emptying, and subjects were given a rest period of approximately 15 minutes to adjust to their surroundings.

Before starting the infusion a baseline EIE reading was obtained, and 2 basal blood samples were taken (-45, -30 min). Subjects also completed the first set of VAS ratings for the assessment of appetite and adverse physiological events (-30 min). Loxiglumide or saline was then infused at the loading dose rate for 10 minutes, and adjusted to supply the maintenance dose for the remainder of the infusion period.
Thirty minutes into the infusion a third blood sample was taken and a VAS rating completed (0 min), and subjects were offered the high fat preload. The preload was consumed at room temperature through a straw, and subjects were instructed to drink steadily to avoid ingesting excess air which may interfere with the EIE.

Following preload consumption post-prandial VAS ratings were completed at 30 minute intervals and venous blood samples were taken at regular intervals (15, 30, 45, 60, 90, 120, 150, 180 min) for the next 3 hours. Gastric emptying of the preload was assessed for 60 minutes by EIE and appearance of paracetamol in plasma, after which time electrodes were removed and subjects were offered an *ad libitum* pasta test meal from which they were able to eat as little or as much as preferred until feeling comfortably full. Subjects were separated from one another during eating to minimise the effects of social interaction upon food intake. From the test meal energy and macronutrient intake were calculated. The infusion continued throughout the test meal and for a further 120 minutes after offering the test meal, giving a total infusion time of 3.5 hours. Following the last VAS and blood sample the infusion was stopped (see figure 7.1 below for outline of study protocol).

Subjects remained in the unit for a short time after the infusion to ensure there were no delayed adverse responses to the infusion, and were supplied with a contact number for the medic on duty. After this time they were free to leave and resume their normal activities.

7.3.5 *Statistical analyses*

Appetite and adverse event ratings were analysed using repeated measures ANCOVA. Adverse events were analysed throughout the infusion period, using the baseline rating (-30 min) as the covariate with infusion and time (0 to 180 min) as repeated measures factors. Appetite ratings were analysed in two sections to assess responses to the preload and responses after the test meal. The baseline rating (0 min) was used as the covariate, with infusion and time (+5 to 60 min) as repeated measures factors following preload consumption. Ratings made following consumption of the buffet test meal were analysed using 90 min as a covariate, with infusion and time (120 to 230 min) as repeated measures factors. However, this analysis was not repeated for high fat preload.
180 min) as repeated measures factors. Buffet test meal intakes and satiety quotients were analysed using a paired, 2 tailed t-test.

Hormone and metabolite responses and gastric emptying data were analysed using repeated measures ANOVA for the period after preload consumption, with preload and time (0 to 60 min) as repeated measures factors. These data were not analysed following test meal consumption as subjects ate differently, and influences upon hormone and metabolite responses cannot be directly determined for this time.

Figure 7.1 Outline of study protocol for investigation into the effects of loxiglumide on appetite, circulating hormones and metabolites, and gastric emptying.

<table>
<thead>
<tr>
<th>Loxiglumide infusion (30 mg/kg/h first 10 min, then 10 mg/kg/h) or saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric emptying</td>
</tr>
<tr>
<td>preload           pasta meal</td>
</tr>
<tr>
<td>-45 -30 -15 0 15 30 45 60 75 90 105 120 135 150 165 180</td>
</tr>
<tr>
<td>VAS ratings       Blood sample</td>
</tr>
</tbody>
</table>

7.4. Results

Due to illness experienced by two subjects during the investigation (one subject was ill during saline infusion, another during loxiglumide infusion) the data presented is from n = 8 subjects.
7.4.1. Occurrence of adverse physiological effects

Subjects were asked to rate any adverse effects during the saline and loxiglumide infusions (figure 7.2). Using repeated measures ANCOVA to analyse any differences in subjective feelings between the infusions, there were found to be no significant differences in ratings of stomach ache ($p = 0.7$), faintness ($p = 0.4$) and thirst ($p = 0.4$). Subjects rated themselves to be slightly more flushed ($p = 0.1$) and jittery ($p = 0.1$), less refreshed ($p = 0.1$), and experience a greater degree of headaches ($p = 0.1$) during the saline infusion compared to the loxiglumide infusion, although these differences were also not significant.

Also during the saline infusion subjects reported feeling significantly more lightheaded ($p = 0.05$), and experienced a significantly greater degree of heartpounding ($p = 0.02$) throughout the infusion. Furthermore, subjects reported feeling significantly more nauseous during the saline infusion ($p = 0.01$), although this is attributable to higher nausea ratings before the start of the infusion, which while decreasing during the first 90 minutes of infusion remained higher than nausea ratings with the loxiglumide infusion.

There was no significant difference in ratings of preload taste (pleasantness) between the saline (4.3 $\pm$ 2.9) and loxiglumide (4.0 $\pm$ 2.9) infusions ($p = 0.2$), and no difference in sweetness ratings of the preload between infusions (saline 4.2 $\pm$ 1.9, loxiglumide 4.8 $\pm$ 2.6, $p = 0.4$).
Figure 7.2  Ratings of adverse physiological events during infusion of loxiglumide against a saline control

Ratings of adverse physiological events (mean, n = 8) during infusion of loxiglumide (■) and saline (■). Subjects reported feeling significantly more lightheaded ($p = 0.05$), and nauseous ($p = 0.01$) and experienced more heartpounding ($p = 0.02$) during the saline infusion.

7.4.2. Appetite responses

7.4.2.1. Hunger ratings

Post-prandial hunger ratings during the saline and loxiglumide infusions are illustrated in figure 7.3. There were no significant differences in hunger ratings during the 60 minutes following preload consumption ($p = 0.4$). It was noted that subjects rated themselves to be more hungry immediately following preload consumption with the loxiglumide infusion (+5 min), although this difference was not significant ($p = 0.16$). Hunger ratings were also higher with saline infusion following
consumption of the pasta test meal, although this difference was not significant ($p = 0.2$).

Figure 7.3 Hunger ratings following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide against a saline control

Hunger ratings (mean ± SEM, $n = 8$) following consumption of a high fat preload and test meal during infusion of loxiglumide (■) and saline (■). There were no significant differences between infusions after the preload ($p = 0.4$) or test meal ($p = 0.2$).

Transformation of data to $z$ scores did not add anything further to the analysis. However, multiplicative adjustment for baseline resulted in the difference between hunger ratings at +5 min approaching significance ($p = 0.08$).

7.4.2.2. Satiety ratings

Satiety ratings during the infusion showed a similar (inverse) pattern to hunger ratings (figure 7.4). Analysis of ratings during the 60 minutes following preload consumption showed no significant differences, although subjects rated themselves to be less satiated immediately following preload consumption with the loxiglumide infusion ($p = 0.1$). Subjects also rated themselves as less satiated following the pasta test meal, but this difference was not significant ($p = 0.2$). Satiety $z$ scores and multiplicative adjustment for baseline highlighted no further differences.
7.4.2.3. Food preference checklist data

The total energy and number of items selected from the food preference checklist showed little difference between infusions (figures 7.5 & 7.6). Subjects selected less energy from the FPC immediately after preload consumption and following pasta meal consumption during the saline infusion, suggesting they felt less hungry at that time, but these differences were not significant \((p > 0.3\) after preload and test meal). Differences in number of items selected reflected those in total energy selected \((p > 0.4\) after preload and test meal).
Figure 7.5: Total energy selected from the food preference checklist following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide against a saline control.

Infusion of loxiglumide or saline

Preload  Pasta test meal

Total energy selected from the FPC (mean ± SEM, n = 8) following consumption of a high fat preload and *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). There were no significant differences between infusions (p > 0.3 after preload and after test meal consumption).
Figure 7.6 Number of items selected from the food preference checklist following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide against a saline control

Number of items selected from the FPC (mean ± SEM, n = 8) following consumption of a high fat preload and *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). There were no significant differences between infusions (*p* > 0.4 after preload and after test meal consumption).

7.4.2.4. *Ad libitum* test meal intake

*Ad libitum* test meal consumption 60 minutes after the preload showed large differences in energy intake between infusions (figure 7.7). Mean test meal energy intake was shown to be significantly higher with the loxiglumide infusion than with the saline infusion (*p* = 0.008). Analysis of individual intakes showed all subjects to eat more during the loxiglumide infusion (figure 7.8).
Test meal energy intake (mean ± SEM, n = 8) during infusion of loxiglumide (■) and saline (■) 60 minutes after consumption of a high fat preload. Intake was significantly higher with loxiglumide infusion ($p = 0.008$).

Test meal energy intake of each subject during infusion of loxiglumide (■) and saline (■) 60 minutes after consumption of a high fat preload. Intake was higher with loxiglumide infusion for all subjects.
Quotients were calculated for the post test meal period (90 to 180 min) for hunger and satiety (figures 7.9 and 7.10 respectively). Hunger quotients were found to be significantly higher immediately after the test meal ($p = 0.06$), and for the remainder of the post test meal period the difference between quotients approached significance ($p = 0.09$). Satiety quotients followed a similar pattern with a higher value immediately after the test meal ($p = 0.01$) and for the duration of the post test meal period ($p = 0.04$) with saline infusion.

Figure 7.9 Quotients calculated from test meal energy intake and hunger ratings before and after test meal consumption during loxiglumide or saline infusion

Mean ± SEM ($n = 8$) hunger quotients following pasta meal consumption during loxiglumide (■) and saline (■) infusion. Difference between infusions approached significance ($p = 0.09$).
Figure 7.10 Quotients calculated from test meal energy intake and satiety ratings before and after test meal consumption during loxiglumide or saline infusion

Mean ± SEM (n = 8) satiety quotients following pasta meal consumption during loxiglumide (■) and saline (■) infusion. Quotients during saline infusion were significantly higher than with loxiglumide infusion (p = 0.04).

7.4.3. Blood metabolite and hormone responses

7.4.3.1. Plasma CCK

As expected circulating CCK remained at basal levels for the first 45 minutes of infusion (-45 to 0 min). Following consumption of the preload an increase in the hormone was observed, and levels remained elevated at approximately 6 - 8 pmol/l for the duration of the study period with saline infusion. Post-prandial CCK concentrations during the loxiglumide infusion were almost double those observed with the saline infusion for the 60 minutes after preload consumption (p = 0.007). Difference in CCK concentrations between infusion was also significant throughout the infusion period (p = 0.004, figure 7.8).
Figure 7.11 Plasma CCK levels following consumption of a high fat preload and an *ad libitum* test meal during loxiglumide or saline infusion

![Graph of Plasma CCK levels](image)

Plasma CCK concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). CCK was significantly higher during loxiglumide infusion after preload consumption (*p = 0.007*) and throughout the infusion period (*p = 0.004*).

### 7.4.3.2. Plasma GLP-1

Circulating GLP-1 concentrations exhibited a dramatic post-prandial increase immediately after preload consumption during the loxiglumide infusion, with a smaller sustained increase during infusion of saline (figure 7.12). For the 60 minutes period following preload consumption GLP-1 remained significantly higher with loxiglumide infusion (*p = 0.008*).
Plasma GLP-1 concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an ad libitum test meal during infusion of loxiglumide (■) and saline (■). GLP-1 was significantly higher following preload consumption with loxiglumide infusion ($p = 0.008$).

7.4.3.3. Plasma GIP

Plasma GIP levels were similar and not significantly different for the 60 minutes following preload consumption (figure 7.13, $p = 0.8$). Following test meal consumption GIP levels were higher with the saline infusion.
Plasma GIP concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an ad libitum test meal during infusion of loxiglumide (■) and saline (■). There was no significant difference in GIP levels for the 60 minutes following preload consumption ($p = 0.8$).

### 7.4.3.4. Plasma insulin

Insulin concentrations during infusion of saline and loxiglumide are illustrated in figure 7.14, and show a similar response with loxiglumide infusion to that observed for GLP-1. Plasma insulin exhibited a large post-prandial rise following preload consumption during the loxiglumide infusion and a more gradual rise with saline infusion. During this time insulin was significantly higher with the loxiglumide infusion (0 to 60 min, $p = 0.04$). Levels remained elevated with loxiglumide infusion following pasta meal consumption.
Plasma insulin concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). Insulin was significantly higher following preload consumption during the loxiglumide infusion (*p* = 0.04).

### 7.4.3.5. Plasma glucose

Plasma glucose levels were similar following consumption of the preload with saline and loxiglumide infusions (figure 7.15) and were not significantly different (*p* = 0.8). The time taken to reach peak glucose levels following consumption of the preload was shorter during the loxiglumide infusion, and the mean difference in time taken to reach peak levels (15 min) resulted in an infusion *x* time interaction (*p* = 0.06).
Plasma glucose concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). Following preload consumption there was no significant difference between infusions (*p* = 0.8), although an infusion × time interaction was observed (*p* = 0.06).

### 7.4.3.6. Plasma TAG

Circulating TAG levels were significantly lower with the loxiglumide infusion than saline infusion following preload consumption (*p* = 0.03, figure 7.16). Consumption of the pasta test meal resulted in a sharp elevation of plasma TAG for the remainder of the study period with saline infusion. Post-prandial TAG response appeared to be suppressed for the duration of the post-prandial period with the loxiglumide infusion compared to the saline infusion.
Plasma TAG concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an \textit{ad libitum} test meal during infusion of loxiglumide (■) and saline (■). Circulating TAG was significantly lower during the loxiglumide infusion following preload consumption ($p = 0.03$).

7.4.3.7. Plasma NEFA

Circulating NEFA levels showed a greater fluctuation following preload consumption during the loxiglumide infusion compared to the saline infusion (figure 7.17). This resulted in a significant infusion \times time interaction following preload consumption ($p = 0.02$), but no main effect of infusion on NEFA levels ($p = 0.5$). Following consumption of the pasta test meal a gradual increase in circulating NEFA was observed with the saline infusion. In contrast little change was observed with the loxiglumide infusion for the rest of the study period.
Figure 7.17 Plasma NEFA levels following consumption of a high fat preload and *ad libitum* test meal during loxiglumide or saline infusion

Plasma NEFA concentrations (mean ± SEM, n = 8) following consumption of a high fat preload and an *ad libitum* test meal during infusion of loxiglumide (■) and saline (■). There was a significant infusion x treatment interaction following preload consumption (p = 0.02).

7.4.4. Gastric emptying response

7.4.4.1. Assessment of paracetamol

Circulating paracetamol concentrations were measured for 60 minutes after consumption of the preload, and rate of appearance in plasma used to assess differences in gastric emptying between the infusions (figure 7.18). Paracetamol was found to peak earlier and was significantly higher with the loxiglumide infusion compared to the saline infusion (p = 0.008), suggesting a faster rate of gastric emptying of the preload during the loxiglumide infusion.
Figure 7.18 Plasma paracetamol levels following consumption of a high fat preload and ad libitum test meal during loxiglumide or saline infusion

Plasma paracetamol concentrations (mean ± SEM, n = 8) following consumption of a high fat preload during infusion of loxiglumide (■) and saline (■). Paracetamol peaked earlier and was significantly higher with loxiglumide infusion ($p = 0.008$).

7.4.4.2. Epigastric impedance epigastrography

The percentage of the preload remaining in the stomach was calculated at 15 minute intervals for 1 hour after consumption (figure 7.19). Although the stomach did not fully empty during this 60 minute period, the rate of gastric emptying was shown to be significantly faster during the loxiglumide infusion than during saline infusion ($p = 0.001$).

From the percentage remaining in the stomach the T50 was calculated, and was significantly shorter with loxiglumide (mean 19.8 ± 4.2 min) than with the saline (mean 29.0 ± 8.1 min) infusion ($p = 0.009$).
Figure 7.19 Percentage of the high fat preload remaining in the stomach measured for 60 minutes during loxiglumide or saline infusion

Percentage of meal remaining in the stomach (mean ± SEM, n = 8) following preload consumption during loxiglumide (■) and saline (■) infusion. Percent remaining was significantly lower, and rate of gastric emptying significantly faster with loxiglumide infusion ($p = 0.001$).

Paracetamol and EIE data was correlated to determine the extent to which paracetamol measurement relates to the gastric emptying rate assessed by EIE. The percentage remaining in the stomach correlated well plasma paracetamol levels when analysing all data ($r = -0.57$, $p < 0.001$), and within each infusion (loxiglumide: $r = -0.48$, $p = 0.002$; saline: $r = -0.75$, $p < 0.001$).

Test meal energy intake was found to correlate strongly with percentage of the preload remaining in the stomach immediately before consumption ($r = 0.76$, $p = 0.001$, all data).

### 7.5. Discussion

Infusion of loxiglumide has previously been shown to block peripheral actions of CCK (Lieverse et al., 1995a) and cause elevated circulating levels of the hormone in a compensatory response to receptor blockade (Drewe et al., 1992). The present study
has shown significantly elevated CCK levels with loxiglumide infusion, supporting previous findings and providing evidence that infusion of the antagonist has blocked peripheral CCK receptors, as increased CCK is secreted in response to receptor blockade. Further evidence for successful action of the loxiglumide infusion comes from the differences in gastric emptying rates between infusions. CCK normally acts to inhibit or delay gastric emptying, however data from the current study show an increased rate of gastric emptying with loxiglumide infusion and supports previous findings (Schwizer et al., 1997). Thus we can assume that CCKA receptor blockade has inhibited the peripheral actions of CCK. No adverse effects were reported during the loxiglumide infusion although subjects reported a greater degree of nausea, lightheadedness and heartpounding with saline infusion. It is unlikely that the saline infusion itself caused these effects, and closer examination of data showed high ratings from two subjects to be the primary reason for the differences seen. Although a randomised crossover design was used both of these subjects had received the saline infusion first, and it is possible that anxiety about the cannulation and infusion procedures was responsible for the results seen. As a similar pattern of food intake response was seen in all subjects the lower mean food intake during saline infusion is unlikely to be attributable to the feelings of nausea observed, although its effect on the above two subjects cannot be ruled out. The exclusion of two different subjects due to illness during the study was justified as one case of illness occurred with both the loxiglumide and saline infusions. Thus illness did not appear to be attributable to one or other of the infusion solutions and was not considered an adverse effect in these subjects.

Although differences in gastric emptying were seen with the loxiglumide infusion no significant effects of the antagonist were apparent on subjective ratings of hunger and satiety, or on food preference checklist data. Immediately following preload consumption (5 min) however subjects rated themselves as more hungry and less satiated with loxiglumide infusion, and while this difference was not significant, the data imply that loxiglumide infusion had some effect on appetite soon after preload consumption. Due to the differences in gastric emptying rates between infusions, and as loxiglumide had been infused for 30 minutes before preload consumption, it is
probable that a faster initial rate of emptying can account for this immediate difference in appetite responses. Dumping of a proportion of the preload may have occurred immediately after ingestion, and if this mechanism were occurring differences in appetite ratings at this time are more likely to be due to differences in gastric fullness and distension (Khan & Read, 1992), than differences in hormonal responses alone.

In spite of the small differences in raw appetite data, adjustment for differences in baseline for satiety ratings did show subjects to consistently rate themselves as less satiated with the loxiglumide infusion following consumption of the preload. Taken together with differences immediately after preload consumption, these data provide tentative evidence for an influence of CCK on satiety, as blockade of peripheral receptors has resulted in lower self rated satiety. However much stronger evidence of a role for CCK in the mediation of satiety comes from test meal intake, with subjects consuming significantly greater energy during the loxiglumide infusion. This supports previous work suggesting that the cessation of eating is mediated by the actions of peripheral endogenous CCK in man (Lieverse et al., 1994c), although the relative effects of differences in gastric emptying and inhibition of any direct peripheral actions of CCK on appetite cannot be determined from these data. Hunger and satiety quotients support the above data, with a smaller hunger quotient during loxiglumide infusion. Thus subjects needed to eat more with loxiglumide infusion to achieve the decrease in hunger observed than during the saline infusion. Similarly satiety quotients show subjects to have eaten more during loxiglumide infusion to elicit the increase in satiety observed, with changes in hunger and satiety relative to ratings before the test meal remaining smaller in relation to test meal intake for the rest of the loxiglumide infusion period. Thus it appears that while ratings of hunger and satiety were not significantly different between infusions appetite differences were likely to have occurred between infusions, and this was expressed in terms of energy intake rather than subjective ratings.

In terms of differences in hormonal responses during loxiglumide infusion, plasma GLP-1 levels were significantly higher after preload consumption with the
loxiglumide infusion compared to the saline infusion. As preload nutrient intake was identical between infusions the difference in GLP-1 must be attributable either to the increased rate of gastric emptying during loxiglumide infusion or the inhibition of the action of CCK. As nutrient contact with the jejunal L-cells will be hastened with an increased rate of gastric emptying, an earlier hormonal response would be predicted with loxiglumide infusion. However while GLP-1 levels were similar after consumption of the pasta test meal, for the 60 minutes following preload consumption peak GLP-1 levels not only occurred earlier, but hormone response was also much greater with loxiglumide infusion. Blockade of the CCKA receptors appears to have potentiated the release of GLP-1, and mean levels after the high fat preload are higher than those previously observed by ourselves (chapter 5, figure 5.8) when taking into account the slight difference in fat intake between this and our previous study.

Another function of CCK is to stimulate gastric motility, and receptor blockade would result in a slower rate of motility. Prolonged nutrient contact with intestinal L-cells may therefore have increased signalling to L-cell in the lower small intestine, which would in turn increase GLP-1 secretion. As the test meal was offered 60 minutes after preload consumption it is difficult to determine whether differences in GLP-1 would persist once a comparable level of nutrient contact with L-cell had occurred during saline infusion. However the similar GLP-1 levels following test meal consumption are not indicative of a difference in GLP-1 secretion due solely to loxiglumide infusion or the blockade of CCKA receptors, as infusion continued during this time and GLP-1 levels were not different. It is possible that GLP-1 secretion was increased to compensate to some extent for the increased rate of gastric emptying with loxiglumide following preload consumption, as GLP-1 acts to delay gastric emptying (Schirra et al., 1997). However a similar effect was not seen following consumption of the pasta test meal, and other than the above speculation, the mechanism through which GLP-1 secretion may have been increased is unknown. As GLP-1 is a potential mediator of satiety (Lambert et al., 1994) it is possible that increased secretion of GLP-1 acts to initiate satiety in the absence of CCK mediated signals. Furthermore as GLP-1 had been recently suggested to act through vagal afferents (Nakabayashi et al., 1996), a related action of these peptides in human appetite regulation would not be surprising. In the present study a satiety effect induced by
Chapter 7

higher levels of GLP-1 with loxiglumide infusion could have occurred in addition to effects attributable to the inhibition of peripheral CCK activity. However subjects ate more with loxiglumide infusion, when GLP-1 levels were highest, and it is therefore likely that the overriding appetite effects in this study were due to CCK mediated actions.

The pattern of insulin secretion between infusions was similar to that of GLP-1, with raised insulin seen during loxiglumide infusion. As with GLP-1, insulin not only peaked earlier but was higher with loxiglumide infusion although venous glucose levels were similar between infusions. Prolonged nutrient contact due to delayed intestinal motility and increased gastric emptying may explain the increased insulin response seen with loxiglumide. In addition it is possible that inhibition of the action of CCK influences the regulation of insulin secretion. However this observation is contrary to previously reported data showing a similar insulin response following ingestion of 100g glucose and mixed meals with and without loxiglumide infusion (Schwarzendrube et al., 1991; Niederau et al., 1992). Thus the increased insulin levels seen with loxiglumide infusion may be attributable to the insulinotropic effects of higher GLP-1 levels during this infusion (Kreymann et al., 1987), or dumping during the early part of gastric emptying which promotes an earlier and higher insulin response. The lower NEFA levels seen with loxiglumide infusion may be attributed to higher insulin levels suppressing the action of hormone sensitive lipase during this infusion, preventing NEFA liberation from adipose tissue in the post-prandial state.

As plasma GIP response to each infusion was similar for the 60 minutes after preload consumption its insulinotropic action is unlikely to account for the difference in circulating insulin. Post-prandial differences in plasma GIP were only observed following the test meal, and the similar GIP levels after preload consumption are probably due to the dependence of GIP secretion upon nutrient absorption rather than the presence of nutrients in the intestinal lumen (Morgan, 1996). As no differences in GIP were apparent following preload consumption there were unlikely to have been differences in nutrient absorption between infusions immediately after the preload.
However as the action of CCK is inhibited in the current study, digestion and absorption of the high fat preload is reduced due to the inhibition of pancreatic enzyme secretions (Schmidt et al., 1991). This lower level of nutrient absorption is probably responsible for the lower GIP levels observed following test meal consumption with this infusion. In spite of the subjects eating more energy from the pasta test meal, TAG levels were lower with loxiglumide infusion, also attributable to lower pancreatic enzyme secretions as the rate of TAG breakdown and subsequent emulsification by bile salts was reduced. Following test meal consumption a smaller rise in TAG levels was observed with loxiglumide infusion which may be attributable to the action of lower small intestinal lipase, stimulated by undigested TAG as the preload moved into the lower small intestine and released by endothelial cells. Although much of the normal pancreatic exocrine secretions are inhibited some pancreatic stimulation may be occurring through the action other peptides (e.g. neurotensin, VIP), resulting in a small amount fat absorption. Evidence for some lipase action during loxiglumide infusion comes from the slight increase in TAG 30 minutes after preload ingestion, with a continued rise in circulating TAG levels following the test meal.

As the peripheral action of CCK was blocked with loxiglumide, the relationship between plasma CCK and gastric emptying could not be directly assessed, other than the observation that rate of emptying was increased during loxiglumide infusion in spite of elevated CCK levels. It was interesting to note the relationship between buffet meal intake and percentage of the preload remaining in the stomach. Although as mentioned previously in this thesis the EIE method of assessing gastric emptying cannot quantify gastric emptying time, this correlation provides evidence for a relationship between gastric fullness and food intake. Thus it appears that in the present study differences in food intake response mediated through CCKA receptor blockade were primarily responsive to variations in gastric fullness. It has previously been shown that plasma CCK does not relate directly to subjective appetite ratings (French et al., 1993), and it is probable that the actions of peripheral CCK upon appetite and food intake control are mediated through its gastrointestinal actions.
Although there were no significant differences in subjective appetite ratings, food intake data from the current study provides good evidence of a role for peripheral CCK in the control of human food intake. As gastric emptying rates were markedly different with and without infusion of loxiglumide, and there was found to be a strong relationship between test meal energy intake and gastric fullness, it is probable that CCK acts primarily through gastric distension and gastric emptying to mediate satiety. However the extent to which peripheral and central CCK are involved in the control of appetite is not apparent from this investigation, and it is possible that while peripheral CCK acts through gastric mechanisms, central CCK acts directly to regulate appetite.
Chapter Eight

8.1. Introduction

There is an increasing amount of research investigating the relationship between physical activity and food intake, with early theories of a direct positive relationship between energy expenditure and energy intake now seeming unlikely to occur in man. In terms of the relationship between energy deficit and energy intake per se, short term feeding studies using the manipulation of preload energy content to alter energy balance have shown there to be differences in hunger and subsequent food intake following high and low energy preloads (Pliner, 1973; Lawton et al., 1993; Green et al., 1994). Thus it would appear the appetite responses are dependent to some extent upon previous energy intake and sensitive to energy deficits induced through differences in intake. However energy deficits induced through alterations in physical activity have not been shown to result in short term increases in hunger ratings or in an elevated energy intake. Previous studies investigating the short term appetite responses to increased energy expenditure have often reported the suppression of self rated hunger immediately after periods of acute exercise (Durrant et al., 1982; King et al., 1994; King & Blundell, 1995; King et al., 1997; Westerterp-Plantenga et al., 1997); these data suggest appetite is unlikely to be directly coupled to energy expenditure. In addition it has been shown that food intake from a test meal offered shortly after a period of exercise was not different from that consumed after no exercise (King & Blundell, 1995), and a delay in meal onset was observed after exercise. A delay in the onset of eating was also reported by King et al. (1994), again with no differences in energy intake between exercise and control. In both of these studies the difference between energy expenditure and energy intake created a negative energy balance. To determine whether there was any compensation for the difference in energy intake and energy expenditure, food intake on the day of and for
one day following prescribed exercise was assessed compared to two rest days (King et al., 1997). There was found to be no difference in energy intake between any of the days investigated, suggesting there was no delayed compensation for energy expenditure the day after exercise. Taken together the above findings do not support a regulation of energy intake to match energy expenditure induced through exercise.

The influence of exercise intensity on the regulation of energy balance also provides evidence for the uncoupling of energy expenditure and energy intake. It has been reported that with no significant changes in short term appetite and food intake following high and low intensity exercise (Imbeault et al., 1997), the relationship between energy expenditure and food intake showed higher exercise intensities to favour negative energy balance (King et al., 1994). While it is possible that energy intake following exercise could be adjusted to compensate for differences in energy expenditure over periods of longer than a few hours, it has been shown that energy intake may remain unaltered for several weeks, and even months (Woo et al., 1982a; Woo et al., 1982b; Westerterp et al., 1991 cited in King, 1999), suggesting no delayed compensatory response to increased energy expenditure. Thus it appears that the dissociation between intake and expenditure may be increased at higher exercise levels.

The mechanisms through which exercise may act to suppress appetite remain unknown. A recent investigation directly comparing the appetite responses to energy deficits induced through exercise and food restriction found increased hunger and food intake in response to food deprivation but not in response to exercise (Hubert et al., 1998). Appetite responses to manipulations of food intake and exercise therefore appear to be regulated differently, and it has been suggested that a low energy meal failed to generate satiety signals, resulting in hunger and elevated food intake, while the metabolic effects of exercise failed to generate the excitatory signals required to promote hunger and food intake (Hubert et al., 1998). While the precise signals through which these mechanisms may act are not yet defined, it has also been suggested that the primary physiological response to exercise is that of increasing fluid intake as opposed to increasing energy intake (King, 1999). This compensation
for energy expenditure shortly after exercise may occur more readily with the provision of energy rich fluids rather than solid foods, and as the majority of investigations use a solid food test meal this could account to some extent for the lack of adjustment in energy intake seen in the investigations reported here. The role of dehydration in the regulation of intake following exercise has recently been more closely investigated by comparing appetite and food intake response following an exercise session to that following a session in a sauna designed to elicit the same degree of dehydration and rise in body temperature (Westerterp-Plantenga et al., 1997). While hunger was shown to decrease only with exercise, the ratio of energy derived from liquid : solid sources was increased after both the sauna and exercise sessions, suggesting thirst to be a primary motivator for intake at this time. However these data still showed an uncoupling between total energy intake and energy expenditure, and it is unlikely that such a weak relationship between intake and expenditure could continue to occur for a prolonged period of time with habitually high levels of energy expenditure. In contrast to the weak coupling between acute energy expenditure and food intake, habitually physically active subjects have been shown to have a suitably high energy intakes (Maughan et al., 1989), suggesting a positive relationship between long term physical activity and food intake for the maintenance of body weight (King, 1999). As a small number of investigations have shown an increase in hunger (Verger et al., 1992) and food intake (Staten, 1991) in response to exercise sessions which have been continued for longer than one day of investigation, these data raise the question of adaptation to different exercise levels which could result in a stronger coupling of energy expenditure and energy intake. The role of psychology in the induction and maintenance of changes in eating behaviour in individuals who exercise frequently must also be considered, and learned cues arising from high energy expenditure and athletic performance probably contribute to the maintenance of energy balance in these individuals (King, 1999). In addition concerns about weight gain or loss, nutritional health and physical performance in individuals who exercise frequently may result in their exhibiting greater control over food intake.
If athletes and other individuals habituated to higher levels of energy expenditure show better coupling of energy expenditure and energy intake, it is possible that the habitual exercise levels in the general population are also able to influence appetite responses. In our previous studies we have noted that subjects who are better able to compensate for differences in preload energy content are those who regularly participate in recreational exercise (chapter 4, section 4.5). In addition recent data showing good compensation for differences in energy intake following periods of exercise in male subjects who undertake regular exercise (non athletes) (King et al., 1999) suggest that exercise may improve the sensitivity of the appetite control system to manipulations of energy intake. If exercise may improve the sensitivity of an individual in detecting differences in energy intake, regular physical activity would be important for the regulation of body weight not only through increases in energy expenditure but also improved appetite regulation.

It is possible that the higher turnover of energy in an active individual as opposed to a sedentary individual is of primary importance in improving the sensitivity of appetite responses, although mechanisms through which this may act have yet to be elucidated. As the incidence of overweight and obesity increases, further research to investigate the importance of habitual exercise levels in appetite regulation and weight maintenance may be beneficial.

8.2. Aim

The present study therefore aimed to investigate appetite responses following high and low energy preloads in individuals who undertake regular exercise compared to those who undertake no exercise. Compensation for preload energy manipulations in subsequent food intake was also investigated to determine whether habitual exercise levels affected the subjects’ ability to detect differences in preload energy manipulations and adjust energy intake accordingly.
8.3. Study design

8.3.1. Subjects

Twenty three healthy male subjects (age range 18 - 40 years) of normal weight for height (mean BMI 23.7 \( \text{sd} \) 2.8 kg/m\(^2\)) were recruited on the basis of their habitual exercise levels. All volunteers were asked to complete the DEBQ during recruitment, and only those subjects with scores of less than 4.0 were recruited for participation in the study. Subjects were initially asked to rate their participation in exercise as one of three categories: one session or less per week (non exercisers), 2 - 3 sessions per week (moderate exercisers) or 4 or more sessions per week (high exercisers). One session was defined as at least 40 minutes of moderate to high intensity activity. In addition to self rated exercise levels, subjects were asked to complete 7 day physical activity diaries on two separate occasions during the study to verify their exercise levels, and were thus assigned to one of the above groups. If any discrepancy occurred between self rated exercise level and that suggested by the physical activity diary a follow-up questionnaire was given to determine exercise levels during the previous three months. Subjects were then assigned to the representative group.

Characteristics of each group are described in table 8.1. While DEBQ scores were similar between each group, the high exercise group had a lower, mean BMI than both the moderate and non exercise group. This difference was not significant. Prior to commencement of the study all volunteers were instructed that they must refrain from alcohol and exercise for the 24 hours before each test occasion.
Table 8.1 Mean (SD) characteristics of subjects assigned to different groups on the basis of their habitual exercise levels

<table>
<thead>
<tr>
<th>Exercise Group</th>
<th>Sessions / week</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Restraint score</th>
<th>Emotional score</th>
<th>External score</th>
</tr>
</thead>
<tbody>
<tr>
<td>non exercisers</td>
<td>≤ 1</td>
<td>22.2 (2.1)</td>
<td>24.3 (3.0)</td>
<td>1.9 (0.5)</td>
<td>2.1 (0.5)</td>
<td>3.1 (0.3)</td>
</tr>
<tr>
<td>moderate exercisers</td>
<td>2 - 3</td>
<td>27.1 (6.8)</td>
<td>24.1 (3.6)</td>
<td>2.1 (0.7)</td>
<td>2.3 (0.8)</td>
<td>3.0 (0.6)</td>
</tr>
<tr>
<td>high exercisers</td>
<td>≥ 4</td>
<td>22.1 (2.8)</td>
<td>22.5 (1.5)</td>
<td>2.0 (0.3)</td>
<td>2.3 (0.6)</td>
<td>3.4 (0.4)</td>
</tr>
</tbody>
</table>

8.3.2. Test meals

Two preload meals were developed to provide high and low energy through the manipulation of carbohydrate content. Each preload was given as a 450 ml milkshake consisting of double cream, maltodextrin and sucrose, and made up to volume with water. Vanilla essence (1.5 ml) was added to each preload to improve palatability. The low energy preload (LE) provided 1008 kJ and the high energy preload (HE) provided 2513 kJ (see table 8.2 for preload composition).

Table 8.2 Macronutrient composition of preloads with low and high energy content. Amounts given are per 450ml serving.

<table>
<thead>
<tr>
<th></th>
<th>Low energy (LE)†</th>
<th>High energy (HE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ (g))</td>
<td>167 (10)</td>
<td>1672 (100)</td>
</tr>
<tr>
<td>Fat (kJ (g))</td>
<td>828 (22)</td>
<td>828 (22)</td>
</tr>
<tr>
<td>Protein (kJ (g))</td>
<td>13 (0.8)</td>
<td>13 (0.8)</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>1008</td>
<td>2513</td>
</tr>
</tbody>
</table>

† LE preload : 46g double cream, 10g sucrose
* HC preload : 46g double cream, 10g sucrose, 90g maltodextrin

Food intake after consumption of the preload was assessed using an *ad libitum* buffet test meal (described in chapter 2, section 2.2.1.3) which enabled subjects to choose from a range of familiar foods, appropriate to the time of day. Subjects were offered their second and third choices of sandwich fillings and food types, and total energy available from the buffet ranged from 10751 to 12611 depending on the food items.
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offered. The number and range of food items presented to each subject was identical on each test occasion.

8.3.3. **Study protocol**

Using a randomised single blind crossover design the appetite and food intake response to each preload was investigated. Subjects were instructed to consume their evening meal before midnight on the night before each study occasion, and to consume the same meal before the next study occasion.

On the morning of each study subjects were asked to consume their normal breakfast before 0930 hours, and to consume the same breakfast on each test occasion. Following breakfast subjects were instructed to eat or drink nothing except water until arrival at the investigation unit at 1115 hours. After arrival at the investigation unit subjects had a 15 minute rest period, and were asked to complete baseline VAS ratings at the end of this rest period. Subjects were then offered either the LE or HE preload and instructed to drink at a steady pace. A second VAS rating was completed after consumption of the preload. VAS ratings were then completed at 20 minute intervals for the next 60 minutes, after which time subjects were offered the *ad libitum* buffet test meal. Subjects were instructed to eat until comfortably full from this meal, and to prevent over consumption due to freely available food subjects were informed they may take away any food they did not want to eat at that time. Energy and macronutrient intakes were calculated from the test meal, and following test meal consumption subjects were free to leave the investigation unit and resume their normal activities.

8.3.4. **Statistical analyses**

Energy intakes from the buffet test meal after the high and low energy preloads were compared within each exercise group using a paired, 2-tailed t-test. To analyse differences in intake between exercise groups following each preload, a one way ANOVA with post hoc testing was used, with group as the independent variable and energy intake as the dependent variable.
Hunger and satiety ratings between the high and low energy preload within each exercise group were analysed using repeated measures ANCOVA. The baseline rating (-5 min) was used as the covariate, with preload and time (10 to 60 min) as the repeated measures factors. Ratings following the buffet test meal were analysed separately using a paired, 2-tailed t-test as they were no longer primarily responsive to preload manipulations. To assess differences in hunger and satiety between exercise groups the difference in ratings between the high and low energy preloads was calculated for each group. These differences were then analysed using ANCOVA, with -5 min as the covariate. Exercise group was an independent variable with time (10 to 60 min) as the repeated measures factor.

8.4. Results

8.4.1. Ad libitum buffet test meal intake

Buffet test meal energy intakes were not significantly different between the LE and HE preloads in the non exercise group ($p = 0.09$, figure 8.1). Subjects in the moderate exercise groups were shown to significantly reduce their intake following the HE preload compared to the LE preload ($p = 0.05$). A similar response was seen in the high exercise group, and the difference in energy intakes was significant at the 6% level ($p = 0.06$).

Energy intake between exercise groups was not significantly different after the LE preload ($p > 0.7$ between all groups). Following the HE preload the non exercise group had a significantly higher energy intake that the moderate exercisers ($p = 0.05$), and a higher intake than the high exercisers ($p = 0.1$). There was no significant difference in energy intake following the HE preload between the moderate and high exercise groups ($p = 0.5$). Differences in macronutrient intakes reflected those seen in energy intake.
Buffet test meal energy intake (mean ± SEM) following consumption of the LE (■) and HE (■) preloads. There was no significant difference in energy intake of the non exercise group (solid bars) between the LE and HE preloads ($p = 0.9$). The moderate exercise group (diagonal striped bars) showed a significantly lower energy intake following the HE preload ($p = 0.05$). The high exercise group (horizontal striped bars) also showed lower intakes after the HE preload ($p = 0.06$).

The differences in buffet test meal energy intakes were used to calculate the compensation within each subject group for the difference in preload energy $((\text{difference in preload energy} \div \text{difference in buffet test meal energy intake}) \times 100)$. The non exercise group were found to have a low level of compensation for the difference in preload energy intake at 7%. However the moderate and high exercise groups were shown to have good levels of compensation at 99% and 82% respectively.

### 8.4.2. Hunger ratings

Hunger ratings following preload consumption are illustrated in figure 8.2. There were no significant differences in hunger ratings following the high and low energy preload in the non exercise group ($p = 0.7$), moderate exercise group ($p = 0.5$) and high exercise group ($p = 0.2$). There was no difference in ratings after the buffet test
meal in any exercise group (p > 0.5 all groups). It was noted that the largest difference in hunger ratings occurred in the high exercise group (figure 8.3), and that ratings in this group were lower after the high energy preload. However there was no significant difference in the difference in hunger ratings between each exercise group (p > 0.2).

Figure 8.2 Hunger ratings following consumption of preloads containing 1008 kJ (LE) and 2513 kJ (HE) in subjects with low, moderate and high habitual exercise levels

Hunger ratings (mean ± SEM) following consumption of the LE (■) and HE (■) preloads. There was no significant difference in hunger ratings between preloads in the non exercise group (p = 0.7, solid lines), moderate exercise group (p = 0.5, dashed lines) and high exercise group (p = 0.2, dotted lines).
Chapter 8

Figure 8.3 Difference in hunger ratings between preloads containing 1008 kJ (LE) and 2513 kJ (HE) in subjects with low, moderate and high habitual exercise levels

![Graph showing the difference in hunger ratings between preloads containing 1008 kJ (LE) and 2513 kJ (HE) in subjects with low, moderate and high habitual exercise levels.](image)

Difference in hunger ratings (mean ± SEM) between the LE and HE preloads in the non exercise (○), moderate exercise (■) and high exercise (▲) groups. There were no significant differences between any group (p > 0.2).

8.4.3 Satiety ratings

Satiety ratings following the high and low energy preloads also showed there to be no differences in subjective ratings between preloads in the non exercise group (p = 0.6) and the moderate exercise group (p = 0.9). However satiety was found to be greater after the high energy preload in the high exercise group, and this difference approached significance (p = 0.07, figure 8.4). There was no difference in ratings after the buffet test meal in any exercise group (p > 0.3 all groups). The difference in satiety ratings between preloads was calculated (figure 8.5), with the greatest difference seen in the high exercise group. There was no significant difference between exercise groups in the difference in satiety ratings between preloads.
Satiety ratings (mean ± SEM) following consumption of the LE (■) and HE (■) preloads. There was no significant difference in satiety ratings between preloads in the non exercise group \((p = 0.6, \text{ solid lines})\) and the moderate exercise group \((p = 0.9, \text{ dashed lines})\). The difference in satiety ratings of the high exercise group approached significance \((p = 0.07, \text{ dotted lines})\).

Difference in satiety ratings (mean ± SEM) between the LE and HE preloads in the non exercise (○), moderate exercise (■) and high exercise (△) groups. There were no significant differences between any group \((p > 0.2)\).
8.5. Discussion

Exercise has recently been shown to result in a good compensatory response to differences in energy intakes (King et al., 1999), suggesting that exercise may improve the sensitivity of the appetite control system in its response to nutritional manipulations. Data from the current investigation support a role for exercise in improving the ability of human subjects to detect differences in energy intake, as differences in habitual exercise levels were shown to result in differences in food intake response. Following consumption of the high and low energy preloads, the non exercise group showed little compensatory response for the difference in preload energy in their subsequent food intake, with test meal energy intake following each preload differing by only 104 kJ (7% compensation for preload energy difference). Subjects with both moderate and high habitual exercise levels compensated for differences in preload energy by adjusting their subsequent food intake; in the moderate exercise group compensation reached almost 100%. An effect of habitual exercise level upon appetite control has not been previously investigated, however it has been suggested that the higher turnover of energy (energy flux) which occurs in individuals who participate in regular exercise may promote the successful regulation of energy intake, and may help explain why more active individuals often find it easier to maintain their body weight.

The almost complete compensation observed in the moderate exercise group was not seen in the high exercise group. Although the difference in compensation between these groups was small, 82% in the high exercise group as opposed to 99% in the moderate exercise group, these data imply that while food intake regulation may be improved at some levels of habitual exercise, dissociation in appetite responses could occur at higher energy levels. This would support the observation of a discordance in energy intake and energy expenditure in very inactive and very active rats, with moderately active animals exhibiting a tight control between the two (Mayer et al., 1954). However it was noted that in the present study although the high exercise group regularly undertook 4 or more exercise sessions per week, this level of energy expenditure may not be high enough to induce dissociation in appetite responses in humans, and to investigate this further a similar investigation could be undertaken
with elite athletes. Although this study was not designed to directly investigate the relationship between energy intake and energy expenditure, it can be speculated that if appetite regulation is improved by the regular turnover of energy associated with regular exercise, highly trained individuals or those who undertake very high levels of recreational exercise may actually imbalance appetite regulation through the frequent induction of an energy deficit. Similarly the poor appetite responses in sedentary individuals may occur due to a low flux of energy and the frequent occurrence of excess available energy.

Self rated appetite responses following each preload suggest that although food intake regulation was improved in subjects with higher habitual exercise levels, this was not directly related to differences in subjective feelings of hunger or satiety. The high exercise group exhibited the largest differences in hunger and satiety ratings between preloads, but did not show the largest difference in test meal intake. In addition the moderate exercise group showed a difference in appetite ratings which was similar to that of the non exercise group, although the moderate exercise group had the largest difference in test meal intake between preloads. Thus it appears that differences in appetite control between the non exercise and exercise groups in the current study manifest themselves primarily as differences in intake. It is therefore possible that exercise increases sensitivity to satiety signals rather than hunger signals, and that food intake is more readily controlled through the cessation of an eating episode. Such a mechanism of appetite regulation would agree with previous work suggesting that obese subjects are less sensitive than lean subjects to short term, post-ingestive satiety signals (Spiegel et al., 1989), and could help explain regular overconsumption in obese persons.

While the present study is unable to elucidate mechanisms which may be involved in the regulation of appetite through exercise, the role of lifestyle and cognitive differences between people who undertake regular exercise and those who do not must be considered as this represents a large potential influence over feeding behaviour. While use of the DEBQ in the current investigation enabled the subjects groups to be balanced in terms of their dietary restraint, emotionality and externality,
the role of other beliefs and cognitive differences was not assessed. As regular exercisers are more frequently aware of the contribution that their lifestyle makes to general health, it is possible that these individuals are more aware of their energy intake and consciously regulate food intake to prevent overconsumption. Although the use of covert preload energy manipulations aimed to minimise the influence of external processes in this investigation, future use of a cross over design, whereby individuals who are habitual non exercisers are prescribed an exercise regime, would enable the effect of exercise itself to be investigated. Thus appetite responses to manipulated preloads before and after an exercise intervention could be assessed.

Regular exercise has been related to the success of weight maintenance after a low calorie diet (Fogelholm et al., 1999), and together with the present study these data provide much support for advocating regular exercise as part of a weight loss programme. However further investigation of a role for habitual exercise in the ability to detect and compensate for differences in preload energy is necessary to determine the relative roles of cognitive differences and exercise level, and to elucidate potential mechanisms of action.
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9. General discussion

The investigations described in this thesis were designed primarily to assess the role of hormonal regulation in the control of food intake in man. During the course of these investigations a number of questions were raised regarding the methodology used in the investigation of appetite. This enabled peripheral investigations into additional factors which may influence appetite response, and may be of importance in the design of future research in this area.

9.1. Appetite

Investigations into the methodologies used in appetite research highlighted the extent to which between- and within-subject variations in self rated appetite and food intake may mask differences in appetite responses to manipulated preloads. As discussed in chapter 3, use of subjects familiar with the self-rating techniques used may help reduce this variability. In addition these data provide a basis for the power calculations needed when determining the number of subjects to use in a study, and also illustrate the benefit of using larger numbers of subjects for appetite investigations. To also reduce the variability in ad libitum test meal intake, use of a more bland, single food test meal was investigated (chapter 3 section 3.4). The difference in food intake following high and low energy preload was shown to increase with the use of a single food test meal compared to a mixed food test meal. Thus this approach may help reduce the variation in test meal intake which could mask differences in appetite response to manipulated preloads, and unless an investigation aims to investigate differences in macronutrient intake and selection following preload manipulations, use of a single food test meal is advocated.

Further evidence of differences in appetite which may arise through different methodologies came from a comparison of the appetite responses to liquid and solid preloads. In the current investigations a liquid preload was used as the EIE method of
assessing gastric emptying was unable to measure emptying rate of a solid preload. As relatively small differences in appetite responses between preload energy manipulations were observed, it was possible that the use of liquid preloads had negative effects upon appetite assessment; possibly as they may be perceived as less satisfying than a solid meal, which would normally be consumed. The results of this investigation raised interesting questions as to the relative role of pre- and post-absorptive factors in the satiety cascade, as the high energy liquid preload was found to be more satiating that its solid counterpart while the low energy liquid preload was less satiating than its solid counterpart. Although these data cannot be used to elucidate potential differences in the satiety mechanisms following liquid and solid preloads, it is speculated that readily absorbed nutrients such as those in the high energy liquid preload may have a greater suppressive effect on short term energy intake than nutrients which would be absorbed more slowly, i.e. those from the solid preload. These studies did not investigate any prolonged differences in subsequent food intake between the liquid and solid preloads, and it is possible that once an equivalent amount of nutrient has been absorbed a similar level of satiety and food intake would be observed. These data highlight differences which may arise when comparing appetite responses between studies which have used different types of preloads. In addition these data also lead to questions regarding the relative roles of nutrient absorption and gastric fullness in the control of food intake, as these are areas where differences in appetite following liquid and solid preloads may arise (Sepple & Read, 1989). The importance of gastric fullness in the mediation of satiety was also illustrated during the infusion of loxiglumide described in chapter 7 (discussed later in this chapter), and data suggest that while hormonal signals are of great importance in the regulation of food intake, some degree of gastric distension is necessary for the mediation of satiety signals.

The appetite responses to graded increases in the amount of energy consumed as fat and carbohydrate have provided evidence that individuals are able to detect differences in energy intake, supporting previous observations (Pliner, 1973; de Graaf et al., 1992). However the compensation for manipulated preloads seen in subsequent energy intake from a test meal rarely matched the difference in preload energy, and
implies that previous energy intake exerts a relatively loose control over energy intake during the next eating episode. Investigations manipulating energy intake through both carbohydrate and fat (chapters 4 and 5 respectively) generally showed subjects to decrease subsequent short term food intake as preload energy increased. Thus it appears that total energy intake, rather than fat or carbohydrate intake, may be the primary determinant of appetite. This is contrary to published observations of a poorer regulation of appetite with only a high fat intake (Lawton et al., 1993; Green et al., 1994), and would appear to question why passive overconsumption is reported to occur only with a high fat diet (Blundell & Tremblay, 1995). As fat is the most energy dense macronutrient, passive overconsumption with a high fat diet probably occurs due to nutrient density rather than as a direct effect of dietary fat upon appetite. In our investigations the high fat and high carbohydrate meals provided the same energy density per ml of preload ingested, and this may account for the similarity in food intake response seen between these sets of manipulated preloads. Thus these data provide evidence that overconsumption with fat is more likely to occur due to the energy density of the nutrient, rather than the effects on appetite of fat per se.

Although a graded response was seen in mean test meal energy intake between each preload manipulation, the difference in intake most closely matched the preload energy manipulation between the high and low energy preloads. Thus it appears that subjects were better able to detect and respond to larger preload energy differences. This supports findings of previous work where good compensation was observed with differences in preload energy intake of 1672 kJ (Pliner, 1973), and this was similar to the difference between the high and low energy preloads in the current investigations. Smaller preload energy differences of 819 kJ (Wooley et al., 1972) have been shown to result in poor compensation for energy intake at a subsequent test meal, and this difference is similar to the energy differences between the low and medium, and medium and high energy preloads in the current investigations. Although these data imply that subjects were better able to detect larger preload energy differences, the variation in test meal energy intakes that may occur should also be considered when interpreting these data. It was noted in chapter 3, section 3.2 that within subject variation in energy intake following an identical preload on three separate test
occasions ranged between 7.2% and 25.8% (327 kJ to 1171 kJ). Thus the fluctuation in *ad libitum* test meal energy intake may mask differences in appetite response, particularly if the preload energy manipulation is less than the above variation in test meal energy intake. These observations therefore provide some explanation as to why better compensation was seen between the lowest and highest preload energy manipulations, and in future appetite investigations the relationship between preload energy manipulations and the fluctuation in test meal intake should be considered. As discussed in chapter 3, section 3.4, the use of a larger number of subjects should also be implemented to increase the statistical power of the experimental design and enable the detection of smaller differences in *ad libitum* test meal intakes.

**9.2. Hormonal responses and gastric emptying rate**

The differences in hormonal responses to each manipulated preload (chapters 4 and 5) were consistent with a link between hormonal and metabolite responses and appetite. However direct correlations between hormone and metabolite responses and appetite were not valid due to the small number of subjects used. In addition there is difficulty in inferring any cause and effect from an ingestive process which inevitably causes a simultaneous change in many circulating post-prandial factors as feelings of hunger and satiety are altered. Levels of the hormones and metabolites assessed generally increased (with the exception of NEFA) as preload energy content increased. In addition satiety levels were generally higher and food intake generally lower following consumption of the high energy preload in both the fat and carbohydrate manipulation studies. Comparison of the glucose responses to preload fat and carbohydrate manipulations do not support a consistent role for glucose in the regulation of satiety, although venous glucose was measured in the current investigations and future assessments of arterialised glucose may provide further elucidation of the role of glucose in appetite regulation. It is more probable that glucose is involved in the mediation of carbohydrate induced satiety through a glucostatic based mechanism which involves the actions of insulin and GLP-1. The higher insulin concentrations observed after the high carbohydrate preload, in conjunction with higher satiety ratings, would also be consistent with a glucostatic
mechanism of appetite control. In addition the elevated GLP-1 levels which accompany the glucose and insulin response may also contribute to the regulation of appetite. As differences in the magnitude of the insulin response were not apparent between preloads of different fat content, and glucose exhibited a much smaller difference between preloads, it is probable that different processes are involved in fat and carbohydrate mediated satiety. Following ingestion of the high fat preload CCK was found to be elevated, accompanying greater satiety and a lower food intake. Thus it is possible that CCK regulated the appetite response to fat ingestion, while GLP-1 would regulate the response to carbohydrate ingestion. As there were small differences in glucose after fat ingestion this metabolite could to some extent be implicated in the satiety mechanism.

Although the exact mechanisms through which glucose, insulin, GLP-1 and CCK may act upon satiety cannot be determined from these data, it seems most likely that these factors would interact to regulate appetite responses. Additional evidence of a synergistic role for glucose and GLP-1 in the regulation of appetite was provided by the GLP-1 infusion study, discussed below, supporting a role for GLP-1 in a glucostatic mechanism of appetite regulation.

9.2.1. GLP-1 and CCK

Investigations of the roles of GLP-1 and CCK in appetite have suggested that these two hormones may compliment each other in their regulation of gastric emptying and satiety. CCK is released following fat ingestion, and may be the primary hormonal regulator of satiety and gastric emptying in this instance. GLP-1 secretion was found to be consistently higher following intakes of the high energy preload, and peak levels were higher following carbohydrate loads compared to isoenergetic fat loads. Thus GLP-1 may act as the primary regulator of appetite and gastric emptying following carbohydrate ingestion. This hypothesis would therefore provide a physiological rationale for the secretion of higher CCK and lower GLP-1 after fat ingestion, and lower CCK / higher GLP-1 following carbohydrate ingestion.
Data from the infusion of GLP-1 did not provide significant evidence of a major role for GLP-1 in the regulation of human appetite. However this data should be interpreted in relation to other research in this area which has shown a positive effect of peripheral GLP-1 administration upon food intake. Differences between the current GLP-1 infusion study and other published work have been discussed previously (chapter 6, section 6.5), and it is possible that circulating glucose concentrations are important in the regulation of appetite by GLP-1. Thus GLP-1 may be a hormonal regulator which acts within a glucostatic mechanism of appetite regulation. Evidence of the stimulation of glucose oxidation by GLP-1 has been reported (Villanueva-Penacarrillo et al., 1994), and supports the hypothesis of an interaction between GLP-1 and glucose. In terms of gastric emptying the effects of GLP-1 upon emptying rate seen in our investigation supports previous findings (Willms et al., 1996), and it is possible that any satiety effects of GLP-1 are mediated through its actions on gastric emptying. This implies that gastric distension is an important factor in GLP-1 mediated satiety, and as positive relationships between gastric distension and appetite have been previously reported (Sepple & Read, 1989; Bergmann et al., 1992; Carbonnel et al., 1994) this mechanism may be the primary determinant of GLP-1 mediated satiety. The current investigations demonstrated lower hunger ratings following test meal consumption with GLP-1 infusion, and this was accompanied by a slower gastric emptying rate. Thus delayed emptying and consequently a greater level of gastric distension could have contributed to lower hunger ratings at this time.

While our study does not support a primary role for GLP-1 in the regulation of appetite, infusion of the CCKA receptor antagonist loxiglumide showed a clear effect on food intake compared to a saline control (chapter 7). In addition the response of increased food intake during CCKA receptor blockade occurred in spite of elevated GLP-1 levels, providing evidence that the influence of CCK is able to override that of GLP-1 in food intake control. Again the role of gastric emptying is likely to be important as a modulator of CCK mediated satiety. Previous work suggests that gastric distension is an important factor for a positive effect of CCK upon appetite (Muurahainen et al., 1991; Melton et al., 1992; Ballinger et al., 1995). Data from our
studies support this hypothesis, as a faster rate of gastric emptying during loxiglumide infusion was accompanied by lower satiety ratings and higher food intake control compared to the saline control. Furthermore the percentage of the preload remaining in the stomach 60 minutes into loxiglumide or saline infusion was related to the energy consumed from the pasta test meal at this time. Thus it is possible there is a direct relationship between gastric distension and food intake in humans.

Use of the epigastric impedance epigastrography technique in these investigations has since been shown to be an inaccurate measure of actual gastric emptying times in comparison to the scintigraphy method (Giouvanoudi et al., 2000). Therefore although the EIE technique may still be used to provide information on relative rates of gastric emptying T50's calculated from this method are actual half emptying times. Thus although the calculation of the percentage of the meal remaining in the stomach may not be quantitatively accurate, the relative amount of the gastric load between infusions and preloads (less or more) still holds. Although GLP-1 and CCK have both been shown to relate to gastric emptying in previous work (Willms et al., 1996; Scarpignato et al., 1996), direct correlations between the circulating concentrations of hormones and gastric emptying could not be made in the current investigations, due in part to the EIE technique. It is probable that the hormonal regulation of gastric emptying operates through a feedback mechanism, supporting a complimentary role for GLP-1 and CCK in gastric emptying regulation. During the first 60 minutes of loxiglumide infusion elevated GLP-1 levels were observed, and as the inhibitory effect of CCK on gastric emptying was blocked by loxiglumide, the secretion of GLP-1 may have been promoted to provide secondary regulation of gastric emptying after fat ingestion. Although there were no direct correlations between either GLP-1 and CCK and gastric emptying during the infusion studies, the alterations in gastric emptying rate observed during both infusion are supportive of the roles of CCK and GLP-1 gastric emptying regulation.
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9.3. The relative importance of exercise in the regulation of appetite

The current research has primarily investigated the roles of hormone secretion and gastric emptying in the regulation of appetite responses. However the preliminary data described in chapter 8 investigating the role of differences in habitual exercise levels in appetite regulation suggest that lifestyle considerations may be extremely important in determining an individual's ability to respond to appetite cues and regulate food intake. These data suggest that participation in regular exercise improves the ability of a subject to compensate for preload energy manipulations in their subsequent food intake. Thus exercise has the potential to aid appetite control and weight maintenance beyond the benefits of increased energy expenditure alone. Additional research is required to determine whether improved appetite regulation can be developed in those people who do not exercise regularly, and to address the mechanisms through which exercise may act to improve appetite regulation. Thus although our research has shown that hormonal response is important in the regulation of food intake and satiety, exercise represents an important focus for research in terms of how an individual may act to improve control over appetite.

9.4. Conclusions

Data from the current investigations provide evidence for some hormonal regulation of satiety and of food intake. Our infusion studies suggested that CCK has a greater effect upon appetite responses than GLP-1, and is likely to be more important than GLP-1 in the regulation of food intake. However a role for GLP-1 in appetite regulation should not be ruled out by the current data, and further research is needed to elucidate the involvement of glucose in the GLP-1 mediated appetite response. It is of course important to put this research into the context of appetite regulation in the free living individual. As CCK and GLP-1 have a number of other physiological functions it is possible that their primary role is not appetite regulation, and thus their effects on appetite may not be immediately apparent in the free living subject. As described above gastric distension plays an important role in the regulation of food intake, and the satiating actions of GLP-1 and CCK may be reliant upon gastric distension. Thus it is clear that the regulation of food intake is through a
multifactorial system, and it is important to continue research into each factor of appetite control. The continual elucidation of mechanisms which may help promote satiety are of importance in a society where increasing obesity and concern over energy intake are well recognised. However at the level of the individual it is also important to explore additional factors which may help in the regulation of appetite and body weight, and as regular recreational exercise has the potential to improve appetite regulation this area would benefit from additional research. A combined physiological and psychological approach to appetite research is therefore vital to maintain a perspective of the application of such research to everyday life.

9.5. Recommendations for future work

Data from these investigations have highlighted a number of other approaches which may be used to further elucidate the role of hormone responses, and the importance of habitual exercise, in food intake regulation.

a) In view of the discrepancy between our study and recent research, infusion of exogenous GLP-1 at a level previously reported to have appetite effects while using a glucose clamping technique to maintain glucose as basal levels would elucidate the role of glucose in the GLP-1 mediated appetite response. Using a saline control and comparing to an infusion of GLP-1 alone, this approach would determine if the drop in glucose observed in our infusion study had any effects upon appetite.

b) To determine the relative effects of GLP-1 and CCK upon appetite, loxiglumide plus exendin (9-39), the GLP-1 receptor antagonist, could be infused against each other and together against a saline control. Simultaneous assessment of gastric emptying would also elucidate the relative role of each of these hormones in the regulation of gastric emptying rate.

c) To investigate whether changes in habitual exercise level improve appetite response to manipulated preloads an exercise intervention could be implemented.
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Recruiting people who do not participate in regular exercise and increasing their exercise level to 3 session per week of cardiovascular exercise provides a basis for the experimental design. The appetite response to manipulated preloads could then be assessed at the beginning and end of the intervention period, along with physiological and psychological assessments to determine any quantifiable changes which may contribute to improved appetite regulation. To determine the length of time needed for increases in exercise to have any effect upon appetite, preload studies could also be implemented at intervals during the intervention.
Publications
Publications

Abstracts


[Oral presentation at Nutrition Society summer meeting, 1998]


[Oral presentation at Nutrition Society summer meeting, 1998]


[Oral presentation at Nutrition Society summer meeting, 1998]


[Oral presentation at Regulatory Peptides conference, Sept. 1998]

[Poster presentation at Regulatory Peptides conference, Sept. 1998]

[Poster presentation at Nutrition Society summer meeting, 1999]

[Poster presentation at Nutrition Society summer meeting, 1999]

[Poster presentation at Nutrition Society summer meeting, 1999]

[Oral presentation at Nutrition Society summer meeting, 1999]

[Oral presentation at joint SEN/NS congress, Sept. 1999]
Papers


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References


References


Appendices
Appendices

Appendix I  The Dutch Eating Behaviour Questionnaire

Please answer each question by circling the most appropriate alternative.

1) If you have put on weight, do you eat less than you usually do?
   Never  Seldom  Sometimes  Often  Very often  Not relevant

2) Do you have a desire to eat when you are irritated?
   Never  Seldom  Sometimes  Often  Very often  Not relevant

3) If food tastes good to you, do you eat more than usual?
   Never  Seldom  Sometimes  Often  Very often

4) Do you try to eat less at mealtimes than you would like to eat?
   Never  Seldom  Sometimes  Often  Very often

5) Do you have a desire to eat when you have nothing to do?
   Never  Seldom  Sometimes  Often  Very often  Not relevant

6) Do you have a desire to eat when you are depressed or discouraged?
   Never  Seldom  Sometimes  Often  Very often  Not relevant

7) If food looks and smells good, do you eat more than usual?
   Never  Seldom  Sometimes  Often  Very often

8) How often do you refuse food or drink offered because you are concerned about your weight?
   Never  Seldom  Sometimes  Often  Very often

9) Do you have a desire to eat when you are lonely?
   Never  Seldom  Sometimes  Often  Very often  Not relevant

10) If you smell something delicious, do you have a desire to eat it?
    Never  Seldom  Sometimes  Often  Very often

11) Do you watch exactly what you eat?
    Never  Seldom  Sometimes  Often  Very often

12) Do you have a desire to eat when someone lets you down?
    Never  Seldom  Sometimes  Often  Very often  Not relevant

13) If you have something delicious to eat, do you eat it straight away?
    Never  Seldom  Sometimes  Often  Very often

14) Do you deliberately eat foods that are slimming?
    Never  Seldom  Sometimes  Often  Very often

15) Do you have a desire to eat when you are cross?
### Appendices

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<tr>
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<th>Question</th>
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<td>Do you have a desire to eat when you are expecting something unpleasant to happen?</td>
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<td>If you walk past the baker, do you have a desire to buy something delicious?</td>
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<td>When you have eaten too much, do you eat less than usual the following days?</td>
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<td>Do you have a desire to eat when you are anxious, worried or tense?</td>
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<td>20</td>
<td>If you walk past a snackbar or cafe, do you have a desire to buy something delicious?</td>
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<td>Do you deliberately eat less in order not to become heavier?</td>
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<td>Do you have a desire to eat when things are going against you, or when things have gone wrong?</td>
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<td>If you see others eating, do you also have a desire to eat?</td>
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<td>How often do you try not to eat between meals because you are watching your weight?</td>
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<td>Do you have a desire to eat when you are frightened?</td>
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<td>26</td>
<td>Can you resist eating delicious foods?</td>
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<td>27</td>
<td>How often in the evening do you try not to eat because you are watching your weight?</td>
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<td>Do you have a desire to eat when you are disappointed?</td>
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<td>29</td>
<td>Do you eat more than usual when you see others eating?</td>
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<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Do you take your weight into account when you eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Do you have a desire to eat when you are emotionally upset?</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td></td>
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<tr>
<td>32</td>
<td>When preparing a meal, are you inclined to eat something?</td>
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<td></td>
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<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td></td>
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<tr>
<td>33</td>
<td>Do you have a desire to eat when you are bored or restless?</td>
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<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
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Appendix II  VAS rating scales used for the assessment of hunger and satiety

How HUNGRY are you?

NOT AT ALL HUNGRY | AS HUNGRY AS I HAVE EVER FELT

How FULL do you feel?

NOT AT ALL FULL | AS FULL AS I HAVE EVER FELT

How THIRSTY do you feel?

NOT AT ALL THIRSTY | AS THIRSTY AS I HAVE EVER FELT
Appendix III  Food preference checklist (FPC) used for the assessment of hunger

____ A medium sized roast chicken breast
____ A currant bun
____ A small pancake (sweet)
____ A medium sized peach
____ A small packet of roasted, salted peanuts
____ A large grilled cod fillet (no batter)
____ A large tomato
____ A small baked potato
____ A small slice of rich cheesecake
____ A small slice of apple pie
____ A small gammon steak
____ A medium portion of boiled rice
____ A slice of melon
____ A plain omelette (2 eggs)
____ Fruit yogurt (thick & creamy type, 150g pot)
____ A large bread roll
____ A large portion of prawns
____ A medium chunk of Cheddar cheese
____ A medium sized apple
____ A plain croissant
____ A large portion of tuna (canned in brine)
____ A medium sized bowl of cornflakes with semi-skimmed milk and sugar
____ A small cream-filled chocolate eclair
____ A large raw carrot (peeled)
____ A cheese scone
____ Two slices of salami
____ Grilled, lean beef rump steak (5oz)
____ A green salad (no dressing)
____ A large banana
____ Three grilled fish fingers (coated in breadcrumbs)
Appendix IV  CCK assay protocol provided by Mark Jordinson, RPMS, Hammersmith.

Reagents

Standards: Obtained from RPMS, Hammersmith and prepared by logarithmic dilution as described in chapter 2, section 2.2.3.13

Assay Diluent: As described in chapter 2, materials and methods.

\(^{125}\text{I} \) CCK8S radiolabel: Purchased from Amersham Pharacia Biotech. Diluted to 1000 cpm / 400 μl.

Antisera: Obtained from RPMS, Hammersmith.

Dextran coated charcoal: Mix 6g Norit PN5 charcoal with 300 mg dextran in 100 ml assay buffer. Solution should be made up and mixed at 4°C for 30 minutes prior to using in the assay.

Day One

Standard tubes
Add volumes of standard and assay diluent as described in table 2.5, chapter 2
Also add 400 μl label (at 1000 cpm / 400 μl), and 100 μl antisera

Sample tubes
Directly to the dried sample in an LP4 tube add the following:
500 μl assay diluent
400 μl label (at 1000 cpm / 400 μl)
100 μl antisera

Vortex and incubate all tubes for 3 days at 4°C.

Day Four

Add 100 μl of dextran coated charcoal to all assay tubes.
Centrifuge at 2000 rpm for 10 minutes at 4°C.
Pour off supernatant and collect for counting.

Count bound (supernatant) and free (pellet) fraction on gamma counter. Calculate B/F ratio to plot standard curve. Read B/F ratio from standard curve and multiply by 2 for unknown samples.
Appendix V  Standard lunch meals provided for subjects before the VAS validation study (chapter 3) and the GLP-1 infusion study (chapter 6).

Standard lunchtime meal to be eaten at 12 noon. Subjects to choose one meal from the options below. The same meal was served on each test occasion.

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<tr>
<th>Item</th>
<th>Energy (kJ)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Fibre (g)</th>
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<tbody>
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<td>Chicken and pasta bake</td>
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</tr>
<tr>
<td>Cheese and onion bake</td>
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<td>14.5</td>
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<td>40.5</td>
<td>1.2</td>
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<tr>
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Values given are per meal
Appendix VI  Coefficients of variation for hunger ratings following consumption of a 1756kJ preload on three separate occasions.

**Within subject CVs**

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**Between subject CVs**

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Appendices

Appendix VII Coefficients of variation for satiety ratings following consumption of a 1756kJ preload on three separate occasions.

**Within subject CVs**

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**Between subject CVs**

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**Within subject CV in ad libitum test meal intake**

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**Between subject CV in ad libitum test meal intake**

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Appendix VIII  Standard evening meal provided for subjects to consume the evening before the carbohydrate manipulation preload study (chapter 4).

Standard evening meal to be eaten before 10.30pm on the evening before each test day. Subjects to choose one meal from the options below. The same meal was served on each test occasion.

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Values given are per meal.

344
Appendix IX  Habitual dietary intakes of subjects participating in the carbohydrate manipulation preload study.

**Habitual daily intake**

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</table>

**Habitual breakfast intake**

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<th>En (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>Pro (g)</th>
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</thead>
<tbody>
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<td>11</td>
<td>102.2</td>
<td>12.6</td>
</tr>
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<td>32.1</td>
<td>13.2</td>
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<td>19.7</td>
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</tr>
<tr>
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<td>71</td>
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<td>52.3</td>
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<td>5.8</td>
<td>21.6</td>
<td>3.6</td>
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<td>9</td>
<td>322</td>
<td>10.5</td>
<td>44.9</td>
<td>9.8</td>
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</table>

**Habitual lunch intake**

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<th>CHO (g)</th>
<th>Pro (g)</th>
</tr>
</thead>
<tbody>
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<td>21.3</td>
<td>95.3</td>
<td>16.7</td>
</tr>
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<td>2</td>
<td>775</td>
<td>34.6</td>
<td>86.8</td>
<td>34.7</td>
</tr>
<tr>
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<td>967</td>
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<td>40.5</td>
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<td>696</td>
<td>36.9</td>
<td>62.4</td>
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<td>1033</td>
<td>26.6</td>
<td>174.7</td>
<td>34.9</td>
</tr>
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<td>681</td>
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<td>68.8</td>
<td>22.3</td>
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<td>963</td>
<td>38.4</td>
<td>133</td>
<td>26.5</td>
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<td>713</td>
<td>33.8</td>
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<td>30.9</td>
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Appendices

Appendix X  Rating scales used to assess the occurrence of any adverse events during infusions of GLP-1 and saline (chapter 6).

1. Are you experiencing any stomach ache?

<table>
<thead>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Faint</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Very severe</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

2. Degree of stomach fullness?

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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
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<tbody>
<tr>
<td>Extremely empty</td>
<td>Very empty</td>
<td>Empty</td>
<td>Full</td>
<td>Very full</td>
<td>Extremely full</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

3. How comfortable do you feel?

<table>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely uncomfortable</td>
<td>Very uncomfortable</td>
<td>Uncomfortable</td>
<td>Comfortable</td>
<td>Very comfortable</td>
<td>Extremely comfortable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Are you experiencing any dizziness?

<table>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Faint</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Very severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. How pleasant do you consider your surrounding?

<table>
<thead>
<tr>
<th>0</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely unpleasant</td>
<td>Very unpleasant</td>
<td>Unpleasant</td>
<td>Pleasant</td>
<td>Very pleasant</td>
<td>Extremely pleasant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Are you experiencing any feelings of sickness or nausea?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Faint</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Very severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendices

Appendix XI  Instructions supplied by Massimo D'Amato, Rotta Research Laboratories, Milan for the preparation of a loxiglumide infusion solution from the sodium salt provided. St Georges Hospital Pharmacy used these instructions to prepare the infusions.

CR 1505 0.5% solution

Preparation of one litre of 0.5% solution of CR 1505:

#1. Dissolve 5 gr of CR 1505 in 11.70 ml of NaOH 1N (the stoichiometrical amount is 10.84 ml, but a slight excess is used in order to facilitate the dissolution of the active drug), then dilute with 700 ml of WATER for injectables.

#2. Dissolve, in another glass beaker, 9 gr of SODIUM CHLORIDE in 200 ml of WATER for injectables and add, under stirring, this solution to that mentioned in the step #1.

#3. Check the pH value of the solution and correct it very carefully to a value of 9.40 - 9.30 by aid of 1N HCl, then bring to the volume of 1000 ml with WATER for injectables.

#4. Filter through a 0.20 microns membrane filter (mixed Cellulose Esthers).

#5. Sterilize at 121°C for 20 minutes.

#6. The final value of pH must lie between 8.10 and 8.50.

Note: if kept in well closed containers, the above solution is stable for at least 48 hours.
Appendix XII  Rating scales used to assess the occurrence of any adverse events during infusions of loxiglumide and saline.

Please answer all of the following questions by placing a vertical mark through the line for each question. Mark the lines according to how you feel at this moment. Regard the end of the lines as indicating the most extreme sensation you have ever felt.

<table>
<thead>
<tr>
<th>Condition</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Stomachache</td>
<td>Not at all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Nausea</td>
<td>Not at all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Heart pounding</td>
<td>Not at all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Feeling flushed</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Faint</td>
<td>Not at all</td>
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<td></td>
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<td></td>
<td>Extremely</td>
</tr>
<tr>
<td>Jittery/shakey</td>
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<td></td>
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<td></td>
<td>Extremely</td>
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<tr>
<td>Light headed</td>
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<td>Extremely</td>
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</tbody>
</table>

How comfortable do you feel?

How pleasant do you consider your surrounding?