GLP-1 AND THE REGULATION OF CARBOHYDRATE INTAKE IN THE RAT

BY

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Master of Science, 2000
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ABSTRACT

The hypothesis that Glucagon-Like Peptide 1 (GLP-1) is a regulator of carbohydrate intake in rats was investigated. The effect of the GLP-1 antagonist, Exendin 9-39, on food intake suppression after carbohydrate, protein or fat preloads and on selection from high protein and high carbohydrate diets was measured. The effect of GLP-1 agonist, Exendin 4, on diet selection was also investigated. Exendin 9-39 significantly enhanced food intake suppression occurring after glucose, but not after corn oil or albumin preloads. When rats selected from high protein and high carbohydrate diets, administration of Exendin 9-39 resulted in a selective decrease in intake of the high carbohydrate diet. In contrast, Exendin 4 caused a selective decrease in intake of the high protein diet. It is concluded that GLP-1 plays a role in macronutrient selection but the results do not support the hypothesis that GLP-1 is involved in carbohydrate intake regulation in the rat.
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LIST OF ABBREVIATIONS

CCK - Cholecystokinin
CRH - Corticotropin Releasing Hormone
DPPIV - Dipeptidyl Peptidase IV
E9-39 - Exendin 9-39
E4 - Exendin 4
EDTA - Ethylene Diamine Tetra Acetate
GHRH - Growth Hormone Releasing Hormone
GIP - Glucose Dependent Insulinotropic Peptide
GLP-1 - Glucagon-Like Peptide-1
GRPP - Glicentin Related PolyPeptide
IP - IntraPeritoneal
ICV - IntraCerebroVentricular
LiCl - Lithium Chloride
MSG - MonoSodium Glutamate
MSH - desacetyl Melanin Stimulating Hormone
NEFA - Non Esterified Fatty Acids
NPY - Neuropeptide Y
NTS - Nucleus of the Tractus Solitarius
PGDP - ProGlucagon Derived Peptides
PVN - ParaVentricular Nucleus
RIA - RadioImmunoAssay
SST - Somatostatin
CHAPTER 1

INTRODUCTION
1. INTRODUCTION

In Canada, obesity is estimated to occur in more than 30% of the population and is increasing every year. Obesity is associated with an increased risk of many diseases including diabetes, hypertension, cancer and cardiovascular disease. Understanding the fundamental mechanisms governing food intake regulation is essential in reducing the prevalence of obesity and its associated health risks.

A number of physiological and psychological signals govern the process of food intake regulation. Both animals and humans are able to adjust their food intake to meet energy requirements. Much research in food intake regulation focuses on the role of the three macronutrients—protein, carbohydrate and fat—in the determination of energy balance.

Carbohydrates form a large percentage of the daily energy intake of North Americans. Therefore, it is of particular importance that the regulation of carbohydrate intake be understood. One aspect of the regulatory mechanism of carbohydrate intake lies in the peptides that regulate food intake. Of primary importance to this thesis research is the observation that peptides exert their effects on food intake in a very macronutrient specific fashion. For example, cholecystokinin mediates the food intake suppression induced by albumin protein, but not by fat or carbohydrate preloads (Trigazis et al 1997). Conversely, neuropeptide Y (NPY) enhances the intake of carbohydrate by the rat.
Glucagon-Like Peptide 1 (GLP-1) release is stimulated by the ingestion of carbohydrates (Ritzel et al. 1997) and certain fatty acids (Brubaker et al. 1995), whereas proteins and amino acids have little or no effect on GLP-1 release (Herrmann et al. 1995).

Contact of carbohydrate with the intestinal mucosa appears to be fundamental to the release of GLP-1 and to the suppression of food intake caused by carbohydrate. In the central nervous system, GLP-1 interacts with NPY and reduces its ability to enhance food intake (Tritos et al. 1998; Turton et al. 1996). Because carbohydrates stimulate GLP-1 secretion and GLP-1 suppresses food intake (Turton et al. 1996), the hypothesis of the present research is that GLP-1 is a component of a control system regulating carbohydrate intake.

Therefore, the present study measured food intake and macronutrient selection of rats after the GLP-1 receptor was either blocked or stimulated in conjunction with preloads of carbohydrate, fat or protein.
CHAPTER 2

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. Introduction

Obesity is a prevalent health problem in Canada. Roughly 31% of the adult population is classified as overweight with a body mass index >27 (Canadian Heart Health Survey 1995). Health risks associated with being overweight include stroke, ischemic heart disease, and diabetes among others. Unfortunately, efforts to lose weight are often unsuccessful with 90-95% of those who lose weight regaining it very quickly (Rosenbaum 1997). Energy balance (intake vs. output) is regulated through complex mechanisms that are not yet fully understood. Obesity results when energy intake exceeds output; small imbalances in intake vs. output can have a large cumulative effect. Therefore, it is critical that the mechanisms underlying the control of appetite, and subsequently food intake be fully understood.

Numerous signals are used by the brain in the regulation of body energy intake, output and, storage. Although cultural and social conventions influence food intake, the focus of this work is on signals arising from ingestion, digestion, and absorption such as metabolic signals to the brain regarding satiety and hunger. The brain integrates these peripheral signals, balancing output and storage of nutrients with food intake (Anderson 1994).

Of particular interest are signals originating from the gut and their influence on satiety. The gut is the interface between the external environment and the internal milieu; it is only logical that the brain receives signals from the first line of contact of food with the body. Prior to absorption, carbohydrate, protein, and fat are in contact with the intestinal lumen and ultimately relay many signals to the brain. Pre-
absorptively, peptides are released from the gut in response to contact with nutrients. The investigation of the effect on satiety of glucagon-like peptide 1 (GLP-1) is proposed in the following work. The objective of this research is to investigate the effects of GLP-1 on food intake and to determine if these effects are carbohydrate specific.

2.2 Appetite Control

Overall energy intake is generally well regulated in normal rats. Rats increase feeding over extended periods of time when energy requirements increase due to a cold environment or adjust feeding volume to the caloric density of the diet in order meet energy requirements (Kraly et al 1976: Sellers et al 1954). Post-absorptively, carbohydrate and protein are rapidly metabolised whereas fat is not. The rapid metabolism of carbohydrate and protein exert a strong effect on appetite (Read 1994). Both the quantity and the composition of food ingested are influenced by these signals (Anderson 1994). The short-term response to energy intake is influenced by the macronutrient composition of food. Carbohydrates given as preload meals suppress later food intake in amounts equivalent to their energy value (Anderson 1995). In general, carbohydrates are more satiating than fat but less satiating than protein (Blundell et al 1994; Trigazis 1997).

In addition to total caloric intake, intake of macronutrients is regulated in the rat. For example, when allowed to choose between two food cups varying greatly in protein content, rats consume a constant percent of protein in their diet (Anderson 1979). Carbohydrate content of the rat diet is also controlled by the rat’s food selection (Geliebter 1979), but fat content is not as well controlled (Geliebter 1979). These observations that rats are able to make choices to control the macronutrient composition
of their diets suggests that the central nervous system receives messages from the ingestion of the individual macronutrients.

2.2.1 Carbohydrates and Food Intake

Rats demonstrate a level of control over carbohydrate intake even though their physiological requirement for carbohydrate is uncertain and thought to be quite low (National Research Council 1995). Several lines of evidence demonstrate that the rat senses the carbohydrate content of food and adjusts both caloric and macronutrient content of the diet accordingly. Rats given a preload of carbohydrate 30 minutes prior to feeding adjust subsequent food choice between a high and low carbohydrate diet to compensate for the macronutrient content of the preload (Li and Anderson 1982). The effect is seen rapidly, mostly within the first hour of feeding. However, the hyperphagic obese Zucker rat compensates more slowly and in a non-selective manner (Van Zeggeren and Li 1990), suggesting aberrations in short-term satiety and macronutrient selection relate to increased body weight.

The effect of carbohydrate preloads in modifying subsequent food intake of the rat is dependent on both dose and source (Trigazis 1997). Affinity of monomeric carbohydrates for the intestinal glucose transporter may explain variation in food intake suppression of carbohydrates. The monomeric glucose analogs, 3-O-methylglucose and alpha methyl glucose are transported by the mucosal sodium/glucose transporter into the intestinal cells but are not metabolised (Landau et al 1962). Both of these analogs significantly reduce food intake (Meyer 1998). Therefore, affinity for the glucose transporter is an important factor in the food intake suppression induced by monomeric carbohydrates separate from their metabolism.
A number of physiological mechanisms have been postulated to account for the satiating effects of carbohydrates. These include signals to the brain arising from both pre-absorptive and post-absorptive actions of carbohydrate. Pre-absorptive signals include gastric emptying, vagal impulses, and the release of regulatory peptides. Of primary interest to this research is the pre-absorptive effects exerted on appetite by gut peptides known to be affected by carbohydrates.

2.2.2 Gut peptides and the control of food intake

Regulatory peptides are classified as "soluble, diffusable, extracellular messengers." Peptides control activities such as secretion of digestive juice, movement of luminal contents, growth of the gut, blood flow, and signalling to and from the central nervous system. Gut peptides are released from epithelial endocrine cells, neurons in the gut, and from other less specialised cells (Dockray 1994). The release of gut peptides is triggered by the presence of nutrients in the gastrointestinal tract.

Food intake is affected by peptides released from the gut. The importance of gut peptides in mediating the satiety of an intestinal infusion of glucose has been measured. Somatostatin (SST) is a substance that inhibits the release of many peptides. Administration of somatostatin abolishes the satiating effect of an intestinal infusion of glucose in human volunteers (Lavin et al 1996, 1998). Because the subjects were maintained on a glucose and insulin clamp, the authors ruled out any effects of these two variables on food intake and thereby isolated the importance of the gut peptides in the feeding response. Concurrent measurement of plasma levels of two gut peptides shows associations with appetite, as will be discussed in a later section.
The importance of gut signals and possibly gut peptides in food intake control is underlined by the effect of transposing intestinal segments in the rat. Transposing either 5 or 10-cm segments of the ileum to mid-duodenum or upper jejunum results in a substantial loss of weight even though total intestinal length is unchanged and absorption is not compromised. Because they eliminated the possibility of infection or bacterial toxins, the authors concluded that the weight loss was accounted for by early release of lower gut hormones from the transposed ileal segment by unabsorbed food (Koopmans and Sclafani 1981). GLP-1 is one gut peptide that is predominantly released from the ileum and is associated with decreases in food intake.

Other gut peptides such as cholecystokinin (CCK), bombesin/gastrin releasing peptide (BBS, GRP) (Geary 1982) and pancreatic glucagon mediate food intake suppression in a dose dependent manner by activating visceral afferent fibers of the vagus nerve (Smith 1992). Bombesin reduces food intake less potently than CCK and is thought to affect food intake by releasing CCK (Kulkosky et al 1982). CCK is found both in gastrointestinal and central nervous systems. Peripheral injections of CCK decrease food intake and produce satiety in a variety of species (Peikin et al 1989).

Clearly, gut peptides are important in food intake regulation.

2.2.3 Brain Peptides and Food Intake

A number of peptides synthesised in the brain have the capacity to regulate appetite. Galanin, Opioids, NPY, Growth Hormone Releasing Hormone (GHRH), and Melanin Concentrating Hormone (MCH) increase food intake when administered centrally (Bray 1992). Brain peptides known to decrease food intake are Anorectin, Corticotropin Releasing Hormone (CRH), Neurotensin and Cyclo-his-pro (derived from
thytropin-releasing hormone) (Arase et al 1987, 1988; Stanley et al 1983; Prasad et al 1989). The focus of this research is on the peptide GLP-1 that is produced in the brain and the gut (Drucker 1998).

2.2.4 Macronutrient Intake and Peptides

Although there is good evidence for the involvement of a large number of peptides in feeding mechanisms that either increase or decrease food intake, it would appear to be a highly redundant system unless some of the peptides are more specifically involved in the regulation of macronutrient specific feeding behavior. There is some evidence that carbohydrate intake is regulated by peptide hormones. NPY has been identified as a stimulator of carbohydrate intake in rats (Leibowitz, 1994). Because hypothalamic NPY synthesis decreases in the presence of high levels of insulin (Sipols et al 1995) it is affected, indirectly through insulin, by carbohydrate intake. Similarly, NPY activity is decreased by GLP-1. Administration of GLP-1 in the brain abolishes the orexigenic effects of NPY (Tritos et al 1998). This relationship between GLP-1 and NPY action suggests that GLP-1 may contribute to the control of carbohydrate intake. Intake of other macronutrients is also affected by various peptides. Galanin in the hypothalamus stimulates feeding and particularly fat intake (Leibowitz 1989). GHRH stimulates intake of protein in the early nocturnal period through a central mechanism involving the suprachiasmatic nucleus (Vaccarino et al 1994).

Peptides not only increase the intake of specific macronutrients, they also decrease food intake in a very nutrient specific way. CCKA receptor antagonists administered peripherally block the food intake suppression caused by albumin ingestion in the rat. Administering the CCKA antagonist concurrently with a gastric load of
albumin weakens the albumin induced food intake suppression. However, the antagonist to CCKA receptors does not affect amino acid, carbohydrate, or fat induced food intake suppression (Trigazis 1997).

Therefore, not only are certain peptides able to increase or decrease overall appetite, but are also able to influence appetite for specific nutrients. This research investigates the role of GLP-1 in carbohydrate induced food intake suppression.

2.3 Glucagon-like Peptide 1

GLP-1 has a number of physiological functions. In the following section an overview of GLP-1 synthesis, function and degradation is provided, followed by a discussion of the role of GLP-1 as a "gut-brain peptide" in appetite and food intake regulation (Campfield 1998; Turton et al 1996; Gutzwiller 1999).

2.3.1 Synthesis of GLP-1

The proglucagon gene gives rise to the proglucagon RNA transcript, which is processed similarly in the brain and intestine (Figure 2.1). GLP-1 is a 30 amino acid peptide chain (Figure 2.2), derived from the cleavage of proglucagon, a 160 amino acid chain. The cleavage of proglucagon differs between pancreas and intestine due to the predominance of different prohormone convertase enzymes in these tissues. In the pancreas, proglucagon cleavage by prohormone convertase 2 produces mainly glicentin-related polypeptide (GRPP), glucagon, and a major proglucagon fragment. A little GLP-1 is produced in the pancreas but not in the active form (Dockray and Walsh 1994), as prohormone convertase 2 is incapable of performing the cleavage necessary to truncate GLP-1 to its active form.
Figure 2.1 Posttranslational processing of proglucagon in pancreas, intestine, and brain.

The abbreviations used are: GRPP- glicentin related pancreatic polypeptide, IP-1- intervening peptide 1, MPGF- major proglucagon fragment, GLP-1- glucagon-like peptide 1, IP-2- intervening peptide 2, GLP-2- glucagon-like peptide 2.

Adapted from Kieffer et al 1999
GLP-1(7-36)  H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R#

Exendin 9-39


Glucagon  H S Q G T F T S D Y S K Y L D S R R A Q D F V Q W L M N T


# carboxyl-terminal amide

One letter notation for amino acids: A = Ala, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, Y = Tyr.

Figure 2.2 Amino acid sequences of GLP-1 and related peptides, the arrow indicates the sequence of Exendin 9-39.
In the intestine and brain prohormone convertase 3 (also known as prohormone convertase 1) predominates (Scopsi et al 1995) and is capable of processing the major proglucagon fragment with a major product being active truncated GLP-1 (Dhanvanteri et al 1996, Rouille et al 1997).

The main site of production of peripheral GLP-1 is in the L cell of the intestine (Drucker 1998). The L cell is exposed to both circulating humoral factors and luminal intestinal contents. Therefore, intestinal proglucagon derived peptides (PGDPs) are subject to both hormonal and nutritional factors. However, GLP-1 release does not occur in response to intravenously induced hyperglycaemia but seems to be dependent on contact of certain nutrients with the intestinal lumen. There is a rapid rise in GLP-1 after nutrient ingestion. This rise is curious due to the location of the L cell in the distal ileum of the gastrointestinal tract. In the rat, early GLP-1 secretion from the L cell is mediated by Glucose-dependent insulinotropic peptide (GIP). Intravenous infusion of GIP increases plasma concentration of the GLP-1 precursor molecule PGDP by two-fold (Roberge et al 1993). Although the process is complex, it is known that GLP-1 secretion is stimulated through a mechanism involving the enteric nervous system, the afferent and efferent vagus nerve and GIP (Rocca et al 1999).

In the brain, the hypothalamus has the highest concentration of PGDPs. The location of GLP-1 in the brain provides indirect evidence that it may play a role in central nervous system regulation of food intake. The thalamus-hypothalamus, pituitary, and medulla oblongata are rich in GLP-1 (Shimizu et al 1987). The highest concentrations of GLP-1 are located in the central hypothalamus, which is an area important in the regulation of feeding (Kreymann et al 1989). Preproglucagon mRNA
(from which the proglucagon protein is translated) is expressed in a single population of neurons in the caudal portion of the nucleus of the tractus solitarius (NTS). GLP-1 NTS neurons project to many targets, one of which is the paraventricular nucleus (PVN). The densest innervation by GLP-1 nerve fibers is in the hypothalamic PVN and dorsomedial nucleus. The processing of GLP-1 in the hypothalamus is similar to that of the gut (Larsen 1997) and a protein kinase pathway (Stobie-Hayes et al 1992) influences secretion.

2.3.2 GLP-1 and Physiological Actions

GLP-1 exerts control of glucose homeostasis after meals not only by stimulating insulin secretion, but also by promoting glucose disposal. GLP-1 is highly potent in stimulating insulin secretion (Orskov et al 1988). Blocking GLP-1 receptors with an antagonist decreases insulin secretion by 48% (Wang et al 1994). GLP-1 decreases glucagon secretion and increases somatostatin secretion (Drucker 1998). The combined effect of raising insulin and decreasing glucagon secretion is to promote the cellular uptake of glucose.

Independent of its insulinotropic effects, GLP-1 potently promotes glycogenesis in the isolated hepatocyte (Valverde et al 1994). In rat skeletal muscle, GLP-1 stimulates both glycogen synthesis and glucose oxidation. At high concentrations, GLP-1 increased the rate of glucose utilisation and lactate formation (an index of glycolysis in this tissue) (Villanueva-Penacarrillo et al 1994). However, the involvement of GLP-1 in glycogenesis versus glycogenolysis is controversial. GLP-1 also directly controls the rate at which glucose is absorbed into the bloodstream from the intestine by inhibiting gastric emptying via vagal afferent mediated central mechanisms (Imeryuz 1997).
2.3.3 GLP-1 secretion

Under normal feeding conditions, glucose concentrations in the ileum are below levels required to stimulate the L cell (Ferraris et al 1990). As stated earlier, GIP and the vagus are the mediators of early phase GLP-1 release in the rat, in response to carbohydrate and fat, but not protein digestion.

Stimulation of GLP-1 release seems to involve intestinal transport of monomeric carbohydrates. Luminal contact with glucose is critical in the release of GLP-1 from intestinal L cells. Intravenous glucose does not elicit a plasma rise in GLP-1, while glucose perfusion of the rat ileum evokes a sodium dependent increase in GLP-1 levels. These results suggest that GLP-1 release is dependent on glucose absorption by the intestinal sodium glucose transporter (Hermann, 1995). 2-deoxy-d-glucose and N-acetyl-d-glucosamine are not substrates of a luminal sodium/glucose or fructose transporter and do not affect GLP-1 release. Fructose perfusion of the ileum with or without sodium greatly increases plasma GLP-1 levels in the rat above those seen with glucose (Ritzel et al 1997). Other studies have found little or no GLP-1 secretion in response to fructose (Shima et al 1990). However, these two studies were done on different species (rat and dog respectively) which likely accounts for the differing results. Still, the importance of fructose transporter mechanisms in GLP-1 secretion is debatable.

3-O-D-methyl glucose and alpha methylglucoside are not metabolised in the intestinal L cell but do stimulate GLP-1 release (Ritzel 1997). The basolateral glucose transporter does not transport alpha methylglucoside (Hopfer 1987). Therefore, affinity for the sodium glucose transport receptor but not the basolateral transporter is an
influential factor in stimulating GLP-1 release. As mentioned earlier, similar mechanisms seem to be at play with the satiety induced by intestinally perfused glucose monomers in the rat.

Oral glucose elicits a large rapid increase in GLP-1 secretion in men (D'Allessio, 1993). GLP-1 secretion in response to carbohydrate is markedly different in obese versus lean subjects (Ranganath et al 1999). An explanation for this observation is based on the results of manipulating circulating non-esterified fatty acids (NEFA). Circulating NEFAs have a great impact on plasma GLP-1 response to carbohydrate. Suppression of NEFAs resulted in increased GLP-1 response. Higher fasting and postprandial NEFA levels in obesity may inhibit nutrient-mediated GLP-1 secretion. GLP-1 secretion in response to carbohydrate ingestion in obese subjects was significantly lower than in lean subjects (Ranganath et al 1999).

Compared to glucose, fat causes a slower but longer lasting increase in GLP-1 plasma concentration (Cordier-Bussat et al 1998). The effect of fat is attributed to monounsaturated fatty acids longer than 14 carbons. Full saturation of the stimulatory fatty acids resulted in a loss of PGDP secretion (a marker of GLP-1 secretion). Neither short nor medium chain fatty acids stimulate PGDP release by the isolated rat L cell (Brubaker et al 1995).

While carbohydrate and certain fatty acids promote GLP-1 secretion, protein has no effect. Perfusion of rat ileum with amino acids had no effect on GLP-1 secretion (Herrmann et al 1995). Human studies showed no GLP-1 secretion in response to a protein meal (D'Allessio 1993).
2.3.4 GLP-1 Receptors

The GLP-1 receptor (Stoffel et al 1993) is a member of seven membrane-spanning, G protein-coupled family of receptors, including glucagon, GIP and calcitonin among others. The receptor consists of 463 amino acids and has eight hydrophobic areas. The N-terminal hydrophobic segment is likely a signal sequence. GLP-1 binding at its receptor activate adenylate cyclase (Hoosein et al 1984), and activate the cAMP signal transduction pathway (Drucker et al 1987) which are thought to mediate GLP-1 actions.

Brain regions found to contain GLP-1 receptor mRNA include the paraventricular, supraoptic, arcuate, and dorsomedial nuclei of the hypothalamus, the NTS, area postrema, dorsal nucleus of the vagus and spinal cord (Merchenthaler 1999). GLP-1 receptors are found in a number of brain tissues but areas of interest to feeding will be emphasised here. The GLP-1 receptors in the brain are the same as those in the periphery (Wei et al 1995). GLP-1 mRNA is localised in the NTS and hypothalamus. In the rat brain the GLP-1 receptor is localised in the thalamus-hypothalamus, pituitary gland and medulla oblongata. Specific high affinity receptors for GLP-1 are located in the pituitary, suggesting GLP-1 may be synthesised in certain parts of the brain and play a role as a neurotransmitter (Shimizu et al 1987). GLP-1 is located in the synaptosome fraction of hypothalamic tissue and is released in a calcium dependent fashion by potassium induced depolarization. Therefore, GLP-1 meets two criteria of a putative neurotransmitter (Kreymann B, 1989).

The NTS receives afferent inputs associated with gustatory sensation. Secondary fiber systems project from the NTS to the medulla, pons, and thalamus/hypothalamus
(Han et al. 1986). Clearly, central GLP-1 receptors are situated in regions of the brain and CNS open to circulating factors and with close contact to neural input from the periphery. Receptors localised in areas known to be important in feeding provide support for GLP-1 function as a satiety inducing peptide.

GLP-1 receptors are distributed in a variety of tissues other than the brain. In the mouse, GLP-1 receptors are located in the pancreas, liver, lung, kidney, and in the large and small intestine. GLP-1 receptor expression is abundant in the mouse colon. The abundance of GLP-1 mRNA transcripts increases with age in RNA from mouse jejunum, ileum and colon (Campos et al. 1994). However, in another study the GLP-1 receptor gene was detected in the pancreas and pancreatic islets but not in liver, kidney or ileum of mouse tissue samples (Yamato et al. 1997). It is likely that GLP-1 receptors also exist in the vagus as GLP-1 administration affects vagal firing (Nakabayashi et al. 1996). GLP-1 receptor gene expression is up regulated in cells cultured in a high versus low glucose medium (Shimizu et al. 1987).

The GLP-1 receptor is very specific for the GLP-1 peptide. Although glucagon, GLP-2 and GLP-1 share a similar structure, their respective receptors are highly selective. The COOH terminal of GLP-1 appears to be crucial in binding to its receptor (Hjorth et al. 1994). However, the N-terminal portion of GLP-1 is essential to confer biological activity. Therefore, N-terminal truncated GLP-1 peptides can still bind to the receptor but not transduce a signal and so may act as antagonists of the intact peptide at the receptor (Grandt et al. 1994).
2.3.5 Degradation of GLP-1

The degradation of active GLP-1 is efficient and swift. In human plasma, active GLP-1 (7-36) is degraded by dipeptidyl peptidase-IV (DPP-IV) to the inactive metabolite GLP-1 (9-36) (Deacon et al 1995). GLP-1 is catabolized in the kidney by a mechanism involving glomerular filtration and tubular catabolism (Ruiz-Grande et al 1990). The kidney has very high DPP-IV activity, while the jejunum and liver also demonstrate significant activity (Mentlein 1999). GLP-1 is stored in the intestinal L cell in the active form GLP-1 (7-36). The capillaries supplying the L cells of the intestine transform most of the GLP-1 (7-36) to inactive GLP-1 (9-36). In fact, DPP-IV positive capillaries are juxtaposed to GLP-1 containing L cells. It is likely that GLP-1 is degraded as it enters the DPP-IV containing blood vessels draining the intestinal mucosa (Hansen et al 1999). In vivo, only 32.9 ± 10.8% of GLP-1 (7-36) is secreted intact into the portal vein. The liver's significant content of DPP-IV probably degrades much of what little active GLP-1 there is entering from the portal vein.

The half-life of the active metabolite is one minute (Deacon et al 1996). Earlier estimates that the half-life was longer appear to have been in error because the radioimmunoassays did not distinguish the intact active peptide and the truncated inactive form (Deacon et al 1995).

2.3.6 Agonist and Antagonist of GLP-1

Antagonists and agonists have assisted investigation of the biological activities of GLP-1. In fact, to truly define the physiological action of a substance, it is necessary to selectively block its action with an antagonist. The amino acid sequences of GLP-1 antagonist and agonists are shown in Figure 2.2.
Exendin 9-39 peptide, a fragment of Exendin 4, acts as an antagonist at the GLP-1 receptor. Exendin 4 was isolated from Heloderma Suspectum venom and has a 53% sequence homology with GLP-1. Exendin 4 shows a high specificity for the GLP-1 receptor and activates cAMP activity upon binding. The t ½ for terminal decay of Exendin 4 injected intravenously in rats was 66.9 minutes. This value was greatly increased in nephrectomized rats, with the kidney appearing to be responsible for 80% of elimination of Exendin 4 in rats (Chen et al 1998). Truncation of the Exendin 4 peptide to Exendin 9-39 removes amino acids critical to GLP-1 receptor activation and results in a peptide which binds to the receptor without activation. A concentration ratio of 10:1 Exendin 9-39 vs. GLP-1 completely abolished GLP-1 mediated insulin secretion (Eng et al 1992). The half-life of the antagonist, Exendin 9-39, in the rat is unclear but is known to be about 1/2 hour in humans (Edwards et al 1999).

2.4 Glucagon-Like Peptide-1 and Food Intake

Animal and human studies show that GLP-1 has a suppressive effect on food intake. Animal studies demonstrating feeding responses related to GLP-1 typically employ injection of peptide into the brain, whereas human studies have delivered the peptide intravenously. Human studies demonstrate effects on satiety and appetite as well as on food intake. The mechanism whereby GLP-1 exerts its influence on appetite, whether central or peripheral in nature has not been satisfactorily answered. The following section reviews current knowledge of GLP-1 and appetite and comments on a) peripheral versus central administration of GLP-1 and appetite and b) GLP-1 and carbohydrate induced appetite suppression.
Little is known concerning the mechanism of peripheral GLP-1 induced satiety. Physiological evidence points to a few possible mechanisms. The vagus serves as a connection between the periphery and the central nervous system. As mentioned earlier, some gut peptides exert their appetite suppressive effects via vagal afferent fibers. GLP-1 acts on the vagus (Nakabayashi et al 1996), but no studies have reported that this is a mechanism by which it induces satiety. GLP-1 may also regulate appetite by an "endocrine" effect on the brain. GLP-1 receptors in the subfornical organ and the area postrema of the rat brain are accessible to circulating GLP-1. The subfornical organ and the area postrema have connections with hypothalamic areas involved in water and appetite homeostasis (Orskov et al 1996).

Peripheral intraperitoneal administration (IP) of GLP-1 was not found to elicit fos-ir (a marker of neural activity) in the rat brain, whereas central administration does (Rowland et al 1997). This suggests that GLP-1 in the periphery does not influence central nervous system activity. However, the study has been criticised because it used a low dosage of GLP-1 given intraperitoneally and a very small number of rats. Considering its very short half-life (Hansen et al 1999) it is unlikely that intraperitoneal GLP-1 possesses the longevity required to alter food intake, though it does have effects on insulin secretion (Tseng et al 1999). Furthermore, intestinally secreted GLP-1 exerts a neural response through the vagus to the central nervous system in delaying gastric emptying. GLP-1 acts via a vagal afferent centrally mediated mechanism in its role in gastric emptying (Imeryuz et al 1997). Administration of GLP-1 is unable to delay gastric emptying in the vagotomized pig.
2.4.1 Animal Studies

When GLP-1 is injected into the brain food intake is decreased (Table 2.1.). The administration of Exendin 9-39 prior to GLP-1 injection completely blocks the decrease in food intake in both fasted and satiated rats. Exendin 9-39 alone has no effect on food intake in fasted rats but doubles food intake in the fed rat and augments the response to the appetite stimulant NPY. Their low levels of GLP-1 and therefore, no effect of GLP-1 receptor blockade (Turton et al 1996) probably explain the lack of effect of Exendin 9-39 on fasted rats.

Several lines of evidence support a physiological role for GLP-1 in food intake regulation. GLP-1 administered in the brain does not act as an anorexigenic agent by creating nausea. PVN infusion of GLP-1 (7-36) in minimal amounts suppresses feeding but does not induce taste aversion or alter locomotion in rats (McMahon 1998). In addition, the argument of taste aversion as a mediator of GLP-1 satiation does not explain the fact that blocking the GLP-1 receptor with Exendin 9-39 significantly increases food intake and body weight in the rat. ICV administration of Exendin 9-39 twice daily for ten days results in rats with significant weight gain and increases in 24-hr intake (Bloom 1997). The long lasting effects of both GLP-1 and Exendin 9-39 after repeated administration argue for a true physiological role in food intake. GLP-1 maintains food intake suppressive effects and decreases body weight when used repeatedly over a number of days, while Exendin 9-39 increases food intake and body weight under the same conditions. Daily ICV injection of GLP-1 over 6 days reduces food intake and decreases body weight by $16 \pm 5$ g compared to control. Exendin 9-39
### Table 2.1. Comparison of Central GLP-1 Feeding Studies in Rats

<table>
<thead>
<tr>
<th>Study</th>
<th>Feeding protocol</th>
<th>Peptide GLP-1 E9-39</th>
<th>Site</th>
<th>Food Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turton et al, Nature 1996 379(Jan4):69</td>
<td>Ad libitum</td>
<td>10 µg</td>
<td>ICV inject onset of Dark period</td>
<td>↓ 0-2 h</td>
</tr>
<tr>
<td></td>
<td>Ad libitum</td>
<td>100 µg</td>
<td>ICV inject before feeding</td>
<td>↑ 0-2 h</td>
</tr>
<tr>
<td></td>
<td>24 hr fasted</td>
<td>3 µg</td>
<td>ICV inject before feeding</td>
<td>↓ 0-2 h</td>
</tr>
<tr>
<td></td>
<td>24 hr fasted</td>
<td>100 µg</td>
<td>ICV inject before feeding</td>
<td>No effect</td>
</tr>
<tr>
<td>Meeran et al, Endocrinology 1999 140(1):244</td>
<td>Schedule fed 9-11am 5-7pm</td>
<td>3nmol</td>
<td>ICV inject before feeding</td>
<td>↓ 0-2 &amp; 0-4 h</td>
</tr>
<tr>
<td></td>
<td>Schedule fed 9am-3pm</td>
<td>30nmol</td>
<td>ICV inject before feeding</td>
<td>↑ 0-6 h</td>
</tr>
<tr>
<td>Tang-Christensen, Diabetes 1998 47(4):530</td>
<td>Schedule fed 9am-12pm</td>
<td>1 µg</td>
<td>lat ventricle</td>
<td>↓ 0-60 min</td>
</tr>
<tr>
<td></td>
<td>Schedule fed 9am-12pm</td>
<td>10 µg</td>
<td>Inject before feeding lat ventricle</td>
<td>No change 60-90 only measure taken</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 µg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donahey et al, Brain Res 1998 779(1-2):75</td>
<td>Fasted 2hr before dark</td>
<td>10 µg</td>
<td>ICV Injection 1hr before dark</td>
<td>↓ 0-1 LongEvan 0-2 Zucker</td>
</tr>
<tr>
<td></td>
<td>Feed at start of dark cycle</td>
<td>100 µg</td>
<td></td>
<td>No change 0-24</td>
</tr>
<tr>
<td>McMahon et al, AMJP 1998 274(43):R23</td>
<td>Fasted 2hr before dark</td>
<td>100ng 200ng</td>
<td>PVN inject before feeding</td>
<td>↓ 0-30 min only measure taken</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asarian et al, Physiol Behav 1998 64(3):367</td>
<td>fasted 17h Sham fed Sucrose solution</td>
<td>3 µg 10 µg 30 µg</td>
<td>lat ventricle just before feeding</td>
<td>↓ 0-45 min Only measure taken</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navarro et al, 1996 67:1982</td>
<td>Fasted 24 hr</td>
<td>100ng 1000ng 2000ng</td>
<td>ICV just before feeding</td>
<td>↑ 0-120min no change 0-120 min only 2hr meas. 'd.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ICV injection for 3 days increases body weight by $7 \pm 2$ g and increases food intake vs. control (Meeran et al 1999).

Location of action is critical in proving GLP-1 is physiologically satiating. Destruction of specific areas of the brain prevents GLP-1 induced feeding suppression but does not affect normal taste aversion response (Tang-Christensen 1998). Although evidence linking GLP-1 induced satiation to specific brain regions is scanty, one region of the brain that GLP-1 seems to act on is the arcuate nucleus. Selective destruction of the arcuate nucleus with monosodium glutamate (MSG) blocks the effect of PVN GLP-1 on food intake. Sensitivity to anorectic agents and normal responses in taste aversion are maintained indicating that GLP-1 mediates satiety via other routes. NPY induced feeding is greatly reduced by GLP-1 in intact rats but not in the MSG lesioned rats. Their data suggests that GLP-1 receptors mediating inhibition of feeding are localised upstream to the NPY sensitive neurones inducing feeding behavior (Tang-Christensen et al 1998).

GLP-1 demonstrates similar effects across a number of different species indicating an important physiological role. GLP-1 decreases food intake in the chicken as well as in man and rats. ICV injection of GLP-1 in the chick greatly reduces food intake (Furuse et al 1997). Truncating GLP-1 at the C terminal by 6 or more amino acids consistently results in abolishing its food intake suppressive effects. Truncation by three C terminal amino acids reduces food intake but only at high dosages, indicating decreased affinity for the receptor (Furuse et al 1998).

Evidence supporting a nausea induced GLP-1 effect of food intake exists but does not successfully establish that GLP-1 is anorexigenic. GLP-1 was administered
ICV at 1.0 or 3.0 micrograms followed by immediate oral infusion of saccharin in rats. When re-exposed to GLP-1 and saccharin several days later a significant number rejected the taste with the 3.0 microgram dose of GLP-1 only. It was concluded that GLP-1 creates a conditioned taste aversion (Van Dijk et al 1997). Injection into the third ventricle of a dose of 10 μg GLP-1 induces taste aversion while a much smaller amount of leptin (3.5 μg) does not (Thiele et al 1997). Ten micrograms is a very large amount of GLP-1, considering that only 100 ng injected in the PVN can induce food intake suppression (Navarro 1996); such supraphysiological concentrations could exert aversive effects not seen at physiological concentrations. The central injection of Exendin 9-39 blocks the suppression of food intake induced by peripheral LiCl, a toxin known to stimulate a range of effects associated with visceral illness, but does not block LiCl induced reduction in NaCl intake. The data may indicate that GLP-1 receptors mediate some effects of visceral illness (Seeley et al 2000). The ability of Exendin 9-39 to increase food intake (Turton et al 1996) and the increased satiety and decreased appetite associated with GLP-1 in human studies (Flint et al 1998) contradict a GLP-1 induced taste aversion.

Although GLP-1 can be shown to be a factor in the regulation of food intake, it is clearly only one of many peptides affecting body weight and food intake. As with other peptides, the absence of GLP-1 receptor in a strain of knockout GLP-1 -/- receptor mice does not affect their ability to maintain body weight and eating patterns, even when fed high fat diets (Scrocchi & Drucker 1997 & 1998). Obviously, these mice compensate for the lack of the GLP-1 receptor by enhancing other appetite control regulators.
It is premature to conclude that GLP-1 regulates food intake solely through a central mechanism. The Exendin 4 agonist to GLP-1 decreases appetite when injected intraperitoneally, but in a much less potent manner than central injection (Bhavsar et al 1998). Exendin 4 has a half-life of approximately 66 minutes after intravenous administration (Chen et al 1998) and provides a more physiologically active response than the more labile GLP-1. It is possible that circulating Exendin 4 was accessible to GLP-1 receptors in areas of the brain with “leaky” blood brain barrier.

2.4.2 Human Studies

In the human, there are two lines of evidence suggesting a physiological role for GLP-1 in food intake. First the release of intestinal hormones is critical to the promotion of satiety after glucose infusions (Lavin et al 1996). Secondly, GLP-1 peripheral infusions decrease food intake suggesting GLP-1 acts by a peripheral mechanism (Flint et al 1998). Different effects on food intake and ratings of hunger are reported by human male subjects given glucose either by infusion in the duodenum or intravenously. Food intake at a test meal 90 minutes post infusion and ratings of hunger at timed intervals are significantly lower when glucose is infused duodenally. Giving octreotide, a somatostatin analog, intravenously to prevent the release of gut hormones to the duodenally glucose infused group reverses the decrease in food intake and hunger. Therefore, the satiety effect of glucose depends on intestinal contact and gut peptides (Lavin et al 1996).

The notion that alterations in plasma insulin and glucose resulting from blocking the release of gut peptides affected food intake in the above study was dispelled by subsequent investigation. Similar results were obtained when using an insulin clamp to
Table 2.2 Comparison of Peripheral GLP-1 Feeding Studies in Humans

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration</th>
<th>Dose/Site</th>
<th>Food Intake/Appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hellstrom et al Appetite 1999</td>
<td>4 hr postbreakfast</td>
<td>0.75 pmol/kg i.v.</td>
<td>$\downarrow$ Food Intake lunch/dinner</td>
</tr>
<tr>
<td>Long et al Br J Nutr. 1999</td>
<td>1 hour</td>
<td>1.2 pmol/kg i.v.</td>
<td>No effect on appetite or FI</td>
</tr>
<tr>
<td>Gutzwiller et al Am J Phys. 1999</td>
<td>2 hours</td>
<td>1.5 pmol/kg/min i.v.</td>
<td>$\uparrow$ Satiety and Fullness, $\downarrow$ Energy Intake</td>
</tr>
<tr>
<td>Toft-Nielsen et al Diab. Care 1999</td>
<td>48 hours</td>
<td>2.4 pmol/kg/min subcutaneous</td>
<td>$\downarrow$ hunger and prosp. FI</td>
</tr>
<tr>
<td>Naslund et al Int J Obes 1999</td>
<td>8 hours</td>
<td>0.75 pmol/kg/min i.v.</td>
<td>$\downarrow$ Energy Intake/Hunger</td>
</tr>
<tr>
<td>Flint et al J Clin Inv 1998</td>
<td>4 hours</td>
<td>50 pmol/kg/hr i.v.</td>
<td>$\downarrow$ Energy Intake/Hunger, $\uparrow$ Fullness/satiety</td>
</tr>
<tr>
<td>Gutzwiller et al Gut 1999</td>
<td>2 hours</td>
<td>0.375, 0.75, 1.5 pmol/kg/min i.v.</td>
<td>$\downarrow$ Food Intake, $\downarrow$ Dose Dependent in obese subjects</td>
</tr>
</tbody>
</table>
maintain plasma concentrations slightly above usual postprandial concentrations. Measurements of plasma GLP-1 show a close association between the increase in GLP-1 and decrease in appetite. Plasma GLP-1 levels were closely temporally associated with the decrease in appetite whereas GIP levels were not (Lavin et al 1998) suggesting a role for peripheral GLP-1 in food intake suppression.

The majority of human studies demonstrate a definite effect of peripheral GLP-1 on energy intake and appetite (Table 2.2.). GLP-1 infusion decreases food intake and increases satiety in lean subjects. Administration of GLP-1 intravenously yields significant decreases in appetite and energy intake at an ad libitum lunch and enhances satiety and fullness (Flint 1998). Similarly, obese subjects receiving an intravenous infusion of GLP-1 showed a significant reduction in hunger and prospective consumption ratings. Hunger ratings immediately and 240 minutes after the meal were significantly lower with GLP-1 infusions, yet there was no difference in food intake at the test meal (Naslund 1998). Obese subjects may not have been as sensitive to satiety cues as subjects with healthy body weights. However, significant reductions in energy intake and appetite in obese men receiving an intravenous infusion of GLP-1 were reported in a subsequent study (Naslund et al 1999).

Type 2 diabetic patients receiving a 48 hour continuous subcutaneous infusion of GLP-1 demonstrated decreased hunger and prospective food intake and increased satiety while fullness was unaffected (Toft-Nielsen et al 1999). Diabetic subjects were required to eat a fixed amount of food (for plasma glucose and insulin analysis) so food intake was not measured. The efficacy of subcutaneous GLP-1 was not as great as intravenous delivery and required longer infusion. When type 2 diabetic patients had access to an ad
libitum meal, they demonstrated increased satiety and decreased food intake with infusion of GLP-1 (Gutzwiler JP et al 1999).

GLP-1 is capable of decreasing food intake at known physiological concentrations. Graded intravenous doses of GLP-1, 0, .375, .75 and 1.5 pmol/kg/min infused in healthy male subjects dose dependently decreases food intake at a test meal one hour following the start of the infusions. The largest dose of GLP-1 caused a 32% reduction in calorie intake. Meal duration was also shortened by the GLP-1 infusions. Therefore, at physiological levels, GLP-1 is capable of decreasing food intake in men (Gutzwiler et al 1999).

Only one study using comparable methods did not find any significant effect of intravenous GLP-1 on short-term satiety and energy intake in lean subjects. Subjects did tend to be less hungry after a buffet meal with GLP-1 infusion but the difference did not reach significance (Long et al 1999). Still the majority of evidence points to a definite effect on appetite with GLP-1.

In comparing the results of animal studies with those of human studies, it is evident that sustained peripheral intravenous delivery of the GLP-1 peptide is active in appetite control in men while single intraperitoneal injections of the same peptide are ineffective in the rat. The extended duration of GLP-1 intravenous infusion allows active GLP-1 to remain in the system longer therefore exerting suppressive effects on food intake and appetite in a variety of subjects ranging from normal weight to the obese and in diabetics. This evidence supports the possibility that a peripherally administered long acting GLP-1 agonist or antagonist could affect food intake in the rat.
2.4.3 GLP-1 and regulation of carbohydrate intake

There is substantial evidence suggesting a physiological role for GLP-1 in the regulation of total food intake. However, it may be that it is a specific signal released by carbohydrate ingestion leading to the regulation of carbohydrate appetite. First, the inhibition that GLP-1 exerts over NPY induced feeding (Tang-Christensen 1998) should logically result in decreased carbohydrate consumption. NPY injection in the PVN of rats leads to a selective increase in the consumption of carbohydrate (Leibowitz 1994). Because GLP-1 inhibits the action of NPY it should lead to a selective decrease in the consumption of carbohydrate.

A second argument in support of the macronutrient specificity of GLP-1 induced satiety is provided by the location of “glucose sensing machinery” with the GLP-1 receptor. GLP-1 receptor mRNA is found in the same hypothalamic neurons as the Glut-2 glucose transporter and the enzyme glucokinase. Glut-2 is a glucose transporter and glucokinase is an enzyme that phosphorylates glucose. In the pancreatic beta cell these three proteins are known to be involved in the process of “glucose sensing”. Therefore, GLP-1 may play a role in glucose sensing centrally due to its colocalisation with Glut-2 and glucokinase in areas of the hypothalamus known to affect carbohydrate intake when injected with NPY. These same hypothalamic areas (median eminence, supraoptic area, and third ventricle wall) are known to contain glucose sensitive and glucose responsive neurons (Navarro 1996).

Finally, because carbohydrate is a potent stimulant of the release of GLP-1, GLP-1 mediated carbohydrate-induced suppression of food intake may explain, in part, carbohydrate's role in feeding behaviour.
2.5 Summary

This literature review has described various appetite control mechanisms. An in depth focus on GLP-1 in satiety was provided. Evidence for a likely carbohydrate satiety/GLP-1 link was provided as support for the hypothesis of the experiments described in the following section.
CHAPTER 3

INTRODUCTION TO EXPERIMENTAL WORK
3. INTRODUCTION TO EXPERIMENTAL WORK

3.1 Hypothesis

GLP-1 is a regulator of carbohydrate intake in rats.

3.2 Objectives

The objectives of this thesis were to determine the role of GLP-1 in carbohydrate induced food intake suppression and in carbohydrate appetite.

The specific objective of this research was to determine:

1. The effect of the GLP-1 receptor antagonist Exendin 9-39 on food intake.
2. The effect of Exendin 9-39 on carbohydrate, fat and protein induced food intake suppression as well as on macronutrient selection.
3. The effect of the GLP-1 receptor agonist Exendin 4 on macronutrient selection as well as on carbohydrate induced food intake suppression.

3.3 Outline of Work

A total of 5 studies, comprising 17 experiments (Table 3.1), were conducted.

In Study 1 (experiments 1-3), the effect of Exendin 9-39 on food intake was measured using two dosages and two feeding schedules. Study 2 (experiments 4-6) investigated the effects of Exendin 9-39 on the food intake suppression induced by glucose using varying times of glucose and Exendin 9-39 delivery. Study 3 (experiments 7-10) examined the effect of Exendin 9-39 on the food intake suppression induced by glucose, corn oil and albumin preloads as well as on macronutrient selection. Study 4 (experiments 11-15) determined the effect of Exendin 4 on macronutrient selection. The effect of Exendin 4 on the food intake suppression
Table 3.1 Summary of Experiments

<table>
<thead>
<tr>
<th>Study</th>
<th>Experiment</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The Effect of Exendin 9-39 on Food Intake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>20 µg with food cups available at all times</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>20 µg with dark cycle feeding</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>50 µg with dark cycle feeding</td>
</tr>
<tr>
<td>2</td>
<td>The Effect of Exendin 9-39 (20 µg) on the Food Intake Suppression Induced by a Glucose Preload</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>Single dose with 1 g glucose preload</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>Two injections with 2 g glucose preload</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>Two injections with 1 g glucose preload</td>
</tr>
<tr>
<td>3</td>
<td>The Effect of Exendin 9-39 on the Food Intake Suppression of Glucose, Corn Oil or Albumin Preloads and on Macronutrient Selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>Glucose preload</td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>Corn oil preload</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>Albumin preload</td>
</tr>
<tr>
<td></td>
<td>3.10</td>
<td>Macronutrient selection after 50 µg</td>
</tr>
</tbody>
</table>
4. The Effect of Exendin 4 on Macronutrient Selection and on Glucose Induced Food Intake Suppression

| 4.11 | Macronutrient selection after 20 μg |
| 4.12 | Macronutrient selection after 10 μg |
| 4.13 | Macronutrient selection after 0.5 μg |
| 4.14 | Macronutrient selection after 0.5 μg with glucose preload |
| 4.15 | Food intake after 0.5 μg with glucose preload |

5. The Effect of Exendin 4 or Exendin 9-39 on the Plasma Glucose and Insulin Response to a Glucose Challenge

| 5.16 | Exendin 4 0.5 μg with samples at 0, 30 and 75 minutes |
| 5.17 | Exendin 9-39 50 μg with samples at 15 minutes |
induced by a glucose preload was also investigated (experiments 14 and 15). Study 5 (experiments 16 and 17) measured the plasma glucose and insulin response to glucose preload after Exendin 4 or Exendin 9-39.

An additional study of interest, "Comparison of the food intake suppression of three different nutrient preloads" is included in the appendix. Although not directly related to the hypothesis and objectives of this thesis, the study provided background for the choice of preloads used in Study 3.

3.4 General Methods

3.4.1 Animals, Housing and General Care

Male Wistar rats weighing 150-175 grams (g) at time of arrival were used for all experiments. The University of Toronto Animal Care Committee approved the protocol, and care and maintenance of the animals conformed to the guidelines of the Canadian Council on Animal Care. Animals were housed in hanging wire mesh caging with free access to water throughout and to pellet chow for the first three days. On the third day following arrival of the rats in the animal facility, pellet chow was removed and replaced with AIN 93G powder diet or with two defined diets containing 50 and 0 % protein (experiments 3.10, 4.11-4.15). A 10:14 hour light/dark cycle (lights on 8am-6pm) was used in Studies 1 and 2. Because the rats ate little in the first hour, the light cycle was changed to shorten the length of the nocturnal feeding period in order to promote greater food intake in the first hour of the dark cycle. Thereafter, (Studies 3 through 5) a 12-hour light/dark cycle was employed with lights on from 6am-6pm. Food was available at all times for experiment 1.1 and during the dark period only for all other experiments.
3.4.2 Experimental Diets

The AIN 93G powder diet (Table 3.2) was used in studies 1 through 3 and in experiment 4.14. In experiments 4.10 - 4.13 and 4.15, a two-cup selection system was used. Diets were formulated to contain 0% protein and 50% protein by weight based on the AIN 93G diet. The proportion of cornstarch and sucrose to casein in the AIN93G diet was altered to achieve the 0 and 50% protein diets. Protein content was calculated accounting for the 87% percent protein content present in the high casein powder (using the casein composition data provided by Harlan Teklad).

For Studies 1 through 3, diet was presented in stainless steel food cups with a screen insert to minimise spillage. In Study 4, glass food jars with wire mesh tube inserts were used. Food intake and spillage were measured to the nearest 0.1 g.

3.4.3 Nutrient Preloads

D-glucose (Sigma Chemical Company, St Louis Missouri) was given as a 40% solution in deionized water (0.4g D-glucose/ml solution) by intragastric gavage. Corn oil (Mazola) 1.5g and crude chicken egg albumin 0.5g containing 85% albumin protein (N x 6.25, Grade II, Sigma Chemical Co, St. Louis, Missouri) were given as preloads brought to equal volumes using deionized water.

3.4.4 GLP-1 Antagonist and Agonist

Exendin 9-39 or Exendin 4 peptides (California Peptide Company, San Jose, California) were diluted in sterile deionized water and divided into aliquots before being quickly frozen on dry ice. The aliquots were lyophilised in a freeze dryer and the resulting dried peptide stored at minus 20 ° C until used. When needed, freeze dried
Table 3.2 Composition of AIN 93G Rodent Diet

<table>
<thead>
<tr>
<th>Ingredients (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein*.................. 200</td>
</tr>
<tr>
<td>Sucrose*.................. 100</td>
</tr>
<tr>
<td>Soybean Oil**............ 70</td>
</tr>
<tr>
<td>AIN 93G MX Mineral Mix.... 35</td>
</tr>
<tr>
<td>L-Cystine***............. 3</td>
</tr>
</tbody>
</table>

AIN 93G MX Mineral Mix (g/kg mixture)***

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>Ingradients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Carbonate........................... 357</td>
<td>Potassium Phosphate monobasic.196</td>
</tr>
<tr>
<td>Potassium Citrate, H2O......................... 70.78</td>
<td>Sodium Chloride...................... 74</td>
</tr>
<tr>
<td>Potassium Sulfate.............................. 46.6</td>
<td>Magnesium Oxide..................... 24</td>
</tr>
<tr>
<td>Ferric Citrate, USP.............................. 6.06</td>
<td>Zinc Carbonate...................... 1.65</td>
</tr>
<tr>
<td>Manganese Carbonate......................... 0.63</td>
<td>Cupric Carbonate..................... 0.3</td>
</tr>
<tr>
<td>Potassium Iodate............................... 0.01</td>
<td>Boric Acid.......................... 0.0815</td>
</tr>
<tr>
<td>Sodium Metasilicate.9H2O.................... 1.45</td>
<td>Nickel Carbonate................... 0.0318</td>
</tr>
<tr>
<td>Chromium Potassium Sulfate.12H2O........... 0.275</td>
<td>Sucrose, finely powdered........ 221.026</td>
</tr>
<tr>
<td>Lithium Chloride.............................. 0.0174</td>
<td></td>
</tr>
<tr>
<td>Sodium Fluoride............................... 0.0635</td>
<td></td>
</tr>
<tr>
<td>Ammonium Vanadate............................. 0.0066</td>
<td></td>
</tr>
<tr>
<td>Sodium Selenate............................... 0.01025</td>
<td></td>
</tr>
<tr>
<td>Ammonium Paramolybdate.4H2O........... 0.00795</td>
<td></td>
</tr>
</tbody>
</table>

AIN 93 VX Vitamin Mix (g/kg mixture)***

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>Ingradients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin................... 3.0</td>
<td>Calcium Pantothenate........ 1.6</td>
</tr>
<tr>
<td>Pyridoxine HCl............... 0.7</td>
<td>Thiamine HCl................. 0.6</td>
</tr>
<tr>
<td>Riboflavin.................. 0.6</td>
<td>Folic Acid....................... 0.2</td>
</tr>
<tr>
<td>Biotin...................... 0.02</td>
<td>Vit. E Acetate (500IU/g).... 15</td>
</tr>
<tr>
<td>Vit. B12 (0.1%)............. 2.5</td>
<td>Vit. D3 (400,000IU/g)........ 0.25</td>
</tr>
<tr>
<td>Vit. A Palmitate (500,000IU/g)..... 0.8</td>
<td>Sucrose, finely powdered..... 967.23</td>
</tr>
<tr>
<td>Vit. K1/Dextrose Premix (10mg/g)... 7.5</td>
<td></td>
</tr>
</tbody>
</table>

*Grain Processing Inc. Scarborough, ON
**Loblaws, Toronto, ON
***Harlan Teklad Test Diets, Madison WI
Reeves et al 1993
peptide was allowed to come to room temperature and reconstituted using phosphate buffered saline pH 7.4 (Sigma Chemical Company, St. Louis Missouri). Reconstituted peptide was generally used within 1 hour of preparation. Sterile saline was the control. All injections were given as bolus intraperitoneal injections in a volume of 0.5 milliliters (ml).

3.4.5 Blood collection

Rats were fully anaesthetised with halothane (Sigma, St. Louis Missouri) in a glass jar kept in a fume hood. A 20 gauge 1 inch needle was used to puncture the heart and collect 3 ml of blood. Rats were immediately returned to the halothane chamber after blood collection and underwent subsequent cervical dislocation to ensure proper euthanasia. Blood samples were aliquoted for plasma insulin and glucose analysis. Samples destined for plasma glucose analysis were placed in 1.5 ml centrifuge tubes containing 20 IU dried heparin (Leo Pharma Inc., Ajax ON). Samples for insulin analysis were placed in tubes containing a 10:1 volume of blood to Trasylol (10,000 KIU/ml Bayer, West Haven CT)/EDTA (Sigma, St. Louis Missouri) solution (1:1 volume of Trasylol: EDTA 2.4g/100 ml distilled water). Each sample was quickly centrifuged (Biofuge A AHSC Inc, Mississauga ON) at 6000 rpm for 5 minutes and plasma was transferred to a second tube. Plasma glucose was immediately tested using a Beckman 2 Glucose Analyser (Beckman Coulter Inc. Fullerton CA USA). All plasma samples were quickly stored in a refrigerator (approx. 3-4 Celsius). Once all blood collection was complete, samples were frozen until analysis for insulin concentrations.
3.4.6 Glucose and Insulin Analysis

Plasma glucose concentrations were measured by the glucose oxidase method. The analyser was calibrated before use and frequently during the experiment with the 150 mg/dl Beckman Certified Glucose Standard. For each sample, 10 µl of plasma containing D-glucose was injected into a solution containing oxygen and glucose oxidase. The glucose reacts with oxygen in the following reaction, which is catalysed by glucose oxidase:

\[
\text{Glucose Oxidase} \\
\text{D-glucose + O}_2 + \text{H}_2\text{O} \rightarrow \text{gluconic acid + H}_2\text{O}_2
\]

In this reaction, oxygen is used at the same rate as glucose to form gluconic acid. A polarographic oxygen sensor is used to detect oxygen consumption, which is directly proportional to the glucose concentration in the sample. Results are obtainable within thirty seconds after sample addition, and the data are accurate to ± 3 mg/dl.

Plasma insulin concentrations were determined by radioimmunoassay using the kit specific for rat insulin (LINCO Research Inc., St. Charles MO USA). Rat insulin RIA kit is a double antibody radioimmunoassay. Insulin in the sample competes with a fixed amount of \(^{125}\text{I}\)-labelled insulin for the binding sites on the specific antibodies. Bound and free insulin are separated by the addition of a second antibody immunoadsorbent followed by centrifugation and aspiration of the supernatant. The radioactivity of the pellet is then measured and is inversely proportional to the quantity of insulin in the sample. A standard curve is first constructed using insulin standards with known concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 ng/ml) in duplicate. After samples are pipetted into appropriate tubes, \(^{125}\text{I}\)-insulin (100 µl to all tubes) is added
followed by the rat insulin antibody (100 µl to all tubes). The tubes are then vortexed to ensure mixing and are incubated overnight (18-24 hours) at 4 degrees Celsius. Then, the tubes are centrifuged at 2500 rpm for 40 minutes. The supernatant was aspirated, and the radioactivity in the pellet was counted for 4 minutes in a gamma counter (Beckman Instruments, Fullerton Ca, USA). The counts (B) for each of the standards and unknowns are expressed as a percentage of the mean counts of the "standard" (Bo):

\[
\text{% activity bound} = \frac{B \text{ (standard or sample)}}{Bo} \times 100\% 
\]

The %B/Bo for each standard is plotted on the y-axis and the known concentration of the standard on the x-axis on a log-log graph paper and a standard curve is constructed. The concentration of the unknown samples are determined by interpolation of the reference curve. The coefficient of interassay variation determined on reference plasma was less than 9%.

3.4.7 Study Design

Studies 1 through 4 used a back to back within subjects design. That is, the experimental treatment was paired with a control for each rat. On day one, half of the rats received treatment while the other half received control. On the next experimental day, treatment delivery was reversed so that those rats that received a treatment on the previous experimental day now received the control and vice versa. In these experiments, there was only one treatment group and one control group. The treatment was an injection of peptide and the control an injection of saline, with all other variables equal between groups.

In experiments involving Exendin 9-39, a one day washout was allowed between treatment and control days and between experiments (when a series was done on the
same group of rats). Two days of washout were used in experiments using Exendin 4 as treatment. The Exendin 4 treatment potently decreased food intake, therefore, an extra washout day was necessary for recovery to normal feeding.

Study 5 required a between subjects design as rats had to be euthanized in the process of collecting blood samples. Therefore, rats were randomly divided into separate groups based on treatment (peptide or saline) and time of sampling and were tested only once as part of their respective group.

3.4.8 Adaptation Procedures

For studies 1 through 4, animals were adapted to experimental procedures prior to experimentation. Animals were injected intraperitoneally with saline and/or gavaged intragastrically with deionized water. The timing of injections and/or gavage during adaptation followed that of the respective experiment. A 16 gauge feeding needle (Fine Science Tools, Victoria BC) was used to gavage 1 ml of deionized water on the first day of adaptation and gradually increased to full experimental volume over four days.

Subsequently, an adaptation test to treatment delivery procedure was conducted to verify that the gavage and/or injection procedure itself did not alter food intake from baseline levels. In a simple back to back design, half of the rats received the gavage and/or injection while the rest did not. On the second day, the order was reversed so that each rat acted as its own control. Experiments began when water gavage and/or saline injection did not alter food intake compared to no treatment.

For macronutrient selection, rats were allowed to select from the 0 and 50% protein diets for ten days prior to experimentation. Food cups were rotated from side to side on a daily basis throughout. A 14 hour measure of food intake was taken for baseline
values five days prior to experimentation. Experimentation proceeded when it was determined that rats were selecting in a consistent fashion between the two food cups.

3.4.9 Statistical Analysis

Food intake was measured at the predetermined intervals of 0-1, 1-2, 0-2, 2-14 and 0-14 hours in experiments 1.2, 1.3, 2.4, 2.5 and 3.7-3.9, or at 0-30 min, 30 min –1, 0-1, 1-2, 0-2, 2-14 and 0-14 hours in experiments 2.6, 3.10 and 4.11-4.15. Food intake was measured for an additional hour (at 2-3 h) in experiments 1.2 and 1.3 in order to determine if longer term effects on feeding were occurring.

Data from the two groups in experiments 3.7, 3.8 and 3.9 were pooled. In macronutrient selection experiments (3.10 and 4.11-4.14) separate analyses of the intake of the 0% diet, 50% diet, and of total food intake were performed. Total spillage was accounted for in analysis of the intake of the 0 and 50% protein diets but individual spillage was not determined. However, the mesh tubes in food jars kept spillage to a minimum in all macronutrient selection experiments. No rat spilled more than 0.5 g and therefore, none were excluded from analysis for excessive spillage.

All analyses of food intake were done using a paired t-test at each time interval. Adaptation tests were also analysed by paired t-test.

In Study 5, the overall treatment effect of Exendin 4 or Exendin 9-39 on plasma glucose and insulin was determined using One Way Analysis of Variance (ANOVA). A post hoc Tukeys test was used to determine specific differences between groups.

All analyses were done with the aid of the Graphpad software package (Graphpad Software Inc, San Diego CA). Statistical significance was declared if p<0.05.
CHAPTER 4

EXPERIMENTAL STUDIES
4. EXPERIMENTAL STUDIES

4.1. Study 1: The Effect of Exendin 9-39 on Food Intake

4.1.1 Introduction

GLP-1 decreases food intake in the rat when administered centrally but not when given intraperitoneally (Turton et al 1996; Table 2.1). Because the half-life of GLP-1 is so limited, it is not surprising that food intake is unaltered after a single intraperitoneal bolus. However, continuous intravenous infusion of GLP-1 in human volunteers is shown to reduce appetite and food intake (Nauck et al 1999; Table 2.2.). Furthermore, the long acting GLP-1 agonist Exendin 4 decreases food intake in rats when administered peripherally, although central injection causes a greater decrease (Bhavsar et al 1998).

The effect on food intake of antagonising the GLP-1 receptor with peripheral Exendin 9-39 injections has not yet been reported. Central injections of Exendin 9-39 increase food intake in the satiated rat but not in the fasted rat (Turton et al 1996). Therefore, the objective of the present study was to determine the effect of antagonising GLP-1 receptors by intraperitoneal injection of Exendin 9-39 on food intake in the rat. Two separate feeding schedules were tested as the fed or unfed state of the rat has been shown to be influential in the rats feeding response to Exendin 9-39 (Turton et al 1996).

4.1.2 Study Design

Five minutes prior to the dark cycle, rats were injected with Exendin 9-39 (20 μg in 1.1 and 1.2 and 50 μg in 1.3) or saline. Food cups were available at all times in experiment 1.1 and during the dark cycle only in experiments 1.2 and 1.3.

A naïve set of 15 rats weighing 216 ± 2 g was used at the start of experiment 1.1.
A naïve set of 21 rats weighing 224 ± 4 g was used in experiment 1.2 and the same set was used again in experiment 1.3 (weighing 243 ± 2 g at the start of experiment 1.3).

**4.1.3 Results**

Food intake was not different between the saline and Exendin 9-39 treatments in any of the experiments (Table 4.1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>0-1h</th>
<th>1-2h</th>
<th>2-2h</th>
<th>2-24 h</th>
<th>0-24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 Hour Fed²</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 µg</td>
<td></td>
<td>1.9 ± 0.2¹</td>
<td>2.4 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>20.3 ± 0.6</td>
<td>24.6 ± 0.6</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>2.0 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>20.5 ± 0.5</td>
<td>24.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-1h</td>
<td>1-2h</td>
<td>2-3h</td>
<td>0-3h</td>
<td>3-14h</td>
</tr>
<tr>
<td><strong>Dark Cycle Feeding³</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 µg</td>
<td></td>
<td>2.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>18.7 ± 0.5</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>2.2 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>19.0 ± 0.6</td>
</tr>
<tr>
<td>Experiment 1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50 µg</td>
<td></td>
<td>1.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>19.6 ± 0.6</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>2.1 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>20.0 ± 0.5</td>
</tr>
</tbody>
</table>

¹ Mean ± SEM (g); n=15. ³21
No significant difference at any time point

**4.1.4 Discussion**

Intraperitoneal injection of Exendin 9-39 did not alter food intake, which is in contrast to reports that central injection of Exendin 9-39 increases food intake. The results may be called into question on the basis that little is known about the absorption of the peptide intraperitoneally. However, Exendin 9-39 is likely well absorbed as the
much larger insulin peptide is absorbed into circulation quite efficiently after intraperitoneal administration (Kelly et al 1996; Wideroe et al 1996; Radziuk et al 1994). Therefore, some other factor must be at play in the lack of effect of Exendin 9-39 on food intake in the present experiments.

The design of all three experiments was such that rats were probably hungry at the time of injection possibly eliminating an Exendin 9-39 feeding response. Although the rats had access to the food cups at all times in experiment 1.1, they would have eaten very little during the day, with a small meal in the afternoon being the major intake (Johnson et al 1979). The rats in experiments 1.2 and 1.3 were prevented from eating during the day by removal of food cups. Because only the satiated rat has been reported to increase food intake with central Exendin 9-39 injection (Turton et al 1996), it is possible that Exendin 9-39 given peripherally would also increase food intake in rats consuming food.

Therefore, the next studies examined food intake after delivery of controlled amounts of nutrient preloads in conjunction with Exendin 9-39 injections. Because glucose stimulates GLP-1 release in the rat (Ritzel et al 1997) it was co-administered with Exendin 9-39.
4.2. Study 2: The Effect of Exendin 9-39 on the Food Intake Suppression of a Glucose Preload

4.2.1 Study Design

The timing of delivery and size of the glucose preload, and the timing and number of Exendin 9-39 injections were varied in three separate experiments. All rats received a glucose preload. The treatment, Exendin 9-39 was given at 20 μg per injection.

In Experiment 2.4, Exendin 9-39 was injected at 35 minutes and 1 g of glucose given 30 minutes before access to food cups.

In Experiment 2.5, rats received Exendin 9-39 injections at 20 and 5 minutes and 2 g of glucose at 15 minutes prior to feeding.

In experiment 2.6, Exendin 9-39 was injected at 35 and 5 minutes and 1 g of glucose given 30 minute before access to food cups.

A naïve set of twelve rats was used in experiment 2.4 (weighing 223 ± 2 g at the start of the experiment) and the same set used again in experiment 2.5 (weighing 285 ± 7 g at the beginning of the experiment). A new set of eight rats was used in experiment 2.6 (weighing 282 ± 6 g).

4.2.2 Results

Food intake was not altered by Exendin 9-39 in experiment 2.4 (Figure 4.1), or in 2.5 (Figure 4.2). There was a trend towards decreased food intake with Exendin 9-39 during the first 30 minutes of feeding in experiment 2.4 (p<0.08), but no change in food intake at 2-14 hours (19.9 ± 0.5 g vs. 19.4 ± 0.6 g).

In experiment 2.6, food intake was significantly decreased by Exendin 9-39 (p<0.001) at 0-1 hours (Figure 4.3) but not at any other time point. However, there was
Figure 4.1 Experiment 2.4 Exendin 9-39 and Glucose (1g) no significant differences were observed.
Figure 4.2 Experiment 2.5 Exendin 9-39 and 2 g glucose
No differences in food intake were seen at any time.
Figure 4.3 Experiment 2.6 Food Intake after Exendin 9-39 (2 injections) and Glucose Preload (1g) *p<0.001
a trend for the Exendin 9-39 treatment to result in greater food intake at 1-2 h (p<0.07) in experiment 2.6.

4.2.3 Discussion

Because GLP-1 (Meeran et al 1999) and its agonist, Exendin 4 (Navarro et al 1996) have been reported to decrease food intake, it was expected that blocking its action would increase food intake. Decreased food intake with Exendin 9-39 in the first hour in experiment 2.6 is contrary to the hypothesis that Exendin 9-39 would increase food intake after glucose preload by antagonising a potential GLP-1 mediated satiety signal.

On the other hand, blocking GLP-1 action may lead to other metabolic effects leading to decreased food intake. Exendin 9-39 decreases the release of insulin in response to a glucose preload resulting in elevated plasma glucose (Tseng et al 1999). Elevations in plasma glucose delay feeding in the rat (Campfield et al 1985). Therefore, Exendin 9-39 may be indirectly decreasing food intake in the presence of a glucose preload by decreasing plasma insulin and thus increasing plasma glucose concentrations.

In light of the opposing findings in food intake behaviour between peripheral and central administration (Meeran et al 1999) of Exendin 9-39, it is likely that intraperitoneal Exendin 9-39 is not stimulating the same feeding regulatory areas as ICV administration.

4.3. Study 3: Effect of Exendin 9-39 on Glucose, Corn Oil or Albumin Induced Food Intake Suppression and on Macronutrient Selection

4.3.1 Study Design

To determine if the effects of Exendin 9-39 on food intake occur only when co-administered with glucose, three separate experiments were conducted with preloads of
glucose (1g), corn oil (1.5g) and albumin (0.5g) (Experiments 3.7, 3.8 and 3.9 respectively). The nutrient preload doses were chosen based on prior studies done in this lab (Trigazis et al 1997). Preloads chosen were similar in their effect on decreasing subsequent food intake (as detailed in Appendix A). Additionally, the effect of Exendin 9-39 on the selection of two diets varying greatly in carbohydrate and protein content was investigated in a separate experiment.

Experiments 3.7-3.9 used the same protocol as in experiment 2.6 except for a change in lighting cycle to a 12:12 hour light dark cycle. Therefore, rats received injections at 35 and 5 minutes and the nutrient preload at 30 minutes before feeding.

In Experiment 3.10, rats were allowed to select between two food cups containing 0% and 50% protein. Treatment was a single Exendin 9-39 injection of 50 μg injected five minutes before feeding. The dose of Exendin 9-39 was increased as only one injection was given and prior experiments showed that a single 20 μg injection did not affect food intake with or without nutrient preload.

A naive set of 20 rats was used at the beginning and experiments 3.7-3.9 were performed on this same set of rats. The twenty rats were divided into two groups of 10, receiving preload experiments in the order of glucose, albumin and corn oil (Group A) or corn oil, albumin and glucose (Group B). Group A rats weighed 225 ± 4 g at the start of the glucose preload experiment and 326 ± 5 g at the start of the corn oil preload experiment. Group B rats weighed 326 ± 7 g at the start of the glucose preload study and 227 ± 3 g at the start of the corn oil preload study. The albumin study was done simultaneously on both groups and combined weights were 294 ± 4.1 g. A new set of fourteen rats (weighing 394 ± 7 g) was used in experiment 3.10.
4.3.2 Results

Exendin 9-39 decreased food intake after the glucose gavage (3.7) during the second hour of feeding (Table 4.2). However, Exendin 9-39 had no effect on food intake after either corn oil or albumin preloads (3.8 & 3.9). No differences in food intake were detected at any other time points in any of the preload treatment groups.

Table 4.2 Food Intake after Exendin 9-39 and Nutrient Preloads

<table>
<thead>
<tr>
<th>Preload</th>
<th>Time(h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1h</td>
</tr>
<tr>
<td>Exp 3.7</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Ex 9-39</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Exp. 3.8</td>
<td></td>
</tr>
<tr>
<td>Corn Oil</td>
<td></td>
</tr>
<tr>
<td>Ex 9-39</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Exp. 3.9</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td>Ex 9-39</td>
<td>1.7 ± 0.2$^1$</td>
</tr>
<tr>
<td>Saline</td>
<td>1.7 ± 0.3</td>
</tr>
</tbody>
</table>

$^1$Mean ± SEM (g); n=20  
$^2$data not available (n/a)  
Significantly different than saline *p<0.05

In Experiment 3.10, rats selected 32 ± 2% protein (by weight) on average over the five baseline days. This is consistent with past selection patterns of rats fed similar diet choices (Musten et al 1974). Exendin 9-39 decreased total food intake over the cumulative measures of 0-2 h and 0-14 h and approached statistical significance for each of the 0-1 and 1-2 h intervals (Table 4.3). Because the food intake was similar between saline and Exendin 9-39 treatments at 2-14 h, the primary effect of Exendin 9-39 in total daily food intake was in the first two hours.
Table 4.3 Experiment 3.10, Effect of Exendin 9-39 on Macronutrient Selection and Total Food Intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-30min</th>
<th>30-1hr</th>
<th>0-1h</th>
<th>1-2h</th>
<th>0-2h</th>
<th>2-14h</th>
<th>0-14h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex 9-39</td>
<td>0.5 ± 0.2*</td>
<td>0.1 ± 0.1</td>
<td>0.6 ± 0.2**</td>
<td>0.4 ± 0.2</td>
<td>1.1 ± 0.2*</td>
<td>9.4 ± 1.1</td>
<td>10.4 ± 1.2</td>
</tr>
<tr>
<td>Saline</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>8.7 ± 0.7</td>
<td>10.7 ± 1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-30min</th>
<th>30-1hr</th>
<th>0-1h</th>
<th>1-2h</th>
<th>0-2h</th>
<th>2-14h</th>
<th>0-14h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex 9-39</td>
<td>2.2 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>10.1 ± 0.9</td>
<td>13.0 ±1.0*</td>
</tr>
<tr>
<td>Saline</td>
<td>2.0 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>2.7 ± 0.5</td>
<td>0.9 ± 0.3</td>
<td>3.5 ± 0.5</td>
<td>10.8 ± 0.6</td>
<td>14.4 ± 0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-30min</th>
<th>30-1hr</th>
<th>0-1h</th>
<th>1-2h</th>
<th>0-2h</th>
<th>2-14h</th>
<th>0-14h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex 9-39</td>
<td>2.7 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>4.0 ±0.3**</td>
<td>19.5 ± 0.6</td>
<td>23.5 ±0.7*</td>
</tr>
<tr>
<td>Saline</td>
<td>3.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>19.5 ± 0.5</td>
<td>25.0 ± 0.6</td>
</tr>
</tbody>
</table>

1 Mean ± SEM (g); n=14
Significantly different than saline* p<0.05.  ** p<0.01

Exendin 9-39 significantly decreased intake of the 0% protein diet at the 0-30 min, 0-1 h, 0-2 h and 0-14 h intervals. Cumulative intake of the 50% protein diet was decreased only for the 0-14 h time interval. The daily percent of protein by weight selected over the two experimental days was 28 ± 2 % with Exendin 9-39 treatment and 29 ± 2 % with saline, while baseline selection was 32 ± 2 % protein.
4.3.3 Discussion

The experiments continue to show that Exendin 9-39 decreases food intake after carbohydrate preloads and that it causes a selective decrease in intake of a carbohydrate diet compared with a high protein diet.

The lack of effect of Exendin 9-39 on food intake after the albumin preload is consistent with the literature. Albumin hydrolysate but not whole albumin is cited as increasing plasma GLP-1 concentrations in the rat (Cordier-Bussat 1998) suggesting that an interaction between whole albumin and Exendin 9-39 would not be expected.

Monounsaturated fats and corn oil (Roberge et al 1993) stimulate GLP-1 release (Brubaker et al 1995), yet Exendin 9-39 failed to modify corn oil induced food intake suppression. Food intake after Exendin 9-39 or saline paired with a corn oil preload at the 0-2 h interval was $3.9 \pm 0.3$ vs. $4.5 \pm 0.4$ g. A sample size calculation ($n= (1.96/difference \text{ in food intake})^2 (\text{variance}_1 + \text{variance}_2)$) (Mendenhall & Sincich 1992) revealed that a sample of 50 rats would be required to demonstrate a significant difference in food intake. However, such a large sample size demonstrates the relative unimportance of any effect of Exendin 9-39 on corn oil induced food intake suppression.

The decrease in food intake with Exendin 9-39 and glucose preload was observed an hour later in experiment 3.7 (12:12 hour light dark cycle) than in 2.6 (10:14 hour light dark cycle). This difference in timing may be due to the shorter dark cycle (and resultant shorter period of feeding) used in experiment 3.7 than in experiment 2.6. This difference in lighting cycle may have resulted in lower plasma glucose concentrations at the onset of feeding in experiment 3.7. If Exendin 9-39 induced increases in plasma glucose contributed to decreased food intake, then a lower fasting
plasma glucose would require greater food intake to bring plasma glucose to levels capable of decreasing food intake.

Exendin 9-39 reduced the total food intake of rats given choice but the primary effect was in the 0-2 h period, and primarily due to decreased intake of the carbohydrate diet. Because the effects of Exendin 9-39 are known to occur within a relatively short period of time, generally within 1/2 hour of administration (Edwards et al 1999, Tseng et al 1999), the early selectivity occurred during the known time course of biological action of Exendin 9-39 (half life 30 minutes as per Edwards et al 1999). There was an overall decrease in intake of the 50% diet, an effect that occurred primarily in the 2-14 h feeding period, and after the expected duration of Exendin 9-39 activity.

4.4. Study 4: The Effect of Exendin 4 on Macronutrient Selection and on Glucose Induced Food intake Suppression

4.4.1 Introduction

Intraperitoneal administration of Exendin 4 potently reduces food intake (Bhavsar 1997). Therefore, Exendin 4 was used to test the hypothesis that GLP-1 functions in specifically regulating intake of carbohydrate.

4.4.2 Study Design

Three doses of Exendin 4 (20, 10 or 0.5 µg) were tested in separate experiments (4.11 - 4.13 respectively). Rats received either an Exendin 4 or a saline injection five minutes prior to selecting between two food cups containing 0% and 50% protein. In experiment 4.14, rats received Exendin 4 (0.5 µg) or saline injections 5 min before glucose glucose gavage (1 g), and the 0% and 50% protein food cups were given 30 minutes after the gavage. Experiment 4.15 followed the same protocol as 4.14 except rats were presented with a single food cup containing the AIN 93G powder diet.
A naive set of fourteen rats was used in experiment 4.11. The same set of rats was used for experiments 4.12 – 4.14. One rat was excluded from experimentation due to consistent avoidance of the 0% protein diet, leaving 13 rats for use in experiments 4.11-4.14. A separate set of fourteen rats was used in experiment 4.15. The weights of rats at the beginning of experiments 4.11 – 4.15 were $231 \pm 6 \text{ g}$, $287 \pm 7 \text{ g}$, $324 \pm 8 \text{ g}$, $352 \pm 8 \text{ g}$ and $370 \pm 8 \text{ g}$ respectively.

4.4.3 Results

The average food intake over four days of baseline measurement was $8.6 \pm 1.0 \text{ g}$ of the 0% protein diet and $14.4 \pm 0.8 \text{ g}$ of the 50% protein diet. The rats selected an average of $31 \pm 3\%$ protein by weight over the baseline period. This is consistent with experiment 3.10.

Daily (0-14 h) food intake was reduced by approximately one-half with Exendin 4 treatments of 10 and 20 μg (Table 4.4.). Exendin 4 (20 μg) reduced food intake during the 0-30, 0-1, 2-14 and 0-14 h intervals. Similarly, 10 μg of Exendin 4 decreased food intake at 0-30, 0-1, 0-2, 2-14 and 0-14 h. Because it was observed that some rats were lethargic after these doses, a much lower dose of 0.5 μg was used in experiment 4.13.

The 0.5 μg Exendin 4 treatment (experiment 4.13) decreased total food intake at 0-30, 1-2, 0-1, 0-2 and 0-14 hours with no lethargy observed. When administered with glucose preload in experiment 4.14, Exendin 4 (0.5 μg) depressed total food intake at 1-2 h which was reflected in a decreased food intake at 0-2 and 0-14 hours. When the single AIN 93G diet cup was provided following the glucose gavage in experiment 4.15, a decrease in food intake occurred at the 2-14 and 0-14 h intervals.
Table 4.4 Effect of Exendin 4 on Total Food Intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-30 min</th>
<th>30-1h</th>
<th>0-1h</th>
<th>1-2h</th>
<th>0-2h</th>
<th>2-14h</th>
<th>0-14h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Two Cup Selection (0&amp;50%protein) g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 μg</td>
<td>0.9 ±0.1**</td>
<td>1.2 ±0.2</td>
<td>2.1 ±0.3*</td>
<td>1.3 ±0.3</td>
<td>3.4 ±0.4</td>
<td>7.3 ±0.9**</td>
<td>10.7± 0.8**</td>
</tr>
<tr>
<td>Saline</td>
<td>2.8 ±0.4</td>
<td>0.8 ±0.2</td>
<td>3.5 ±0.3</td>
<td>1.1 ±0.3</td>
<td>4.6 ±0.4</td>
<td>17.5±1.0</td>
<td>22.1±1.0</td>
</tr>
<tr>
<td>Experiment 4.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μg</td>
<td>0.8 ±0.1**</td>
<td>1.0 ±0.1</td>
<td>1.8 ±0.2**</td>
<td>1.1 ±0.2</td>
<td>3.0 ±0.3**</td>
<td>8.6 ±0.5**</td>
<td>11.5± 0.7**</td>
</tr>
<tr>
<td>Saline</td>
<td>2.6 ±0.2</td>
<td>1.1 ±0.3</td>
<td>3.7 ±0.2</td>
<td>1.8 ±0.3</td>
<td>5.5 ±0.3</td>
<td>18.0±0.9</td>
<td>23.4±0.8</td>
</tr>
<tr>
<td>Experiment 4.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 μg</td>
<td>2.7 ±0.2**</td>
<td>1.2 ±0.1</td>
<td>4.0 ±0.3**</td>
<td>0.9 ±0.2**</td>
<td>4.7 ±0.4**</td>
<td>15.2±0.9</td>
<td>19.2± 1.3**</td>
</tr>
<tr>
<td>Saline</td>
<td>4.4 ±0.3</td>
<td>1.2 ±0.3</td>
<td>5.5 ±0.3</td>
<td>1.7 ±0.3</td>
<td>7.3 ±0.4</td>
<td>15.2±0.9</td>
<td>22.5±0.7</td>
</tr>
<tr>
<td>Experiment 4.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 μg+glucose</td>
<td>2.1 ±0.2</td>
<td>1.2 ±0.2</td>
<td>3.3 ±0.3</td>
<td>1.0 ±0.2**</td>
<td>4.3 ±0.3**</td>
<td>15.1±0.7</td>
<td>19.4± 0.7*</td>
</tr>
<tr>
<td>Saline+glucose</td>
<td>2.3 ±0.3</td>
<td>1.7 ±0.3</td>
<td>4.0 ±0.4</td>
<td>2.1 ±0.3</td>
<td>6.1 ±0.5</td>
<td>15.0±0.9</td>
<td>21.1± 0.6</td>
</tr>
<tr>
<td><strong>AIN 93G Diet g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 μg+glucose</td>
<td>1.9 ±0.2</td>
<td>1.3 ±0.2</td>
<td>3.2 ±0.4</td>
<td>1.4 ±0.3</td>
<td>4.5 ±0.4</td>
<td>17.2±0.6**</td>
<td>21.7± 0.6**</td>
</tr>
<tr>
<td>Saline+glucose</td>
<td>2.2 ±0.2</td>
<td>0.8 ±0.3</td>
<td>3.0 ±0.3</td>
<td>1.2 ±0.2</td>
<td>4.2 ±0.3</td>
<td>19.5±0.6</td>
<td>23.8± 0.6</td>
</tr>
</tbody>
</table>

1 Mean ± SEM; food intake (g)
significantly different than saline *p<0.05,**p<0.01
The highest dose of Exendin 4 (20 µg experiment 4.11) significantly increased intake of the 0% protein diet in the first two hours, but this effect was absent with all other treatments (Table 4.5). A decreased intake of the 0% protein diet was observed during the 2-14 h interval after both the Exendin 4 10 & 20 µg treatments. Intake of the 0% protein diet was not altered by 0.5 µg of Exendin 4 (experiment 4.13).

Intake of the 50% protein diet was decreased by Exendin 4 at all times except for 30-1 and 1-2 h after the 10 & 20 µg treatments (4.11 & 4.12). With 0.5 µg of Exendin 4 (4.13), intake of the 50% protein diet was decreased during the first 0-30 minute time period and carried through to the 0-1, 0-2 and 0-14 h intervals. When Exendin 4 (0.5 µg) was given with a glucose preload (4.14), intake of the 50% protein diet was decreased at the 30-1 and 1-2 h time points and carried through in the 0-1, 0-2 and 0-14 h intervals.
Table 4.5 Effect of Exendin 4 on Macronutrient Selection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-30min</th>
<th>30-1h</th>
<th>Time(h) 0% Protein Diet</th>
<th>Time(h) 50% Protein Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-1h</td>
<td>0-1h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-2h</td>
<td>0-2h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-14h</td>
<td>0-14h</td>
</tr>
<tr>
<td>Experiment 4.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 µg</td>
<td>0.5 ± 0.1(^1)</td>
<td>0.9 ± 0.2(*)</td>
<td>1.4 ± 0.3</td>
<td>1.0 ± 0.3(*)</td>
</tr>
<tr>
<td>Saline</td>
<td>0.6 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Experiment 4.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µg</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Experiment 4.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 µg</td>
<td>1.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>1.7 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Experiment 4.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 µg+Gluc</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2(†)</td>
<td>2.0 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Saline+Gluc</td>
<td>1.0 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>1.5 ± 0.4</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SEM; intake (g); n=13; significantly different from saline\(*p<0.05, **p<0.01, \(†p<0.07\).
The daily percent protein selected by weight during experimental days were generally within ranges seen during the baseline period (Table 4.6).

Table 4.6 Protein Concentration Selected by Weight with Exendin 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 4.11</td>
<td></td>
</tr>
<tr>
<td>20 µg</td>
<td>30 ± 2(^1)</td>
</tr>
<tr>
<td>Saline</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Experiment 4.12</td>
<td></td>
</tr>
<tr>
<td>10 µg</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Experiment 4.13</td>
<td></td>
</tr>
<tr>
<td>0.5 µg</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Experiment 4.14</td>
<td></td>
</tr>
<tr>
<td>0.5 µg+glucose</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>31 ± 2(^1)</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM

4.4.4 Discussion

The results of this study show that Exendin 4 potently decreases food intake and that the effect is directed mainly to the protein component of the diet. When rats were allowed to select between a high protein or high carbohydrate diet and given an amount of Exendin 4 (0.5 µg) that did not cause obvious lethargy, intake of the high protein diet, but not the carbohydrate diet was decreased. This was surprising given that ICV GLP-1 has an inhibitory action on NPY (Turton et al. 1996; Tritos et al. 1998) which is known to increase carbohydrate intake (Leibowitz 1992). Therefore, it was expected that intake of the carbohydrate diet would decrease with the Exendin 4 agonist.

Only at very high doses (20 and 10 µg) of Exendin 4 was the intake of the carbohydrate diet affected. A brief increase in intake of the carbohydrate diet occurred
in the first two hours with the largest amount of Exendin 4 given (20 μg), followed by a decrease in intake at 2-14 h (decrease also seen with 10 μg). However, the large doses likely created physiological and behavioural effects that may have impacted on feeding and so did not reflect the true role of Exendin 4 in macronutrient selection. For example, Exendin 4 has been shown to increase plasma insulin concentrations in fasted animals (Kolligs et al 1995; Greig et al 1999). It may be that rats increased their carbohydrate intake in response to an insulin influx that lowered plasma glucose concentrations in response to such large amounts of Exendin 4.

Behavioural effects induced by the large dose Exendin 4 treatments were clearly evident and may have impacted on feeding behaviour. Although not quantitatively measured, clear behavioural abnormalities were observed after 10 & 20 μg of Exendin 4. Rats became listless; moving little compared to control rats. They tended to sit in position above the food cups sluggishly attempting to eat, and did not move to the back of the cage during measurement of food intake but did so with the saline treatment. In fact, they sometimes had to be picked up off of the food cups and moved in order to obtain measurements. Consistent with these observations, others have found that ICV administration of a large amount of GLP-1 (10 μg) reduced locomotor activity as defined by frequency of line-crossing, to 41% of control activity (Turton et al 1996). These behavioural effects were not obvious when the dosage of Exendin 4 was reduced to 0.5 μg.

In order to examine the importance of glucose disposal in modifying food intake after Exendin 4, rats were given a glucose preload along with Exendin 4. If Exendin 4 induced alterations in glucose disposal were influencing food intake, it could be assumed
that intake of the carbohydrate diet would be affected. However, when the 0.5 μg dose was combined with glucose preload, the only effect observed was a decrease in intake of the protein diet that was slightly delayed from 0-30 minutes without glucose to 30 minutes- 1h with the glucose preload.

Substituting the AIN 93G diet for the two food cup selection paradigm resulted in a further time delay in the decrease in food intake caused by Exendin 4 with glucose preload. The AIN 93G diet is approximately 18% protein by weight. The rats tended to select between 30-34% protein in the two cup selection design. Therefore, they were consuming a lower protein diet in the AIN 93G experiment. Perhaps, the cumulative small reduction in intake of AIN 93G diet was also based on its protein content, but because of the low protein content of the AIN 93G diet, was only apparent over a longer time of 2-14 hours.

Finally, the decrease in food intake induced by all doses of Exendin 4 on test day was not compensated for over the next two days. In fact, a continued decrease in food intake on the first day following administration of 10 μg of Exendin 4 was observed. Also, there was a trend towards decreased intake on the first washout day following 20 μg Exendin 4 treatment (Figure 4.6). Others have found that repeated long-term administration of Exendin 4 has resulted in significant losses of body weight in diabetic fatty rats (Young et al 1999; Szayna et al 2000).
Figure 4.6 Food Intake on Days following Exendin 4 Administration

*p<0.001 n=11 (0.5 & 10 ug) and n=10 (20 ug)
4.5. Study 5: Effect of Exendin 4 or Exendin 9-39 on the Plasma Glucose and Insulin Response to a Glucose Challenge

4.5.1 Introduction

To explore the links between the metabolic effects of Exendin 4 and Exendin 9-39 and their effects on feeding behaviour, plasma glucose and insulin responses are of interest. Exendin 9-39 increases plasma glucose and decreases insulin in the rat (Tseng et al 1999). Exendin 4 increases fasted insulin levels in the rat (Kolligs et al 1995).

4.5.2 Study Design

Animals were fasted for at least 6 hours prior to administration of an oral glucose tolerance test. Rats received an injection of saline or peptide five minutes prior to administration of a 1 g glucose preload. Blood collection times are indicated as minutes after administration of the glucose gavage. The times for blood sampling were based on the time intervals when Exendin 9-39 and Exendin 4 exerted their effects on food intake in other experiments. Rats were divided into groups based on treatment and time of blood collection and were euthanized after sampling.

In Experiment 5.16, an injection of 0.5 μg Exendin 4 or saline was given. Twenty-three rats (weighing 369 ± 8 g) were divided into the following groups: 30 minutes blood collection (saline n=4, Exendin 4 n=5) and 75 minutes blood collection (saline n=5 Exendin 4 n=5). A single baseline group receiving neither injection nor gavage was also tested (n=4).

In experiment 5.17, thirteen rats (weighing 436 ± 4 g) were divided into two groups based on one 15-minute blood sampling post gavage, one receiving 50 μg Exendin 9-39 (n=6) and the other receiving saline (n=7).
4.5.3 Results

In Experiment 5.16, there was an overall treatment effect on plasma glucose after Exendin 4 treatment (p<0.0005, F 8.84). Plasma glucose in the baseline group was 164.5 ± 3.1 mg/dl. There was no difference in plasma glucose between Exendin 4 and saline groups at 30 minutes (228 ± 20 mg/dl vs. 205 ± 7 mg/dl). However, at 75 minutes, Exendin 4 administration resulted in significantly higher plasma glucose (238 ± 2 mg/dl) than saline (192 ± 9 mg/dl p<0.05, figure 4.5). Plasma insulin concentrations were not affected at any time point by Exendin 4 treatment. Baseline insulin concentration was 0.75 ± 0.05 ng/ml. Plasma insulin concentrations were not different between Exendin 4 treatment versus saline at either 30 minutes (1.30 ± 0.30 ng/ml vs. 1.20 ± 0.2 ng/ml) or at 75 minutes (1.22 ± 0.1 ng/ml vs. 1.30 ± 0.2 ng/ml, figure 4.6).

In experiment 5.17, there was no effect of Exendin 9-39 treatment at 15 minutes on plasma glucose (206 ± 13 mg/dl vs. 197 ± 13 mg/dl saline), nor on plasma insulin concentrations (2.1 ± 0.5 ng/ml vs. 3.1 ± 0.6 ng/ml) of 50 µg Exendin 9-39 vs. saline respectively (figure 4.7).

4.5.4 Discussion

The expected effects of Exendin 4 and Exendin 9-39 on plasma insulin and glucose were not observed. Exendin 4 resulted in an increase in plasma glucose, but no effect on plasma insulin, while no effect on plasma glucose or insulin was observed with Exendin 9-39 treatment. It was expected that the agonist, Exendin 4, would increase plasma insulin and decrease plasma glucose concentrations, and conversely, that the antagonist, Exendin 9-39, would decrease plasma insulin and increase plasma glucose concentrations for several reasons. First, GLP-1 has an insulinotropic action (Drucker
Figure 4.5 Experiment 5.16 The Effect of Exendin 4 on Plasma Glucose. * p<0.05
Figure 4.6 Experiment 5.16 The effect of Exendin 4 on Plasma Insulin. No significant difference at any time point.
Figure 4.7 Experiment 5.17 The Effect of Exendin 9-39 on Plasma Glucose and Insulin at 15 minutes. No significant effect was observed.
1998). Second, Exendin 4 treatments have been shown to increase fasting plasma insulin concentrations and improve HbA1c in diabetic rats (Greig et al 1999, Young et al 1999), while Exendin 9-39 was shown to increase plasma glucose and decrease plasma insulin response to a glucose challenge (Tseng et al 1999).

The surprising finding that the agonist of the GLP-1 receptor, Exendin 4 increased plasma glucose concentrations may relate to a possible decrease in gastric emptying of the glucose preload. Exendin 4 administration inhibits gastric emptying (Wettergren et al 1993). The presence of nutrients in the intestine is influential in the plasma insulin and glucose response to GLP-1. Although no information is available on the rat, studies of humans have reported that plasma insulin concentrations decrease in response to GLP-1 infusion after a mixed meal. Inconsistent with the decrease in plasma insulin was a decrease, rather than increase, in plasma glucose concentration (Nauck et al 1997). Others have found decreases in plasma glucose (Fineman et al 1999; Buse et al 1999) but did not report on plasma insulin response to a Sustacal™ meal in the presence of synthetic Exendin 4. Therefore, the direction of the plasma insulin and glucose response to Exendin 4 may also vary depending on the presence or absence of nutrients in the intestine. Therefore, decreased gastric emptying and subsequent delayed absorption of glucose due to Exendin 4 administration may have contributed to the lack of insulin response and to the later increase in plasma glucose seen here.

Intraperitoneal injections of Exendin 9-39 have been reported to increase plasma glucose concentrations in response to luminal carbohydrate in the rat (Tseng et al 1999) but had no effect in the present study. A few aspects of the experimental design used here may have decreased the ability to detect changes in insulin and glucose in response
to Exendin 9-39 and glucose challenge. Prior studies have used smaller glucose preloads based on the rats' weight, and the increases in plasma glucose detected were in the order of 11.8 (Tseng et al 1999) and 23 mg/dl (Kolligs et al 1995) at 15 and 20 minutes post glucose administration respectively. The bolus glucose preload, used here to simulate the plasma insulin and glucose response of the rat during the consumption of a small meal, may have increased variability in plasma insulin and glucose response. Differences in body weight between individual rats combined with a single bolus glucose preload translate into varying g/kg glucose dosages. Therefore, a combination of small sample size (n=6 or 7 per group), possible increased variability in response to a bolus preload and a small expected effect of Exendin 9-39 on insulin and glucose, are possible factors in the lack of significant outcome in Experiment 5.17.
CHAPTER 5

GENERAL DISCUSSION
5. GENERAL DISCUSSION

The results of this research do not support the hypothesis that GLP-1 is a regulator of carbohydrate intake in rats. Overall, however, the results of the study support a role for GLP-1 in the regulation of food intake and diet selection. The GLP-1 antagonist, Exendin 9-39, decreased food intake when given with a glucose preload and selectively decreased intake of a carbohydrate diet when rats were allowed to choose between a high carbohydrate or high protein diet. However, the GLP-1 agonist, Exendin 4 decreased food intake with evidence of selectivity against the protein but not the carbohydrate component of the diet.

The original hypothesis of this research was based on evidence that GLP-1 suppresses food intake (Turton et al 1996; Meeran et al 1999), increases glucose disposal (Tseng et al 1999) and reduces the action of NPY (Tritos et al 1998), a peptide that selectively increases carbohydrate intake (Leibowitz et al 1992). Therefore, blocking the action of GLP-1 was expected to increase food intake in the presence of a carbohydrate preload and enhance selection of carbohydrate. Certainly, the glucoregulatory properties and the rapid secretory response to carbohydrates would seem to make GLP-1 an ideal candidate for alerting feeding regulatory centers to the availability of carbohydrate.

The lack of results in support of the original hypothesis may have been due to a couple of issues. First of all, indirect actions unrelated to direct effects on centers of food intake regulation, of Exendin 9-39 and Exendin 4 may have impacted on the feeding response. For example, effects on gastric emptying and pancreatic secretion, plasma glucose, and interactions with other peptides are possible contributors to the
results obtained. Secondly, a recent report indicates that the peptide agonist and antagonist used here may not have mediated vagal responses associated to GLP-1.

Possibly, slowing of protein digestion and absorption due to Exendin 4 is the cause of the selective decreases in protein intake. GLP-1 decreases gastric acid secretion, gastric emptying and pancreatic secretion (Wettergren et al 1998). As such, Exendin 4 may have decreased the rate of digestion and absorption of protein specifically, increasing the amount of time intact/peptide forms of protein are present in the intestine. A prolonged presence of protein and peptides in the intestine would be predicted to prolong the release of CCK (Douglas et al 1988). Because CCK mediates the satiating effects of certain proteins (Trigazis et al 1997), it is possible that Exendin 4 indirectly stimulates a decrease in protein intake via this mechanism. Although gastric emptying may be an important factor in mediating feeding behaviour, its importance as the mechanism responsible for GLP-1 induced decreases in feeding is uncertain. Damage to the arcuate nucleus and sensory circumventricular organs by monosodium glutamate administration to rats completely abolish the inhibitory effect of central GLP-1 on feeding behaviour (Tang-Christensen et al 1998). However, this effect in abolishing GLP-1 action on feeding may still involve gastric emptying as GLP-1 is thought to mediate gastric emptying via a central mechanism (Imeryuz et al 1997).

Plasma insulin concentration has been associated with changes in food intake but no evidence was obtained to support its role in this capacity in this thesis. Exendin 9-39 was predicted to decrease (Kolligs et al 1995) and Exendin 4 was predicted to increase (Greig et al 1999, Young et al 1999) plasma insulin concentrations but neither had effect in the present work. Elevated insulin concentrations in plasma are associated with
decreased food intake. Insulin crosses the blood brain barrier and gains access to neurons containing insulin receptors that are important in the control of feeding and metabolism (Woods et al 1996). Macronutrient intake may also be influenced by insulin, as strong increases in hypothalamic immuno-reactive insulin (microdialysis measure) occur in response to carbohydrate but not protein ingestion (Gerozissis et al 1998). However, since no alterations in plasma insulin were detected in either experiment 5.16 or in 5.17 involving Exendin 4 and Exendin 9-39 respectively, insulin may not be a strong influence on the present findings.

Plasma glucose concentrations influence food intake and were expected to increase with Exendin 9-39 (Kolligs et al 1995, Tseng et al 1999) and decrease with Exendin 4 (Greig et al 1999, Young et al 1999). Small decreases in blood glucose are noted prior to meal initiation in rats (Campfield et al 1985). Glucose sensitive and glucose responsive brain neurons alter their firing rate in response to fluctuations in brain glucose. Such neurons are found in the VMH, LH and nucleus of the solitary tract, areas known to alter feeding behaviour. Alterations of plasma glucose of 2mM alter brain glucose levels by 0.2 - 0.3 mM within 10 minutes and are associated with changes in firing of glucose sensing neurons (Silver et al 1998 as cited in Levin et al 1999). Peripheral glucose levels stimulate portal vein glucosensors that, in turn, provide direct neural input (Hevener et al 1997 as cited in Levin et al 1999). As well, reductions in cellular glucose uptake associated with decreased insulin release may lead to increased food intake. For instance, glucoprivation induced by 2-deoxyglucose leads to increases in food intake (Thompson and Campbell 1977). Yet no effect of Exendin 9-39 on plasma glucose was observed here. However, increases in plasma glucose after testing
rats with Exendin 9-39 are small (Tseng et al 1999, Kolligs et al 1995), and difficult to detect (Tseng personal communication). Adding to the difficulty in detecting small changes in plasma glucose was the between subjects design, bolus preload and small sample size used in the present study, while others have used within subject repeated measures designs and smaller weight based glucose preloads (Kolligs et al 1995). Although Exendin 9-39 enhanced the food intake suppression occurring after a glucose preload and selectively decreased intake of a nearly pure carbohydrate diet, it is questionable if this effect is related to alterations in plasma glucose.

An increase in plasma glucose was observed after Exendin 4, but is an improbable mechanism for the feeding response observed as Exendin 4 induced decreases in feeding were specific to protein intake. In diet selection studies in rats an increase in plasma glucose is associated with a decrease in carbohydrate preference but not in protein intake (Van Zeggeren and Li 1990). Because the primary effect of Exendin 4 on food intake was to decrease intake of the protein but not the carbohydrate diet, the relevance of the plasma glucose response to feeding behaviour is brought into question. Therefore, plasma glucose response is an uncertain mechanism for the results obtained here.

Although definitive evidence was accumulated indicating that the Exendin 4 agonist of GLP-1 receptors selectively decreases protein intake, the same selection scenario was not tested for fat. Peptides such as Galanin (Leibowitz 1989) and opioids (Bray 1993) have been shown to regulate fat intake in the rat. GLP-1 effects on gastric emptying, pancreatic and insulin secretion, and on lipogenesis in adipose tissue (Kieffer et al 1999) show GLP-1 to exert regulation over the digestion, absorption and
metabolism of ingested fat. Additionally, fatty acids potently stimulate GLP-1 secretion (Brubaker et al 1995). Therefore, GLP-1 could potentially be seen as an ideal signal of fat availability to feeding regulatory centers. Before it can be concluded that Exendin 4 is exclusively selective in altering protein intake, future avenues of research should involve measures of fat selection after GLP-1. Additionally, should these studies be undertaken the rats weights and ages should be held constant as ageing has been found to affect GLP-1 secretory response. GLP-1 concentrations were found to be higher in older postmenopausal human subjects, perhaps as a compensatory mechanism to increase plasma insulin concentrations (Ranganath et al 1998). There were large differences in rat weights between but not within studies of this thesis. Weights ranged between 200-400 grams. However, all rats were still in or just nearing the end of a growth phase, therefore, it is questionable as to whether ageing had a significant impact here.

Interactions of Exendin 4 with other peptides may also explain the decreases in protein selection and in total food intake. Central infusion of glucagon has been found to alter macronutrient intake (Bray 1992; Nagai et al 1991; Thibault et al 1996). GLP-1 inhibits glucagon secretion in vivo but stimulates its secretion in vitro (Fridolph et al 1991; Heller et al 1997 as cited in Habener et al 1999). Decreased glucagon release in vivo experiments involving GLP-1 is possibly due to an interaction with insulin. However, the primary action at an isolated tissue level seems to be stimulatory to glucagon secretion. Therefore, stimulation of glucagon by GLP-1 is an uncertain mediator in the selective decrease in protein intake.
In the brain, GLP-1 is inhibitory to NPY and MCH (Tritos et al 1998) and stimulatory to TSH and Calcitonin (Beak et al 1998, 1996; as cited in Habener et al 1999). NPY and MCH increase food intake, while TSH (Lin et al 1983) and Calcitonin (Chait et al 1995) decrease food intake in the rat. A recent report indicated that subcutaneous administration of Exendin 4 and of GLP-1 significantly decreased food intake in rats with Exendin 4 being more potent. Exendin 4 also increased circulating corticosterone concentrations (Rodriquez de Fonseca et al 2000), an effect that may be related to decreased feeding (Merali et al 1998 as cited in Rodriguez de Fonseca et al 2000). Therefore, evidence exists to support the ability of Exendin 4 to decrease food intake and this effect may be contributed to indirectly by influences other than a direct satiating effect (Figure 5.1).

Adding to the difficulty in interpreting the mechanism by which Exendin 4 and Exendin 9-39 (and hence the role of GLP-1) affected feeding behaviour in the present study is a recent report showing that they do not act on hepatic vagal afferents and therefore, may be incomplete tools with which to investigate the role of GLP-1 in feeding behaviour. Intraportal injection of physiological and pharmacological doses of GLP-1 facilitated significantly the afferent impulse discharge rate of the hepatic vagus. Conversely, intraportal injection of Exendin 4 did not facilitate the vagal afferents at all. Injection of Exendin 9-39 shortly prior to or shortly after injection of GLP-1 failed to modify the GLP-1 induced facilitation of the afferents (Nishizawa et al 2000). If the Exendin peptides do not act at the vagus, as the aforementioned report indicates, then a prominent feeding regulatory mechanism may have been untested. The vagus is an important communication link between the brain and the periphery and has an important
role in feeding behaviour. Many peptides such as CCK require intact vagal afferents in order to mediate satiety (Peikin 1989). Therefore, an important site whereby GLP-1 may have exerted peripheral effects on appetite may not have been evaluated with the experimental design of this thesis. However, this was a single report and conclusions regarding receptor specificity of the Exendin peptides cannot be made without further investigation.

The lack of effect of Exendin peptides on vagal discharge indicates that they may be acting at alternate GLP-1 receptor sites, such as the stomach, intestine, pancreatic islets (Kieffer et al 1999) or in regions of the brain where receptors have access to circulating peptides such as the NTS (Shimizu et al 1987). However, the ability of the Exendin peptides to access central GLP-1 receptors involved in the feeding response may be called into question based on the results obtained here. Intraperitoneal Exendin 9-39 affected feeding in a manner opposite to that of centrally administered Exendin 9-39 with decreased feeding peripherally and increased feeding with central administration (Meeran et al 1999, Turton et al 1996). Although there were differences in study design between the present study and central feeding studies involving Exendin 9-39, the results are still suggestive of a lack of effect on central feeding regulatory centers with peripherally administered Exendin 9-39.
Figure 5.1 Biological actions of GLP-1, which may impact on feeding.
LHRH= Leutinizing Hormone Releasing Hormone, MCH= Melanin Concentrating Hormone, NPY= Neuropeptide Y, SST= Somatostatin TSH= Thyrotropin Stimulating Hormone, CHO= Carbohydrate, FI= Food Intake, PRO= Protein

1 Based on: Habener et al Endocrine Reviews 1999 20(6): 876-913
The factors discussed earlier relating to protein specific satiety most probably account for the results obtained. It is unlikely that the results reflect true GLP-1 mediated macronutrient specific satiety for several reasons. First of all, GLP-1's primary function seems to be in the regulation of the digestion, absorption and metabolism of carbohydrate. Ingested carbohydrates and fat potently stimulate GLP-1 secretion while proteins have little impact. Therefore, GLP-1 makes a very improbable candidate in the regulation of protein intake. In addition, the peptides and mode of administration used did not seem to support known effects of the peptides on food intake when given centrally. The administration of intraperitoneal Exendin 9-39 peptide decreased food intake whereas, Exendin 9-39 administered centrally increased intake. Therefore, it cannot be assumed that either Exendin peptide administered intraperitoneally is reflecting the effects of GLP-1 on feeding regulatory centers as when given centrally.

In summary, the results obtained indicating selective decreases in protein intake with Exendin 4 and selective decreases in carbohydrate intake with Exendin 9-39 do not support the hypothesis that GLP-1 is a regulator of carbohydrate intake in rats. A lack of responsiveness to the Exendin peptides at vagal afferents may have contributed to the absence of supportive evidence for the original hypothesis. Physiological actions involving gastric emptying and pancreatic secretion, plasma insulin and glucose responses and interactions with other peptides may have also impacted on the feeding response observed in the present work.
Future Directions

Clearly, there exist several areas of study needed in the future to address questions arising from this thesis. Following are some suggested experiments addressing fundamental questions involving carbohydrate and GLP-1 induced satiety.

1. Evaluate the importance of Exendin 4 induced inhibition of protein degradation and absorption in selectively decreasing protein intake. Measuring the rate of absorption of protein after Exendin 4 administration will aid in determining if this is a factor in decreasing food intake. If so, providing a completely hydrolysed casein protein as a component of a protein selection paradigm will eliminate the presence of intact peptides in the intestine and aid in determining if this is the mechanism by which Exendin 4 selectively alters protein intake.

2. The ability of methylglucose and 3-O-methylglucose to decrease food intake has not yet been evaluated qualitatively in terms of macronutrient selection. These non-metabolisable glucose analogues provide no energy yet decrease food intake. Therefore, they may be accessing a carbohydrate sensitive satiety mechanism not related to metabolism. As methylglucose is specific for the sodium glucose transporter but not the basolateral glucose transporter, this mechanism may involve intestinal glucose transport. Employing a macronutrient selection paradigm after a preload of methylglucose and/or 3-O-methylglucose will help determine if the mechanism by which these analogues decrease food intake is macronutrient specific.

3. Measure the effect of Exendin or GLP-1 peptides on fat selection using a food cup selection paradigm.

4. Directly compare central vs. peripheral injections of Exendin 4 or 9-39 on feeding.
CHAPTER 6

SUMMARY AND CONCLUSIONS
6.0. SUMMARY AND CONCLUSIONS

6.1. Summary

A. The GLP-1 receptor antagonist Exendin 9-39, administered intraperitoneally, did not alter intake of a mixed diet in fasted rats but did alter macronutrient selection by selectively decreasing intake of a high carbohydrate diet. Food intake suppression was enhanced when Exendin 9-39 was co-administered with glucose but no effect was observed on fat or protein induced food intake suppression.

B. Exendin 4, the GLP-1 agonist, selectively and potently decreased intake of a high protein diet while leaving intake of the carbohydrate diet unaltered. This effect was delayed with the addition of a carbohydrate preload. Decreases in food intake were weaker and delayed when a single mixed diet was fed.

C. Exendin 4 in conjunction with a glucose preload resulted in elevated plasma concentration of glucose but had no effect on insulin. Exendin 9-39 did not affect plasma glucose or insulin response in rats given a bolus glucose preload.

6.2. Conclusion

The GLP-1 agonist, Exendin 4 and antagonist, Exendin 9-39 affect diet selection in the rat, suggesting GLP-1 plays a role in regulating macronutrient intake. However, the results do not support a role for GLP-1 as a selective regulator of carbohydrate intake.
CHAPTER 7

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7. REFERENCES


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APPENDIX 1

COMPARISON OF THE FOOD INTAKE SUPPRESSION OF THREE NUTRIENT PRELOADS

Introduction

The objective of this experiment was to determine the extent to which three nutrient preloads suppress subsequent food intake. The preloads, glucose, corn oil and albumin were to be used in studies defining the effect of Exendin 9-39 on the ability of each preload to suppress food intake. Therefore, it was desirable that each preload suppress food intake to a similar extent. The present experiment compared the food intake suppression of each of the three nutrient preloads.

Methods

In a simple repeated measures design each rat (n=14) received all three nutrient preloads (corn oil 1.5 g, D-glucose 1 g, and chicken egg albumin 0.5 g) as well as a water control by gavage in random order over the course of four experimental days. One washout day was given between each treatment. All preloads were brought to a volume of 2.5 ml using laboratory grade distilled water. Preloads were given exactly 30 minutes prior to feeding. Intake of the AIN 93G diet was measured at 0, 1, 2 & 14 hours.

Statistical Analysis

The following time points were analysed 0-1, 1-2, 0-2 & 0-14 hrs by one way repeated measures analysis of variance. Food intake in grams and % caloric compensation: (Kcal intake Control H2O – Kcal intake Tx) / Kcal in preload were analyzed. A post hoc Tukeys test was used
to determine differences between each treatment. A value of p<0.05 was used to declare significance.

Results

Food Intake (g)

Intake of chow was significantly less in all preloads as compared to water control during the 0-1 and 0-2 hour comparisons, with no differences seen between the three nutrient preloads. At 1-2 hours glucose and water treatments had intakes greater than corn oil and albumin (which were not different). There were no differences in food intake between any of the treatments during the 2-14 hour time point. Daily food intake (0-14hr) was significantly greater with water control as compared to glucose and albumin, while intake with corn oil was lower than any of the other three preloads (Table A-1).

Percent caloric suppression

Caloric suppression was significantly higher for the albumin preload as compared to glucose and corn oil for 0-14 hours (p<0.05). There was no difference between glucose and corn oil preloads in their 0-14 hour caloric suppression. Albumin and glucose induced greater caloric suppression as compared to corn oil during the first hour. At the 1-2 hour time point, albumin and corn oil induced greater caloric suppression than glucose, while glucose preload actually showed an increase in caloric intake. Caloric suppression during 0-2 hours followed
TABLE A-1

Food Intake and Percent Caloric Suppression Induced by Preloads

<table>
<thead>
<tr>
<th>Time</th>
<th>Preload</th>
<th>Food Intake (g)</th>
<th>% Caloric Supp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1hr</td>
<td>Water</td>
<td>4.3 ± 0.4 A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>2.1 ± 0.3 B</td>
<td>208 ± 40 A</td>
</tr>
<tr>
<td></td>
<td>Corn Oil</td>
<td>2.7 ± 0.3 B</td>
<td>41 ± 18 B</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>2.7 ± 0.3 B</td>
<td>256 ± 63 A</td>
</tr>
<tr>
<td>1-2hr</td>
<td>Water</td>
<td>1.2 ± 0.3 A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>1.9 ± 0.3 A</td>
<td>-74 ± 35 A</td>
</tr>
<tr>
<td></td>
<td>Corn Oil</td>
<td>0.6 ± 0.2 B</td>
<td>12 ± 10 B</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>1.0 ± 0.3 B</td>
<td>28 ± 51 B</td>
</tr>
<tr>
<td>0-2hr</td>
<td>Water</td>
<td>5.5 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>3.9 ± 0.4</td>
<td>134 ± 40 A</td>
</tr>
<tr>
<td></td>
<td>Corn Oil</td>
<td>3.3 ± 0.3</td>
<td>53 ± 17 B</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>3.7 ± 0.4</td>
<td>284 ± 50 C</td>
</tr>
<tr>
<td>2-14hr</td>
<td>Water</td>
<td>19.8 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>19.7 ± 0.6</td>
<td>22 ± 42</td>
</tr>
<tr>
<td></td>
<td>Corn Oil</td>
<td>18.6 ± 0.4</td>
<td>41 ± 14</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>19.6 ± 0.6</td>
<td>17 ± 80</td>
</tr>
<tr>
<td>0-14hr</td>
<td>Water</td>
<td>25.2 ± 0.5 A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>23.6 ± 0.8 B</td>
<td>156 ± 42 A</td>
</tr>
<tr>
<td></td>
<td>Corn Oil</td>
<td>21.9 ± 0.5 C</td>
<td>94 ± 15 A</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>23.3 ± 0.7 B</td>
<td>301 ± 60 B</td>
</tr>
</tbody>
</table>

Rows baring different letters are significantly different
the order of albumin greater than glucose greater than corn oil. There was no difference in percent caloric suppression during the final 2-14 hour time period (Table A-1).

Discussion

The objective of experiment 4 was to determine whether or not the three macronutrient preloads chosen for study were equally suppressive of food intake in the rat. The preloads chosen did demonstrate similar effects on food intake suppression in the first hour, but had different time patterns of intake suppression. Glucose induced a rapid large decrease in food intake which dissipated quickly. Albumin also induced a rapid decrease in food intake but continued to decrease food intake throughout all time periods. Corn oil showed a very different pattern by inducing moderate constant depression of food intake across all time points.

Total (0-14h) percent caloric suppression of the three preloads showed great differences. Albumin induced the greatest percent caloric suppression followed by glucose. Corn oil showed the lowest caloric suppression. The findings are not surprising and agree with past studies of nutrient preload intake suppression in the rat (Van Zeggeren and Li 199; Warwick et al 1994). Differences in digestion and absorption patterns of fats versus carbohydrates versus proteins likely account for the differences in time course of food intake suppression.

This preload experiment employed different quantities (both calorically and quantatively) of each preload yet demonstrated a fairly similar ability to suppress food intake (g) in the crucial first hours of feeding. This allows for a more direct comparison of the time course pattern of caloric suppression of the three preloads with mimimised variability in total food intake (g)
suppression. If similar gram or caloric quantities of each preload were used, then it would be
difficult to separate the time course pattern of percent caloric suppression from the total food
intake suppression by weight.

In conclusion, although the three nutrient preloads demonstrated different time course
patterns of caloric compensation, the food intake suppression by weight of these three preloads
was similar during the first two hours and, therefore, the preloads are suitable for use in the main
study.