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Glucagon-like peptide 1 stimulation in relation to body weight

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Glucagon-like Peptide 1 Stimulation in Relation to Body Weight

The studies presented in this thesis were performed at the Nutrition and Toxicology Research Institute Maastricht (NUTRIM), which participated in the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences) accredited by the Royal Netherlands Academy of Arts and Sciences.

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Es muß das Herz bei jedem Lebensrufe
Bereit zum Abschied sein und Neubeginne.
Um sich in Tapferkeit und ohne Trauern
In andre, neue Bindungen zu geben.
Und jedem Anfang wohnt ein Zauber inne
Der uns beschützt und der uns hilft, zu leben
.....

Contents

		Page
1.	General Introduction	9
2.	Galactose in combination with guar gum stimulates GLP-1 release in healthy, normal-weight subjects	27
3.	Nutrient-stimulated GLP-1 release in normal-weight men and women	43
4.	Activity-induced GLP-1 release in lean and obese subjects	61
5.	GLP-1 release and satiety after a nutrient challenge in normal-weight and obese subjects	77
6.	Decreased GLP-1 release after weight loss in obese subjects	95
7.	Nutrient stimulated GLP-1 release after body-weight loss and weight maintenance in humans	111
8.	General Discussion	129
	Summary	151
	Samenvatting	155
	Zusammenfassung	159
	Dankwoord	163
	Publications	167
	Curriculum vitae	169

Chapter 1

General Introduction

Body weight regulation

The way to maintain a stable body weight is to balance the daily energy intake with its expenditure. Whether this balance is achieved through the control of intake or by the control of expenditure has been at issue (1). The control of food intake, reflected in the pattern of eating and overall energy intake, makes a contribution to the maintenance of a healthy weight. It follows that poor regulation can lead to weight gain and obesity (2). In humans, body weight tends to remain within a relatively narrow range, despite a large day-to-day fluctuation in the amount of food consumed. Although major changes of energy intake can be induced in humans by restricting energy intake or by overfeeding, body weight returns very close to baseline levels when ad libitum feeding is resumed (1, 3).

Short-term and long-term food intake and energy balance are regulated through distinct, but interacting mechanisms. In this context neural and hormonal signals (i.e. gastrointestinal hormones) are involved (Fig. 1). This thesis deals with one particular gastrointestinal hormone, glucagon-like peptide 1 (GLP-1), which is believed to play a role depending on body weight status (3, 4).

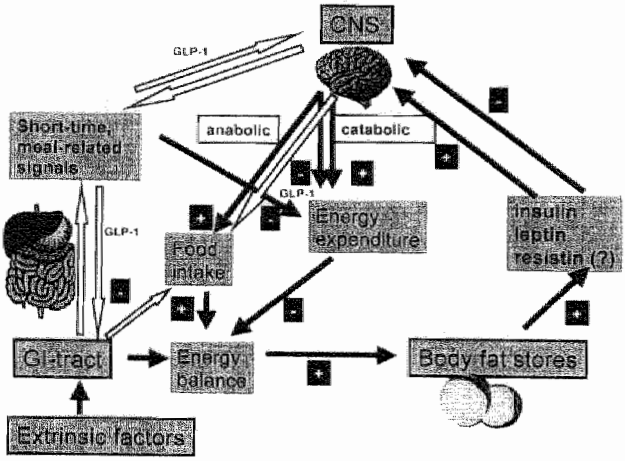


***Figure 1**. Regulation of energy balance and obesity (4)*
Neural systems and hormonal signals of energy balance represent major controllers of food intake. Anabolic effector pathways promote feeding and suppress energy expenditure, whereas catabolic effector pathways have the opposite effect. Short-term, meal-related hormonal (i.e. GLP-1) and neuronal (i.e. via distension of the stomach) signals from the gut exert further effects on central nervous regulation of feeding and energy balance. Biological effects of GLP-1 are shown by open arrows

Obesity

Obesity is defined as an excessive accumulation of body fat. For classification of overweight and obesity the body mass index (BMI = body weight (kg)/ height (m)2) is commonly used. A classification of normal weight, overweight and obesity based on BMI has been published by the World Health Organization (WHO) (5). For each BMI class the severity of associated health risks have been described (Table 1).

Table 1. *The WHO classification by BMI in adults (5)*

BMI (kg/m2)	Classification	Associated health risks
<18.5	Underweight	low (but risk of other clinical problems increased)
18.5-24.9	Normal range	average
≥25.0	Overweight	
25.0-29.9	Pre-obese	increased
30.0-34.9	Obese class I	moderately increased
35.0-39.9	Obese class II	severely increased
≥40.0	Obese class III	very severely increased

The incidence and prevalence of obesity is reaching alarming levels in children (6) as well as adults (7) in and outside the United States (8). Obesity is strongly associated with insulin resistance, dyslipidaemia, hyperinsulinemia, hypertension, cardiovascular disease and non-insulin-dependent diabetes (NIDDM) (9, 10). This cluster of metabolic disorders is also known as metabolic syndrom (11). The health risks, that are associated with obesity relate to the amount of fat and to the distribution of fat. Obesity has been found to be a major determinant of health care costs, varying between 1-5% of total health care expenditure depending on the definition of obesity (12).

Diet-induced obesity is the result of a long-term imbalance of energy expenditure and energy intake. Energy intake, thus food intake regulation is a complex process that involves physiological as well as social and psychological components. It is a form of behaviour that is subject to conscious control although many obese or weight gaining individuals claim that in practice their eating is out of their own control (2).

Satiety

Satiety can be described as the inhibition of eating and hunger as a consequence of food consumption. It needs to be distinguished from satiation, which is the process that brings a period of eating to a halt. Satiety and satiation act together and determine the pattern of eating behavior (13). For a better understanding of satiety, it is useful to distinguish the different phases of satiety and the different mechanisms that characterize them. Extent and capacity of food to suppress hunger and inhibit further eating is called satiating efficiency of a food (14, 15). Food causes this effect by mediating processes

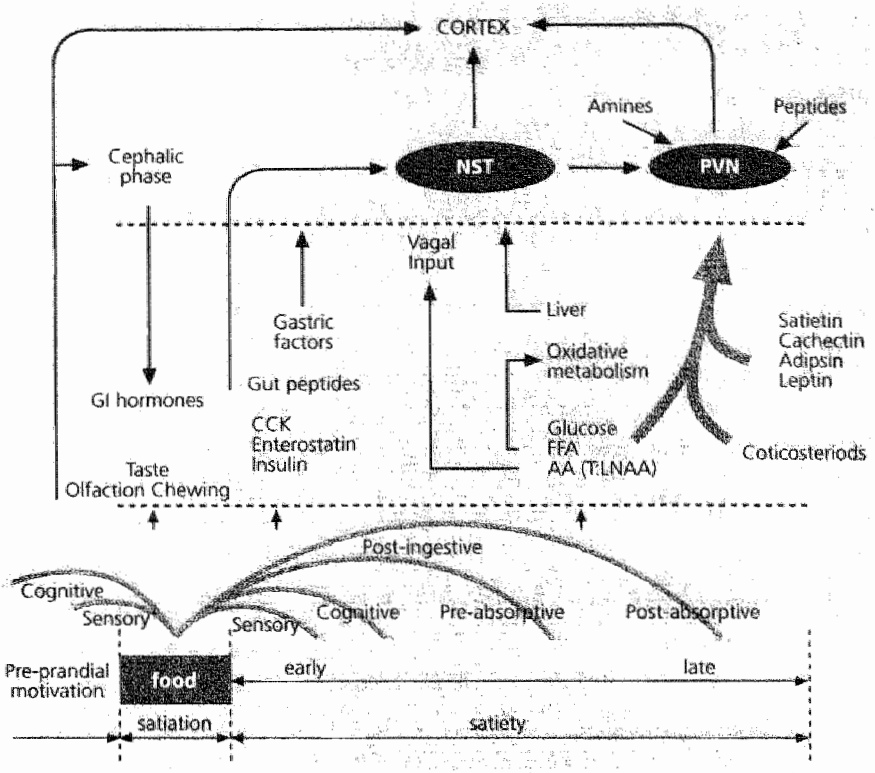
that can be classified as sensory, cognitive, postingestive and postabsorptive. These processes are operated by the impact of food on physiological and biochemical mechanisms (16) and have been referred to as the satiety cascade. The satiety cascade relates to three levels of operation of the psychobiological system: the behavioural pattern, the peripheral physiology and metabolism and brain activity (Fig. 2) (17). The concept of the satiety cascade implies that foods that vary in nutrient composition will correspond differently with the satiety mediating processes and will therefore exert different effects on satiation and satiety.

The way in which food is sensed and processed by the biological system generates neural and humoral signals, which are used to control appetite.
One part of the system involves signals of body energy reserves. These include insulin and leptin, the long-term signals that contribute to feeding behaviour and body weight regulation (3). Another component of the system is the control of feeding by the gastrointestinal tract. Specific triggers for hunger and satiety, such as gut hormones, that are released in response to nutrient ingestion control frequency of eating, meal size and hence total energy intake. GLP-1 is one of those gut hormones. In addition the gut hormones ghrelin (18), Peptide YY (19), pancreatic polypeptide and oxyntomodulin (20) may all play a role.

GLP-1

Biosynthesis & Metabolism

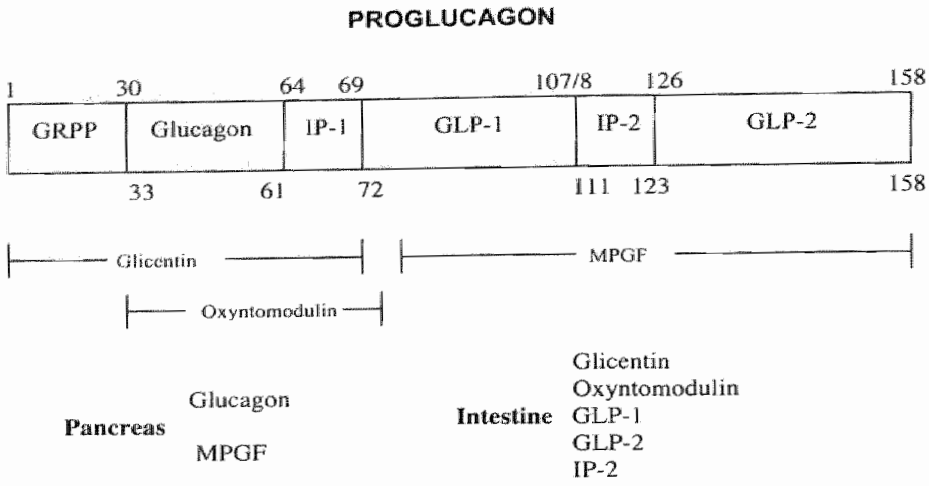
Glucagon-like peptide (GLP) 1 is a 30 amino acid peptide hormone derived from the glucagon precursor proglucagon. The mammalian glucagon precursor is produced both in the a cells of the islets of Langerhans and in the L cells of the intestinal mucosa (21). Proglucagon contains sequences for GLP-1 and glucagon and GLP-2. All three domains are structurally related, whereas GLP-1 and glucagon show a 50% sequence homology by sharing 14 amino acids in identical positions. Pancreatic and intestinal glucagon-related peptides are encoded by the same single gene, leading to an identical proglucagon RNA transcript, but to different processing in brain, pancreas and gut (Fig. 3), which is probably due to different prohormone convertases (21, 22). In the pancreas, glucagon is cleaved from its precursor as the major biologically active hormone, whereas in the gut GLP-1 (7-36 amide) is the major active peptide, due to a different cleavage site.



PVN = paraventricular nucleus, NST = nucleus of the tractus solitarius, CCK = cholecystokinin, AA = amino acids, T = tryptophan, LNAA = large neutral amino acids.

Figure 2. The satiety cascade in relation to three levels of operation of the psychophysiological system : The behavioral pattern, peripheral physiology and metabolism and brain activity ((17) after Blundell)

Glucagon-like peptide 1 occurs as a non-amidated form (GLP-1 (7-37amide)), also called insulinotropin, and as an amidated form (GLP-1 (7-36)), which predominantly is produced in the ileum. The latter one is physiologically more important, because 80% of plasma activity seems to be related to that form of GLP-1 (23). This thesis will deal with GLP-1 (7-36), unless otherwise specified.



GRPP = glicentin-related pancreatic polypeptide, IP = intervening peptide, MPGF = major proglucagon fragment.

Figure 3. *Structural organization of mammalian proglucagon (22). The numbers refer to the amino acid sequences in proglucagon. The peptides released by posttranslational processing in the pancreas and intestines are shown.*

GLP-1 has a short half-life, due to a rapid degradation by dipeptidyl-peptidase IV, representing the intravascular route of elimination. By removing two amino acid residues from the N-terminal of the intact peptide it transforms the biologically active GLP-1 (7-36) into an inactive or antagonistic GLP-1 (9-36). This transformation happens very rapidly and leads to a half-life time of less than three minutes (24). In addition to the vascular component there seems to be an organ-related mechanism playing a central role in the degradation of the active GLP-1, a mechanism that probably involves the kidney. Measuring active GLP-1 requires that the method, which is used, should be directed to the NH2 terminal of the molecule. If only COOH terminated methods are used, active as well as inactive GLP-1 will be measured. If a C- as well as a N-terminated assay is used, it is possible to make the measurement even more precise and determine N-terminally truncated metabolites. The N-terminus of the peptide is important for receptor binding, the C-terminus is important for biological activity (25).

Postprandially, the level of GLP-1 (9-36) released, exceeds that of the bioactive 7-36 amide molecule. The inactive form also binds to the GLP-1 receptor but with a lower affinity than the active form (24).

Secretion and Reception

GLP-1 releasing L cells are distributed throughout the intestine but the greatest numbers are found in the jejunum, distal ileum and colon (26). The GLP-1 amino acid sequence is identical across different mammalian species and is highly conserved throughout evolution. Concentrations show a pulsatile pattern and ranges of basal concentrations depend on the assays used. Deacon et al. found fasting active GLP-1 (7-36 amide) levels of 3.3 ± 0.7 pmol/L rising up to 9.9 ± 1.2 pmol/L in a postprandial state (24).
GLP-1 is released in a biphasic pattern after food intake, with a first peak about 15-30 minutes after nutrient ingestion and a second peak approximately one hour later (27). Elliot et al. (27) reported GLP-1 peaks 30 min after a carbohydrate and protein meal and 150 min after a fat meal. Results reported by Hermann et al. (28) as well as Ørskov et al. (29) confirmed the pattern of GLP-1 response to a meal. Since most of the L cells are localized in the distal small intestine, it seems likely, that the GLP-1 secretion after a meal additionally is induced by signals from the proximal gut, rather than by direct L cell stimulation through luminal nutrients.
Two different systems for the release of GLP-1 have been suggested. The first phase is likely to be caused by a mediated response, whereas the second phase is initiated by nutrient sensation in the distal ileum (30).
One explanation for the early phase of GLP-1 release after the administration of nutrients into the duodenum is the indirect GLP-1 stimulation through a pathway involving the glucose-dependent insulinotropic polypeptide (GIP) secretion by endocrine K cells, which in turn stimulates the afferent vagus nerve to the central nervous system, thereby activating the efferent vagus to the distal gut (30-33).
These findings underline the possible importance of mediating influences of peptides, endocrine and neural mechanisms in GLP-1 secretion.

Both, GLP-1 secretion and GLP-1 reception have been the focus of recent interest because of their central role in glucose metabolism (34).
Although the sequence homology of amino acids between glucagon and GLP-1 is quite high, they both have specific receptors, which also show an extensive sequence homology, but hardly interact with each other (34). In rats GLP-1 receptors have been identified in thalamic and hypothalamic regions and in the brainstem. Since high concentrations of GLP-1 immunoreactive fibers and cells are localized in areas where food intake is controlled, like i.e.the periventricular areas, it seems likely that GLP-1 has an influence on food intake and satiety. This has been confirmed by experiments using c-fos as a marker of neuronal activation (35).
GLP-1 receptor mRNA transcripts have been found in human pancreas-, lung-, kidney-, stomach-, heart- and brain tissues. GLP-1 exerts its activity through binding to a G-protein-linked receptor, which is expressed on the islet b-cells (22). A 90% homology has been detected between the human and rat receptors. The possibility of cloning the

receptor allows the investigation of specific structure functions and signal transduction studies (36).

Biological activities of GLP-1

Pathways, loops or mechanisms by which GLP-1 exerts its effects on insulin, glucagon, glucose, possibly in relation to hunger, satiety or energy intake are still unclear for a large part. The question whether GLP-1 affects food intake regulation via a direct effect on the brain by crossing the blood-brain barrier or an indirect effect by slowing gastric emptying or neural signaling, is still under discussion. There is evidence that the peptide is able to reach the brain by gaining access to subfornical organs and the area postrema (37, 38). These areas lack a perivascular blood-brain barrier and allow free exchange of molecules between blood and cerebrospinal fluid.

There are several ways to manipulate active GLP-1 concentrations experimentally. One is to prevent degradation of active GLP-1 by applying a peptidase inhibitor (DPP-IV inhibitor) to avoid truncation of the peptide. Secondly, many studies have applied GLP-1 exogenously, mostly intravenously (iv) or intracerebroventricular (icv).

In line with the suggestion that peripheral GLP-1 might not have fully access to receptors in the brain are the results of rat experiments, in which effects on food and water intake were only obtained by centrally administered GLP-1 and not by peripherally given GLP-1 (39).

In healthy humans, peripheral GLP-1 turned out to have an effect on satiety and appetite sensations as well as on glucagon, insulin and blood glucose levels (40). The same results for appetite and energy intake have been reported for subjects with obesity as well as those with NIDDM, in whom energy intake was reduced by 27% by GLP-1 compared to saline infusion (41). Actions of GLP-1 are summarized in Fig. 4.

The incretin effect

According to Creutzfeldt (42) an incretin is defined as an endocrine transmitter produced in the gastrointestinal tract, that is released after stimulation by nutrients (especially carbohydrates), and that stimulates insulin release at physiological concentrations. Incretins work through a sensitization of beta cells to glucose stimulation, thereby leading to an augmented insulin response to absorbed glucose (42) and contribute to postprandial control of substrate concentrations, especially glucose. Many peptides were discussed as incretings. The two peptide hormones that qualify as incretins, are glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) (43).

Their effects on insulin secretion is additive and the effect of both of them seem to account for the whole incretin effect in humans. When GLP-1 (7-36) and GIP are infused at physiological doses, GLP-1 turns out to be more powerful than GIP concerning insulin stimulation (44).

Studies using exendin (9-39 amide), a GLP-1 receptor antagonist, provide evidence for GLP-1 as an important physiological incretin by attenuating insulin response and eliciting increased plasma glucose levels after intraduodenal glucose infusion (22).

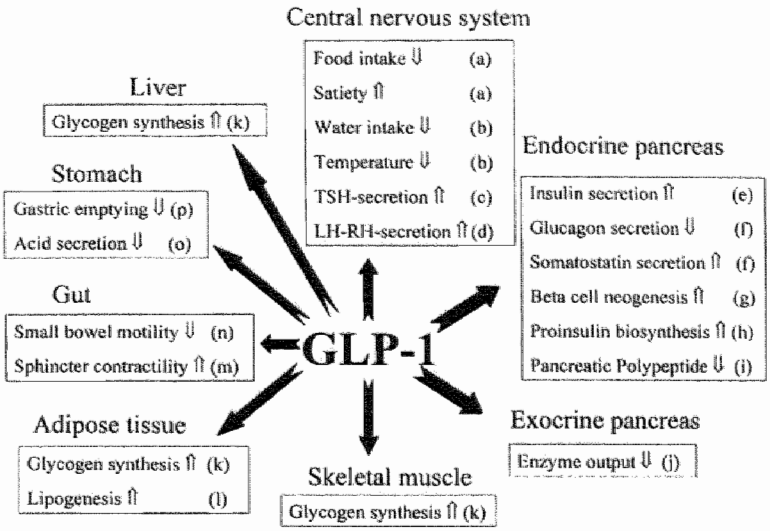


***Figure 4**. Actions of GLP-1 in various organs and tissues in vivo (4 (modified according to (80)). Small letters indicate references documenting the quoted effect:*
(a) (40); (b) (81); (c) (82); (d) (83); (e) (84); (f) (46); (g) (85); (h) (86); (i) (87); (j) (61); (k) (88); (l) (89); (m) (90); (n) (91); (o) (59); (p) (61).

Glucagon suppression

It has been reported that GLP-1 in physiological doses inhibits glucagon secretion in animals as well as in humans (45). Furthermore, the peptide stimulates somatostatin secretion. This has been shown by experiments in the perfused pig pancreas (46). Somatostatin in turn has been proposed, like GLP-1, to lower or moreover inhibit glucagon secretion. Additionally, it prolongs the absorption of nutrients by decreasing the motility of the small intestine (42). To answer the question whether glucagon suppression is primarily due to the influence of GLP-1 or is mediated by its influence on somatostatin, we may look at experiments which have shown, that GLP-1 induces inhibition of glucagon release from isolated human cells, but not from a culture of pancreatic islets (47). This suggests that the effect on glucagon-producing cells is indirect (23).

Glucose-tolerance improvement

The prevalence of insulin resistance, a decreased ability of insulin to stimulate glucose uptake in peripheral tissues is increased in obese individuals. Although the majority of individuals in the general population that can be considered insulin resistant are also obese, not all obese individuals are insulin resistant (48, 49). A failure of b-cells to compensate for the insulin resistance then leads to both, fasting and postprandial hyperglyceamia or glucose intolerance. Criteria for the diagnosis of various forms of hyperglycemia, such as impaired glucose tolerance, impaired fasting glucose, and finally diabetes have been developed by the WHO (50).
An improvement of glucose tolerance through i.e. exogenous administration of GLP-1 has been reported in animal as well as in human studies, in healthy subjects as well as in obese subjects and subjects suffering from NIDDM (51-55).
An explanation for the improved glucose tolerance caused by GLP-1 is, that GLP-1 might accelerate glucose elimination through its insulinotropic effect (51, 56).
Experiments in humans with the GLP-1 receptor antagonist exendin (9-39) have shown a significant deterioration in postprandial glycemic control, which suggests, that GLP-1 is an important factor in mediating the physiological regulation of postprandial glucose concentrations (54).

Gastric actions

Glucagon and related peptides, such as GLP-1 are potent pharmacological inhibitors of gastric acid secretion in humans (57-59). GLP-1 stimulates cAMP production in gastric cells as well as adenylate cyclase activity, the corresponding enzyme that is localized in the cell membrane. This suggests that GLP-1 plays a pivotal role in the enteric control of gastric acid secretion.
Schjoldjager et al (59) were able to support this suggestion by finding an inhibition of gastric acid secretion in humans by truncated GLP-1. It also has been shown, that gastric acid output during a GLP-1 (7-36) infusion close to postprandial levels (44) decreased pentagastrin stimulated acid output in man by 47-49% (57).
Effects on gastric emptying are controversial (43, 60). The inhibitory action of GLP-1 on gastric emptying has been described in normal subjects and in type 2 diabetic subjects (61). Using paracetamol as a marker, Balkan et al. (43) did not find any effect of GLP-1 on gastric emptying in rats.

Effects on satiety and food intake

GLP-1 is supposed to affect hunger, satiety and appetite. Although findings concerning the reduction of sensations of hunger and satiety are controversial (62), most studies found a significant reduction of energy intake in healthy (40), as well as in diabetic (41) and obese subjects (63). For an overview see Table 2.

The role of the vagus nerve in signal transduction, suggests, that GLP-1 affects satiety in terms of an inter-meal interval related satiety rather than by satiation (62).
GLP-1 receptors are localized in brain areas, which are strongly involved in the control of feeding (40). With the administration of exendin (9-39), the GLP-1 receptor antagonist, humans increase their food intake when having access to an ad libitum meal (35). These results contribute to the suggestion of a role for GLP-1 in the control of hunger and satiety. The mechanisms by which GLP-1 leads to a regulation of appetite and food intake are not clear yet. One of the current explanations is, that the effect of GLP-1 on satiety might be due to its inhibition of gastric emptying and thereby leading to a prolonged period of gastric distension (64). This again leads to a prolonged period of release of various other gastrointestinal hormones and a longer simulation of gastrointestinal vagal receptors that are involved in the control of food intake (62).

GLP-1 and obesity

The role of GLP-1 in relation to obesity is not clear yet. One study has been reporting hypersecretion of GLP-1 in obese subjects. Others have found lower basal as well as attenuated responses to carbohydrate stimulation in the obese (65, 66).
According to latter it can be speculated that the lower postprandial GLP-1 level in obese subjects may lead to a shorter inter-meal interval related satiety and thereby leading to a more frequent food intake to reach appropriate individual satiety levels (20). Several studies have shown that GLP-1 administration prolongs postprandial satiety and improves glycaemic control in the obese (63). Observations concerning stimulated GLP-1 release in obesity seem to be strongly related to the class of obesity (63), the health status (67) that is being investigated and the kind of macronutrient that is used as a stimulus (65). Studies investigating GLP-1 release in relation to obesity have been assessing subjects with an BMI of 38 or higher concerning the postprandial GLP-1 release (65, 66). With respect to treatment and prevention it is important though that pre-obese and class I obesity are being investigated as well. Studies investigating the effect of GLP-1 on appetite parameters mostly have been assessing the effect of pharmacological dosages of GLP-1 (35, 63). When physiological concentrations were used, only hunger and prospective food consumption were affected, all other ratings and ad libitum energy intake were not different compared to a placebo condition (68). Assessment of the effect of weight loss on GLP-1 led to ambivalent results. Strong improvement of GLP-1 concentrations was shown after jejunoileal bypass surgery, when subjects were investigated twenty years after surgery (69). Short-term effects of surgery on GLP-1 concentrations could not be observed (70). Only very few studies have been investigating the effect of diet-induced weight loss on GLP-1 concentrations. Results in the study from Verdich et al. show differences in GLP-1 concentrations between obese and lean subjects, but no improvement due to weight loss in the obese subjects (71). Others report a decrease in GLP-1 concentrations after weight loss (72).

Table 2. Effects of GLP-1 on appetite, feeding behavior and body weight in humans (4)

References	Dose (pmol kg min −1)	Duration (hours)	Route	Subjects	Effects	P-value
Flint et al., 1998	0.7	4	i.v.	20 healthy subj.	reduction of energy intake:21%	0.0002
					decreased feeling of hunger	0.012
					enhanced fullness	0.028
					enhanced satiety	0.013
Gutzwiller et al., 1999b	0-1.5	2	i.v.	16 healthy subj.	max. reduction of food intake:35%	<0.001
					max. reduction of caloric intake:32%	<0.001
					max. reduction of fluid intake: 18%	<0.01
Gutzwiller et al., 1999a	1.5	2	i.v.	12 Type 2 diabetic patients	enhanced satiety	0.026
					enhanced fullness	0.028
					reduction of energy intake:27%	0.034
					enhanced satiety	<0.05
Toft-Nielsen et al., 1999	2.4	48	s.c.	6 Type 2 diabetic patients	decreased feeling of hunger	<0.05
					decreased prospective food consumption	<0.05
					fullness not affected	-
Hellström and Näslund, 1999	0.75	8	i.v.	obese subjects	reduced caloric intake	-
					reduced feeling of hunger	-
Zander et al., 2001	2.4	6weeks	s.c.	10 Type 2 diabetic patients	reduction of body weight:1.9%	0.02
					reduction of appetite:21%	0.01

i.v. = intravenous; s.c. = subcutanous

Outline of the thesis

Many questions remain unanswered concerning the role of GLP-1 in obesity and the potential role of GLP-1 for prevention and treatment of obesity. This thesis describes a series of experiments that aim to study nutrient and physical activity stimulated GLP-1 release in relation to body weight and to which extent GLP-1 can be considered a metabolic target for the treatment of obesity.

In order to answer these questions we firstly assessed whether basal and postprandial GLP-1 concentrations are related to body weight and thus are different between lean and overweight/ obese subjects (Chapter 2, 3, 5). Second we explored ways in which GLP-1 release can be stimulated in normal-weight subjects (Chapter 2, 3, 4). It is known that glucose is a potent stimulus for GLP-1 release (73). With respect to glyceamic index and treatment or prevention of obesity (74), it seems reasonable to test non-glucose carbohydrates as well, like galactose (28, 75, 76) and dietary fiber and their potential to stimulate GLP-1 release. Dietary fiber, such as guar gum have been suggested to act on satiety by stimulating gastrointestinal peptides (77) and fermentable dietary fiber has been shown to increase GLP-1 release in animals (78).

After investigating ways of stimulating GLP-1 release in normal-weight subjects, we assessed, whether stimulation of GLP-1 release observed in normal-weight subjects also appeared in obese subjects (Chapter 4, 5).

Since the autonomic nervous system has been suggested to be involved in the release of GLP-1 as well (28, 79) we have been investigating the effect of physical exercise on GLP-1 secretion in obese and normal-weight subjects (Chapter 4).

Not much is known yet about the influence of weight loss on GLP-1 release. Increased concentrations of GLP-1 have been found (69), while other studies observed decreased GLP-1 concentrations after weight loss (72). These results might depend on the amount of weight loss or the way weight was lost and need further investigation (Chapter 4, 6).

Finally it is important not only to look at the effect of weight loss on GLP-1 release, but also to investigate how GLP-1 concentrations change after weight loss, during a weight maintenance period (Chapter 7). Time of investigation after weight loss, seems to play an important role for the conclusion concerning GLP-1 concentrations (69, 70, 72).

The results of the studies described above are discussed (Chapter 8) with the purpose of coming to a general conclusion on the role of GLP-1 as a metabolic target for the treatment of obesity.

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Chapter 2

Galactose in combination with guar gum stimulates GLP-1 release in healthy, normal-weight subjects

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Abstract

The stimulation of gut hormones like glucagon-like peptide 1 (GLP-1) seems to play a major role in triggering satiety, but seems to differ between different monosaccharides. Guar gum is known for its effects on satiety as well. The present study investigates whether galactose or galactose in combination with guar gum is sufficient for stimulation of GLP-1 compared to glucose and glucose with guar gum.

20 subjects (12 women and 8 men) were investigated in a randomized, cross-over design with five different test conditions. Subjects received either 75g glucose, 75g glucose in combination with 2.5g guar gum, 50g galactose, 50g galactose with 2.5g guar gum or 250 ml water. Blood samples were taken for determining GLP-1, insulin, glucose and free fatty acids. The appetite profile was obtained with visual analogue scales.

Galactose in combination with guar gum was sufficient in stimulating GLP-1 release different from galactose at 60 ($P = 0.03$) and 90 minutes ($P = 0.03$). The area under the curve of GLP-1 release for galactose/guar gum was not different from glucose/guar gum. Plasma insulin and glucose concentrations were significantly lower in the galactose conditions compared to the glucose conditions ($P < 0.01$).

Satiety ratings were higher in both guar gum conditions compared to no guar gum ($P = 0.01$). Galactose in combination with guar gum was a trigger for GLP-1 release and satiety similar to glucose in combination with guar gum in healthy, normal-weight subjects, but accompanied by lower glucose and insulin concentrations.

Introduction

Glucagon-like peptide 1 (GLP-1) is a 30 amino acid peptide hormone secreted from intestinal L cells after ingestion of glucose or mixed meals (1-3). Secretion of GLP-1 in response to food intake is known to stimulate insulin secretion in pancreatic beta-cells (4, 5) and intravenous GLP-1 administration reduced energy intake in normal-weight subjects (6). There is evidence that the peptide reaches the brain by areas lacking a perivascular blood-brain area, like subfornical organs and the area postrema, which allow free exchange of molecules between blood and cerebrospinal fluid (7). The area postrema as well as the subfornical organ are known to be involved in the regulation of feeding and drinking by projecting to the hypothalamus (8). The stimulation of gut hormone release by oral carbohydrates seems to play a major role in triggering satiation, but may differ between different monosaccarides (9).

Galactose has been shown to stimulate GLP-1 secretion (8, 10, 11) with a committant increase in plasma insulin secretion (12), but a lower glycaemic index (GI = 20) compared to glucose. There is evidence that foods with higher glycaemic indices are associated with less subjective satiety (13, 14) although results are contradictory (15, 16). Furthermore, guar gum, a soluble fiber, has been shown to prolong satiety by slowing the absorption of glucose from the small intestine, and thereby possibly leading to a prolonged stimulation of gut hormones (17, 18). Fermentable fiber has been shown to increase GLP-1 secretion in dogs (19). The aim of the present study was to investigate the effects of galactose and guar gum together on GLP-1 release and satiety compared to glucose in combination with guar gum.

We hypothesize that galactose in combination with guar gum leads to profound GLP-1 stimulation, with prolonged satiety compared to glucose and guar gum, due to lower glycaemic impact.

Materials and Methods

First, a dose-response study (study A) was done in eight subjects (5 women and 3 men). Four loads were tested in randomized order on different test days, with at least three days between trials. 2,5g guar gum (20), 12g galactose +2,5g guar gum (200 kJ or 48 kcal), 25g galactose +2,5g guar gum (418 kJ or 101 kcal), or 50g galactose +2,5g guar gum (836 kJ or 202 kcal). All ingredients were dissolved in 250 ml water. 50g of galactose was the maximum amount that could be dissolved in 250 ml water without being perceived as unpleasant by the subjects.

After the dose-response study, a randomized, crossover design was applied (study B) to twenty subjects. (12 women and 8 men). Five conditions were applied single blind and in random order one week apart from each other. Subjects received either 75g glucose (1254 kJ); 75g glucose + 2.5g guar gum; 50g galactose (836 kJ); 50g galactose+2.5g guar gum, dissolved in 250 ml water, or water alone. 75g glucose was chosen since this is a standard solution in similar tests (21). The addition of guar gum did not increase

caloric content of the glucose or galactose loads. Since the energy content of the galactose and glucose loads was different, data were controlled for energy content. Procedures were the same for study A and B.

Subjects

Subjects were recruited by means of advertisements in local newspapers. Informed consent was obtained from all subjects. For study A subjects underwent a medical screening, including a medical history, and a two-hour oral glucose tolerance test, conducted according to the guidelines of the World Health Organization (1999)(22). Body weight was measured on a digital balance (Seca, model 707, Hamburg, Germany). Height was measured using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Body mass index (BMI) was calculated as weight (in kg) / height (in meters squared). Body composition was determined by underwater weighing using the method of Siri (23). Dietary habits were assessed using a validated Dutch translation of the Three Factor Eating Questionnaire (TFEQ) (24, 25). The study was approved by the Maastricht University Medical Ethics Committee.
Subject characteristics are shown in table 1.

***Table 1**. Subject characteristics*

	Women (n=12)	Men (n=8)
Age (y)	41±14	39±12
Weight (kg)	62±8.8	76±8.6
Height (cm)	167±10	178±8
BMI (kg/m^2)	22±2	24±1
Bodyfat (%)	28.5±7	18.7±7

values shown as means ± sd

Procedure

After arrival in the morning, a catheter (Baxter BV, Utrecht, The Netherlands) was inserted in an antecubital vein. Subjects were recumbent during trials.
In study A, blood samples were taken at 0; +30 and +60 minutes, relative to oral intake. In study B, after a baseline sample at 0 minutes, blood samples were taken at +30, +60, +90, +120 and +240 minutes relative to oral intake, for determining GLP-1, insulin, and glucose concentrations. Free fatty acids were measured in samples taken at time points 0, +30, +60 and +120 minutes. Prior to blood sampling, subjects rated their feelings of hunger, fullness, satiety and desire to eat on 100-mm visual analogue scales (VAS) (26). Subjects were instructed to report discomfort or nausea at any time during the trial. Resting metabolic rate (RMR) was measured ten minutes after the subject

arrived, using a ventilated hood system (Oxycon b, Mijnhardt, Bunnik, The Netherlands) (27). Diet-induced thermogenesis was measured using the same apparatus four times after treatment and was expressed as percentage energy intake (Table 2). O_2 consumption and CO_2 production were measured and energy expenditure was calculated according to Weir (28). At the end of each test day, subjects were offered an ad-libitum meal on a plate placed on the Universal eating monitor (29, 30) consisting of pasta, peas, green beans, corn and cheese with a vinegar dressing. Energy density was 10,8 kJ per gram.

Analytical methods

Blood samples for free fatty acids and glucose analysis were mixed with EDTA to prevent clotting. Samples for GLP-1 analysis were collected into chilled syringes and mixed with EDTA and 40 µl of a DPP-IV (DPP-IV403: EC 3.4.14.5) inhibitor (Linco Research, St. Charles, USA) to prevent degradation. The plasma samples were centrifuged at 2500 x g at 4 °C for 10 minutes. To blood samples for insulin no anticoagulant was added and samples were allowed to clot for 30 minutes, then centrifuged at 2000 x g at 4 °C for 10 minutes. Aliquots of plasma and serum were frozen immediately in liquid nitrogen and stored at −20 °C. Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France). The WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations. GLP-1 concentrations were measured using an ELISA kit (EGLP −35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less and sensitivity is 2pmol/l (31). Insulin was measured using a fluoroimmunometric sandwich assay (AutoDELFIATM assay; Perkin Elmer, Finland).

Statistical Analyses

In the dose response study, GLP-1 release was compared among four conditions using a repeated measurement analysis of variance (ANOVA) and Sheffe-F or Fisher PLSD post-hoc test. In study B, GLP-1, insulin, glucose, free fatty acids as well as hunger, satiety, desire to eat, fullness and RMR were compared among conditions using a repeated measurement analysis of variance (ANOVA) and Sheffe-F or Fisher PLSD post-hoc test with a condition x time interaction. Diet-induced thermogenesis was expressed as percentage of energy intake. It was compared among the four nutrient conditions in a repeated measurement analysis of variance (ANOVA) and a Sheffe −F post-hoc test.

Regression analysis was used to study the relationship between satiety ratings and GLP-1 increase. STATVIEW+GRAPHICS software (Abacus Concepts Inc, Berkeley, CA) was used for statistical analysis. Significance was defined as $P < 0.05$

Results

Dose-response study

The effect of 12g galactose as well as 25g galactose in combination with guar on GLP-1 release was not different from guar gum alone. Only 50g galactose in combination with 2,5g guar gum evoked a significantly different GLP-1 increase at 30 ($F\ 3,21 = 15.81$; $P = 0.0001$) and 60 ($F\ 3,21 = 5.68$; $P = 0.005$) minutes compared to all other conditions (Fig. 1)

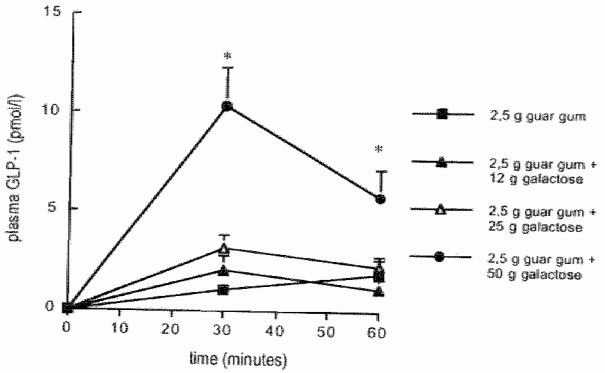

***Figure. 1**. Plasma GLP-1 concentrations expressed as means (±SEM) after ingestion of 2,5 g guar gum alone (filled squares) or in combination with 12 g galactose (filled triangles), 25 g galactose (open triangles) or 50 g galactose (filled circles).*
** significantly different compared to all other dosages at $P<0.05$.*

Plasma GLP-1 levels and insulin concentrations (study B)

Compared to water, maximum plasma GLP-1 concentrations were reached at 30 minutes in the glucose as well as in the galactose conditions ($F_{4,76} = 11.27$; $P < 0.05$; Fig. 2A). Δ-GLP-1 plasma concentrations were significantly higher in the galactose/ guar gum condition compared to galactose alone at 60 ($F_{1,19} = 5.19$; $P = 0.03$) and 90 minutes ($F_{1,19} = 4.78$; $P = 0.03$). Δ-GLP-1 concentrations between glucose conditions were not different at any time. Calculating the area under the curve, there was no significant difference between the glucose+guar gum or galactose+guar gum treatment (Fig. 2B). Serum insulin concentrations peaked at 30 minutes in the galactose conditions and at 60 minutes in the glucose conditions (Fig. 3). Insulin concentrations in the galactose/ guar gum condition were not different from galactose alone, but both galactose conditions lead to significantly lower insulin concentrations at 30 ($F_{3,57} = 26.43$; $P < 0.01$), 60 ($F_{3,57} = 34.37$; $P < 0.01$), 90 ($F_{3,57} = 16.21$; $P < 0.01$) and 120 minutes ($F_{3,57} = 16.98$; $P < 0.01$) compared to both glucose conditions.

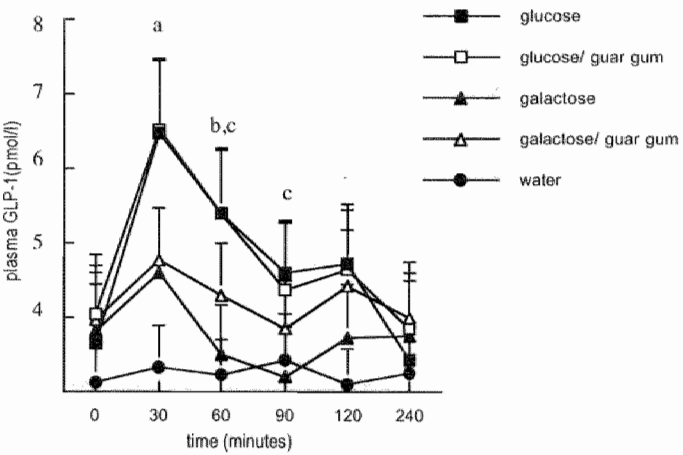

***Figure 2A**. Plasma GLP-1 concentrations expressed as means (±SEM) after ingestion of glucose (filled squares), glucose/ guar gum (open squares), galactose (filled triangles), galactose/ guar gum (open triangles) or water (filled circles).*
a: glucose, glucose/ guar gum, galactose and galactose/ guar gum are significantly different from water at $P < 0.05$.
b: glucose, glucose/ guar gum and galactose/ guar gum are significantly different from water at $P < 0.05$.
c: Δ- GLP-1 (compared to baseline) after galactose/ guar gum is significantly different from galactose at $P < 0.05$.

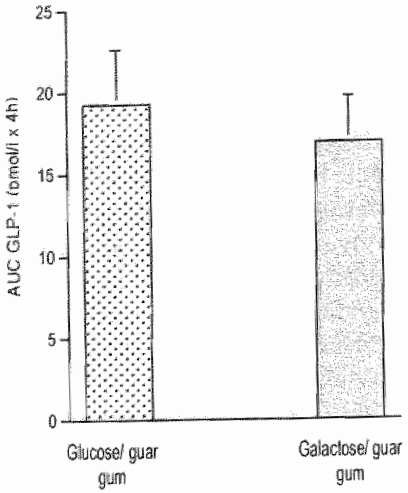

***Figure. 2B**. AUC (pmol/l x 4h) for GLP-1 release after ingestion of glucose/ guar gum or galactose/guar gum (n.s.)*

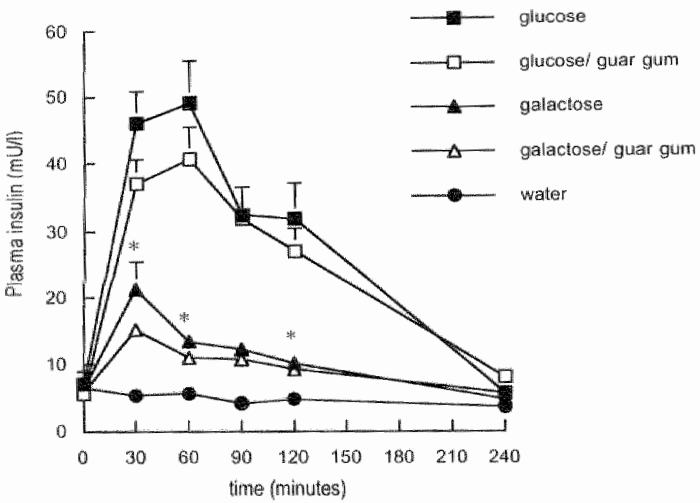

***Figure 3**. Serum insulin concentration (mU/l) expressed as means (±SEM)after ingestion of glucose (filled squares), glucose/ guar gum (open squares), galactose (filled triangles), galactose/ guar gum (open triangles) or water.*
** both galactose conditions are significantly different from both glucose conditions at P <0.01.*

Plasma glucose and free fatty acid concentrations (study B)

Plasma glucose peaked at 30 minutes in all conditions compared to water (Fig. 4). Glucose concentrations were not different between the galactose conditions. Both galactose conditions lead to significantly lower glucose concentrations at 30 ($F_{3,57} = 26.61$; $P < 0.01$), 60 ($F_{3,57} = 30.41$; $P < 0.01$), 90 ($F_{3,57} = 14.45$; $P < 0.01$) and 120 minutes ($F_{3,57} = 17.09$; $P < 0.01$) compared to both the glucose conditions.

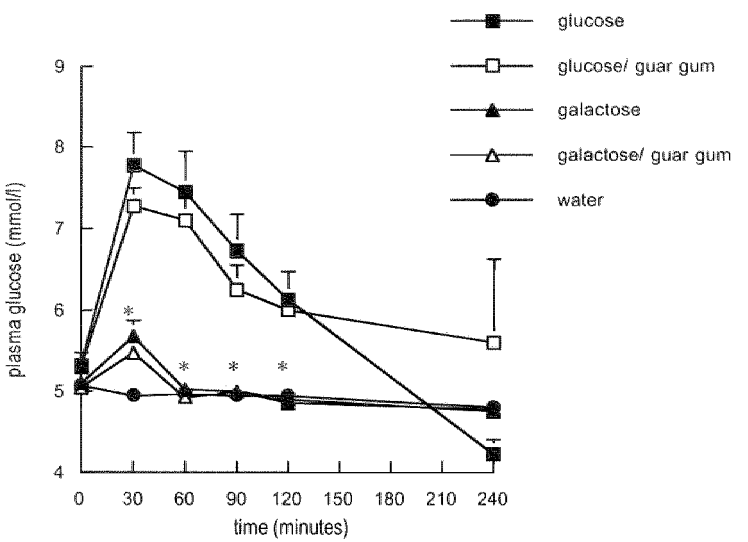

***Figure 4**. Plasma glucose concentrations (mmol/l) expressed as means (±SEM) after ingestion of glucose (filled squares), glucose/ guar gum (open squares), galactose (filled triangles), galactose/ guar gum (open triangles) or water.*
**both galactose and galactose/ guar gum are significantly different from both glucose conditions at $P < 0.05$.*

Plasma free fatty acids decreased over time in all nutrient conditions except for water (Fig. 5). After ingestion of galactose/ guar gum, free fatty acid concentrations were significantly higher compared to both glucose conditions at 60 minutes ($F_{3,57} = 6.59$; $P < 0.01$). Both galactose conditions had higher free fatty acid concentrations at 120 minutes compared to both glucose conditions ($F_{3,57} = 11.72$; $P < 0.01$).

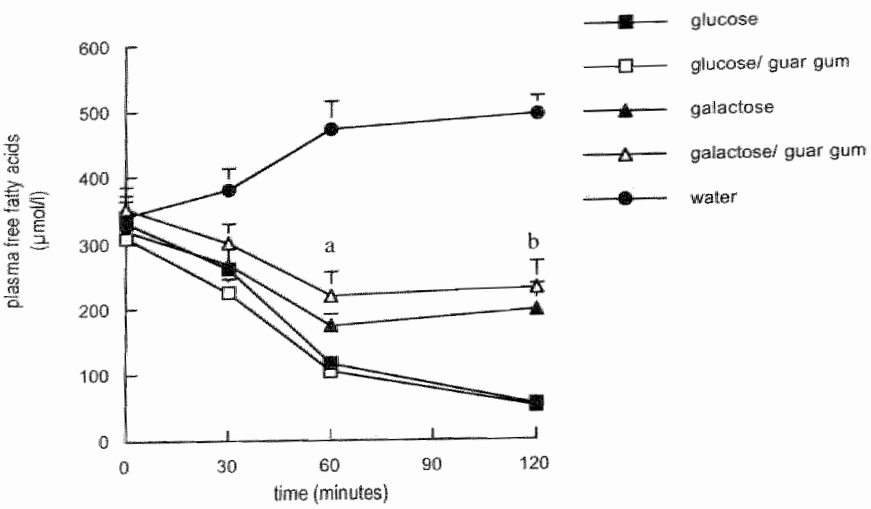

Figure 5. *Plasma free fatty acid concentration (µmol/l)) expressed as means (±SEM)after ingestion of glucose (filled squares), glucose/ guar gum (open squares), galactose (filled triangles), galactose/ guar gum (open triangles) or water.*
a: galactose/ guar gum is different from both glucose conditions at p <0.01
b: both galactose conditions are different from both glucose conditions at p <0.01

Appetite sensations, resting metabolic rate and diet-induced thermogenesis (study B)

Δ-satiety ratings (compared to baseline) were higher in both the guar gum/ nutrient conditions (Fig. 6A,B), with significantly higher ratings after ingestion of glucose/ guar gum compared to glucose alone at 60 minutes ($F_{1,19} = 7.48$; $P = 0.01$). Fullness ratings were similar to the satiety ratings (data not shown). Hunger ratings were similar to the desire to eat ratings (data not shown). Average (mean value of each point of measurement) satiety was related to GLP-1 release at 30 ($r^2 = .23$; $P = 0.03$) and 120 minutes after ingestion of the loads ($r^2 = .22$; $P = 0.03$), independent of condition, excluding the water condition.

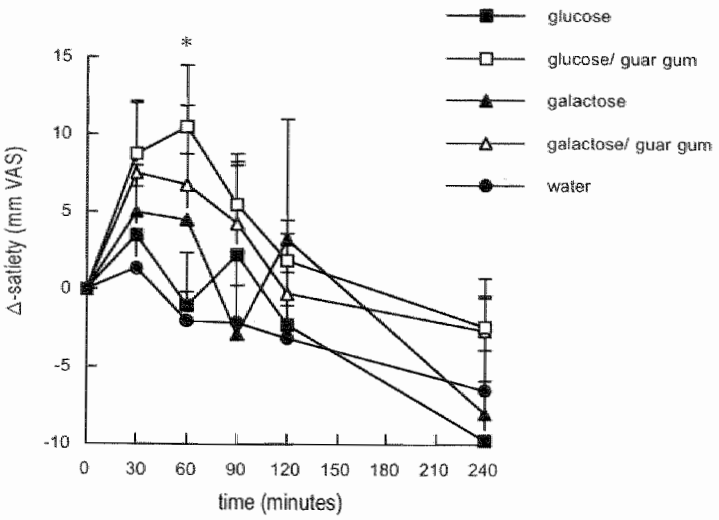

***Figure 6A**. Δ satiety (mm VAS) relative to baseline, expressed as means (±SEM) after ingestion of glucose (filled squares), glucose/ guar gum (open squares), galactose (filled triangles), galactose/ guar gum (open triangles) or water (filled circles).*
** glucose/ guar gum is different from glucose at P = 0.01.*

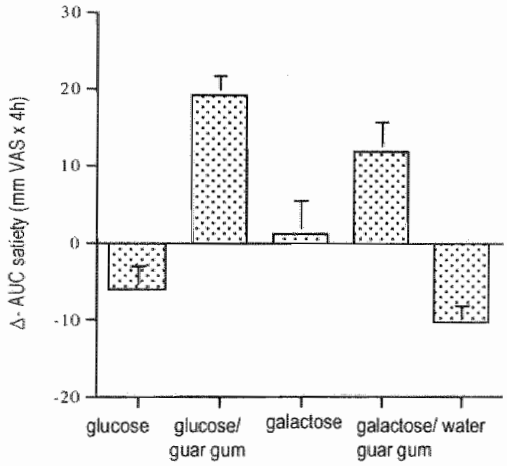

***Figure 6B**. Δ-Area under the curve (compared to baseline, average 0-240 minutes) of satiety ratings (mm VAS) after ingestion of glucose (75g), glucose/ guar gum (75g/2.5g), galactose (50g), galactose/ guar gum (50g/ 2.5g) or water (250 ml).*

No subject discomfort was reported during the trials. Resting metabolic rate was not significantly different among conditions. Diet-induced thermogenesis (as a percentage of total energy intake) was not significantly different among the conditions (Table 2). The

subjects consumed an average of 3.5±1.3 MJ in the test meal, with no difference among the treatment groups (F 4,36 = 1.65; ns.)

***Table 2**. Diet induced thermogenesis (DIT) and resting metabolic rate (RMR) for 20 subjects (12 women, 8 men); values are means±s.d.*

Condition	*DIT(% of energy intake)*			*RMR*		
Glucose	*6.2*	±	*5.6*	*6.33*	±	*1.1*
Glucose/ guar gum	*4.7*	±	*4.7*	*6.48*	±	*1*
Galactose	*9.1*	±	*13.5*	*6.33*	±	*1.1*
Galactose/ guar gum	*6.4*	±	*8.1*	*6.33*	±	*1.1*

Discussion

In healthy, normal-weight subjects, glucose and galactose, with or without guar gum showed maximum GLP-1 stimulation 30 minutes after ingestion. Galactose in combination with guar gum appeared to be more sufficient in stimulating GLP-1 than galactose alone. Surprisingly, the area under the curve for GLP-1 release in the galactose/ guar gum combination was not different from glucose with guar gum, despite of a higher caloric content in the glucose conditions. Like glucose, galactose is absorbed by the liver and converted to glucose. Subsequently it is stored as glycogen or released into the circulation. Compared to lower dosages only the dosage of 50g of galactose in combination with guar gum had a stimulating effect on GLP-1 release (Fig. 1).

Guar gum, a soluble viscous fiber, has been suggested to decrease the rate of gastric emptying and transit time through the small intestine (32, 33). A decrease in the rate of gastric emptying might delay nutrient absorption and thus lead to prolonged stimulation of GLP-1 release (34). In the dose response study we found that the amount guar gum used did not affect GLP-1 release. Previously we have shown that the dosage of guar gum we used did not affect gastrointestinal transit, or gastric emptying rate (35), but did affect satiety (36). If gastric emptying was affected it is likely that it has been by the effect of GLP-1 itself on gastric emptying (37-39). Similar to previous observations (6, 40) GLP-1 appeared to be related to satiety. The effect on satiety in this study seems to be influenced by the addition of guar gum rather than glycaemic index (Fig. 6B).

Stimulation of insulin release is blunted in both glucose and galactose conditions, when guar gum is added. This effect has been found in other studies about the effect of fiber on insulin release as well and has been suggested as probably due to the slowed absorption of nutrients by fiber (17, 41). The effects of guar gum on insulin and satiety seem likely to be explained by the increased contact of the carbohydrates with receptors in the small intestine (17, 18).

Since galactose, as well as fiber such as guar gum are known for their low glycaemic index compared to glucose (42), it seems relevant to investigate the nutrient composition with respect to obesity.

We did not find any differences among treatments in the amount of food eaten after the treatments, or any differences in eating rate. The satiety effect was expressed in mm VAS during four hours after the load. After four hours, when the test-meal was given, it was obvious that the difference in satiety had disappeared.
There was no significant difference in diet-induced thermogenesis (DIT) between the fiber and non-fiber conditions. This result is not necessarily contradictory to other findings that found a reduction in diet-induced thermogenesis after consumption of a high-fiber meal (43), possibly due to the low energy content of the nutrients compared to the time subjects were fasted during the trial.
In conclusion, 50g galactose in combination with 2.5g guar gum dissolved in 250 ml water, was a trigger for GLP-1 release and satiety similar to GLP-1 release caused by glucose with guar gum, but was accompanied by lower glucose and insulin concentrations in healthy, normal-weight subjects. The question remains, whether this can be seen in obese subjects as well.

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Hughes, TE, Westerterp-Plantenga MS, Adam TCM.
"Nutritional compositions comprising a non-glucose carbohydrate or pectine and soluble fiber". WO 2004/ 022074 A1, 2004

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Chapter 3

Nutrient-stimulated GLP-1 release in normal-weight men and women

Tanja CM Adam & Margriet Westerterp-Plantenga

Hormone and Metabolic Research (in press)

Abstract

Aim of the present study was to investigate the effect of a monosaccharide/ fiber preload (galactose/ guar gum) in combination with a standard breakfast (GG) on plasma GLP-1, insulin, glucose, free fatty acid concentrations and appetite ratings (satiety, hunger, fullness and desire to eat) in 30 normal-weight subjects. GG was compared to water in combination with the standard breakfast (W). Furthermore we assessed whether GLP-1 release in the described conditions is different in relation to gender.

In the women postprandial plasma GLP-1 concentrations after GG were significantly increased compared to W ($P < 0.05$). The Δ GLP-1 area under the curve (AUC) for GG was related to body fat (% BF) in women ($r = .48$; $P = 0.02$), but not in men. The rise in plasma insulin was delayed and plasma glucose concentration was blunted with GG. Δ- satiety was significantly related to Δ- GLP-1 (W).

In conclusion galactose with guar gum increased and extended the GLP-1 release due to breakfast in women, but not in men. This may be partly explained by % BF, which is larger in women than in men, since GLP-1 release appeared to be related to %BF.

Introduction

Glucagon-like peptide 1 (GLP-1) is a 30 amino acid peptide hormone, secreted from L cells in the intestinal mucosa after ingestion of carbohydrates and after mixed meals in humans (1-3). Peripheral GLP-1 probably gains access to the subfornical organs and the area postrema, two of the circumventricular organs in the brain that may communicate to appetite regulating centers in the hypothalamus (4). The stimulation of gut hormone release, such as GLP-1 by oral carbohydrate seems to play a major role in triggering satiety, but may differ among different monosaccharides. Not much is known about the effect of galactose on GLP-1 release yet. Carbohydrates with a low glyceamic index (GI), thus with low postprandial glucose and insulin responses are considered beneficial for health (5, 6). Galactose is one of the major monosaccharides (GI=20) (7). In the liver it is rapidly converted to glucose-1-phosphate and is subsequently stored as glucose in glycogen. It also may be converted to glucose with subsequent release into the circulation (8). A few studies demonstrated a stimulating effect of galactose on GLP-1 release, but without evaluating concomitant effects on satiety (9-11).

Guar gum, a viscous polysaccharide, was found to prolong satiety by slowing the absorption of carbohydrates from the small intestine, extending the period of contact between nutrients and small intestinal epithelium, thereby possibly leading to a prolonged stimulation of gut hormones (12, 13). Results about the effect of fiber on GLP-1 release are inconsistent. Some studies found stimulating effects on GLP-1 release (14-16), others showed that the effect of fiber on GLP-1 release might rather be related to the form and botanical structure of food (17).

In a former experiment we found a dose of 50g galactose in combination with 2.5g guar gum effective in stimulating GLP-1 release in normal weight subjects in a manner similar to glucose in combination with guar gum (18). Recent work has been showing that leptin is able to stimulate GLP-1 secretion (19). Gender differences have been described for leptin and other peptides so far (20-22). Nothing is known yet whether stimulated GLP-1 concentrations are different in relation to gender.

Therefore we investigated the effect of galactose in combination with guar gum in combination with a standard breakfast on GLP-1 secretion, and satiety in healthy, normal- weight subjects and second, whether this is related to gender.

Materials and Methods

Subjects

36 normal-weight subjects were recruited by means of advertisements in local newspapers. After they had given their written, informed consent, subjects underwent a medical screening. The study was approved by the Medical Ethics Committee of the Maastricht University. The screening included a medical history and a two-hour oral glucose tolerance test, conducted according to the guidelines of the World Health Organization. Systolic and diastolic blood pressures were measured during screening by an automatic blood pressure monitor (Omron 705 CP, Omron Healthcare GmbH, Hamburg, Germany). After screening, 30 healthy subjects, with a normal glucose tolerance (23) and normal blood pressure (24) (15 women, 15 men) were selected. Subject characteristics are depicted in Table 1.

Table 1. *Basic characteristics of subjects (n=30; 15men, 15 women) (means± sd)*

	Women (n=15)			Men (n=15)			*P*
Age (y)	34.6	±	13.5	27.3	±	11.1	n.s.
Weight (kg)	65.2	±	6.5	74.2	±	3.9	<0.05
Height (cm)	169	±	1	182	±	1	<0.05
BMI[1] (kg/m^2)	23	±	1.6	22	±	1.3	n.s.
Bodyfat (%)	27.1	±	7	15	±	6.8	<0.05
TFEQ[2] I (restraint)	7	±	3.3	4	±	2	<0.05
TFEQ[2] II (disinhibition)	4.1	±	2.5	3.7	±	1.4	n.s.
TFEQ[2] III (hunger)	4.5	±	3.2	3.6	±	2.4	n.s.

Anthropometry

Body weight was measured on a digital balance (Seca, model 707, Hamburg, Germany; weighing accuracy of 0.1 kg) with subjects in underwear, in a fasted state. Height was measured using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). The body mass index was calculated as BW/ $height^2$ (kg/ m^2).

Whole body composition was determined by underwater-weighing with simultaneous assessment of residual lung volume with the helium dilution technique using a spirometer (Volugraph 2000, Mijnhardt, the Netherlands). Measurements were performed in triplicate and the average was used to calculate body density. Body composition was calculated according to Siri (25).

Eating behavior

Eating behavior was analyzed during screening using a validated Dutch translation of the Three Factor Eating Questionnaire (TFEQ) (26, 27). Cognitive restrained and unrestrained eating behavior (factor 1), emotional eating and disinhibition (factor 2) and the subjective feeling of hunger (factor 3) were scored.

Study design and protocol

A crossover, randomized design was applied in 15 women and 15 men on two test days that were at least one week apart. After arriving at the laboratory in a fasted state, a catheter (Baxter BV, Utrecht, The Netherlands) was placed for blood sampling in an antecubital vein. Subjects stayed in a supine position for the rest of the test day. After the catheter was placed, subjects were allowed to rest for 30 minutes before the baseline blood sample was taken. Subjects then received a preload of 50g galactose+2.5g guar gum (836 kJ) dissolved in 250 ml water and a standard breakfast (28) (GG) or the same breakfast with 250 ml water as preload (W). The two test conditions were applied in random order. Subjects were asked to drink the preload in less than five minutes. Subjects received the standard breakfast 15 minutes after the preload. Then they had 15 minutes again for finishing the breakfast. The breakfast (1.9 MJ) had an energy density of 3.9 kJ/g and consisted of two slices of brown bread (100g), baked egg (85g) and 300 ml skim milk. The distribution of energy (ED=3.9kJ/g) was 48.8 energy-percent (E%) from carbohydrates, 28.5 E% from protein and 22.6 E% from fat. All subjects reported that the breakfast size is more than what they habitually have as a breakfast.

Total available energy content of the breakfast was calculated (29) to be 20% of energy requirements for men and 22% of energy requirements for women, adjusted to the nearest 0.5 MJ.

After a fasted blood sample, samples were taken five times during 2 hours, to determine plasma GLP-1, insulin, glucose and free fatty acid concentrations at 30, 45, 60, 90 and 120 minutes relative to completion of the preload (thus immediately after breakfast, 15, 30, 60 and 90 minutes after breakfast). Prior to blood sampling subjects were instructed to rate their feeling of hunger, fullness, sensations of satiety and desire to eat on 100-mm visual analogue scales (VAS) with anchors expressing the most positive and the most negative rating at each end (30). Furthermore subjects were instructed to report possible discomfort or nausea at any time during the trial immediately.

Laboratory analyses

Blood samples for GLP-1 were taken in iced syringes and mixed with EDTA and 40 µl of DPP-IV inhibitor to prevent degradation (Linco Research, St. Charles, USA). Blood samples for other blood parameters were mixed with EDTA to prevent clotting. Plasma

was obtained by centrifugation for 10 minutes at 2800 g at 4 °C. Plasma was collected, frozen in liquid nitrogen and stored at –20 °C for analysis.
GLP-1 concentrations were measured using an ELISA kit (EGLP –35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less and sensitivity is 2pmol/l (31).
Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France). The WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations.
Insulin concentrations were measured using a radioimmunoassay kit (Insulin RIA-100; Pharmacia, Uppsala, Sweden).

Statistics

Data are presented as means ± standard error of the means (SEM). Differences between the treatments were determined by analysis of variance for repeated measures (ANOVA) and Sheffe-F post-hoc test (Statview SE graphics TM). Gender differences were analyzed with factorial analysis of variance (ANOVA) and Sheffe-F post-hoc test (Statview SE graphics TM).
Pearson correlation coefficients, r, were calculated to determine the relationship between GLP-1, anthropometrical data and appetite ratings.
The level of significance was set at $P < 0.05$.

Results

Blood parameters

Blood parameters are given as means ± SEM. Δ was calculated as difference from fasted concentrations at 0 minutes. To control GLP-1 release for the difference in energy content of the preloads, Δ GLP-1 release has been calculated per MJ.
Baseline plasma GLP-1 concentrations were not different between conditions ($P = 0.31$). Plasma Δ- GLP-1 concentration (compared to baseline) after ingestion of GG was significantly increased compared to W at 30 minutes ($F\ 1;29 = 32.5$; $P = 0.0001$), 45 minutes ($F\ 1;29 = 8.85$; $P = 0.006$) and 60 minutes ($F\ 1;29 = 6.19$; $P = 0.01$) (Fig. 1A).
The addition of GG to breakfast increased the AUC (x h) for plasma GLP-1 concentrations by 57% compared to the AUC for W, while the energy content was increased by 40%.
The Δ-AUC (increase in GLP-1 compared to baseline x h) for GG (9.4±.66) and breakfast was significantly higher compared to W (6.32±.57; $P = 0.008$).

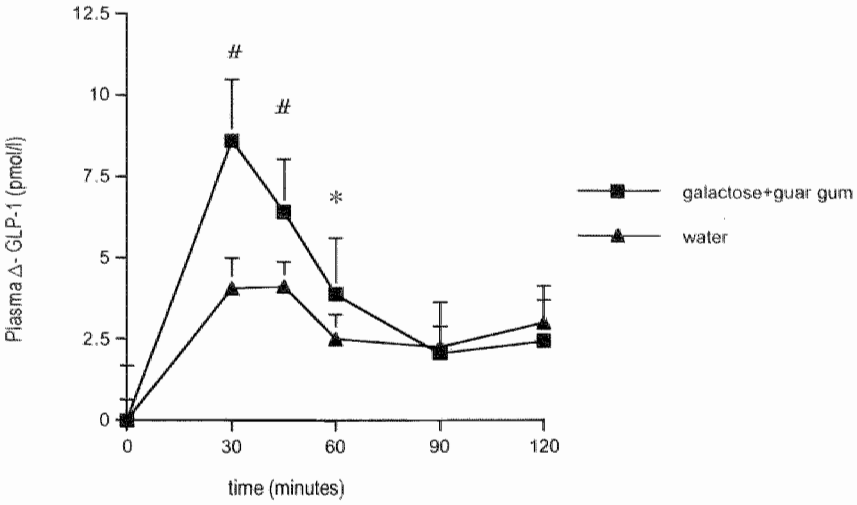

***Figure 1A**. Δ- GLP-1(change from fasted concentrations) plasma concentrations expressed as means±SEM after ingestion of galactose/ guar gum and a standard breakfast (GG) (squares), or the same breakfast with water as a preload (W) (triangles).*
** Significant difference at P = 0.01 comparing GG and W*
 # Significant difference at P < 0.01 comparing GG and W

Δ- GLP-1 release (compared to baseline) after GG was significantly higher for women compared to men at 30 (F 1;28 = 4.77; P = 0.03), 45 (F 1;27 = 19.99; P = 0.0001), 60 (F 1;28 = 8.86; P = 0.005), 90 (F 1;28 = 11.07; P = 0.002) and 120 minutes (F 1;27 = 3.9; P = 0.05). This was not seen in the W condition (Fig 1B). This can be seen in the AUC for Δ-GLP-1 release as well (Fig 1C). Fasted GLP-1 concentrations were not different between men and women. Gender only affected plasma GLP-1 concentrations. None of the other blood parameters differed between men and women.

Plasma insulin concentrations were not different between conditions in the fasted state or after 30 minutes (Table 2). Ingestion of GG before breakfast led to significantly lower insulin concentrations at 45 (F 1;25 = 5.20; P = 0.02) and 60 (F 1;25 = 12.54; P = 0.002) minutes compared to W and breakfast. At 90 minutes (F 1;25 = 5.50; P = 0.03) and 120 minutes (F 1;25 = 18.55; P = 0.0004) plasma insulin concentrations were increased in the GG condition compared to W.

Plasma glucose concentrations were not different between conditions in the fasted state or at 30 minutes (Table 2). At 45 minutes (F 1;28 = 10.44; P = 0.003) as well as 60 minutes (F 1;29 = 11.64; P = 0.001) glucose was significantly elevated after W compared to GG. Glucose concentrations in the GG condition were not significantly different from baseline concentration at any time.

Free fatty acids decreased continuously over time in both conditions. No differences were observed between conditions (Table 2).

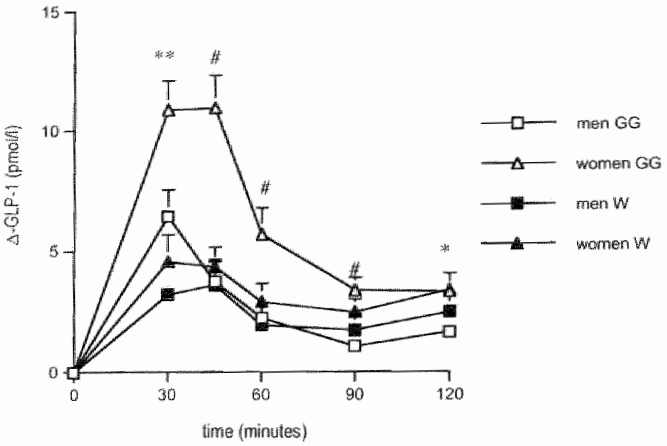

***Figure 1B**. Δ- GLP-1 (change from fasted concentrations) expressed as means ± SEM separately for men (squares) and women (triangles) after ingestion of GG (open squares and triangles) or W (filled squares and triangles).*
** Significant difference at P = 0.05 comparing GG and W in women*
*** Significant difference at P < 0.05 comparing GG and W in women*
 # Significant difference at P < 0.01 comparing GG and W in women

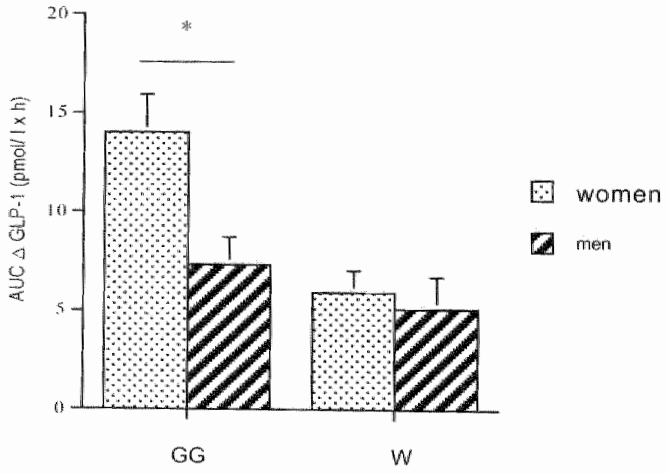

***Figure 1C**. AUC (pmol/l x h) for Δ-GLP-1 release (change from fasted concentrations) for men (n=15) and women (n=15) after ingestion of galactose/ guar gum and breakfast (GG) or water and breakfast (W).*
** = significant difference after GG between men and women at P = 0.003*

Appetite ratings

Δ- satiety ratings were significantly related to Δ- GLP-1 release in the W condition at 60 (r = .42; P = 0.02) and 90 (r = .39; P = 0.03) minutes (Fig. 2). Appetite ratings were not different between W and GG in the men or in the women (data not shown). That subjects were able do distinguish between the preloads did not lead to differences in appetite ratings.

Anthropometry

AUC (x 2 h) of Δ - GLP-1 (increase from fasted concentrations) was positively related to bodyfat (%), (r = .71; P = 0.02). Looking at the effect separately for men and women, it becomes obvious that only women contributed to that effect (r = .48; P = 0.02). No relationship between the AUC for Δ- GLP-1 and bodyfat was found for men (r = .08; n.s.), (Fig. 3).

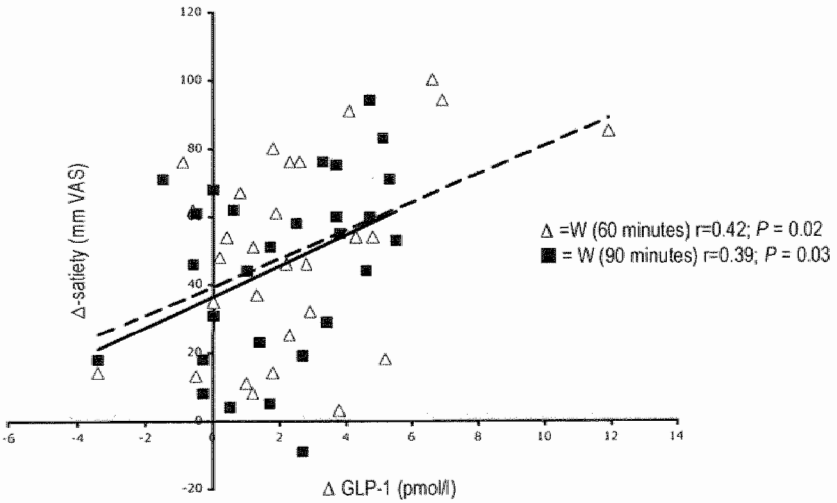

***Figure 2**. Δ- GLP-1 (change from fasted concentrations) is significantly related to Δ- satiety (change from fasted ratings) after ingestion of water and breakfast (W) at 60 minutes (open triangles, dashed line: y = 4.14x+39.4; r = 0.42; P = 0.02) and 90 minutes (filled squares, solid line: y = 4.55x+36.5; r = 0.39; P = 0.03).*

Table 2. *Plasma concentrations of insulin (mU/l), glucose (mmol/l) and free fatty acids (µmol/l) in 30 normal-weight subjects (15male, 15 female) after ingestion of galactose/guar gum (GG) or water (W) in combination with a standard breakfast at 0, 30, 45, 60, 90 and 120 minutes. Values are means (±SEM)*
** significant difference between GG and W at P < 0.05*
 # significant difference between GG and W at P < 0.0

		0	30	45	60	90	120
Insulin (mU/l)	GG	5.9±0.5	19.0±1.7	23.9±2.9*	23.9±2.8#	37.6±4.9*	33.9±2.9#
	W	6.3±0.5	18.9±2.6	37.7±4.4	40.2±4.6	30.0±3.8	24.4±2.8
Glucose (mmol/l)	GG	5.23±0.1	5.24±0.1	5.27±0.1#	5.3±0.1#	5.54±0.1	5.03±0.1
	W	5.24±0.1	5.22±0.1	6.14±0.2	6.16±0.2	5.04±0.2	4.79±0.2
Free fatty acids(µmol/l)	GG	338.5±32.2	232.7±23.4	214.8±23.8	147.9±13.9	84.0±8	63.3±5.6
	W	366.4±41.1	295.3±24.1	294.0±22.1	184.6±20.0	99.2±11.9	86.7±7.6

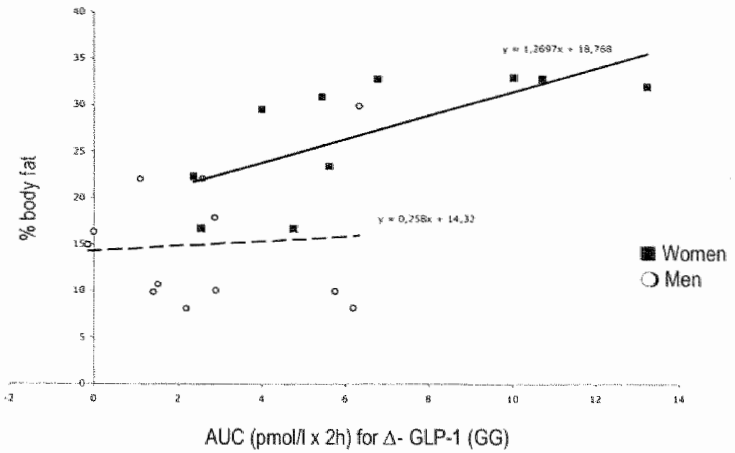

***Figure 3**. AUC (pmol/l x h) for Δ- GLP-1 (change from fasted concentrations) in the GG condition related to % body fat separately for men (open circles, dashed line) and women (filled squares, solid line).*

Discussion

The addition of a galactose/ guar gum preload to a standard breakfast led to a 57% increase of the GLP-1 release compared to breakfast alone. The GLP-1 response to the breakfast was also extended to 90 minutes in the women by the addition of the GG load. These observations confirm results of a former study which showed, that the galactose/ guar gum combination stimulates GLP-1 release in a manner similar to glucose/ guar gum (18). Different from glucose, which has been shown to stimulate GLP-1 release sufficiently before (2, 32), not much is known about galactose and GLP-1 secretion yet. Compared to glucose galactose has a lower glycaemic index (7), that might be beneficial and relevant for prevention and treatment of obesity (33), diabetes (34) and cardiovascular disease.

Insulin and glucose concentrations are lower in the GG condition, although the ingestion of galactose beside the breakfast adds up on the total carbohydrate intake. It has been shown though, that the amount galactose used as a preload in the present study results in a mean increase of the peripheral circulating glucose of only 1mmol/L or less in healthy as well as in type 2 diabetic subjects (35, 36).

The potential role of gastric emptying on the increase and extension of GLP-1 release (37) in the present study is not clear. Carbohydrates as well as fiber have been shown to

affect gastric emptying (13, 38). However, it has been found that the amount of guar gum used in the current study does not seem to affect gastric emptying (39).
From the results of the present study no final conclusions can be drawn about the role of fiber or galactose on GLP-1 release alone, since fiber always is presented in combination with galactose. However, in a pilot experiment, preceding the present study, the dosage of galactose and guar gum used have been tested separately and did not evoke GLP-1 release differently from water. Only the combination of both was effective (18).
Not much is known about the relationship of gender and GLP-1 release yet.
GG led to a significant increase and extended GLP-1 release compared to W. In the current study this effect was primarily present in women and appeared to be partly explained by the relationship of GLP-1 and body-fat mass. Gender has been reported as the major independent covariate for incremental GLP-1 response (40), yet clear gender differences in stimulated GLP-1 release have not been shown before. Gender differences have been described so far for other peptides, i.e. resistin, adiponectin (41, 42), CCK (20, 21) and for leptin (21, 22). The gender difference for CCK is somehow related to sex steroids (20, 43). As far as GLP-1 concentrations, it still needs to be determined whether sex steroids might play a direct or indirect role in the gender related differences.

DPP-IV hyperactivity leading to an accelerated degradation of GLP-1 was found in obese subjects compared to normal controls (44). Nothing is known about potential gender differences in DPP-IV activity, but should be a topic for future research, since that might contribute to the interpretation of the present results.
Physiological concentrations of leptin stimulated GLP-1 release from mouse, rat and human L cells in a dose-dependent manner (19) . The authors found the leptin receptor to be present on the membrane of all cells of the epithelium, including all rodent and human L cells. They suggested leptin as a physiological regulator of GLP-1 secretion under both basal and stimulated conditions (19). Since leptin has been reported to play a more important role in food intake regulation in women than in men (45), a stronger stimulation of GLP-1 in women in the present study may be due to leptin. This explanation seems to be at odds with the reports on lower GLP-1 concentrations and decreased GLP-1 release in obese compared to normal-weight subjects (46), since leptin is produced in direct proportion to the amount of fat stored in the adipocytes (47). However, it has been stated before, that the leptin resistance in particular present in obesity might account for lower GLP-1 secretion (19).
It is unlikely that the size of the breakfast was the cause for a difference in GLP-1 release in the men and women. Due to the small non-significant difference in BMI the energy requirements of these men and women differed on average by 1 MJ. The intake at breakfast was more than all subjects consumed habitually and the difference in the percentage of total energy requirements covered was only 2%.

The caloric content of the two preloads differ. Therefore Δ- GLP-1 release has been calculated per MJ (3.15 pmol/MJ after GG compared to 1.84 pmol/MJ after W ($p = 0.003$) at 30 minutes). The increase in GLP-1 stayed significant when adjusted for caloric content, suggesting that the difference in caloric content is not is not the only possible explanation for differences in GLP-1 release between conditions.
Due to the difference in texture it was not possible to blind this study. However, appetite ratings between conditions were not different.
In accordance with other work, we found a reduced rise in postprandial plasma glucose concentrations following the guar gum condition compared to the condition without guar gum (48-50). Due to lower glucose concentrations, insulin requirements were lower in the GG condition compared to W. The increase in GLP-1 was significantly related to satiety ratings in the W condition, this observation reinforces the possible role of GLP-1 as a short-term satiety hormone (51).
In conclusion the addition of galactose and guar gum to a standard breakfast increased and extended plasma GLP-1 release compared to water and a standard breakfast to a greater extent than expected based upon the increase in energy intake. This increase in GLP-1 release was observed in particular in women. Since the Δ- AUC (GG) for GLP-1 was significantly related to percent body fat in women, and since percent body fat is the most discriminating variable between men and women in the present study, it seems likely, that body fat is a crucial variable for GLP-1 release in normal-weight subjects. Possible relationships of GLP-1 with leptin and other peptides as well as with sex steroids need to be further elucidated. A significant relationship between the increase in satiety and the increase in GLP-1 concentrations supports the view of GLP-1 as a short-term satiety regulating peptide.

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Chapter 4

Activity-induced GLP-1 release in lean and obese subjects

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Physiology & Behavior (in press)

Abstract

Aim of the study was to determine whether physical activity stimulates GLP-1 release on the short-term in normal-weight and in obese subjects compared to rest and furthermore, whether modest weight loss affects GLP-1 release or sensitivity in the obese.

Normal-weight (n=28; 12 male, 16 female; BMI 22.9±1.4; age 35±12.7), as well as obese subjects (n=27; 21 male, 6 female; BMI 30.9±2.7; age 47.1±11.86) were tested in a resting and a physical activity condition. Obese subjects were matched over two groups for a weight loss period of three months. After weight loss the tests were repeated.

The area under the curve (AUC pmol/l x min) for GLP-1 concentrations was significantly increased in the physical activity condition compared to rest in lean subjects ($p = 0.05$) as well as in the obese subjects after weight loss ($p < 0.05$), but not in the obese subjects before weight loss.

Physical activity stimulated GLP-1 release in lean and obese subjects after a weight loss period supports the idea of a neuroendocrine loop in addition to distal- intestinal stimulation of GLP-1 release. Modest weight loss might be effective for increasing GLP-1 sensitivity to acute stimulation.

Introduction

Glucagon-like peptide 1 (GLP-1) is a physiological incretin hormone, secreted by intestinal L cells in response to nutrient ingestion(1, 2). GLP-1 has been shown to increase postprandial satiety sensations in lean (3, 4) as well as in obese subjects (5, 6). The GLP-1 secretion in response to nutrient ingestion seems attenuated as well as delayed in obese subjects compared to lean, healthy controls (7). Since diminished GLP-1 release in obese subjects may be an effect of obesity (7) body weight loss might affect GLP-1 concentrations. There is evidence that weight loss, whether dietary induced or after surgery, improves GLP-1 concentrations in obese subjects (8, 9).

Secretion of the peptide is biphasic with levels rising quickly (15-30 min) after ingestion of a mixed meal before nutrients can reach the ileum (10), where the GLP-1 producing L cells are predominantly located. These observations make it unlikely that GLP-1 release is stimulated only by direct action of nutrients on L cells (11). Several peptides and neurotransmitters –in line with the idea of a neuroendocrine loop- have been suggested to act as triggers for GLP-1 secretion as well. Gastrointestinal polypeptide (GIP) i.e. was shown to be a potent stimulus for GLP-1 secretion, GIP is released when nutrients are present in the upper intestine (11). Furthermore it is believed that GLP-1 release is triggered by the autonomic nervous system (11, 12) . For peptide YY (PYY), which is cosynthezised with GLP-1 from intestinal L cells, it has been shown, that the sympathetic nervous system affects its release (13). The modulation of GLP-1 secretion by the nervous system has been poorly investigated in humans. Meal-induced activation of the sympathetic nervous system has been mentioned as a factor to account for the early secretion of GLP-1 (14). Cholinergic agonists enhanced GLP-1 secretion, when arterially infused (15), and epinephrine has been shown to induce GLP-1 release in the isolated perfused rat ileum (11).

One way to stimulate the sympathetic nervous system is physical activity (16). GLP-1 was shown to be increased in marathon runners after a race (17).

With respect to the treatment of obesity and the metabolic syndrome, increased physical activity is one of the first-line treatments next to weight reduction (18). Although a body of literature has shown that exercise as such has little effect on weight loss, its effect on fat loss and preservation of fat free mass makes is a major component in weight-loss programs (19). There is a two- to sixfold increase in norepinephrine levels during light to maximal exercise. Epinephrine output from the adrenal medulla increases with exercise as well, and the magnitude of increase is directly related to the intensity and duration of effort (16).

The present study aims to assess, whether there is an acute GLP-1 release following physical activity of low intensity in normal weight subjects. Secondly, we assessed whether this would occur in overweight/ obese subjects. If, as expected, GLP-1 release differs between lean and obese subjects (7, 8), we aimed to assess GLP-1 release in the overweight/ obese subjects after modest weight loss.

Materials and methods

Subjects

Subjects (n=65) were recruited by means of advertisements in local newspapers or by advertisements in the university. 55 subjects were selected following the criteria of being in good health, not taking any medications, and no history of diabetes, eating disorders or any chronic disease. 28 (12 male, 16 female) normal-weight (BMI 19-25) volunteers (Caucasian) and 27 (21 male, 6 female) visceral overweight/obese subjects (Caucasian) as defined by a BMI between 25 and 30, and by a waist-hip ratio above .85 (20, 21), were selected. Informed written consent was obtained. The study was approved by the Medical Ethics Committee of the Maastricht University. Subject characteristics are depicted in Table 1.

Screening

Before participating in the study all subjects underwent a medical screening, and performed an oral glucose tolerance test, conducted according to the guidelines of the World Health Organization (22).

Anthropometry

For all participants body weight was measured on a digital balance (Seca, model 707, Hamburg, Germany; weighing accurancy of 0.1 kg) with subjects in underwear, in a fasted state. Height was measured using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Body mass index was calculated as BW/height^2 (kg/m^2).

Physical fitness

For investigating the individual appropriate power for the moderate physical exercise condition lean, as well as overweight/obese subjects had to perform an incremental exercise test on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands) with open-circuit indirect calorimetry (Oxycon b, Mijnhardt, Bunnik, The Netherlands) and heart rate monitoring (Polar, Kempele, Finland). After an initial warm up period of 7.5 min (5 min at 50 W and 2.5 min 100 W), the workload was increased every two and a half minutes with 25 W until exhaustion. The maximal power output was calculated using the total time cycled at the exercise test. The highest workload completed for 2.5 minutes (Wcompleted) and the number of seconds (X) that the final increase of 25 W was maintained, were added, according to the following formula: Wmax = Wcompleted + ((X/150) *25 (23). On the test day, subjects cycled on 25% of their maximal power output for 60 minutes. The reason for applying a bout of 25% of maximum power output to subjects during the test was that metabolic rate with that

workload is increased by approximately 3- to 4-fold above rest, which is a requirement that is primarily met by an increase in uptake and oxidation of plasma fatty acids and is reflected in the results on free fatty acids (24).

Blood sample collection and processing

At the test day subjects arrived at the laboratory in a fasted state. An indwelling cannulae (Baxter BV, Utrecht, The Netherlands) was inserted in an antecubital vein for blood sampling. At every time point of blood sampling plasma concentrations were measured for: GLP-1, insulin, free fatty acids and glucose. All subject were in a supined position for thirty minutes after placing the cannula and before a fasted blood sample was taken. After the first blood sample subjects either continued resting or started cycling on the same electromagnetically braked cycle ergometer on which they performed the maximal power output test. They cycled for one hour on 25% of their maximal power output. Blood samples were taken immediately after 60 minutes of physical activity and 30 minutes after cycling. After 60 minutes on the ergometer, subjects stayed in a supine position again for the rest of the trial. In the resting condition blood samples were taken at the same time-points, while subjects stayed in a supined position. In between blood sample 0 and 60 all subjects got 250 ml of water to drink to prevent dehydration.

GLP-1 plasma (4 ml) was collected into iced vacutainer tubes (Becton Dickinson, UK) prepared with EDTA and 40 µl of DPP-IV inhibitor (Linco Research, St. Charles, USA) for preventing active GLP-1 (7-36 amide) degradation into truncated, inactive GLP-1 (9-36 amide). Dipeptidyl peptidase IV (DPP-IV: EC3.4.14.5) is an amino peptidase present in serum, which causes degradation resulting in the formation of an inactive metabolite of the former active hormone.

Insulin and free fatty acids were measured in plasma from a 4ml blood sample collected into EDTA vacutainer tubes (Becton Dickinson, UK). Blood for glucose was collected into vacutainers prepared with potassium (Becton Dickinson, UK).

Analytical methods

Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France) and free fatty acid concentrations were analyzed with the WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany). GLP-1 samples were analyzed by LINCO (USA) using ELISA (EGLP-35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. It is highly specific for the immunological measurement of active GLP-1 and does not detect other forms of GLP-1.

The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less and sensitivity is 2pmol/l (25).

Insulin was measured using RIA for quantitative measurement of insulin from serum, EDTA-plasma and heparin plasma (Insulin RIA-100; Pharmacia, Uppsala, Sweden).

Adverse events

Adverse events during treatment were recorded and severity and outcome specified. Subjects were instructed to report discomfort or nausea at any time during trials immediately.

Weight loss period

To investigate whether weight loss affects the results obtained in overweight/obese subjects at the first test session and based on reported improvement of GLP-1 concentrations after weight loss (8, 9), a weight loss period of three months was executed.

Based on physical activity levels, maximum power output and subject characteristics, the obese subjects were matched over two groups for the weight loss period of three months.

One group was instructed to increase their daily physical activity level in order to lose weight, the other group was instructed to keep their habitual daily activity pattern. All subjects were told not to change their habitual diet. Monthly subjects received instructions on how to increase their physical activity, e.g. thirty minutes of walking every day, working in the garden, cycling, or walking the dog. Once a month subjects came to the lab to have their body weight determined and for receiving new activity instructions. At the same time subjects reported their activities during the last month.

After the three months weight loss period, all overweight/obese subjects repeated the two test trials of rest and physical activity, which have been described above.

Basal metabolic rate and energy expenditure

Basal metabolic rate (BMR) was estimated according to the Harris-Benedict equation (26):

BMR women (MJ/d) = 2.74 + (0.774 x height (m)) + (0.04 x bodyweight (kg)) - (0.02 x age (years)).

BMR men (MJ/d) = 0.28 + (2.093 x height (m)) + (0.058 x bodyweight (kg)) - (0.028 x age (years)).

Estimation of energy expenditure (EE) in obese subjects was based upon the equation of Harris & Benedict (26) and the physical activity level (PAL), calculated as:

Total energy expenditure (TEE) = BMR x PAL.

Physical activity

Daily Physical activity level of the obese subjects was measured before and after weight loss with either tri-axial accelerometers (Tracmor) (27) or uni-axial accelerometers (computer science and application monitor, CSA) (28, 29) and the Baecke questionnaire (30). Subjects wore the CSA or Tracmor during waking hours on a belt at the back of the waist for one week before and after the weight loss period.

Physical activity level was calculated using the following equation for the CSA (29):

physical activity level = (0.000001379 x (counts (n) per day x 5)) + 1.113

and the following equation for the Tracmor (27):

total EE = -1.259 + (1.552 x resting EE) + (0.076 x counts (n) per min),

and then:

physical activity level (PAL) = total EE (MJ/d) / resting EE (MJ/d).

The accelerometers were randomized over the two groups. Subjects received the same accelerometers for measurements before and after weight loss.

Statistical Analyses

The data in text and figures are presented as means ±SEM. Data in the table are presented as mean ± standard deviation (s.d.). AUC is calculated as pmol/l x 90 minutes. All statistics were calculated with STATVIEW+GRAPHICS (Abacus Concepts Inc, Berkeley, CA). GLP-1, insulin, glucose and free fatty acids were compared between conditions with factorial analyses of variance (ANOVA) and Sheffe-F or Fisher PLSD as a post hoc test. The area under the curve between conditions was compared with Student's t test for paired data. A value of $p<0.05$ was considered significant.

Results

Since it appeared that weight loss did not differ between the two groups (one group with the activity instructions, the other one with the instruction to keep their habitual daily activity level), both groups were taken together for further analysis.
Also, no difference between men and women was observed, therefore they were taken together for further analysis as well.

OGTT

Glucose plasma concentrations during the OGTT were significantly different between lean and overweight/obese subject at 0 (P = 0.0001), 30 (P = 0.0002), 60 (P = 0.0001) and 120 minutes (P = 0.0001) with increased glucose concentrations in obese subjects compared to lean subjects (Table 1). Overweight/obese subjects did not match the criteria for impaired glucose tolerance (22).
The OGTT in overweight/obese subjects was not different after the weight loss period compared to before weight loss (Table 1).

***Table 1**. Subject characteristics of lean subjects (n = 28) and overweight/obese subjects (n = 27) before and after weight loss*

	Lean subjects (12 male, 16 female)	Obese subjects (21male, 6 female)		
		before weight loss	after weight loss	*p*
Weight (kg)	69.34 ± 7.09	98 ± 5.1#	94.5 ± 4.5*#	0.02
BMI (kg/m2)	22.9 ± 1.4	30.9 ± 2.7#	29.3 ± 2.5*#	0.01
Wmax	187.7 ± 48.3	205.6 ± 10	220 ± 10	0.49
Waist (cm)		103.9 ± 1.7	101 ± 1.2*	0.02
Baecke		8.03 ± 2.4	8.37 ± 1.2	0.39
PAL (physical activity level)		1.5	1.5	0.17
BMR (MJ/d) (basal metabolic rate, calculated)		7.89 ± 1	7.7 ± 1*	0.04
EE (MJ/d) (energy expenditure)		12.13	11.6*	0.05
OGTT 0 (mmol/l)	5.12 ± 0.07	6.06 ± 0.16#	5.89 ± 0.15#	0.0001
OGTT 30 (mmol/l)	7.43 ± 0.23	9.13 ± 0.39#	9.41 ± 0.26#	0.0002
OGTT 60 (mmol/l)	6.52 ± 0.30	9.54 ± 0.58#	9.96 ± 0.41#	0.0001
OGTT 120 (mmol/l)	5.24 ± 0.19	7.29 ± 0.46#	7.02 ± 0.36#	0.0002

Values are means ± s.d. OGTT glucose concentrations are means±sem.
* significant difference in overweight/ obese subjects after weight loss compared to before weight loss.
# significant difference between lean and overweight/ obese subjects

Results for lean subjects and overweight/obese subjects before weight loss

First, in lean subjects the physical activity condition was compared to the resting condition. The concentration of basal plasma GLP-1 (7-36) amide in lean subjects was 4.5 ± 0.6 pmol/l (mean ± SEM). With physical activity, GLP-1 release was significantly increased at 60 minutes (5.9 ±1.08 pmol/l) compared to rest (3.62 ± .56 pmol/l), ($F\ 1;26 = 5.50$; $P < 0.05$), as well as compared to baseline ($F\ 1;26 = 5.26$; $P = 0.03$). Immediately after the cycling period GLP-1 levels went back to almost basal concentrations (4.2 ± .81pmol/l), comparable to 4.8 ± 1.02 pmol/l at rest.

The AUC (pmol/l x minutes) for GLP-1 was different between activity and rest (F 1;26 = 5.52; P = 0.05) (Figure 1).
Before weight loss, in the overweight/obese subjects, the AUC (pmol/l x minutes) for GLP-1 concentrations was not different between the resting and the activity condition. (Figure 2A).

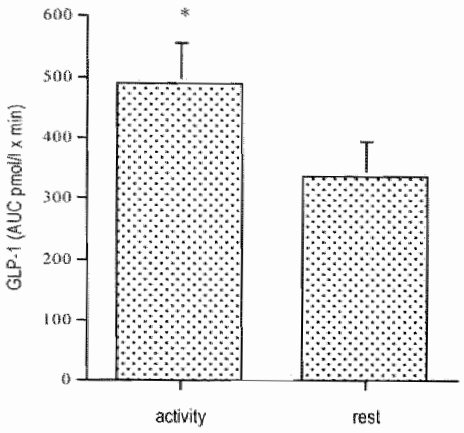

***Figure 1**. Comparison between AUC (pmol/l x min) of GLP-1 release in normal-weight subjects (n=28) during rest and activity*
** different at p = 0.05*

In the lean subjects insulin concentrations after physical activity were significantly increased compared to rest at 90 minutes (F 1;24 = 7.55; P = 0.01) (Table 2).
For the obese group insulin concentrations were not different between the resting condition and the activity condition before weight loss.

Glucose concentrations in lean subjects were elevated during physical activity compared to rest at 60 minutes (F 1;24 = 10.16; P = 0.003). (Table 2).
Glucose concentrations in the obese group were not different between the two conditions before weight loss.

Free fatty acids significantly increased in the lean subjects during physical activity and peaked at 60 minutes (F 1;26 = 4.97; P = 0.03). After activity, free fatty acids declined rapidly and approached resting concentrations (Table 2).
Before weight loss, free fatty acid concentrations were significantly increased in obese subjects during physical activity compared to rest at 60 (F 1;25 = 10.98; P = 0.002) and 90 minutes (F 1;21 = 8.48; P = 0.008) (Table 2).
Baseline concentrations between physical activity and rest for insulin, glucose and free fatty acids were not different in obese subjects.

Results for overweight/ obese subjects after weight loss

After three months all overweight/obese subjects had reduced their weight and their waist circumference significantly, without any differences between the two groups that received different activity instructions (Table 1).

In all overweight/obese subjects physical activity indicated by the Baecke score, Wmax or PAL remained unchanged. Since no difference was observed between the two overweight/obese sub-groups, these groups were taken together for further analysis.

After weight loss the AUC (pmol/l x 90 minutes) for GLP-1 release was significantly different between physical activity and rest (375.2 pmol/l ± 75.2 compared to 267.2 pmol/l ± 30.9; P= 0.03), (Figure 2 B).

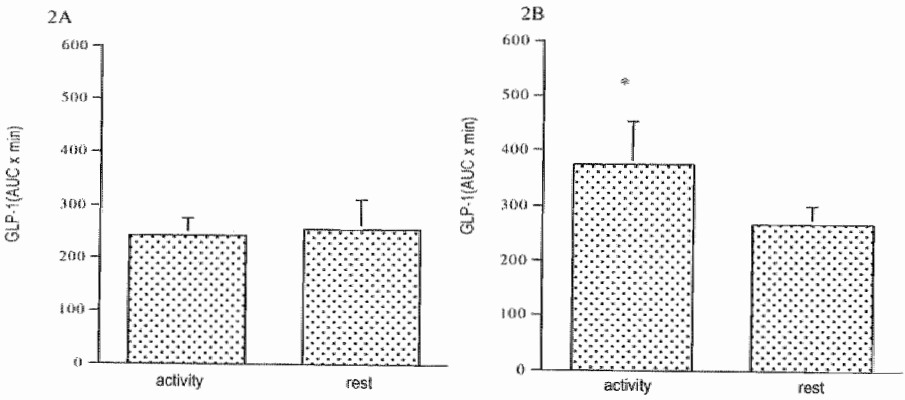

***Figure 2 A, B**. Comparison between AUC (pmol/l x min) of GLP-1 release in obese subjects (n=27) during rest and activity, before weight loss (A) and after modest weight loss (B).*

* different at p < 0.05

Baseline concentrations for insulin, glucose and free fatty acids between physical activity and rest were not different in obese subjects.

In the obese group after weight loss no difference in plasma insulin or glucose concentrations was observed between the resting and the activity condition (Table 2).

Free fatty acid concentrations were different between activity and rest at 60 ($F_{1;20}$ = 15.2; P = 0.0009) and 90 minutes ($F_{1;13}$ = 21.9; P = 0.0004).

***Table 2**. Plasma concentrations of insulin (mU/l), glucose (mmol/l) and free fatty acids (µmol/l) in lean (n = 28; 12 male, 16 female) and in overweight/obese subjects (n = 27) before and after weight loss (21 male, 6 female) in rest and with physical activity at 0, 60 and 90 minutes. Values are means ±SEM*

			Physical Activity						Rest					
			0	60	90	0	60	90
lean		Insulin (mU/l)	6.95 ± 0.74	7.28 ± 1.26	6.38 ± 0.92*	7.91 ± 2.4	5.27 ± 1.04	3.65 ± 0.47
		Glucose (mmol/l)	5.21 ± 0.15	5.26 ± 0.10#	5.19 ± 0.15	5.10 ± 0.08	4.87 ± 0.07	4.90 ± 0.07
		Free fatty acids (µmol/l)	362.3 ± 39.3	707.5 ± 80.6#	551.2 ± 52.4	310.7 ± 29.9	490.3 ± 54.6	490.4 ± 35.8
obese	before weight loss	Insulin (mU/l)	13.3 ± 2.44	9.31 ± 1.98	10.71 ± 1.17	11.67 ± 1.13	9.78 ± 1.07	9.65 ± 1.02
		Glucose (mmol/l)	5.93 ± 0.16	5.51 ± 0.12	5.56 ± 0.11	5.71 ± 0.07	5.49 ± 0.07	5.52 ± 0.09
		Free fatty acids (µmol/l)	391.8 ± 34.1	558.9 ± 42.4#	545.7 ± 32.1#	379.3 ± 19.4	386.6 ± 19.1	422.5 ± 23.1
obese	after weight loss	Insulin (mU/l)	12.64 ± 2.7	12.69 ± 4.2	11.96 ± 3.0	10.21 ± 2.4	8.66 ± 1.8	7.76 ± 1.6
		Glucose (mmol/l)	6.08 ± 0.23	5.66 ± 0.19	5.61 ± 0.18	5.68 ± 0.14	5.36 ± 0.13	5.48 ± 0.09
		Free fatty acids (µmol/l)	332.4 ± 35.6	526.8 ± 38.4#	486.3 ± 34.2*	351.6 ± 32.0	348.2 ± 37.2	363.9 ± 33.4

* significantly different from concentrations in rest at $P < 0.05$.
\# significantly different from concentrations in rest at $P < 0.01$.

Discussion

Whereas the effects of nutrients on GLP-1 secretion have been studied extensively, modulation of this secretion by physical activity or by the nervous system has been poorly investigated in humans.

On the short term, after 60 minutes of low intensity activity, GLP-1 release was significantly increased in fasted, normal-weight subjects, but not in overweight/obese subjects. The area under the curve in a physical activity condition was significantly different from a resting condition in lean subjects. However, this phenomenon of increased GLP-1 concentration during activity compared to rest appeared as well in the overweight/obese subjects after weight loss.

We speculate, that the short-term stimulation of GLP-1 secretion in these subjects is due to the stimulation of the sympathetic nervous system during exercise. GLP-1 induces inhibition of gastrointestinal motor activity (31). Colonic sympathetic afferents that control the gastroduodenal area have been described (32) and GLP-1 action on these fibers has been suggested to activate an ileal brake mechanism (32, 33).

With respect to the intended physical activity induced weight-loss, it is unlikely that weight-loss was activity-induced, since the scores on the Baecke questionnaire remained unchanged after the weight loss period compared to before weight loss. The same holds for the Wmax. Although the lack of increase in PAL is partly due to the weight-loss induced decrease in BMR, and a small increase in activity induced energy expenditure, the achieved weight loss seems not to be completely attributable to increased physical acitivity. It has been shown before that other factors may be responsible for the actual weight loss, such as an increase in dietary restraint (34, 35).

Epinephrine (11) and isoproterenol (36) were shown to stimulate GLP-1 release in the isolated rat ileum. Together with other studies that show a blockade of the effect of GLP-1 on gastric intestinal motor activity by phentolamine and propranolol, the present data suggest a possible involvement of adrenergic pathways in GLP-1 actions (32). The stimulation of the sympathetic nervous system during exercise leads to epinephrine as well as norepinephrine release, possibly triggering GLP-1 release in lean as well as post-obese subjects. Exercise stimulates b-adrenergic receptors (37). There is evidence from animal studies, that stimulation of b-adrenergic receptors stimulates ileal L cell secretion (38, 39). Epinephrine has not been measured in this experiment. The quick but rather short stimulation of GLP-1 release is in line with experiments showing transient peptide release in response to adrenergic agonists and support the hypothesis that neurotransmitters modulate intestinal endocrine cell secretion for brief time periods (36, 39, 40).

There is evidence for attenuated postprandial GLP-1 release in obese subjects (7, 8). It might be possible that obese subjects are less sensitive to any kind of GLP-1 stimulation in line with other metabolic changes and disturbances caused by obesity (21, 41), and that diminished GLP-1 concentrations in the obese may indeed be an effect of obesity,

and may be reversible after weight loss. The difference in GLP-1 concentrations after physical activity was not observed in obese subjects before weight loss, following the same protocol as lean subjects, suggesting that weight loss contributes to an improvement of metabolic fitness (42, 43) and to an improvement of GLP-1 sensitivity. A gradual postprandial normalization of GLP-1 response has been reported in obese subjects after weight loss (8).

Leptin, an adiposity signal that is secreted in proportion to the amount of fat stored in the adipocytes, was shown to stimulate GLP-1 secretion from rat, mouse and human L cells dose dependently and to contribute to the improvement of GLP-1 release after weight loss (44). Leptin resistance is common in obese humans (45) and leptin sensitivity improves with weight loss (46). In a murine model leptin resistance impaired GLP-1 release. These results give support to the idea of leptin as a causative factor for impaired GLP-1 release in obese humans (44). Although leptin was not measured in the present study, improved leptin sensitivity due to weight loss might be one possible mechanism underlying improved GLP-1 release following weight loss.

Based on the present study it seems that even modest weight loss affects GLP-1 release. Further studies are necessary for investigating effects of severe weight loss on GLP-1 release. Insulin concentrations appeared as expected (47).

Free fatty acids are a major oxidative fuel during rest and activity. Sub-threshold power output physical activity increases the proportion of free fatty acid oxidation. As expected, this is reflected in the results, comparing activity induced increase in free fatty acids to rest.

Taken together, GLP-1 release is stimulated by physical activity of 25 % Wmax after one hour in normal weight subjects, suggesting a role of a neuroendocrine loop in the regulation of the entero-insular axis and GLP-1 release, that may be mediated by the sympathetic nervous system. A significant difference between the activity condition and rest after weight loss was observed in overweight/obese subjects. That difference was not seen before weight loss, indicating a diminished acute GLP-1 response due to obesity related insensitivity.

From the tendency of improved GLP-1 concentrations in the post-weight loss vs. the pre-weight loss state, we conclude that the diminished GLP-1 concentrations in the obese may be reversible, and therefore may be rather an effect than a cause of obesity.

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Chapter 5

GLP-1 release and satiety after a nutrient challenge in normal-weight and obese subjects

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British Journal of Nutrition (In Press)

Abstract

The present study was conducted to assess whether Glucagon-like peptide (GLP)-1 release and appetite after a breakfast with or without an additional galactose/guar gum stimulation is different in normal-weight as compared to overweight/obese subjects. 28 overweight/obese (BMI: 30.3±2.7 kg/m^2; age: 44.3±9.7y) and 30 normal-weight subjects (BMI: 22.8±1.4; age: 31.5±12.8y] participated in a crossover study. Fasting and postprandial plasma GLP-1, insulin, glucose and free fatty acid concentrations were measured in response to either a galactose (50g)/guar gum (2,5g) load (836 kJ), and a standard breakfast (1,9 MJ) (GG), or water (250ml) and the standard breakfast (W) every 30 minutes relative to the ingestion for 120 minutes. Appetite was assessed using 100 mm visual analogue scales.

GLP-1 concentrations were significantly increased after GG at 30 and 60 minutes compared to W in both groups.

Plasma GLP-1 concentrations in the W condition were higher in normal-weight as compared to overweight/obese subjects (p=0.03). No difference was observed in the GG condition between groups. Satiety was increased in normal-weight subjects compared to the overweight/obese in the GG condition at 30 (p=0.02) and 60 minutes (p=0.04). We conclude, that after a standard breakfast with water, GLP-1 release was lower in the overweight/obese compared to normal-weight subjects. However, postprandial GLP-1 release in overweight/obese subjects was not different from normal-weight subjects, when galactose/guar gum was added to the breakfast. The latter was not mirrored by subjective feelings of satiety. Disturbed perception of physiological feedback of a satiety hormone rather than disturbed feedback itself might contribute to obesity.

Introduction

Food intake regulation is a complex process that involves physiological as well as social and psychological components. The way food is sensed and processed by the biological system generates and activates neural and humoral signals that control appetite. Glucagon-like peptide 1 (7-36) amide (GLP-1) is believed to be one of the gut peptides that are involved in satiety signaling in addition to other signals that operate via gastric and small-intestinal vagal afferent nerve fibers (1-3).

GLP-1 is a 30 amino acid peptide hormone that is released from intestinal L-cells of the intestinal mucosa in response to nutrients and mixed meals (4, 5). It increases satiety and suppresses appetite in normal weight subjects (6, 7). GLP-1 release in response to nutrient sensing is known to stimulate insulin release in pancreatic beta-cells (6-9). Findings on basal GLP-1 concentration and the effect of food intake on GLP-1 release and satiety in obese subjects are contradictory. While one study reports hypersecretion of truncated GLP-1 in obese subjects in response to a glucose load (10), others find attenuated release of GLP-1 in response to a meal (11). Peripheral administration of GLP-1 in obese subjects decreased hunger ratings and reduced energy intake (12).

Although dietary fiber such as guar gum was found to effectively increase satiety and fullness ratings and reduce hunger and desire to eat in obese as well as normal weight subjects in the short-term (13, 14), the evidence for effects on weight loss is poor (15). The effect of fiber on GLP-1 release seems not clear yet and has been found to be a matter of amount (16, 17), but also has been suggested to depend on structural food properties rather than the amount of fiber

ingested (18). In an earlier study we found that galactose in combination with guar gum before breakfast increased GLP-1 release in normal-weight subjects (19). The aim of the present study was to investigate whether GLP-1 release will be increased postprandially in response to galactose with guar gum consumed before a standard breakfast (GG) in obese subjects compared to normal- weight subjects. Furthermore we examined whether this is reflected in appetite ratings.

Subjects and Methods

Subjects

70 subjects between the ages of 20 and 60 years were recruited by means of advertisements in local newspapers. Of the 58 included subjects, 28 subjects were overweight/ obese (9 male and 19 female) according to the classification of the World Health Organization (WHO) (20). Thirty subjects were normal weight controls (15 male and 15 female), (Table 1). Subjects were used to a laboratory environment, since they participate frequently in experiments that are executed at the Maastricht University. Selection criteria included being in good health, not taking any medications, and no history of diabetes or chronic disease and no participation in other ongoing studies or former studies that would influence the outcome of the present study.

The power calculation for the present study is based on former results that are assumed as a scientifically important difference (19) and has been calculated for a sensitivity of 0.90 and a two-sided significance level of 0.05 according to the standard equations (21). Based on a difference between conditions of 8.6 pmol/l and a standard deviation of 10 we calculated 26 subjects. If a Mann-Whitney U test is applied, the N needs to be increased by 5%, which added up to 28 subjects.
Informed written consent was obtained and the study was approved by the Medical Ethics Committee of the Maastricht University.

Body weight and BMI

For all subjects body weight (BW) was measured on a digital balance (Seca, model 707, Hamburg, Germany; weighing accuracy of 0.1 kg) and height was measured using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Body mass index was calculated as BW/ height^2 (kg/m^2) (Table 1).

***Table 1**. Subject characteristics are expressed as means ± sd.*
** significant difference between normal-weight (n=30) and overweight/ obese subjects (n=28), (factorial ANOVA).*

	Normal weight (n = 30)	Obese (n = 28)	p
Age (years)	31.58±12.84	44.38±9.76	0.0002*
Height (m)	1.75±0.09	1.71±0.06	0.10
Weight (kg)	69.34±7.09	89.32±8.92	0.0001*
BMI(kg/m^{-2})	22.89±1.49	30.35±2.70	0.0001*
%body fat	20.49±9.02	38.15±6.13	0.0001*

Body composition

Total body water was measured using the ^{2}H (^2H_2O)- dilution technique (22, 23). The evening prior to the first test day subjects drank a deuterium dilution (70g water with an enrichment of 5 atom% excess ^{2}H) after voiding. Deuterium enrichment was measured in urine from the second voiding of the following morning. ^{2}H concentrations in the urine samples were measured using an isotope ratio MS (Micromass Optima, Manchester, UK). Total body water was determined by dividing the measured ^{2}H dilution space by 1.04 (23). Fat-free mass (FFM) was calculated by dividing the total body water by the hydration factor 0.73. By subtracting FFM from body weight, fat mass (FM) was obtained. Body fat (%) was calculated as FM expressed as % body weight (Table 1).

Study protocol and meal

Subjects came to the laboratory for two visits, separated by at least one week. The subjects were instructed to fast from 10 pm the night prior to each visit. After arrival at 8:00 in the morning, an indwelling cannulae (Baxter BV, Utrecht, The Netherlands) was inserted in an antecubital vein. After a baseline blood sample was collected, subjects consumed a nutrient load (836 kJ) that consisted of either 50 g galactose (D-(+)-galactose; Fagron Farmaceuticals, The Netherlands) and 2.5 g guar gum (Meyprofin, Switzerland), dissolved in 250 ml water, or they drank 250 ml water alone, in randomized order. After drinking the load subjects had to eat a standard breakfast. Subjects were given fifteen minutes to finish the meal. The breakfast (1.9 MJ) had an energy density of 3.9 kJ/g and consisted of two slices of brown bread (100 g), baked egg (85 g) and 300 ml skim milk. The distribution of energy was 48.8 energy-percent (E %) carbohydrates, 28.5 E % protein and 22.6 E % fat. All subjects reported the breakfast as much bigger than what they habitually would eat for breakfast.
Blood samples were taken for a total of two hours, every half hour relative to ingestion.

Pre- and post-absorptive appetite profile

To determine the appetite profile, satiety and desire to eat were rated on anchored 100 mm visual analogue scales before the meal (timepoint 0), immediately after the meal (timepoint 30) and during the rest of the test day every 30 minutes relative to the measurement after the meal for two hours. For the increase in satiety due to the meal delta-satiety was calculated as increase from the fasted rating at timepoint 0.

Blood sample collection and processing

Blood samples were taken to measure plasma GLP-1, insulin and glucose concentrations. Blood samples for GLP-1 were taken in iced syringes and mixed with EDTA and 40 µl of DPP-IV inhibitor to prevent degradation (Linco Research, St. Charles, USA). Blood samples for other blood parameters were mixed with EDTA to prevent clotting. Plasma was obtained by centrifugation for 10 minutes at 2800 g at 4 °C. Plasma was collected, frozen in liquid nitrogen and stored at –20 °C for analysis.
GLP-1 concentrations were measured using an ELISA kit (EGLP –35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less. Sensitivity of the analysis is 2pmol/l (24).
Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France). The WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations.
Insulin concentrations were measured using a radioimmunoassay method (Insulin RIA-100; Pharmacia, Uppsala, Sweden).

Statistical analysis

For test of normality, data were tested with the Shapiro-Wilk test of normality.
A one-way repeated-measures ANOVA was carried out to determine the hormonal and appetite differences between GG and water per group. Hormonal parameters and area under the curve (AUC) were tested with a non-parametric Mann-Whitney U test for differences between groups. AUC was calculated as incremental area under the curve over time (2h). Appetite differences in GG and W between obese and lean subjects were tested with factorial ANOVA.
The relationship between age and blood parameters such as GLP-1, insulin, glucose and free fatty acids was tested with a multiple regression analysis.
Results are presented as mean values and standard error of the means (SEM) or medians and ranges as appropriate. Statistical procedures were performed by using Statview SE+Graphics (1988; Abacus Concepts, Berkely, CA, USA).
For all statistical tests the level of significance was set to $p < 0.05$.

Results

Differences between the conditions GG and W

Fasting GLP-1 concentrations were not different between conditions, neither in the normal-weight group, nor in the obese group. In the normal-weight group plasma GLP-1 was significantly increased in the GG condition compared to W at 30 min ($F1,28 = 30.09$; $p = 0.0001$) and 60 min ($F1, 28 = 6.10$; $p = 0.02$) after ingestion of the load.
Similarly, in the overweight/ obese group, GLP-1 concentration in response to GG was higher at 30 min ($F1,27 = 20.94$; $p = 0.0001$), 60 min ($F1,27 = 4.38$; $p = 0.045$) and 90 min ($F1,28 = 6.39$; $p = 0.017$), compared to W (Figure 1).
In lean as well as in the overweight/ obese subjects Δ-plasma insulin concentrations peaked at 60 minutes in the W condition. In lean subjects Δ-insulin concentrations were significantly higher in the W condition compared to GG at 60 minutes ($F1, 25 = 9.51$; $p < 0.05$) and were lower compared to GG at 90 ($F1, 24 = 5.48$; $p < 0.05$) and 120 minutes ($F1, 23 = 15.87$; $p < 0.05$).

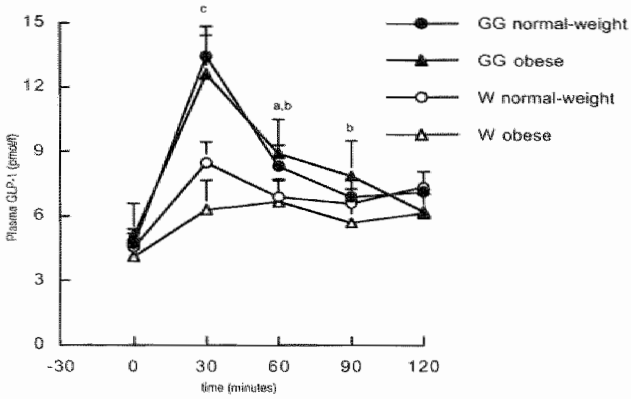

Figure 1. *GLP-1 plasma concentrations after ingestion of galactose/guar gum (GG) or water (W) and a standard breakfast in normal-weight and obese subjects. Values are means (±SEM).*
a : GG normal-weight different from W normal-weight at $p < 0.05$
b: GG obese different from W obese at $p < 0.05$
c: GG different from water in obese and normal-weight subjects at $p = 0.0001$

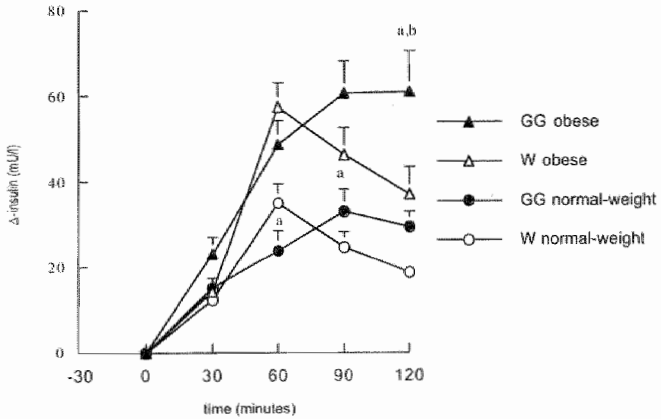

Figure 2. *Δ-insulin plasma concentrations (mU/l) after ingestion of galactose/guar gum (GG) or water (W) and a standard breakfast in normal-weight and obese subjects. Values are means (±SEM).*
a : GG normal-weight different from W normal-weight at $p < 0.05$
b: GG obese different from W obese at $p < 0.05$

In overweight/ obese subjects insulin concentrations were significantly different at 120 minutes (F1, 17 = 5.46; p < 0.05) with lower plasma insulin concentrations in the W condition compared to GG (Figure 2).
In normal-weight subjects, plasma glucose concentrations (Figure 3) were significantly higher after ingestion of water compared to GG at 60 min (F1, 28 = 10.3; p = 0.003). Glucose concentration in the overweight/ obese group did not differ between GG and W at any point of measurement.

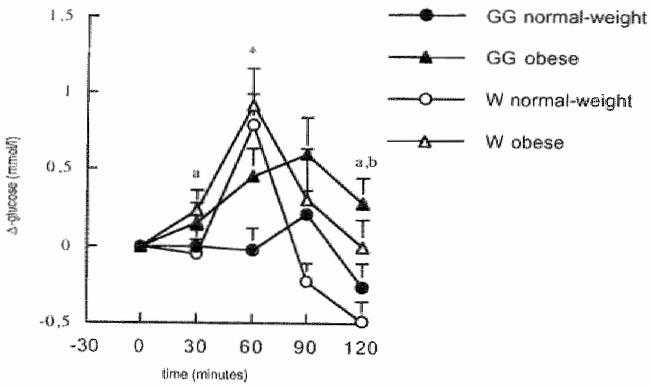

***Figure 3**. Δ-plasma glucose concentration (change from fasted concentrations) after ingestion of galactose/guar gum and breakfast in normal-weight (filled circles) and obese subjects (filled triangles), compared to water and breakfast in normal-weight (open circles) and obese subjects (open triangles).*
** GG normal weight different from W normal weight at p = 0.003*
a: Median difference of W obese is significantly different from W normal weight at 30 (p = 0.04) and 120 minutes (p = 0.05; Mann-Whitney U test for two groups).
b: Median difference of GG obese is significantly different from GG normal weight at 60 (p = 0.02) and 120 minutes (p = 0.04; Mann-Whitney U test for two groups).
Values are means (± SEM).

Plasma free fatty acid concentration (Figure 4) was higher in the normal-weight group at 30 min (F1, 25 = 5.90; p = 0.02) during W compared to GG. There were no differences between W and GG at any other point of measurement. No differences were found in free fatty acid concentration in the overweight/ obese group comparing W and GG.

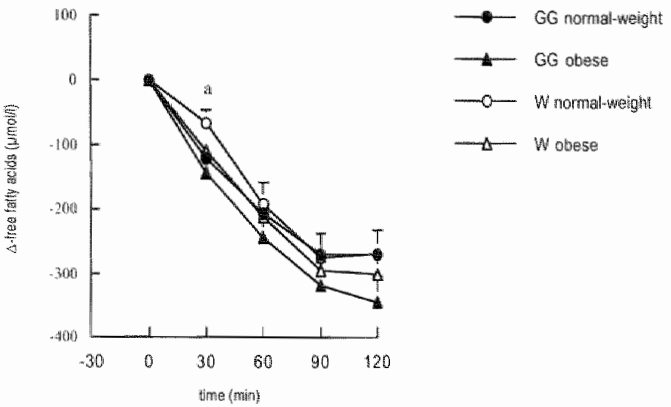

Figure 4. *Δ-plasma free fatty acid concentration (change from fasted concentrations) after ingestion of galactose/guar gum and breakfast in normal-weight (filled circles) and obese subjects (filled triangles), compared to water and breakfast in normal-weight (open circles) and obese subjects (open triangles).*
a: Median difference of W obese is significantly different from W normal weight at 30 (p = 0.03; Mann-Whitney U test for two groups).

Differences between normal-weight and obese subjects

The overweight/ obese subjects were on average older than the lean subjects. However, as tested none of the blood parameters assessed were related to age.
Fasted GLP-1 concentrations were not different between the groups, neither in the GG nor in the W condition. Normal-weight subjects had significantly higher GLP-1 concentrations after W at 30 min compared to the overweight/ obese group (p = 0.02) (Table 2). The area under the curve (pmol/l x h) for GLP-1 concentrations (Figure 5A) after W was significantly different in the normal-weight group compared to the overweight/ obese group [6.42 pmol/l x h (4.52-9.13) compared to 4.2 pmol/l x h (2.2-6.8)]; (p = 0.03).
The area under the curve (pmol/l x h) for GLP-1 concentrations after GG was not different between groups (Figure 5B).
Median fasted insulin concentrations were significantly different for GG as well as for W between lean and overweight/ obese subjects with overweight/ obese subjects having significantly higher fasted insulin concentrations in the W and in the GG condition (p = 0.0001) (Table 2).

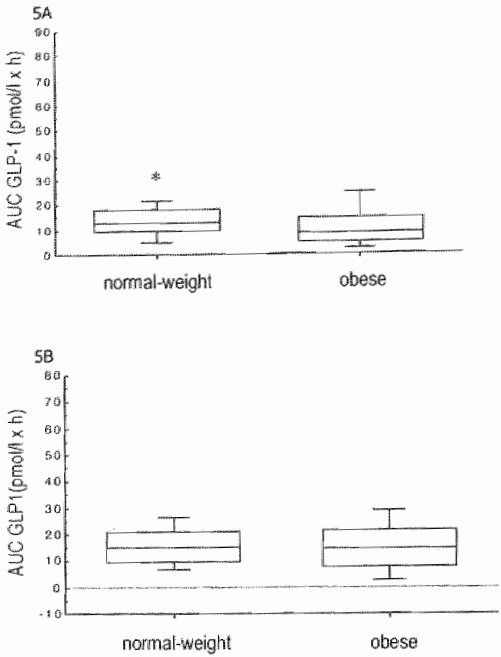

***Figure 5A, B**. Median (horizontal bar within box) of AUC (pmol/l x h) for GLP-1 concentrations in normal-weight and obese subjects after ingestion of W; (A), or GG; (B). The median difference for GLP-1 concentrations was significantly higher for normal-weight subjects compared to the obese after W (p = 0.03, Mann-Whitney U test for two groups). This was not the case for ingestion of galactose/guar gum and breakfast. 75th percentile, upper limit of box; 25th percentile, lower limit of box; 90th percentile, edge of upper limit; 10th percentile, edge of lower limit.*

Median differences between the overweight/ obese and normal weight group for Δ-glucose concentrations were significant at 30 min (p = 0.04) and 120 min in the W condition (p = 0.05) (Table 3). Values were different in the sense that overweight/ obese subjects had significantly higher glucose concentrations compared to the normal-weight group after ingestion of W. Median differences for Δ- glucose concentrations were significant at 60 min (p = 0.02) and 120 min (p = 0.04) between groups in the GG condition, with significantly higher glucose concentrations in the overweight/ obese group compared to the normal-weight group (Table 3).
In the W condition Δ-free fatty acid concentrations in the normal-weight group were significantly less decreased compared to the overweight/ obese at 30 minutes (p = 0.03) (Table 3). There were no differences between groups in Δ-free fatty acid concentrations in the GG condition.

Table 2. Concentrations of GLP-1 (pmol/l) and insulin (mU/l) in normal-weight (n=30) and overweight/obese subjects (n=28) after ingestion of galactose/ guar gum and a standard breakfast (GG) or water and a standard breakfast (W). Values are expressed as median and range (25th and 75th percentile)
* significant difference between normal-weight and overweight/obese subjects at p = 0.02.
# significant difference between normal-weight and overweight/obese subjects at p = 0.0001

Minutes		0		30		60		90		120	
		MD	RG	MD	RG	MD	RG	MD	RG	MD	RG
Lean	GLP-1(GG)	3.8	2.0-5.9	13.7	7.7-17.7	7.5	5.0-10.1	5.8	4.2-8.5	6.4	3.2-9.8
	GLP-1(W)	4.1	2.0-5.6	7.3	4.3-11.1	5.6	3.8-9.1	5.7	4.1-8.6	7.2	5.0-9.3
Overweight/ obese	GLP-1(GG)	2.0	1.0-6.5	10.9	5.0-15.6	6.8	4.0-12.0	5.7	2.9-11.5	6.0	2.0-9.2
	GLP-1(W)	2.0	1.0-4.0	5.2	1.0-8.8	5.0	2.8-9.0	5.0	1.0-7.0	4.2	2.0-7.9
Lean	Insulin(GG)	6.3	4.6-7.6	18.0	14.3-27.4	23.9	16.2-33.6	27.4	22.7-43.4	34.4	25.1-41.6
	Insulin(W)	6.0	4.8-7.0	12.4	9.2-22.1	35.8	28.1-45.9	23.1	17.9-35.6	21.4	16.0-27.4
Overweight/ obese	Insulin(GG)	12.4	9.9-13.5	30.5	22.9-38.5	62.1	36.2-72.8	59.4	44.3-82.8	60.5	45.5-102.4
	Insulin(W)	10.5	7.9-11.7	25.6	16.9-34.0	62.0	42.5-92.2	43.0	29.0-74.7	39.2	22.2-60.9

Table 3. Concentrations of glucose (mmol/l) and free fatty acid (µmol/l) in normal-weight (n=30) and overweight/obese subjects (n=28) after ingestion of galactose/ guar gum and a standard breakfast (GG) or water and a standard breakfast (W), expressed as change from fasted values (Δ). Values are median and range (25th and 75th percentile)
* significant difference between normal-weight and overweight/ obese subjects at $p < 0.05$.
\# significant difference between normal-weight and overweight/obese subjects at $p = 0.04$

Minutes		30		60		90		120	
		MD	RG	MD	RG	MD	RG	MD	RG
Lean	Δ glucose (GG)	0.04	-0.3-0.2	-0.2*	-0.4-0.2	0.3	-0.3-0.8	-0.4*	-0.6-0.3
	Δ glucose (W)	0.0*	-0.3-0.2	0.7	0.2-1.5	-0.3*	-0.5-(-0.3)	-0.3*	-0.9-(-0.1)
Overweight/ obese	Δ glucose (GG)	0.3	-0.2-0.6	0.3	-0.1-1.0	0.4	-0.1-1.3	0.3	-0.4-0.7
	Δ glucose (W)	0.2	-0.1-0.6	0.8	-0.1-0.6	-0.2	-1.0-1.1	0.0	-0,4-0.4
Lean	Δ ffa (GG)	-104	-154-(-38)	-185	-226-(-137)	-228	-276-(-208)	-252	-289-(-214)
	Δ ffa (W)	-33#	-100-(-2.5)	-163	-201-(-101)	-233	-290-(-179)	-239	-294-(-175)
Overweight/ obese	Δ ffa (GG)	-148	-210-(-75)	-218	-327-(-168)	-262	-393-(-220)	-283	-395-(-238)
	Δ ffa (W)	-103	-155-(-52)	-213	-286-(-156)	-279	-337-(-213)	-283	-345-(-237)

Ratings of satiety (AUC) were related to GLP-1 concentrations (AUC) in the normal-weight group after ingestion of GG ($r = 0.20$; $p = 0.01$), but not in the overweight/ obese group ($r = .07$; $p = 0.74$).
Ratings of satiety and desire to eat were not different between groups in the W condition. After ingestion of GG the increase in feelings of satiety were significantly higher in normal-weight subjects at 30 min ($F1, 53 = 5.28$; $p = 0.02$) and 60 min ($F1, 52 = 4.21$; $p = 0.04$) compared to the overweight/ obese group (Figure 6).

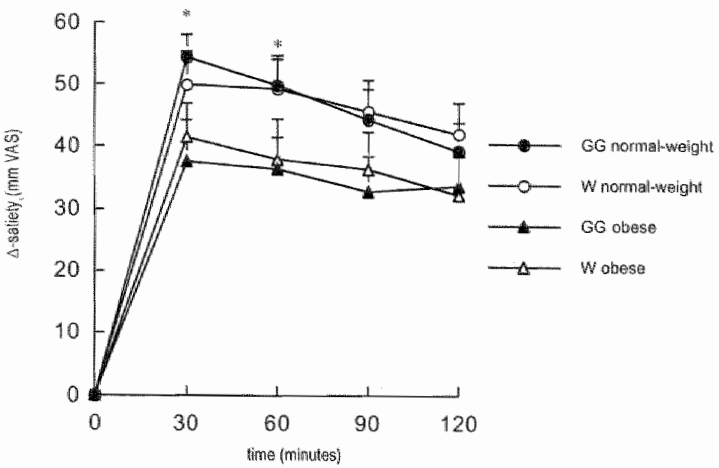

Figure 6. Δ-satiety (mm VAS) in normal weight (circles) and obese (triangles) subjects after ingestion of galactose/guar gum and a standard breakfast (filled circles; filled triangles) or water and a standard breakfast (open circles; open triangles).
** significantly different at $p < 0.05$ from obese subjects in the GG condition.*

Discussion

The results of the current study show a significant difference in postprandial GLP-1 stimulation in normal-weight subjects compared to overweight/ obese subjects after ingestion of water and a standard breakfast. Ingestion of a galactose/ guar gum load seems to outweigh this difference. Galactose in combination with guar gum has been shown to sufficiently stimulate GLP-1 release in normal weight subjects. The area under the curve for GLP-1 release stimulated by galactose/ guar gum was similar to the area under the curve for stimulation by glucose/guar gum (19). The question remained whether this could be seen in overweight/ obese subjects as well.
The sensitivity for a GLP-1 response seems slightly higher in the normal-weight compared to the overweight/ obese subjects, as shown by the difference in the GLP-1 response to water and breakfast. However, baseline GLP-1 appeared not to be different between the subjects, nor the increase in GLP-1 release due to a stronger trigger such as GG. With the GG load, the effect of additional energy intake also may have stimulated GLP-1 release in the overweight/ obese subjects. It has to be taken into consideration

that a decreased L cell stimulation in the overweight/ obese due to a relatively lower kJ stimulation per kg body weight might possibly contribute to the difference in GLP-1 release in normal-weight and overweight/ obese subjects. However, a between as well as a within subject design has been applied in the present study. The difference between overweight/ obese and normal-weight subjects, when subtracting GLP-1 release after W from GLP-1 release after GG was not significant, suggesting a lower sensitivity rather than a decreased L cell stimulation.

The present findings are different from observations by Ranganath et al. (11, 25) and Verdich et al. (27) who reported a pronounced attenuation of postprandial GLP-1 response in obese subjects (11, 25, 26) In those studies obese subjects with a higher BMI were assessed (38-40 kg/m^2) (11, 25, 26), in which the GLP-1 release may be lower than in our obese subjects with a BMI of 30 kg/m^2. It was suggested before that GLP-1 response to a nutrient trigger normalizes gradually with weight loss (27). According to the WHO classification the subjects investigated in the present study can be classified as overweight/ obese class I compared to obese class II subjects in the other studies.

GLP-1 has been shown to reduce energy intake, enhance sensations of fullness and decrease feelings of hunger in lean (6), as well as in obese subjects (12, 27). Therefore one would expect that higher GLP-1 concentrations in the GG condition compared to W in both groups would be mirrored in appetite ratings being related to GLP-1 concentration.

This only is the case in normal-weight subjects, where we found a weak relationship between satiety and GLP-1 release. Also the almost similar GLP-1 concentrations in normal- weight and obese subjects in the GG condition would be expected to be reflected in similar appetite ratings. But, despite no difference in GLP-1 stimulation with galactose/ guar gum and breakfast between the obese and the normal weight, perceived satiety was increased only in the normal weight, and not in the obese. This could be an example of an inappropriate feedback in a situation of energy imbalance (28).

Two groups with significantly different body weight status were investigated in the present study. Leptin is considered an important adiposity signal and is secreted in direct proportion to the amount of fat stored in individual adipocytes (29). Leptin was shown to stimulate GLP-1 release and it has been suggested that leptin resistance may account for decreased GLP-1 concentrations in obese humans (30). Leptin was not measured in this experiment, yet no difference in GLP-1 release after GG between overweight/ obese and normal-weight subjects suggests, that the subjects are probably not leptin resistant. The difference in GLP-1 release between the groups after W though might indicate a starting development of leptin resistance. A stronger trigger, such as GG still seems to be able to compensate for that.

Insulin release parallels glucose release. Higher plasma insulin concentrations in both groups at 90 and 120 minutes in the GG condition compared to W are probably due to a reduced rate of glucose absorption, which will lead to a prolonged influence on insulin concentrations (31).

As has been shown before (14) the addition of guar gum decreased insulin and glucose release compared to the condition without guar gum in both the normal-weight as well as the overweight/ obese group. Insulin is an important adiposity signal (29) and has been reported to produce anorexic responses, including reduced food intake and body weight (32, 33). Higher scores of satiety in the normal-weight group are corresponding with lower insulin release compared to the overweight/ obese group. In the present study, no differences between the GG and W condition have been observed that would support the idea of increased insulin concentrations contribute to increased satiety in the short term. However, unlike other hormones, such as Cholecystokinin where hypophagia has a rapid onset and lasts for only a few minutes after administration, the hypophagia following insulin develops much more slowly and has been shown to last hours to days (34), in line with the view of insulin as a long-term adiposity and satiety signal (35).
In conclusion, obese subjects seem to have a slightly lower sensitivity for GLP-1 release in response to a standard nutrient challenge like a standard breakfast compared to normal-weight subjects. The sensitivity can be improved to a level comparable to normal-weight subjects by addition of a stronger challenge like a galactose/guar gum nutrient load. However, since the improvement is not reflected in subjective sensations of satiety, it seems likely that in obese subjects, disturbance of appropriate perception of the feedback rather than primarily the physiological feedback disturbance may contribute to obesity.

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Chapter 6

Decreased GLP-1 release after weight loss in obese subjects

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submitted for publication

Abstract

Objective: Postprandial GLP-1 release seems to be attenuated in obese subjects. Results on whether weight loss improves GLP-1 release are contradictory.

Aim of the present study was to further clarify the effect of weight loss on basal and postprandial GLP-1 release in obese subjects.

Research methods and procedures: 32 obese subjects participated in a repeated measurement design before [BMI (kg/m^2): 30.3±2.8; waist circumference (cm): 92.6±7.8; hip circumference (cm): 111.1±7.4] and after a weight loss period of six weeks [BMI (kg/m^2): 28.2±2.7; waist circumference (cm): 85.5±8.5; hip circumference (cm): 102.1±9.2].

During weight loss subjects received a very low energy diet (Optifast) in order to replace three meals/ day. Subjects came to the laboratory fasted and after a baseline blood sample they received a standard breakfast (1.9 MJ). Postprandially, blood samples were taken every half hour relative to intake for 120 minutes, in order to determine GLP-1, insulin, glucose and free fatty acids from plasma. Appetite ratings were obtained with visual analogue scales.

Results: After weight loss, postprandial GLP-1 concentrations at 30 and 60 minutes were significantly lower than before weight loss ($p < 0.05$). Glucose concentrations were also lower and free fatty acids were higher compared to before weight loss.

Ratings of satiety were increased and hunger scores were decreased after weight loss ($p < 0.05$).

Discussion: In obese subjects GLP-1 concentrations after weight loss were decreased compared to before weight loss and nutrient related stimulation was abolished. This might be a response to a proceeding negative energy balance. Satiety and GLP-1 seem to be unrelated on the long term.

Introduction

Obesity is associated with increased risks of cardiovascular diseases, impaired glucose tolerance and insulin resistance (1, 2).
Two of the factors that are suggested to be related to the development of obesity are increased energy intake and decreased energy expenditure (3, 4).
Energy intake is partly controlled by neural and humoral signals that are generated by a biological system that senses and processes food (5). There is evidence that glucagon-like peptide 1 (GLP-1) is one of the mediators that is involved in the post-meal satiety response (6). Together with glucose-dependent insulinotropic polypeptide (GIP) GLP-1 acts as an incretin and has synergistic effects on insulin release after food ingestion.
GLP-1 is a 30 amino acid peptide hormone that is secreted from intestinal L-cells after intake of a mixed meal (7, 8) and has been shown to affect appetite ratings and food intake. Peripheral GLP-1 administration reduced food intake and suppressed appetite in normal-weight subjects (9). Intravenous GLP-1 infusion in obese subjects led to significantly lower hunger ratings compared to a saline infusion and reduced ad libitum energy intake (8).
Basal GLP-1 concentrations and postprandial GLP-1 release seem to be attenuated in obese subjects, although statistical significance is unclear (10, 11). It has been suggested that this is related to increased concentrations of non-esterified fatty acids, which are associated with obesity (12). Not much is known yet about the effect of weight loss on GLP-1 release. Only few studies have been investigating the effect of weight reduction on GLP-1 concentrations and found an increase of GLP-1 response to a level between that of obese and lean subjects (11).
The purpose of the present study was to further investigate and clarify the effect of weight loss on GLP-1 release in obese subjects.

Methods and Procedures

Subjects

Forty subjects were recruited by means of advertisements in local newspapers.
Thirty-two obese (BMI: 30.1±2.6) subjects (23 women and 9 men), aged between 20 and 60 participated in the study. Selection criteria included being in good health, not taking any medications, and no history of diabetes or chronic disease.
Informed written consent was obtained and the study was approved by the Medical Ethics Committee of Maastricht University.

Experimental design

A repeated measurement design (two visits T1 and T2) was applied to the 32 selected subjects before (T1) and after a six week weight loss period (T2) with a very-low-energy-diet (VLED). Subjects came to the laboratory in the morning in a fasted state. They were instructed to fast from 10 pm the night prior to the test day. After filling in the questionnaires, collecting urine samples and measurement of resting energy expenditure, an indwelling cannulae (Baxter BV, Utrecht, The Netherlands) was inserted in an antecubital vein. After 20 minutes of rest, subjects received a standard breakfast. The breakfast (1.9 MJ) had an energy density of 3.9 kJ/g and consisted of two slices of brown bread (100 g), baked egg (85 g) and 300 ml skim milk. The distribution of energy was 48.8 energy-percent (E %) carbohydrates, 28.5 E % protein and 22.6 E % fat.
Blood samples were taken for a total of two hours, every half hour after ingestion of the breakfast, for determining GLP-1, insulin, glucose and free fatty acids.
To determine the appetite profile, subjects rated their subjective feelings of hunger and satiety on anchored 100 mm visual analog scales prior to each blood sample (13).
After the baseline measurements, a VLED intervention followed for 6 weeks in order to produce weight loss in the subjects. The VLED (Optifast®; Novartis Consumer Health, Osthofen, The Netherlands) was supplied in three sachets per day, dissolved in water to obtain a milk shake, pudding or soup. Three sachets provided 2540 kJ/d, consisting of 52.5 g protein (35 E%), 13.5 g fat (20 E%) and 67.5 g carbohydrate (45 E%). 200 g of vegetable or fruit were allowed in addition to the VLED.
After the six weeks of the VLED, all measurements were repeated.

Anthropometry

For all subjects, body weight (BW) was measured on a digital balance (Seca, Hamburg, Germany), with subjects in underwear, in a fasted state, and after voiding their bladder. Height was measured using a wall-mounted stadiometer (Seca, Hamburg, Germany). The body mass index (BMI) was calculated as BW/ height^2 (kg/m^2). Systolic and diastolic blood pressures were measured by an automatic blood pressure monitor (Omron 705 CP, Omron Healthcare GmbH, Hamburg, Germany).
The distribution of fat was determined by measuring the waist and hip circumferences and calculation of the waist-hip ratio (WHR). The waist circumference was measured at the site of the smallest circumference between the rib cage and the ileac crest, with the subjects in standing position. The hip circumference was measured at the side of the largest circumference between the waist and the thighs. The WHR was calculated by dividing the waist circumference by the hip circumference.
Body composition was measured by using the deuterium (^2H_2O) dilution technique (14). The dilution of the deuterium isotope is a measure for total body water (TBW) (15). The evening prior to the two test days, subjects drank a deuterium dilution (70g water with an enrichment of 5 atom% excess ^2H) after voiding. Deuterium enrichment was

measured in urine from the second voiding of the following morning. ^{2}H concentrations in the urine samples were measured using an isotope ratio mass spectrometer (Micromass Optima, Manchester, UK). Total body water was determined by dividing the measured ^{2}H dilution space by 1.04 (14).
Fat-free mass (FFM) was calculated by dividing the total body water by the hydration factor 0.73. By subtracting FFM from body weight, fat mass (FM) was obtained. Percentage of body fat (%BF) was calculated according to the equation of Siri (16).

Resting Energy Expenditure and substrate oxidation

Resting energy expenditure (REE) was measured by means of an open-circuit ventilated hood system. Subjects came to the laboratory in the morning by car or by bus to minimize the amount of physical activity before the test. REE was measured at the beginning of each of the two test days with subjects in a fasted state while lying supine for 30 minutes. Gas analyses were performed by a paramagnetic O_2 analyzer (Servomex type 500A; Servomex Controls Ltd, Crowborough, Sussex, UK) and an IR CO2 analyzer (Servomex type 500A), similar to the analysis system described by Schoffelen et al. (17). Calculation of REE was based upon Weir's formula (18). Respiratory quotient (RQ) was calculated as CO_2 produced/ O_2 consumed.
Fat oxidation was calculated using the following equation (19):
Fat oxidation (g/h) = (1.695 x VO_2 (l/min) −1.701 x V CO_2 (l/min) x 60.

Insulin resistance

Measures for insulin resistance were obtained from fasting plasma insulin and glucose concentrations using the 'homeostasis model assessment' (HOMA). It is assumed that normal-weight subjects with an age under 35 years have an insulin resistance of 1. Based on that assumption resistance can be calculated according to the following equation (20):

$$\text{resistance} = \text{insulin}/ (22.5e - \ln \text{glucose})$$

Attitude towards eating

Eating behavior was analyzed at the beginning of each test day using a validated Dutch translation of the Three-Factor Eating Questionnaire (TFEQ) (21, 22). Cognitive restrained and unrestrained eating behavior (factor 1), emotional eating and disinhibition (factor 2) and the subjective feeling of hunger (factor 3) were scored.

Blood parameters

Blood samples for GLP-1 were taken in iced syringes and mixed with EDTA and 40 µl of DPP-IV inhibitor to prevent degradation (Linco Research, St. Charles, USA). Blood samples for other blood parameters were mixed with EDTA to prevent clotting. Plasma

was obtained by centrifugation for 10 minutes at 2800 g at 4 °C. Plasma was collected, frozen in liquid nitrogen and stored at –20 °C for analysis.
GLP-1 concentrations were measured using an ELISA kit (EGLP –35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less. Sensitivity of the analysis is 2pmol/l (23).
Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France). The WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations.
Insulin concentrations were measured using a radioimmunoassay kit (Insulin RIA-100; Pharmacia, Uppsala, Sweden).

Statistical procedures

Data are presented as mean ± standard error of the mean (SEM) or as mean ± standard deviation (sd). Differences for blood parameters, appetite profile and anthropometric data between T1 and T2 were determined by analysis of variance for repeated measures (ANOVA) and Sheffe-F post-hoc tests (Statview SE Graphics TM). Area under the curve (AUC) was calculated as incremental area under the curve over time (2h). Pearson correlation coefficients, r, were calculated to determine the relationship between fat free mass (FFM) and resting energy expenditure (REE).
The level of significance was set at $p < 0.05$.

Results

Anthropometrical data for T1 and T2 are shown in Table 1.
During the six-week weight loss period subjects lost a significant amount of weight, reducing their BMI as well as waist and hip circumferences.

***Table 1**. Subject characteristics (n= 32) before and after weight loss (means ± sd)*

	before weight loss	after weight loss	*p*
Body weight (kg)	85.59±9.8	79.50±10.2	<0.05
BMI (kg/m^2)	30.3±2.8	28.2±2.7	<0.05
Waist circumference (cm)	92.6±7.8	85.5±2.5	<0.05
Hip circumference (cm)	111.1±7.4	102.1±9.2	<0.05
Waist-hip ratio	.83±.05	.84±.08	*ns*
% body fat	37.8±6.4	33.4±7.7	<0.05
FFM (kg)	55.0±8.5	54.1±8.3	*ns*
RQ	.83±.04	.78±.05	<0.05
REE (MJ/d)	7.0±.8	6.9±.9	*ns*
F1 (cognitive restraint)[1]	6.5±4.3	11.2±4.4	<0.05
F3 (hunger)[1]	5.3±3.9	3.6±2.7	<0.05

[1] dietary restraint and hunger measured with Factor 1 or Factor 3 respectively of the Three-Factor Eating Questionnaire

Cognitive restraint (factor 1, TFEQ) was significantly increased after weight loss and general hunger scores (factor 3, TFEQ) were decreased (Table 1).
Respiratory quotient was significantly lower after weight loss compared to before weight loss, due to increased fat oxidation (4.16 g/h (sd = 1.31) before weight loss compared to 5.49 g/h (sd = 1.73) after weight loss; $p < 0.05$. REE (MJ/d) was a function of FFM (kg) before weight loss ($r = .81$; $p < 0.05$) and after weight loss ($r = .43$; $p < 0.05$) (Fig. 1).

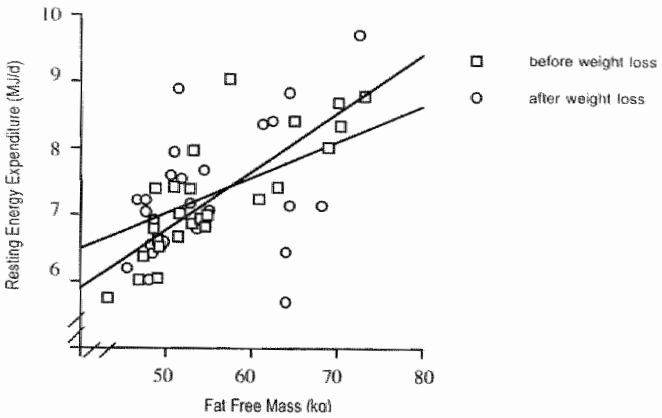

Figure 1. *Resting energy expenditure before (squares) and after (circles) weight loss as a function of fat-free mass. Regression equation before weight loss is: resting energy expenditure (MJ/d) = 0.08 x fat-free mass (kg) + 2.3 ($p < 0.05$; $r = .81$).*
Regression equation after weight loss is: resting energy expenditure (MJ/d) = 0.05 x fat-free mass (kg) + 4.3 ($p < 0.05$; $r = .43$).

Comparison of GLP-1 concentrations before (T1) and after (T2) weight loss showed the following. Although only approaching significance, fasting GLP-1 concentrations at T2 tended to be lower compared to T1 ($p = 0.07$).
After weight loss, stimulated GLP-1 concentrations were on a level below that of T1. GLP-1 concentrations were significantly different between T1 and T2 at 30 minutes ($F1, 31 = 5.39$; $p < 0.05$) and 60 minutes ($F1, 31 = 8.27$; $p < 0.05$). The area under the curve for GLP-1 concentration at T1 was significantly higher compared to T2 (Fig. 2 A). While at T1 stimulated GLP-1 release was significantly different from fasting concentrations at 30 ($F1, 31 = 10.19$; $p < 0.05$), 60 ($F1, 31 = 23.05$; $p < 0.05$) and 120 minutes ($F1, 31 = 16.07$; $p < 0.05$), the stimulated GLP-1 concentrations measured at T2 did not differ significantly from fasting concentrations at T2 any time (Fig 2 A).

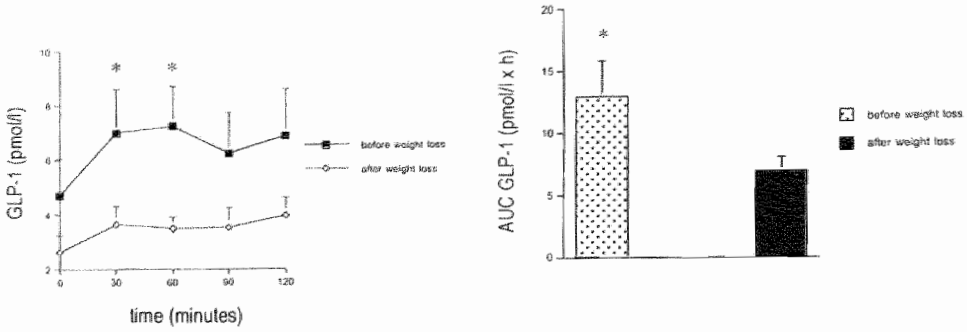

***Figure 2 A**. Plasma GLP-1 concentrations at baseline and postprandially after ingestion of a standard breakfast before and after a six-week weight loss period. Data are presented as means (± SEM).*
Left panel: plasma concentrations before weight loss (filled squares) and after weight loss (open diamonds).
** significant difference between T1 and T2 at p < 0.05 (ANOVA for repeated measurement).*
Right panel: AUC (average x 2h) before (left bar) and after (right bar) weight loss.
** significantly different at p < 0.05 (ANOVA for repeated measurement).*

After ingestion of the breakfast, insulin concentrations increased, peaking at 60 minutes at T1 and T2. There were no differences in baseline or postprandial insulin concentrations between T1 and T2 (Fig. 2 B). Insulin resistance, calculated using the homeostasis model assessment (HOMA), was not different between T1 and T2 (mean T1: 2.54 ±..25 compared to 2.53 ± .26 at T2).
Glucose concentrations increased after ingestion of the breakfast and peaked at 60 minutes at T1 and T2. In contrast to the insulin results, glucose concentrations at T2 were always below the concentrations at T1 with significant differences between T1 and T2 at baseline ($F1, 27 = 14.44$; $p < 0.05$) and 30 minutes ($F1, 26 = 9.46$; $p < 0.05$) (Fig. 2 C).

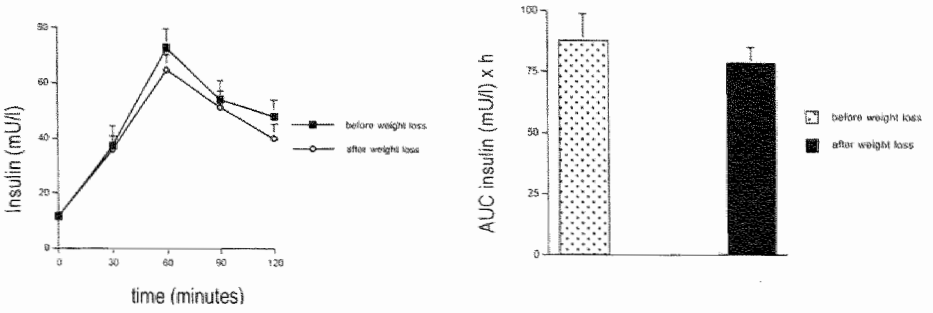

***Figure 2 B**. Plasma insulin concentrations at baseline and postprandially after ingestion of a standard breakfast before and after a six-week weight loss period. Data are presented as means (± SEM).*
Left panel: plasma concentrations before weight loss (filled squares) and after weight loss (open diamonds).
Right panel: AUC (average x 2h) before (left bar) and after (right bar) weight loss.

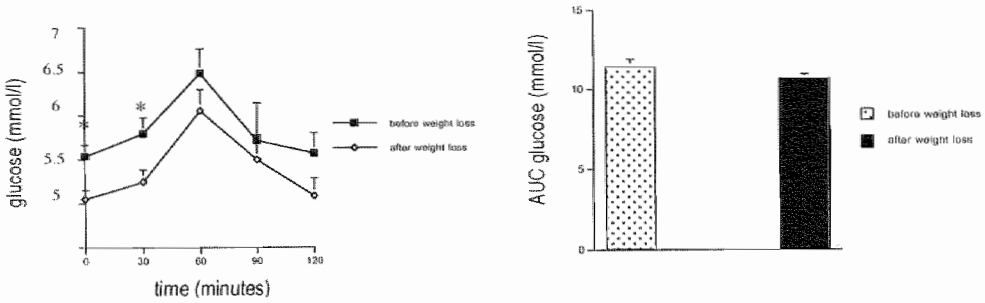

***Figure 2 C**. Plasma glucose concentrations at baseline and postprandially after ingestion of a standard breakfast before and after a six-week weight loss period. Data are presented as means (± SEM).*
Left panel: plasma concentrations before weight loss (filled squares) and after weight loss (open diamonds).
** significant difference between T1 and T2 at $p < 0.05$ (ANOVA for repeated measurement).*
Right panel: AUC (average x 2h) before (left bar) and after (right bar) weight loss.

Free fatty acid concentrations decreased over time after breakfast. At every blood draw, concentrations were increased after weight loss compared to before weight loss. Differences between T1 and T2 were significant at baseline ($F1, 26 = 5.24$; $p < 0.05$) and postprandially at 30 ($F1, 26 = 6.07$; $p < 0.05$), 60 ($F1, 26 = 11.22$; $p < 0.05$), 90 ($F1, 26 = 6.27$; $p < 0.05$) and 120 minutes ($F1, 26 = 9.87$; $p < 0.05$) (Fig. 2 D).

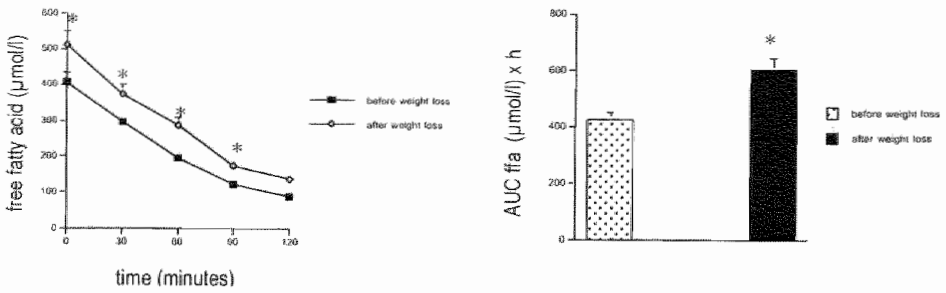

***Figure 2 D**. Plasma free fatty acid concentrations at baseline and postprandially after ingestion of a standard breakfast before and after a six-week weight loss period. Data are presented as means (± SEM).*
Left panel: plasma concentrations before weight loss (filled squares) and after weight loss (open diamonds).
** significant difference between T1 and T2 at $p < 0.05$ (ANOVA for repeated measurement).*
Right panel: AUC (average x 2h) before (left bar) and after (right bar) weight loss.
** significantly different at $p < 0.05$ (ANOVA for repeated measurement).*

Baseline satiety ratings were not different between T1 and T2. Postprandial satiety ratings were significantly increased after the weight loss period compared to T1 at 120 minutes ($F1, 22 = 5$; $p < 0.05$) (Fig 3 A). Hunger ratings were not different at baseline comparing T1 and T2, but were decreased after weight loss compared to before weight loss at 90 minutes ($F1, 21 = 4.52$; $p < 0.05$) and 120 minutes ($F1, 22 = 4.64$; $p < 0.05$) (Fig 3 B). Satiety- or hunger ratings were not related to the increase in GLP-1 release.

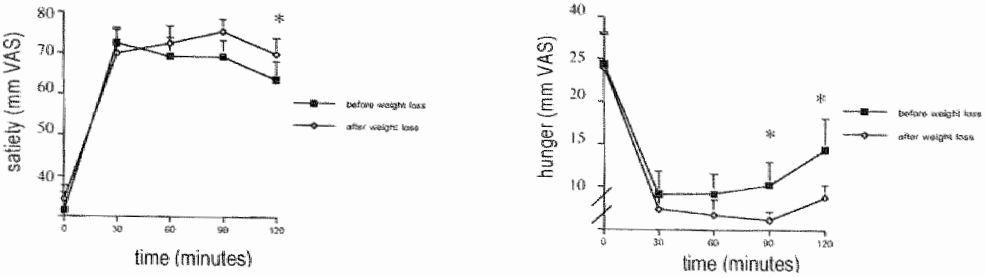

Figure 3 A-B.
Subjective satiety (3 A) and hunger (3 B) ratings before (filled squares) and after (open diamonds) a 6 week weight loss period. Data are presented as means (± SEM).
** significant difference between T1 and T2 at $p < 0.05$ (ANOVA for repeated measurement).*

Discussion

The current study shows decreased GLP-1 concentrations with weight loss in a group of obese subjects. Not only were fasting GLP-1 concentrations decreased, but it appears that secretion due to nutrient ingestion is abolished after weight loss as well. This result is similar to a study reporting decreased levels of GLP-1 after weight loss in severely obese subjects (24) but partly different from a study showing a marginal increase in GLP-1 concentrations after weight loss in obese subjects. However, incremental GLP-1 concentrations were not improved after weight loss (11, 25). In contrast to these studies, the present study has been investigating subjects with a BMI of 30±2.68, compared to an average BMI of 38 (11) or an average BMI of 44 (25) in the other studies. This may partly explain the inconsistent results. Since in the current study subjects lost less weight than in other studies (11, 24, 25) and the weight loss period was shorter, our results rather should be described as effects of the first phase of weight loss on GLP-1 concentrations, likely being induced by a strong negative energy balance. Free fatty acid concentrations were increased during weight loss, as has been shown before (26).

GLP-1 secretion may be inhibited by circulating non-esterified-fatty acids (10, 12, 27), especially during the first phase of weight loss, when energy balance is strongly negative. It has been suggested that the postprandial fall in plasma non-esterified-fatty acids is an important mechanism by which GLP-1 release is stimulated and that an impaired postprandial suppression of non-esterified-fatty acids may in part be responsible for the impaired GLP-1 secretion in obesity (12) This mechanism might have played a role in this study. An alternative explanation for decreased GLP-1 concentrations with weight loss may be an alteration in the autonomic nervous system

(ANS) due to obesity or weight loss. There is no consensus yet on how the ANS is changed and/or possibly contributes to obesity (28). Findings of decreased levels of vagally mediated gastrointestinal hormones after weight loss suggest that the effect of weight loss on vagal tone in the sense of either depression or hyperstimulation might play an important role in decreased GLP-1 concentrations after weight loss (24).
The decrease of GLP-1 concentrations during weight loss might be a response to a proceeding negative energy balance, thereby playing a role as a neuroendocrine factor signaling energy deficiency.
After weight loss satiety ratings were increased and hunger ratings were decreased compared to before weight loss. After two hours subjects felt more satiated and less hungry, which may be explained by less energy requirements due to weight loss, although the test meal provided the same energy contents as before weight loss. No relationship was found between GLP-1 and satiety ratings before, nor after weight loss. Previously, a positive relationship between satiety and GLP-1 release in normal-weight subjects was shown by Flint et al. (9). Näslund et al. showed a relationship between satiety and GLP-1 in obese men, when GLP-1 was infused (8, 29). In a previous study we showed an increase in GLP-1 release in obese subjects, when stimulated with a galactose and guar gum solution. However, a corresponding increase in satiety did not occur. We suggest, given the results of the present study, that GLP-1 is a satiety regulator in the short term, and that a possible relationship between satiety and GLP-1 release is weaker in obese subjects compared to normal weight subjects.
It has been suggested that GLP-1 secretion normalizes gradually when overweight is reduced, as concluded based upon increased fasting concentrations after weight loss. However, incremental AUC does not seem to be normalized after weight loss (11). More research needs to be done to clarify how the reduced GLP-1 concentrations after weight loss change during weight maintenance and regain. It is likely that during the first phase of weight loss, when energy balance is very negative, GLP-1 decreases, and that it normalizes subsequently. Similar changes in other hormones during different phases of weight loss have been shown before (24).
As expected, fasting and postprandial glucose concentrations were lower and free fatty acid concentrations were increased after weight loss (26, 30). Insulin sensitivity did not change, which was not surprising, since subjects were normally insulin sensitive before as well as after weight loss.
REE as a function of FFM decreased due to weight loss and restraint score increased significantly during weight loss as expected. Subjects lost a little less weight than expected based on the three sachets a day. Weight loss was six kilograms on average. This means that subjects consumed an average energy of 4.2 MJ/day, providing evidence for a very negative energy balance, but also showing that subjects did not stick to the VLED completely. The difference of 1.6 MJ/day cannot be explained by the additional intake of 200 grams of fruit or vegetables.

In conclusion the present study shows that in the presence of a very negative energy balance, fasting GLP-1 release decreases in modestly obese subjects. Even the ability of nutrients to stimulate GLP-1 release seems to be abolished. The question remains how GLP-1 concentrations develop when subjects are back in energy balance.

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Chapter 7

Nutrient stimulated GLP-1 release after body-weight loss and weight maintenance in humans

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submitted for publication

Abstract

Background: Glucagon-like peptide 1 (GLP-1) is a peptide hormone, which is released in response to nutrient ingestion. Postprandial GLP-1 release has been reported as being attenuated in obese subjects. Reports on the effect of weight loss on GLP-1 are conflicting.

Objective: The aim of the current study was to clarify the effect of a weight loss period and a consecutive weight maintenance period on nutrient stimulated GLP-1 release in obese subjects.

Design: Nutrient stimulated (standard breakfast,1.9 MJ) GLP-1 release was investigated in 32 obese subjects at three occasions: before weight loss (T1) [BMI(kg/m^2):30.0±2.5], after a six-week very-low-energy diet (VLED) (T2) [BMI:27.6±2.3] and after a 3-month weight maintenance period (T3) [BMI:27.9±2.3]. At each of these occasions, following a fasting blood sample the test meal was fed and blood was drawn every 30 minutes for two hours relative to ingestion in order to determine plasma GLP-1, insulin, glucose and free fatty acid concentrations.

Results: Subjects lost 7±3.4kg during the VLED ($p<0.0001$) and regained 1±3.2kg during the weight maintenance period (ns). The area under the curve (AUC) for nutrient-stimulated plasma GLP-1 (pmol/l x h) was significantly decreased ($P=0.01$) at T2(6.8±1) compared to T1(12.8±2.9;) and T3(11.1±1.5).

Conclusions: Nutrient-stimulated plasma GLP-1 concentrations decreased after weight loss. Since we found a rebound of concentrations after a weight maintenance period, decrease after weight loss seems to be transient and more due to a very negative energy balance.

Introduction

The increasing incidence of obesity is a recognized medical problem in developed countries (1). Obesity is associated with insulin resistance and hyperinsulinemia, hypertension, dyslipidaemia, cardiovascular disease, type 2 diabetes mellitus, and gallbladder disease(2, 3). Cancer mortality has been shown to increase with increasing BMI among women (4).

The treatment of obesity is beneficial, since modest weight loss of 5 to 10% of the initial body weight is associated with a marked health improvement (5). Factors suggested as being related to the development of obesity are decreased physical activity and/or increased energy intake. Weight loss can thus be achieved by reducing energy intake and/ or increasing energy expenditure.

Energy intake is partly controlled by neural and humoral signals that are generated by a biological system that senses and processes food and that has impact on the satiating efficiency of food (6). One of the supposed mediators that is involved in the development of post-meal satiety and possibly contributes to satiation, is glucagon-like peptide 1 (GLP-1) (7).

GLP-1 is a thirty amino acid peptide hormone that is released from intestinal L cells into the circulation after a mixed meal (8, 9). Peripheral GLP-1 administration compared to saline infusions reduced food intake and suppressed appetite in normal weight-subjects (10) and led to lower hunger ratings and decreased energy intake during an ad libitum meal in obese subjects (9). The role of GLP-1 and its possible contribution to obesity is not clear yet. One study has been reporting hypersecretion of GLP-1 in obese subjects (11). However, there also is evidence that postprandial GLP-1 release in response to carbohydrate and mixed meal ingestion seems to be attenuated in obese subjects (12, 13). Results about the effect of weight loss on GLP-1 concentrations in obese subjects are ambivalent. While increased concentrations have been found after weight loss (12, 13), others report decreased concentrations of appetite related peptides after weight loss (14). The aim of the present study is to clarify the effect of diet-induced weight loss on GLP-1 concentrations in obese subjects and in particular the development of GLP-1 concentrations during consecutive weight maintenance following weight loss.

Subjects and Methods

Subjects

Forty subjects were recruited by means of advertisements in local newspapers.

Thirty-two overweight/ obese class1 (BMI: 30.1±2.6) subjects with and average age of 44±9 years (23 women; mean age 43±8 years, ranging between 20 and 59, and 9 men; mean age 46±12 years, ranging between 28 and 60) according to the classification of the World Health Organization (BMI ≥ 25 and < 34.9 (kg/m^2)) (15) , participated in the study. Selection criteria included being in good health, not taking any medications, and no history of diabetes or chronic disease.

Informed written consent was obtained and the study was approved by the Medical Ethics Committee of the Maastricht University

Experimental design

A repeated measurement design was applied to the 32 selected subjects including three visits: before weight loss (T1), after a six week weight loss period (T2) with a very-low-energy-diet (VLED) and after a 12 week weight maintenance period (T3). Each time subjects came to the laboratory in the morning in a fasted state. They were instructed to fast from 10 pm the night prior to the test day. After the questionnaires were completed, urine samples collected, resting energy expenditure measured, an indwelling cannulae (Baxter BV, Utrecht, The Netherlands) was inserted in an antecubital vein. After 20 minutes of rest, subjects received a standard breakfast. The breakfast (1.9 MJ) had an energy density of 3.9 kJ/g and consisted of two slices of brown bread (100 g), baked egg (85 g) and 300 ml skim milk. The distribution of energy was 48.8 energy-percent (E %) carbohydrates, 28.5 E % protein and 22.6 E % fat.
Blood samples were taken for a total of two hours, every half hour relative to ingestion of the breakfast for determining plasma GLP-1, insulin, glucose and free fatty acid concentrations.
To determine the appetite profile, subjects rated their subjective feeling of hunger and satiety on anchored 100 mm visual analog scales 15 minutes before the meal (timepoint 0), immediately after the meal (timepoint 30), and during the rest of the test day every 30 minutes relative to the measurement after the meal for a total of 120 minutes (16).
After the baseline measurements at the first appointment, a VLED intervention followed for 6 weeks in order to let the subjects lose weight. The VLED (Optifast®; Novartis Consumer Health, Osthofen, Germany) was supplied in three sachets per day, dissolved in water to obtain a milk shake, pudding or soup. Three sachets provided 2540 kJ/d, consisting of 52.5 g protein (35 E%), 13.5 g fat (20 E%) and 67.5 g carbohydrate (45 E%). A maximum of 200 g of vegetable or fruit were allowed in addition to the VLED.
After the six weeks of the VLED all measurements were repeated. The last appointment followed after a 12-week weight maintenance period. During the weight maintenance period subjects had no specific instructions about diet or physical activity.

Anthropometry

All anthropometrical data were obtained three times, before and after weight loss and after weight maintenance. Subject characteristics are depicted in Table 1.
For all subjects, body weight (BW) was measured on a digital balance (Seca, Hamburg, Germany), with subjects in underwear, in a fasted state, and after voiding their bladder. Height was measured using a wall-mounted stadiometer (Seca, Hamburg, Germany). The body mass index (BMI) was calculated as BW/ height^2 (kg/m^2).

Table 1. *Subject (n=32; 23 women, 9men) characteristics (Data are presented as means ± s.d. Statistical significance was determined by ANOVA for repeated measures*

	before weight loss (T1)	after weight loss (T2)	after weight maintenance (T3)	*P*
Body weight (kg)	86.7 ± 8.8	79.7 ± 8.4 [a]	80.7 ± 9.6[a]	0.0001
BMI (kg/m^2)	30.0 ± 2.5	27.6 ± 2.3 [a]	27.9 ± 2.3 [a]	0.0001
%body fat	38.1 ± 6.1	33.8 ± 7.7 [a]	33.6 ± 7.6 [a]	0.0001
FFM (kg)	54.3 ± 7.9	53.4 ± 7.7	53.9 ± 8.8	*ns*
RQ	.83 ± .04	0.78 ± 0.05 [a]	0.86 ± 0.06[b]	0.0001
Fat oxidation (g/h)	4.0 ± 1.3	5.4 ± 1.8 [a]	3.3 ± 2.0 [b]	0.0001
REE (MJ/d)	7.3 ± 0.9	7.1 ± 1	6.9 ± 0.88	*ns*
F1 (cognitive restraint)[1]	6.5 ± 4.3	11.2 ± 4.4 [a]	10.5 ± 4.3 [a]	0.0001
F2 (disinhibition)[1]	6.1 ± 3.1	4.9 ± 2.5 [a]	4.8 ± 2.9 [a]	0.005
F3 (hunger) [1]	5.3 ± 3.9	3.6 ± 2.7 [a]	3.1 ± 2.7 [a]	0.0004

[1] dietary restraint, disinhibition and hunger measured with factor 1, 2 or factor 3 respectively of the Three Factor Eating Questionnaire
[a] significantly different compared to before weight loss
[b] significantly different compared to after weight loss

The distribution of fat was determined by measuring the waist and hip circumferences and calculation of the waist-hip ratio (WHR). The waist circumference was measured at the site of the smallest circumference between the rib cage and the ileac crest, with the subjects in standing position. The hip circumference was measured at the side of the largest circumference between the waist and the thighs. The WHR was calculated by dividing the waist circumference by the hip circumference.

Body composition was measured by using the deuterium (^2H_2O) dilution technique (17). The dilution of the deuterium isotope is a measure for total body water (TBW) (18). The evening prior to the three test days subjects drank a deuterium dilution (70g water with an enrichment of 5 atom% excess ^2H) after voiding. Deuterium enrichment was measured in urine from the second voiding of the following morning. ^2H concentrations in the urine samples were measured using an isotope ratio MS (Micromass Optima, Manchester, UK). Total body water was determined by dividing the measured ^2H dilution space by 1.04 (17).

Fat-free mass (FFM) was calculated by dividing the total body water by the hydration factor 0.73. By subtracting FFM from body weight, fat mass (FM) was obtained. Percentage of body fat (%BF) was calculated according to the equation of Siri (19).

Resting Energy Expenditure and substrate oxidation

Resting energy expenditure (REE) was measured by means of an open-circuit ventilated hood system. Subjects came to the laboratory in the morning by car or by bus to minimize the amount of physical activity before the test. REE was measured at the beginning of each of the test days with subjects in a fasted state while lying supine for 30 minutes. Gas analyses were performed by a paramagnetic O_2 analyser (Servomex type 500A; Servomex Dontrols Ltd, Crowborough, Sussex, UK) and an i.r. CO_2 analyser

(Servomex type 500A), similar to the analysis system described by Schoffelen et al. (20). Calculation of REE was based upon Weir's formula (21). Respiratory quotient (RQ) was calculated as CO_2 produced/ O_2 consumed.
Fat oxidation was calculated using the following equation (22):
Fat oxidation (g/h) = 1.695 x VO_2 (l/min) −1.701 x V CO_2 (l/min) x 60.

Ambient temperature

Data on 24-h average ambient temperature were supplied by the Royal Dutch Meteorological Institute and were collected at a location near the university (Maastricht, Beek; 51° North, 6° East).

Attitude towards eating

Eating behavior was analyzed at the beginning of each test day using a validated Dutch translation of the Three-Factor Eating Questionnaire (TFEQ) (23, 24). Cognitive restrained and unrestrained eating behavior (factor 1), emotional eating and disinhibition (factor 2) and the subjective feeling of hunger (factor 3) were scored.

Blood parameters

Blood samples for GLP-1 were taken in iced syringes and mixed with EDTA and 40 µl of DPP-IV inhibitor to prevent degradation (Linco Research, St. Charles, USA). Blood samples for other blood parameters were mixed with EDTA to prevent clotting. Plasma was obtained by centrifugation for 10 minutes at 2800 g at 4 °C. Plasma was collected, frozen in liquid nitrogen and stored at −20 °C for analysis.
GLP-1 concentrations were measured using an ELISA (EGLP −35K; Linco Research Inc., St Charles, MO, USA) for non-radioactive quantification of biologically active forms of glucagon-like peptide. The assay has an intra-assay coefficient of variation (CV) of 8% or less and an inter CV of 12% or less. Sensitivity of the analysis is 2 pmol/l (25).
Plasma glucose concentrations were determined using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montepellier, France). The WAKO NEFA C-kit (Wako Chemicals, Neuss, Germany) was used to determine free fatty acid (FFA) concentrations.
Insulin concentrations were measured using a radioimmunoassay kit (Insulin RIA-100; Pharmacia, Uppsala, Sweden).

Statistical procedures

Data are presented as means (± standard error of the mean, SEM) or as means ± standard deviation (s.d.). All figures show changes from fasting plasma concentrations at each time of blood sampling (Δ) at the three testing occasions T1, T2 and T3 or area under the curve. Differences for blood parameters, appetite profile and anthropometrical data between T1, T2 and T3 were determined by analysis of variance for repeated

measures (ANOVA) and Sheffe-F post-hoc test (Statview SE Graphics TM). Area under the curve (AUC) was calculated as incremental area under the curve over time (2h), using the trapezoidal method. Pearson correlation coefficients, r, were calculated to determine the relationship between resting energy expenditure (REE) and fat free mass (FFM).
The relationship between anthropometrical data such as age, sex and Δ body weight with the Δ of GLP-1 release and other blood parameters was tested with a multiple regression analysis. The decrease in GLP-1 release after weight loss compared to before weight loss was related to Δ body fat, Δ body weight, sex and age with the same method.
The level of significance was set at $P < 0.05$.

Results

Anthropometrical results

Anthropometrical data are shown in Table 1 and are expressed as means (± s.d.).
During the six-week VLED subjects had lost 8% (range 1% - 14.8 %) of their weight. This was followed by a not statistically significant1.2% regain during the twelve-week weight maintenance period. All anthropometrical results were compared between men and women. There was no gender effect with respect to age ($r = 0.15$; n.s.), F1 ($r = 0.11$; n.s.), F2 ($r = 0.31$; n.s.), F3 ($r = 0.02$; n.s.) and RQ (0.04; n.s.). Women and men lost a significantly different amount of weight (women -5.8±2.7 vs. men: -9.8±2.8; $P = 0.001$). Subjects mainly lost fat mass during the VLED (Figure 1).
Body-weight status after weight loss as well as after weight maintenance both were significantly lower compared to body-weight before weight loss ($P=0.0001$).
Percentage body fat was significantly reduced after weight loss as well as after weight maintenance compared to before weight loss ($P = 0.0001$). Fat free mass decreased slightly, but not statistically significant over time. Factor one of the three-factor eating questionnaire (restraint scores) was significantly increased at T2 and T3 compared to T1 ($P = 0.0001$), while factor two (disinhibition) and three (hunger) were significantly reduced ($P < 0.05$).
Respiratory quotient (RQ) was significantly decreased at T2 compared to T1, yet significantly increased at T3 compared to T2 ($P = 0.0001$). Resting energy expenditure was significantly related to fat free mass before weight loss ($r = .81$; $P < 0.0001$), after weight loss ($r = .43$; $P < 0.05$) and after weight maintenance ($r = .65$; $P < 0.05$) (Figure 2).

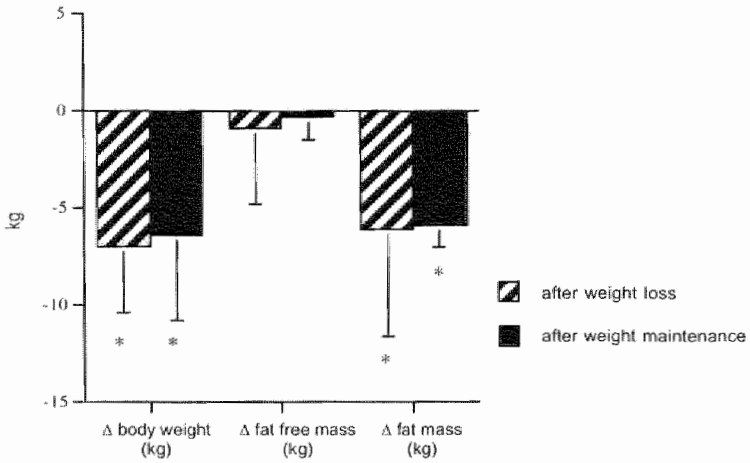


Figure 1. *Changes in body weight(average for 32 subjects), FFM and FM (kg±s.d.) after a six-week weight loss period (striped bars) and a consecutive three months weight maintenance period (black bars) expressed as change from before weight loss(Δ).*
** significantly different from before weight loss at P = 0.0001*

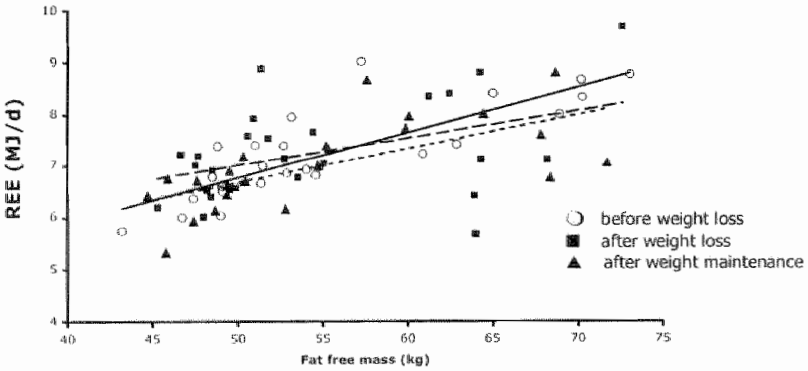


Figure 2. *Resting energy expenditure (REE) as a function of fat free mass (n = 32; 23 women, 9 men) before weight loss (circles, solid line, y = 0.09x + 2.38; P = 0.0001), after weight loss (squares, dashed line, y = 0.05x + 4.33; P = 0.02) and after weight maintenance (triangles, dotted line, y = 0.07x + 3.37; P = 0.001).*

Blood parameters

Since men and women lost a different amount of weight all blood parameters were investigated for men and women separately first. Since no differences for sex were observed, results on blood parameters are given for the whole group.

The decrease in GLP-1 after weight loss compared to before weight loss was not related to the decrease in body weight (r = 0.12; n.s.), the decrease in body weight (r = 0.15; n.s.), gender (r = 0.11; n.s.) or age (r = 0.06; n.s.).

After weight loss, mean plasma GLP-1 (pmol/l) concentration in the fasting blood sample tended to be lower compared to before weight loss (2.6 (SEM 0.61) vs. 4.7 (SEM 1.3), approaching significance (P = 0.06).

Plasma GLP-1 concentrations expressed as change from the fasting blood sample (Δ) were significantly increased after ingestion of the breakfast before weight loss (T1) and after weight maintenance (T3). Post hoc testing revealed a significant increase (P< 0.05) at 30, 60, 90 and 120 minutes at both occasions. After weight loss (T2), plasma GLP-1 concentrations were not significantly different from fasting plasma concentrations after breakfast.

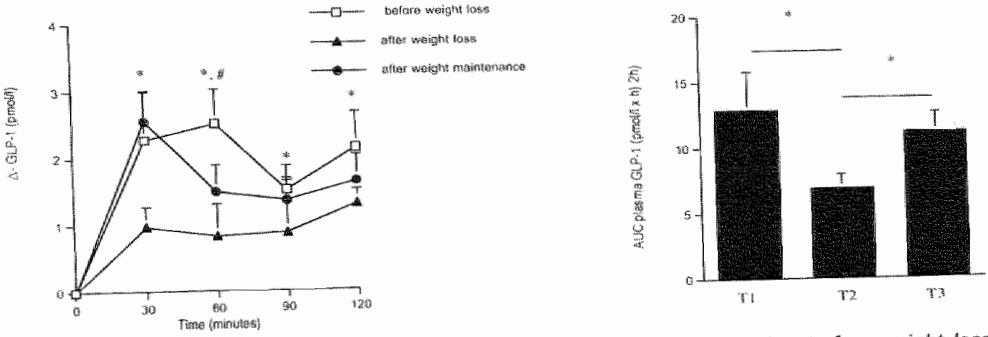


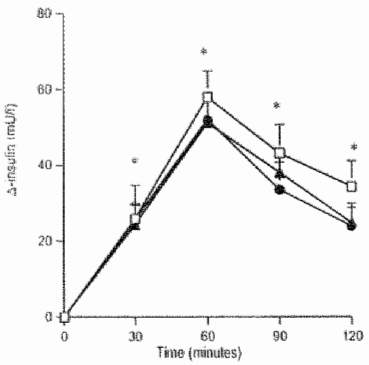
***Figure 3 A**. Plasma GLP-1 concentrations in response to a standard breakfast before weight loss, after weight loss and after weight maintenance. Data are presented as means ± SEM.*
Left panel: Plasma concentrations expressed as change from the fasted sample at time point 0 (Δ) before weight loss (squares), after weight loss (triangles) and after weight maintenance (circles).
** significantly different concentrations compared to fasting concentrations before weight loss and after weight maintenance. P < 0.05*
 # significant difference between plasma concentrations before weight loss and after weight loss. P < 0.05
Right panel: Area under the curve (AUC) calculated as incremental AUC x 2h before weight loss (T1), after weight loss (T2) and after weight maintenance (T3).
** significantly different at P < 0.05*

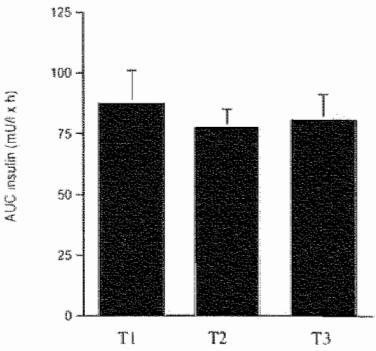
Δ- plasma GLP-1 concentrations were significantly lower after weight loss compared to before weight loss at 60 minutes after breakfast (.83 (SEM 0.48) vs. 2.5 (SEM 0.52), (P = 0.003).
The AUC (pmol/l x 2h) for T1, T2 and T3, the AUC for plasma GLP-1 concentrations before weight loss (12.8 (SEM 2.9)) and after weight maintenance (11.1 (SEM 1.5) were significantly higher compared to after weight loss (6.8 (SEM 1.0)) (P = 0.01) (Figure 3A).
Plasma insulin concentrations were significantly increased compared to fasting concentrations after breakfast at T1, T2 and T3 (P < 0.05). No differences were observed between T1, T2 and T3 in insulin concentrations expressed as change from fasting concentrations or comparing the AUC (mU/l x 2h) (Figure 3B).

Plasma glucose concentrations were significantly increased compared to fasting concentrations after ingestion of the breakfast at 60 minutes (P < 0.05) at T1 (5.52 (SEM 0.12) vs. 6.44 (SEM 0.26), T2 (5.07 (SEM 0.10) vs. 6.08 (SEM 0.23) and T3 (5.36 (SEM 0.12) vs. 6.61 (SEM 0.32). No differences were observed between T1, T2 and T3. Change from fasting concentrations for glucose (Δ) are shown in Figure 3C.

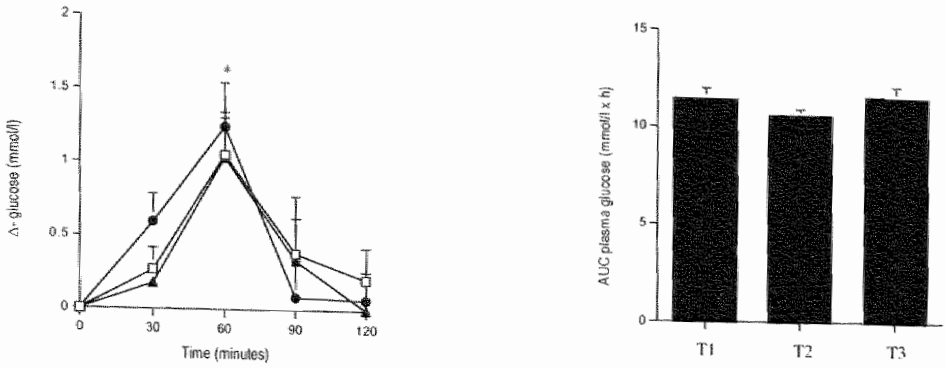
Plasma free fatty acid concentrations decreased over time at T1, T2 and T3. The decrease compared to fasting concentrations (Δ) was significant before weight loss and after weight loss at every time point except for 30 minutes after weight maintenance (P < 0.05). The AUC for free fatty acid concentrations (μmol/l x 2h) was significantly higher at T2 (598.2±47.9) compared to T1 (435.7±23.2) and T3 (471.8±34.1), (P = 0.004) (Figure 3D)

3B





3C



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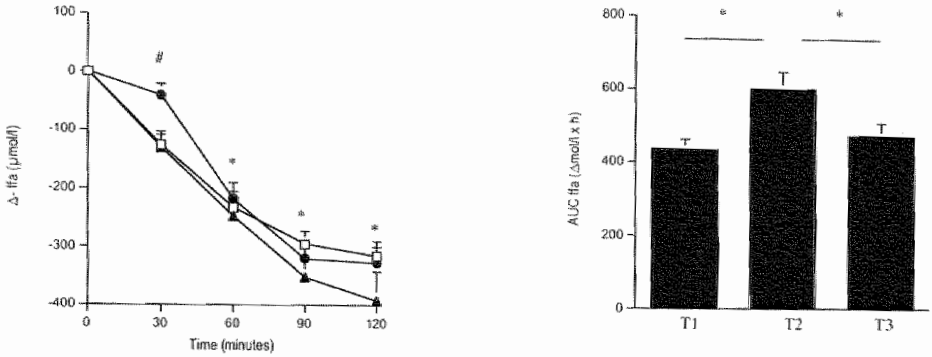

Figure 3 B-D. . *Plasma concentrations of insulin (3B), glucose (3C) and free fatty acids (3D) after ingestion of a standard breakfast before weight loss, after weight loss and after weight maintenance. Data are presented as means ± SEM.*
Left panel: Plasma concentrations expressed as change from the fasting sample at time point 0 (Δ) before weight loss (squares), after weight loss (triangles) and after weight maintenance (circles).
** significantly different concentrations compared to fasting concentrations at 0 minutes after ingestion of a standard breakfast before weight loss, after weight loss and after weight maintenance. P < 0.05*
 # significant difference compared to baseline plasma concentrations after weight maintenance compared to before weight loss and after weight loss. P < 0.05
Right panel: Area under the curve (AUC) calculated as incremental AUC x 2h before weight loss (T1), after weight loss (T2) and after weight maintenance (T3).
** significantly different at P < 0.05*

***Table 2**. Appetite ratings of 32 subjects (23 women; 9 men) before weight loss, after weight loss and after weight maintenance. Values are means ± sem. Statistical significance was determined by ANOVA for repeated measures.*

	time (minutes)	before weight loss	after weight loss	after weight maintenance
Hunger (mm VAS)	0	24.37 ± 3.7	23.8 ± 3.9	33.2 ± 3.9
	30	9.03 ± 2.7	6.9 ± 5.7	6.7 ± 1.8
	60	9.22 ± 2.3	6.7 ± 1.6	9.9 ± 2.2
	90	10.2 ± 2.7	6.1 ± 1.0	11.6 ± 1.6
	120	14.48 ± 3.6	8.8* ± 1.4	14.33 ± 2.6
Satiety (mm VAS)	0	31.33 ± 4.3	34.1 ± 3.3	30.86 ± 3.8
	30	72.38 ± 3.8	69.95 ± 5.7	79.36 ± 3.4
	60	69.14 ± 4.6	72.37 ± 4.3	75.72 ± 3.8
	90	69.04 ± 4.2	75.5 ± 2.9	67.0 ± 4.1
	120	63.57 ± 4.7	69.7 ± 4.1	61.55 ± 4.5

* significantly different compared to before weight loss at $P = 0.03$.

Appetite ratings

Hunger ratings were not different between T1, T2 and T3 at baseline, at 30, 60 and 90 minutes. Hunger scores decreased after breakfast at T1, T2 and T3. The decrease in hunger was significantly different at 120 minutes (P = 0.03) between T1 and T2 with lower hunger ratings after weight loss compared to before weight loss (Table 2).
Satiety ratings increased after breakfast compared to fasted ratings at T1, T2 and T3 and stayed elevated compared to the fasted ratings throughout the two hours of measurement. No differences between T1, T2 and T3 were observed over time (Table 2). Appetite ratings were not related to plasma GLP-1 concentrations.

Discussion

The present study shows a tendency of decreased plasma GLP-1 concentrations after modest weight loss in overweight/ obese subjects. After a weight maintenance period of three months GLP-1 concentrations rebound to a level similar to before weight loss. After weight loss not only fasting GLP-1 concentrations seemed to be decreased compared to before weight loss, but it appears that nutrient-stimulated GLP-1 release was disturbed after weight loss as well.
This observation might be an effect of the semi-solid VLED that the subjects followed during weight loss. A study comparing a liquid and a solid test meal in healthy, normal-weight subjects revealed a difference in gastric-emptying time, but not in small-bowel transit time for a liquid and a solid test meal (26). Altered gastric emptying rates, and increased proximal absorption rates of a liquid test meal in obese subjects (27), possibly resulting in less food reaching the distal intestine, are suggested as a possible explanation for decreased GLP-1 release (28). Thus, the absorption rate of the semi-solid VLED in the presence of a negative energy balance might have lead to the decrease of GLP-1 secretion after weight loss in the overweight/ obese subjects. It

needs to be taken into consideration that the reported results in the present study also might be due to a decreased stimulation of the L-cells. Peptide YY (PYY) is a peptide colocalized with GLP-1 in the L-cells of the gut. GLP-1 infusion studies showed significantly decreased PYY concentrations as a response to GLP-1 infusion, giving evidence that L-cell secretion is regulated in part by GLP-1 itself through negative feedback regulation (29, 30). Since in those studies GLP-1 concentrations were elevated to supraphysiological levels (30), the question remains in how far the results apply to rather a low range of physiological GLP-1 concentrations in the present study.

During the weight maintenance period subjects appeared to have stable body weight. This might have been achieved since the body weight lost can be described as modest, but in accordance with reports from other weight loss studies (31, 32)

Surprisingly, after the three months weight maintenance period GLP-1 concentrations were rebounded back to a level comparable to before weight loss. The decrease of GLP-1 secretion after weight loss might be due to a negative energy balance and may suggest that GLP-1 might play a role as a neuroendocrine factor signaling energy deficiency.

The overall pattern of GLP-1 release during T1, T2 and T3, is following the biphasic pattern, that has been described before (33). GLP-1 release has been reported as attenuated in obese subjects compared to normal-weight subjects (12). This observation has been explained by increased DPP-IV activity for example (34). However, based upon the literature on DPP-IV activity in obese subjects, where abnormally increased DPP-IV activity could be observed in the obese even after substantial weight loss, it seems unlikely that altered DPP-IV activity is an explanation for different GLP-1 release at T1, T3 and T2 in the present study

Due to the negative energy balance during weight loss, free fatty acid concentrations were increased after weight loss compared to before weight loss. An increase in plasma non-esterified fatty acids has been found to inhibit carbohydrate-mediated GLP-1 secretion (12, 35), so that increased plasma free fatty acids after weight loss in the current study, might in part contribute to decreased GLP-1 secretion after the standard breakfast.

As expected, the RQ measured after a VLED was reduced (32), indicating increased fat oxidation. RQ was increased again after three months weight maintenance. REE as a function of FFM did not change over time. Since the main determinant of REE is FFM (36) and FFM did not change over time either, this result is not surprising. Subjects mainly lost fat mass, as can be seen in the significant reduction in percentage fat mass. Weight lost during the VLED consisting mainly of fat mass seems to be favorable for the prevention of weight regain, and the result is in line with studies showing that weight regain was slower, when body weight regained consisted of a greater FFM (37, 38).

During a weight maintenance period subjects usually regain weight (32, 39). Subjects in the current study did not regain weight significantly during weight maintenance. Part of an explanation for this might be either individual motivation or the weather (40). The summer 2003 was an exceptionally warm one and June was with 17.8° Celsius the

warmest June since 1901. Subjects had their weight maintenance period in the months between April and July 2003.

Another important factor in successful weight maintenance is the restraint status of the subjects, which was increased significantly after weight loss and stayed increased after weight maintenance. It has been shown, that on the individual level, differences in successful weight maintenance after an energy restriction period are related to increases in cognitive restrained eating behavior during energy restriction phase (39, 41, 42). This was supported by a decrease in disinhibition and hunger during weight loss and weight maintenance.

Differences in hunger and satiety ratings were observed at only one timepoint after weight loss compared to before weight loss, an observation that is similar to reports from other studies before (32). Ratings were not different after the weight maintenance period either. Again, this might be due to the weather or could be explained by less energy requirements due to weight loss.

Evaluating the hunger and satiety ratings as well as the blood parameters it is important to mention that the size of the test meal was the same at every occasion, although energy requirements changed. This is a procedure similar to other dietary induced weight loss studies (44). It is unlikely that this had an effect on GLP-1 concentrations, since if at all an increased GLP-1 release would have been expected due to more energy relative to requirements after weight loss compared to before weight loss.

No relationship between satiety ratings and GLP-1 concentrations were observed in the present study. Decrease in hunger ratings was not related to the increase in GLP-1 concentrations after weight maintenance. Both rather seem to reflect a new state of energy balance achieved.

In conclusion nutrient stimulated GLP-1 release was decreased after weight loss, but rebounded to a level similar to before weight loss after a weight maintenance period. The rebound in nutrient stimulated GLP-1 release makes it likely that the absence of nutrient stimulated GLP-1 release after weight loss is transient and might be due to a negative energy balance.

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Chapter 8

General Discussion

The research presented in this thesis focused on the investigation of both endogenous GLP-1 stimulation and effects of GLP-1 stimulation in lean and obese subjects. First, galactose as a non-glucose carbohydrate in combination with guar gum as a fiber has been assessed concerning its ability to stimulate GLP-1 release. As a second stimulus for GLP-1 release physical activity has been investigated. Physical activity as well as galactose and guar gum as a nutrient combination were shown to stimulate GLP-1 release in lean subjects. Secondly, it has been assessed whether GLP-1 release can be triggered by the same nutrient combination, respectively physical activity in overweight/ obese subjects. As for the stimulation with galactose and guar gum, no differences were observed between lean and obese subjects concerning GLP-1 release. Physical activity only stimulated GLP-1 release after a period of modest weight loss in the overweight/ obese subjects.
Thirdly, parameters were investigated that might influence GLP-1 dynamics. Particularly gender was found to be a major determinant of GLP-1 release in lean subjects in one of the studies.
Furthermore the effects of weight loss and weight maintenance on GLP-1 release have been examined. Weight loss was shown to improve sensitivity of GLP-1 release to physical activity and to stimulation by nutrients, yet only after a weight maintenance period when a new point of energy balance had developed. Otherwise, weight loss induced by a very-low-energy-diet, initially led to decreased GLP-1 release and diminished sensitivity.

Stimulation of GLP-1 release

GLP-1 is released into the circulation after a meal (1-3). Macronutrients have been tested distinctively in their potential to stimulate GLP-1 release. As far as protein it has been found that GLP-1 secretion is stimulated, when protein is part of a mixed meal, but protein alone did not consistently increase GLP-1 release in humans (2, 4, 5). GLP-1 is increased by the ingestion of mixed fats or triglycerides in humans (2, 6).
Carbohydrates, especially glucose, are a sufficient stimulus for GLP-1 secretion (2-5).
This thesis focused on non-glucose carbohydrates and their ability to stimulate GLP-1 release. With respect to obesity and diabetes non-glucose carbohydrates such as galactose may stimulate GLP-1 release sufficiently with a lower glycaemic index (11).

Carbohydrates

Oral glucose has been shown to stimulate GLP-1 release consistently (2, 4). It has been suggested, that the effect of oral carbohydrates on gut hormone release may vary between different monosaccharides (7). The monosaccharide that was investigated in the studies of this thesis was galactose in combination with guar gum.
The release of GLP-1 from the ileum requires sodium (2), implicating a role for the brush-border sodium/ glucose co-transporter in the effect of glucose on GLP-1 release

(8). Other sugars that utilize the same co-transporter as glucose for the absorption across the intestinal epithelium such as galactose, are expected to stimulate GLP-1 release as well (2). Not much is known about the effect of galactose on GLP-1 secretion. The results of the present studies, particularly described in Chapter 2 demonstrate that galactose, when combined with fiber such as guar gum, is a potent stimulus for GLP-1 release in lean subjects. The area under the curve for GLP-1 concentrations after ingestion of galactose/ guar gum was not significantly different compared to glucose/ guar gum; both showed stronger GLP-1 release in comparison to the single nutrient or to water.

With respect to diabetes and obesity, galactose seems to be an interesting ingredient. Oral galactose was shown to increase insulin concentrations most likely due to an incretin effect. That means that galactose affects insulin concentrations via GLP-1 or GIP, the two major incretins in men (9). Galactose also has a lower glycemic index compared to glucose, which is thought to be beneficial with respect to the risk for obesity, type 2 diabetes and cardiovascular disease (10, 11).

It has been suggested, that sugars using a different mechanism of transport compared to glucose, such as fructose and lactose do not to stimulate GLP-1 release (12).

Fructose has been investigated concerning its ability to stimulate GLP-1 release. Effects of fructose on GLP-1 secretion are contradictory. While no difference between fructose and glucose has been observed concerning the stimulation of GLP-1 (13, 14), other results show a smaller GLP-1 release in response to fructose as compared to glucose (7, 15). Because of the positive effects on insulin release, glucose concentrations and the utilization of the same co-transporter as glucose, galactose was used as one nutrient in the studies described in this thesis, when it was the intention to investigate nutrient stimulated GLP-1 release.

Fiber

Observations on the effect of fiber alone on GLP-1 release are conflicting. Results from animal studies show an increase in GLP-1 concentrations along with the consumption of fiber (16, 17). A human study revealed no effect of 1.7g physillium on GLP-1 concentrations compared to a control condition (18). In the present studies guar gum has been used as a fiber component. Guar gum is a polysaccharide, which on contact with water forms a highly viscous gel (19).

The addition of guar gum has been shown to delay absorption of a meal, delay small bowel transit time and to prolong the contact between nutrients and small intestinal epithelium (20). The intention for the studies presented in this thesis was to use the properties of guar gum in order to enhance and extend the GLP-1 stimulation caused by galactose. The results of Chapter 2 show that galactose in combination with guar gum led to GLP-1 concentrations that were higher than concentrations evoked by galactose alone. In comparison with other results on the effect of fiber on GLP-1 release(18), guar gum in combination with a nutrient such as galactose increased GLP-1 release. The

underlying mechanism may be an increased contact of galacose with receptors in the small intestine due to the properties of guar gum.

Physical activity

Nutrient stimulation is not the only mechanism to regulate GLP-1 release, since the GLP-1 releasing cells are located mainly in the distal part of the intestine and the early peak of GLP-1 secretion occurs before nutrients reach the distal ileum (21, 22). Therefore it is believed that GLP-1 release is triggered by putative humoral mediators, like i.e. gastrointestinal polypeptide (GIP), which are released when nutrients are present in the upper intestine and by stimulation of the autonomic nervous system (23). Meal-induced activation of the sympathetic nervous system has been mentioned as a potential factor to account for the early secretion of GLP-1 (24). There is evidence that release of peptide YY (PYY), a peptide that is co-synthesized with GLP-1 in the intestinal L cells as well as co-secreted with GLP-1 in response to ingestion of mixed meals is affected by the sympathetic nervous system (25, 26). Until now the effect of sympathetic stimulation on GLP-1 release has only been investigated in animal or cell line studies (27-29).

Only one study has investigated the effect of physical activity on GLP-1 concentrations and other gastrointestinal peptides (30). In that study marathon runners were investigated concerning their GLP-1 concentrations during a race. Significantly increased concentrations were reported post-race, but mechanisms remained unclear.

In Chapter 4 the results on GLP-1 release after stimulation by means of physical activity are reported. Indeed, increased GLP-1 concentrations after physical activity compared to rest were observed in lean subjects and in obese subjects after modest weight loss. Subjects were in a fasted state. Physical activity is known to increase norepinephrine concentrations by two- to six-fold depending on the intensity (31), thus we may speculate that the increase in GLP-1 release is related to activation of the sympathetic nervous system. Stimulation of adrenergic pathways mediates the inhibition (32, 33) of gastrointestinal motility by GLP-1 (29, 34). Colonic sympathetic afferents controlling the gastroduodenal area have been described and as a consequence, a GLP-1 action on these fibers which could activate an ileal brake mechanism has been suggested (29, 34). Furthermore, GLP-1 receptor stimulation was shown to increase blood pressure and heart rate (35). Taken together, GLP-1 release during physical activity may be due to a decrease in splanchnic blood flow, which may lead to gut ischaemia. Gastrointestinal hormones may be released in conditions where increased blood flow is needed (30).

The results presented in this thesis show that not only endurance, but also daily physical activity increased GLP-1 concentrations, which may be either related to catecholamine concentrations or to gut ischaemia, or both in lean subjects. This novel result supports the suggestion of a mediating role for the GLP-1 system in multiple stress responses (36).

Parameters of importance for the stimulation of GLP-1 release

GLP-1 release and age

Aging is associated with decreased appetite and food intake (37, 38). The causes for this so called anorexia of aging are unknown but are likely to be multifactorial, possibly including changes in gastrointestinal peptides associated with hunger and satiety (39). Differences in GLP-1 concentrations in relation to age hardly have been investigated.
Age related differences have been found for Cholecystokinin (CCK), after indraduodenal glucose or lipid infusion, but not for GLP-1 or PYY concentrations (39). CCK concentrations appeared to be higher in older compared to younger subjects as a result of higher baseline values and a greater increase from baseline during lipid infusion. Chapter 5 reports on a group of subjects significantly different ages showing no difference in fasted or postprandial GLP-1 concentrations, similar to the observations by MacIntosh et al. (39). However, due to their study design, no clear conclusions on age-related difference after oral or intragastric ingestion of nutrients could be drawn after intraduodenal infusions of lipid or carbohydrate (39). Another study by Ranganath et al. found significantly increased GLP-1 concentrations in older subjects compared to younger subjects after administration of oral glucose (40). Results of Chapter 5 are due to oral ingestion and show no age-related difference in GLP-1 release. Different from Ranganath et al. (40) active GLP-1 (7-36 amide) was measured in the studies of this thesis. The immunoassay Ranganath et al. (40) used does not reliably differentiate between active GLP-1 (7-36 amide) and the inactive metabolite GLP-1 (9-36 amide). If older subjects would indeed have inappropriate accumulation of active GLP-1 concentrations, this should lead to lower glucose and higher insulin responses, which have not been observed in the older subjects. Comparing our findings with those from Ranganath et al. (40) it is likely that older subjects might have increased total GLP-1 *concentrations but not active* GLP-1 concentrations. Nevertheless this would raise the question whether age might be related to progressive receptor insensitivity for the GLP-1 receptors. To our knowledge this has not been investigated yet.
Thusfar, based on the results presented in Chapter 5 and the results from MacIntosh et al. (39) age seems to affect other gastrointestinal hormones like CCK rather than GLP-1 and it seems unlikely that changes in circulating GLP-1 concentrations contribute to the reduced appetite accompanying normal aging.

GLP-1 release and weight status

GLP-1 concentrations appear to be affected by weight status. Results on how GLP-1 concentrations differ in obese subjects compared to normal-weight subjects are contradictory. One study found a hypersecretion of GLP-1 in the obese after a glucose challenge (41). GLP-1 has been shown to be strongly insulinotropic (3). It has been suggested that an exaggerated 'incretin' factor – GLP-1- might play a pathophysiological role in obesity (41) and it seems possible, that an increased secretion of GLP-1

contributes to hyperinsulinaemia in obese patients. Similar results have been reported for an obese group of African-Americans (42, 43). Higher fasting as well as higher stimulated GLP-1 concentrations were observed after an oral glucose challenge, possibly contributing to a higher prevalence of hyperinsulinaemia-related disorders in African-Americans.

Most of the studies executed in Caucasian subjects show attenuated GLP-1 concentrations in the obese compared to normal-weight controls (44, 45). It has been shown that hyperglycaemia, a feature of obesity, due to a condition of either insulin resistance or insulin deficiency could induce a progressive desensitization of intestinal L cells with a consequent peptide failure response to stimulation (46). Another explanation for attenuated GLP-1 concentration in obesity is the observation that GLP- 1 degradation by the enzyme DPP-IV is accelerated compared to lean subjects, leading to the attenuated GLP-1 concentrations. Furthermore higher plasma levels of free fatty acids in obesity have been mentioned as a possible reason for lower GLP-1 concentrations in obese subjects (45).

Results of Chapter 5 show no differences in baseline GLP-1 concentrations when comparing lean and obese subjects. However, weight status appeared to affect GLP-1 sensitivity to nutrient ingestion rather than baseline concentrations, since stimulated GLP-1 concentrations were different between lean and obese subjects after ingestion of water and a standard breakfast. When water was replaced with galactose/ guar gum instead, the difference between the groups disappeared, showing a sensitivity in the obese to a stronger trigger, resulting in a similar GLP-1 release as in the lean subjects. Free fatty acid concentrations were not different between obese and lean subjects in the study of Chapter 5. The results of the studies in this thesis suggest that attenuated baseline GLP-1 concentrations do not yet occur in subjects in a class 1 stadium of obesity and that the difference between lean and obese subjects in nutrient-stimulated GLP-1 concentrations is related to the strength of the nutrient stimulus.

GLP-1 and weight loss

Since GLP-1 concentrations have been described as attenuated in obesity (44, 45, 47), it is relevant to investigate how GLP-1 concentrations may change with weight loss. The effect of weight loss on GLP-1 concentrations has been mainly investigated after weight loss induced by surgery (jejunoileal bypass (48), gastric bypass (49) or gastric pacing (50)). Bypass surgeries have in common that parts of the gastrointestinal tract are connected by bypassing others, such as parts of the stomach or the duodenum. With gastric pacing a gastric stimulator system is implanted to provide electrical stimulation of the stomach (50). With surgically induced weight loss it is difficult to evaluate whether changes in peptide hormones are due to weight loss or result from a manipulated stimulation of secretory cells due to surgery. Two surgically induced weight loss studies report improved GLP-1 concentrations in relation to either subjects own baseline (51) or compared to a control group (48). For the latter one the question remains, whether

baseline concentrations differed between the treatment and the control groups before surgery and whether GLP-1 concentrations in the weight loss group improved compared to their own baseline.

Chapter 6 shows initially decreased GLP-1 concentrations after weight loss in a group of obese subjects (BMI 30.3), probably caused by the VLED induced lack of nutrient stimulation. These results confirm the observations concerning the effect of gastric pacing on gastrointestinal hormone concentrations (50). GLP-1 concentrations were decreased after gastric pacing induced weight loss. Since GLP-1 is released in response to vagal nerve stimulation, a decreased vagal tone due to gastric pacing has been considered as a possible explanation for decreased gastrointestinal hormone secretion after weight loss (50). The mechanisms are not fully understood yet, but either vagal depression or excessive stimulation may play a role in decreased GLP-1 concentrations after weight loss (50). Only one study investigated the effect of diet induced weight loss on GLP-1 concentrations (52). Another study induced weight loss pharmacologically with Orlistat (53).

When GLP-1 concentrations were investigated after dietary induced weight loss (52), improved GLP-1 concentrations have been reported compared to before weight loss, although the reported increase in GLP-1 release was not statistically significant. This seems to contradict the findings of decreased GLP-1 concentrations after weight loss reported in this thesis. The most striking difference between the study presented in this thesis and other studies that investigated effect of weight loss on GLP-1 concentrations, is the duration of the weight loss period and the time between baseline measurement and measurements after weight loss. Verdich et al. (52) investigated subjects after six months of weight loss, while subjects in the present thesis were investigated after six weeks of weight loss with a VLED.

In addition to decreased baseline GLP-1 concentrations, meal-induced postprandial GLP-1 concentrations after weight loss also failed to modify (Chapter 6). Lugari et al. (51) reported similar results after surgically induced weight loss. The group of subjects investigated in this thesis showed meal induced sensitivity before weight loss, which is most likely due to a lack of nutrients stimulating GLP-1 release associated with weight loss caused by the VLED.

The suggestion of an initial decrease in GLP-1 concentrations due to a VLED and the importance of the duration of the weight loss period is supported by the rebounded meal induced GLP-1 concentrations to a level similar to before weight loss, after a weight maintenance period (Chapter 7). Subjects from those studies that report improved GLP-1 concentrations after weight loss (52), may be adjusted to a new set-point of energy balance at the time of re-investigation and may have passed the decrease in hormones already.

Ghrelin, an orexigenic hormone that seems to play a role in the regulation of hunger and satiety has been given much attention lately concerning its concentrations before and after weight loss. Ghrelin appears to be inversely related to GLP-1 secretion and ghrelin and GLP-1 seem to have opposite effects on satiety (54, 55).

Diet-induced weight loss was shown to increase plasma ghrelin concentrations, while gastric bypass surgery suppressed ghrelin concentrations (49). This may be explained by the possible role of ghrelin in fat oxidation (56). Suppressed ghrelin concentrations seem to be the effect of enhanced fat oxidation, which is stronger after gastric bypass surgery than after diet-induced weight loss (56). In addition decreased ghrelin levels after surgery may be caused by the surgical technique that is applied, as recently shown for bariatric surgery (57). Ghrelin concentrations were only decreased after Roux- en –Y gastric bypass, yet not after gastric banding and biliopancreatic diversion.

Taken together the results on initially decreased GLP-1 concentrations after weight loss in a group of obese subjects and a subsequent rebound in GLP-1 concentrations after a weight maintenance period up to a level similar to concentrations before weight loss (Chapter 7) are not necessarily contradicting observations by Cigaina et al (50), who reported decreased GLP-1 concentrations after weight loss, nor by Verdich et al (52), who reported slightly increased GLP-1 concentrations after six months of gradual weight loss.

In our studies GLP-1 concentrations rebounded while significant weight loss was maintained during weight maintenance, indicating an improved sensitivity in obese subjects after weight loss. Improved sensitivity due to weight loss is supported by the results in chapter 4, where modest weight loss caused sensitivity to activity induced GLP-1 release. The results show that a clear conclusion on the effects of weight loss has to imply the final effect when weight stabilization is reached.

GLP-1 and gender

The relationship between gender and GLP-1 release has not explicitly been studied before. In Chapter 3 a clear relationship between gender and GLP-1 release was observed. The effect of increased GLP-1 concentrations after ingestion of galactose/ guar gum and breakfast compared to water and breakfast was almost exclusively observed in female subjects. (Chapter 3)

GLP-1 release was positively related to %body fat and negatively with fat free mass respectively. Since women in the present experiment (Chapter 3) had significantly more body fat and less fat free mass than men, body composition seems to be an explaining factor for the gender related difference in GLP-1 release. Leptin and insulin are released in direct proportion to body fat as well (58). One other study reported similar results concerning the difference between men and women in GLP-1 release (59). Vaag et al. (59) reported higher values in women for GLP-1, GIP, glucose and insulin concentrations after a glucose challenge. Glucose and insulin concentrations were not different for men and women in our study, although, due to the insulinotropic action of GLP-1, higher insulin concentrations and lower glucose concentrations would be expected for women. Since we stimulated GLP-1 endogenously in healthy subjects with a difference in peak concentrations of about 5 pmol/l between men and women, the effect of GLP-1 on

insulin and glucose concentrations supposively is less pronounced in our group of subjects.
Body fat content is highly correlated with circulating leptin concentrations (60). Recently it has been shown that leptin significantly stimulated GLP-1 secretion in vitro and in vivo and leptin resistance in mice was associated with impaired GLP-1 release (61).
Therefore different leptin concentrations due to differences in body fat mass are a likely explanation for the difference in GLP-1 concentrations.
GLP-1 and leptin exert counteregulatory actions in the periphery, but centrally actions overlap. While GLP-1 simulates insulin biosynthesis, leptin inhibits glucose-dependent insulin release in the endocrine pancreas (62). In order to create a homeostatic condition women would have to show higher plasma GLP-1 concentrations due to higher circulating leptin levels.
Taken together the present results show that gender may be a determinant of GLP-1 concentrations due to different body fat mass. There is evidence that leptin as a hormonal indicator of body fat is involved in GLP-1 secretion and contributes to higher plasma concentrations in women.

Effects of GLP-1 release on satiety

It has been stated that the primary cause of obesity lays in the control of appetite (63). Energy balance is regulated very precisely, although daily energy intake is highly variable (64).
Long-term and short-term hormonal signals from adipose tissue and the gastrointestinal tract act in concert to regulate feeding behavior and body weight regulation.
Long-term signals have different functions than the short-term regulators. Insulin and leptin are considered to be long-term regulators of energy homeostasis that are activated in proportion to both adipose stores and the amount of energy consumed over a prolonged period of time to regulate food intake and energy expenditure in order to keep body adiposity relatively constant and to maintain energy homeostasis.
In contrast to the long term signals, short-term signals can be overridden by the long-term signals and are not primary determinants of body adiposity (65).
Short-term signals like GLP-1, CCK or PYY primarily act as determinants of satiety to limit the size of individual meals and to sustain the intermeal-interval (65).
This thesis focuses on the effect of GLP-1 as a short-term satiety regulator. Findings on GLP-1 as a short-term satiety regulating peptide are contradictory (66, 67). Studies that found an effect on satiety mostly have been applying GLP-1 infusions in supraphysiological doses (68-70). One study investigated the paradigm of endogenous GLP-1 stimulation with an energy fixed meal like it is described in this thesis (52). Subjects had a sandwich based standard breakfast, and meal related GLP-1 release was investigated before and after a weight loss period. Hunger and satiety was assessed before and after the test meal with visual analog scales. Only weak correlations were reported for GLP-1 release and subjective appetite ratings in that study. The results of

chapter 3 and chapter 5 show a relationship between GLP-1 release and satiety in normal-weight subjects yet not in obese subjects, when GLP-1 was stimulated endogenously by a standard meal. Taken together, the question remains whether the pharmacological effect of GLP-1 on satiety and food intake is always clearly present as a physiological effect. In Chapter 3 a relationship between the increase in GLP-1 and the increase in satiety at 60 and 90 minutes in the water and breakfast condition was described. In Chapter 5 a relationship for the area under the curve in the galactose/ guar gum and breakfast condition and GLP-1 was found for the lean subjects. The relationship between GLP-1 and satiety in this thesis is also less pronounced as with exogenous supraphysiological GLP-1 infusions (68-70). GLP-1 has been shown to even induce nausea when administered in higher doses (71). Furthermore, there is evidence that GLP-1 might be involved in the development of taste aversion and that the anorexic actions may partially be caused by the activation of hypothalamic stress pathways (72-74). Thus, the effect of GLP-1 on appetite in the studies using exogenous pharmacological doses of GLP-1 might be an effect exceeding satiety, resulting in nausea, respectively taste aversion.

Results of chapter 5, chapter 6 and chapter 7 show that the relationship between GLP-1 and satiety does not appear in the obese subjects.

Since no differences in basal GLP-1 concentrations between lean and obese subjects were observed, lower satiety ratings in the obese group might be rather an effect of a disturbed perception of a physiological feedback. Other hormones that are involved in the regulation of hunger and satiety i.e. ghrelin or CCK might play a role as well.

The suppressing effect of exogenous CCK administration on food intake in humans was first described by Kissileff et al. (75). Pharmacological doses of CCK8 decreased intake of a liquid meal in humans (75). That the pharmacological effect of CCK on satiety in humans may not always be clearly present as a physiological effect is supported by other authors (76). Studies on CCK have been reviewed and the conclusion was drawn that central CCK plays a role in the control of food intake, yet peripheral endogenous CCK is not a major satiety factor (77).

Results on GLP-1 are similar to the results on CCK. Clear central effects of GLP-1 on food intake have been reported in animal studies (78). When human subjects are investigated it appears that pharmacological doses of GLP-1 are necessary to show a clear relationship between GLP-1 release and satiety and food intake (66). Based on the results presented in this thesis GLP-1 appears to have some effect on satiety (Chapter 3 and 5) yet cannot be seen as the major satiety factor when stimulated endogenously in lean subjects and should be further investigated in combination with other satiety factors.

Practical relevance of GLP-1

Based on the actions of GLP-1 that have been described, GLP-1 became interesting for the treatment of non-insulin-dependent Diabetes Mellitus (NIDDM). NIDDM is characterized by an increase in basal glucose concentrations and exaggerated postprandial glucose excursions, induced by a combination of beta-cell dysfunction and impaired insulin sensitivity (79). NIDDM patients, who are often obese, share a lot of features with obese subjects and obese subjects in turn are at high risk to develop NIDDM.

GLP-1 is considered as a potential treatment in NIDDM and obesity especially because of its insulinotropic potency and the effect on food intake. Different from GIP, which has lost most of its insulinotropic activities in NIDDM (80), GLP-1 still appears to stimulate insulin release in these subjects (1). In contrast to other insulinotropic agents, i.e. sulfonylureas, GLP-1 acts fully glucose-dependent, thus prevents hypoglycaemia (81). A significant relationship between the increase in GLP-1 and the increase in insulin was observed in the lean subjects from Chapter 3, but not in the obese subjects in Chapter 6 (Figure 1). This result suggests that the incretin effect is present in lean subjects when GLP-1 is stimulated endogenously, yet concentrations are not sufficient for causing an effect in obese subjects. Physiologically stimulated GLP-1 concentrations may be too low to show an effect in obese subjects. However, the addition of guar gum that has been shown to lower insulin concentrations (82) limits conclusions about the effect of GLP-1 on insulin release. Different from the water and breakfast condition, a relationship between GLP-1 and insulin was observed in the guar gum and breakfast condition. Guar gum may interfere with the actions of GLP-1 on insulin release by delaying nutrient absorption and slowing access to the absorptive epithelium (82).

The effect of GLP-1 on satiety and food intake has been discussed before. There is a body of evidence showing that GLP-1 plays a role in satiety and body weight loss when administered exogenously and in high doses (66, 68, 83). The question remains whether the effect of GLP-1 on satiety and body weight may be transitory or persists during long-term treatment as well ((84). To date, work dealing with this research question came to contradictory results.

In animal as well as in human studies effects on hunger and prospective food consumption (85) or reduced food intake (86) were observed for the duration of GLP-1 administration, but no effects on total food consumption after a longer period of time (86) or during an ad libitum lunch five hours after GLP-1 infusions respectively were reported (85). The results described in this thesis suggest a short-term role of GLP-1 in satiety. After endogenous GLP-1 stimulation with different nutrients initial differences in satiety were observed, yet no differences were observed in ad libitum food intake four hours later (Chapter 1).

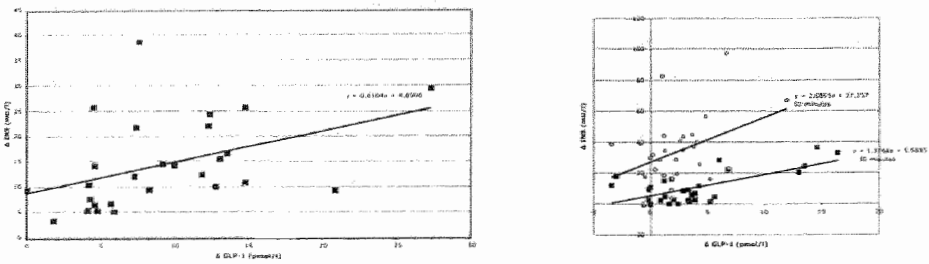

***Figure 1**. Increase in insulin is significantly related to the increase in GLP-1 concentrations in 30 normal-weight subjects at 30 minutes (R^2 = 0.17; p = 0.02) after ingestion of a galactose guar gum preload and a standard breakfast (left side figure) and at 30 (R^2 = 0.43; p = 0.0001) and 60 minutes (R^2 = 0.15; p = 0.03) after ingestion of water in combination with the same standard breakfast (right side figure).*

Other groups have reported reduced body weight after long-term sustained treatment with GLP-1 (87).

Taken together, it appears that GLP-1 plasma concentrations need to be maintained elevated constantly for long-term effects. That raises the question about a decrease in postsynaptic receptor sensitivity and adaption (tachyphylaxis). No occurrence of tachyphylaxis has been reported for short-term investigations (88), but not much is known about long-term effects yet.

Results from chapter 5 show that satiety ratings differed between lean and obese subjects despite similar GLP-1 concentrations in the water and breakfast conditions. This would give evidence to the suggestion of receptor insensitivity or that pharmacological doses are needed to see effects of GLP-1 on satiety in obese subjects.

Another problem for the therapeutic suitability of GLP-1 in addition to finding the appropriate dosage, is its short half-life time. Most of the peptide is cleaved in-between two minutes after administration at the N-terminus and thereby yielding the biologically inactive fragment GLP-1 (9 – 36 amide). When plasma concentrations are measured, like described in this thesis, peptidase inhibitors are used to inhibit degradation.

For practical use of GLP-1 this thesis revealed that weight loss programs should be combined with increased physical activity in order to improve GLP-1 sensitivity, thus contributing to metabolic fitness. Both, activity and weight loss increase sensitivity for GLP-1 release in obese subjects.

Pharmacologically, GLP-1 may be potent as a therapeutic agent in NIDDM and obesity when administered in high doses steady state in order to maintain constantly elevated plasma concentrations. Furthermore suitable modes of administration are needed to prevent early degradation.

Implications for future research

This thesis has demonstrated that galactose and guar gum is an effective nutrient combination for endogenous stimulation of GLP-1 release. This nutrient combination is very specific and has been applied through all the experiments in the same dosage.

For therapeutic purpose of enhancing GLP-1 release endogenously in diabetes and obesity it is important to evaluate more nutrients with a low glycaemic index to prevent hyperglycaemia and still have sufficient GLP-1 release. Otherwise only exogenous GLP-1 application seems useful in this disease area, which is accompanied by diverse shortcomings.

Suitable modes of GLP-1 application need to be evaluated. Since the peptide is inactivated immediately by gastric acid it cannot be ingested orally. A problem with intravenous or subcutaneous injection is the short half-life time of GLP-1 (89, 90). Therefore another approach is to inhibit the degradation of GLP-1 by DPP-IV or use analogues of the peptide that have extended half-lives (91).

Due to the current state of development of these modes of GLP-1 application it is important to further evaluate the possibilities of endogenous GLP-1 stimulation, not only for treatment, but also for prevention.

More research is needed to investigate the effect of the stimulation of GLP-1 in physiological concentrations to draw final conclusions on the effect of GLP-1 on satiety. Studies in humans are mostly investigating the effect of exogenously administered supraphysiological doses of GLP-1 (66, 68, 69). Since it has been shown that the GLP-1 system is related to anxiety and plays a role in stress responses (36), the effect on satiety might be mediated by these circuits rather than being a direct effect. It is not clear yet, whether GLP-1 specifically acts on inhibitory feeding centers in the brain to evoke satiety or rather is a mediator of aversive stimuli (35). Taste aversion has been reported in response to agents that serve visceral illness such as lithium chloride (92) and central as well as pheripheral GLP-1 receptor agonist were found to increase blood pressure and heart rate (35). These models can be described as internal stressors and have been defined as interoceptive stress (93). Interoceptive stress may be mediated by central GLP-1 systems (74).

We have shown a stimulation of GLP-1 release by physical activity. Our study is one of the very few reports on the relationship between physical activity and GLP-1 release. More work needs to be done to elucidate the mechanism for that relationship. Taken together our results and the described possible role for GLP-1 in the mediation of interoceptive stress the question remains, whether GLP-1 release during physical activity is a cause or a consequence in the regulation of sympathetic outflow.

This thesis shows that decreased GLP-1 concentrations after weight loss are transient and rebound after a weight maintenance period. The present results and the results from the literature (48, 52) show, that the timepoint of investigation after weight loss seems to play a critical role for the interpretation of the results. Although GLP-1 sensitivity was improved after weight maintenance, future research is needed to clarify

the decrease in GLP-1 concentrations and the impaired GLP-1 release after a nutrient stimulus after weight loss.

Conclusions

From the results presented in this thesis and the results from the literature several conclusions can be drawn.

- In addition to nutrients, physical activity is a suitable way to stimulate GLP-1 release and increase GLP-1 plasma concentrations.
- Results on GLP-1 concentrations need to be analyzed for gender differences, since differences in body fat, fat free mass and related hormones appear to affect GLP-1 concentrations.
- Weight loss decreases GLP-1 concentrations transiently, yet increases the overall sensitivity for GLP-1 release in the longer term
- Basal GLP-1 concentrations are not different between lean and modestly obese subjects, but stimulated GLP-1 release is different depending on the stimulus that is used.
- Weight loss affects the sensitivity for GLP-1 release in modestly obese subjects.
- Pharmacological effects of GLP-1, which appear to correlate with satiety and food intake are not always clearly present as physiological effects in humans.
- For increasing GLP-1 release endogenously and therapeutic use, weight loss and activity should be combined.

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Summary

Summary

During the last decades the prevalence of obesity dramatically increased. Weight gain occurs when energy intake is increased or energy expenditure is decreased. Acute and long-term food intake and energy balance are regulated through distinct, but interacting mechanisms, such as neural and hormonal signals. Hormonal signals are for example gastrointestinal hormones that are released by the intestinal L cells, like peptide YY 3-36 or glucagon-like peptide 1 (GLP-1) that are released in response to nutrient sensing in the intestine. Those hormones have peripheral as well as central effects in areas that are supposed to be involved in the sensation of hunger and satiety. Via such actions the hormones mentioned are expected to play a role in both the frequency and amount of energy intake. For a hormone like GLP-1, that is believed to be involved in the regulation of hunger and satiety it has been suggested that basal concentrations as well as the release in response to a meal are decreased in overweight and obese subjects compared to normal-weight subjects. This has been considered a cause for the development of obesity. However, most of the work showing clear relationships between these gastrointestinal hormones and satiety were executed with exogenous hormone application in supraphysiological doses.The research described in this thesis focused on one gastrointestinal peptide in particular, namely GLP-1.

One approach for the therapeutic us of GLP-1 is to enhance its endogenous release, in order to avoid compliance issues that are related to the necessary injections of peptide hormones. Therefore different ways to stimulate GLP-1 release endogenously in normal-weight as well as in overweight and obese subjects were investigated as well as the effect of gender on these findings. Furthermore, the effects of weight loss and weight maintenance on GLP-1 release were explored. In addition it was investigated whether endogenously stimulated GLP-1 release affects satiety sensations in subjects with different weight status.

GLP-1 is believed to play a role in the regulation of food intake and satiety, but also functions as an incretin. Incretins stimulate insulin release and improve glucose tolerance and thereby GLP-1 presently is discussed as a target peptide for the treatment of type II diabetes. With regard to nutrients, carbohydrates, especially glucose, was shown to stimulate GLP-1. With respect to the role of GLP-1 for diabetes and obesity another carbohydrate than glucose, i.e. galactose and the ability to stimulate GLP-1 release has been tested in the present thesis. Soluble viscous fiber (modified guar gum) is believed to increase the contact of carbohydrates with receptors in the small intestine. Therefore a study was performed where galactose in combination with guar gum was tested as a potential stimulus for GLP-1 release.

In combination with guar gum galactose stimulated GLP-1 release in a manner similar to glucose in combination with guar gum. All tested nutrient combinations showed a relationship between GLP-1 release and satiety after 30 and 120 minutes of ingestion. After finding the combination of galactose and guar gum effective in stimulating GLP-1, the mixture was combined with a standard breakfast. The effect of the galactose with

guar gum and breakfast combination was significantly more effective in stimulating GLP-1 release than breakfast alone. The effect on GLP-1 release has been analyzed for men and women separately. Body fat was the most discriminative variable between men and women in the experiment and was significantly related to GLP-1 release in women. Since leptin is an adiposity signal that is secreted in direct proportion to the amount of body fat stored in the adipocytes and since in animal as well as in human studies leptin was shown to stimulate GLP-1 release, leptin may be a possible explanation for the gender related difference in GLP-1 release. Furthermore the increase in satiety was related to the increase in GLP-1 release at 60 and 90 minutes after ingestion, independent of gender and only present when the breakfast was combined with water instead of galactose and guar gum.

GLP-1 receptor stimulation also has been shown to play a role in the physiological adaption to a stressor by increasing blood pressure and heart rate. As an alternative to nutrients GLP-1 release was stimulated with physical activity as a physiological stressor. Most of the work investigating the effect of stress, activity and the nervous system on GLP-1 release has been done in animals and cell lines. In this thesis it is shown that physical activity stimulates GLP-1 release in lean subjects and in overweight and obese subjects, yet only after modest weight loss suggesting a role of body weight for GLP-1 sensitivity.

Since weight status seemed to be important for GLP-1 release, the role of body weight was further investigated. Comparing a normal-weight group with an overweight and obese group concerning their GLP-1 release after a nutrient trigger, it was observed that weight status did not affect GLP-1 release after the galactose with guar gum and breakfast combination. When breakfast was combined with water instead of the galactose and guar gum mixture, the overweight and obese group had significantly lower GLP-1 release compared to the normal-weight group, indicating that stimulated GLP-1 release is different for different weight categories depending on the stimulus that is used for triggering the release of the peptide. To further clarify the impact of weight status on GLP-1 release a weight loss experiment with a subsequent weight maintenance period was executed. Since GLP-1 release has been suggested to gradually improve with weight loss and was indeed increased after modest weight loss in the experiment with physical activity, a six-week very-low-energy diet (VLED) was applied to a group of obese subjects. Unexpectedly, GLP-1 release initially was decreased after substantial weight loss and was not triggered anymore by the galactose with guar gum and breakfast combination. However, after a subsequent weight maintenance period of three months, GLP-1 release rebounded to a level comparable to before weight loss. Satiety ratings were not related to GLP-1 release in these subjects neither before weight loss, nor after weight loss or weight maintenance.

In conclusion, it was found that a galactose with guar gum mixture alone, as well as in combination with a standard breakfast was effective in stimulating GLP-1 release in lean and obese subjects. In lean subjects, replacement of the galactose with guar gum mixture by water in combination with the same breakfast showed significantly increased GLP-1 release compared to obese subjects, indicating a slightly lower sensitivity for

GLP-1 release in the obese, depending on the nutrient challenge that is used. However, the stimulus-related difference in GLP-1 release in the obese was not mirrored in their subjective satiety sensations. That leads to the suggestion that a disturbance of appropriate perception of the satiety feedback rather than a disturbed feedback itself might contribute to obesity.

In addition to the nutrient combination physical activity was found to be a GLP-1 stimulus as well, yet depending on the weight status of the subjects, leading to the conclusion that attenuated GLP-1 release in the obese may be reversible and might be rather an effect than a cause of obesity. However, in the presence of a negative energy balance, after significant weight loss, GLP-1 release was blunted and decreased compared to before weight loss. The rebound of GLP-1 release to a level similar to before weight loss after a subsequent weight maintenance period though leads to the conclusion that the effects of our weight loss experiment on GLP-1 release are transient.

Samenvatting

Samenvatting

De prevalentie van overgewicht en obesitas is in de Westerse welvaartsmaatschappij de laatste decennia drastisch gestegen. Gewichtstoename is het resultaat van een positieve energiebalans, veroorzaakt door een verhoogde energie-inname en/of een verlaagd energiegebruik.
Neurale en hormonale signalen reguleren de energiebalans, en met name de energie-inname op korte en lange termijn. Hormonale signalen zijn bijvoorbeeld gastro-intestinale hormonen, zoals peptide YY3-36 (PYY3-36), en glucagon-like peptide 1 (GLP-1) die afgegeven worden door intestinale L-cellen. Deze hormonen worden in de dunne darm vrijgemaakt wanneer daar nutriënten aanwezig zijn. Genoemde hormonen hebben zowel perifere als centrale effecten die betrokken blijken te zijn bij de signalering van honger en verzadiging. Alszodanig dragen deze hormonen bij aan de maaltijd-frequentie en de totale energie-inname. Voor GLP-1 is er enige evidentie dat niet alleen de basale concentratie, maar ook de afgifte na een maaltijd verlaagd is bij overgewichtige/obese ten opzichte van normaal-gewichtige individuen. Op deze manier zou het een rol kunnen spelen bij het ontstaan van obesitas. De relatie tussen deze hormonen en verzadiging is echter alleen aangetoond bij studies die met een exogene toediening van hormonen in supra-fysiologische doseringen gewerkt hebben.
Het onderzoek beschreven in dit proefschrift richt zich op een gastro-intestinaal peptide in het bijzonder, namelijk GLP-1. Om complicaties, die aan de exogene toediening van GLP-1 zijn gekoppeld te voorkomen, dient ten behoeve van mogelijk therapeutisch gebruik, de GLP-1 afgifte endogeen gestimuleerd te worden. Verschillende mogelijkheden om de GLP-1 afgifte in normaal-gewichtige en obese individuen endogeen te stimuleren zijn onderzocht, evenals het mogelijke effect van geslachtsverschil hierop en het effect van gewichtsverlies en gewichtsbehoud. Tevens werd de relatie tussen GLP-1 afgifte en verzadiging bij proefpersonen met een verschillend uitgangsgewicht onderzocht.
GLP-1 zou niet alleen een rol spelen bij de regulatie van de voedsel-inname en verzadiging, maar wordt ook geacht te functioneren als 'incretine'. Incretinen zijn hormonen die de insuline afgifte op een glucose afhankelijke manier stimuleren en de glucose tolerantie verbeteren. Daarom staat GLP-1 tegenwoordig ook in de belangstelling als een target peptide voor de behandeling van type II diabetes.
Voor nutriënten is aangetoond dat koolhydraten, met name glucose, de GLP-1 afgifte stimuleren. Gezien de rol van GLP-1 afgifte bij obesitas en diabetes werd in dit proefschrift tevens een ander koolhydrate dan glucose op het vermogen om de GLP-1 afgifte te bevorderen onderzocht, te weten galactose.
Van oplosbare, visceuze voedingsvezels zoals guar gum wordt verondersteld dat ze het contact van koolhydraten met receptoren in de dunne darm verlengen. Om die reden is in de beschreven experimenten galactose met guar gum gecombineerd als potentiele stimulus voor GLP-1 afgifte. In het eerste experiment bleek de combinatie van guar gum en galactose de GLP-1 afgifte op een vergelijkbare manier te stimuleren als glucose in

combinatie met guar gum. Alle nutriënten die in dit experiment werden getest toonden een relatie van GLP-1 afgifte met verzadiging op 30 en 120 minuten na inname van het drankje.
Nadat de combinatie van galactose en guar gum als effectief voor de stimulering van de GLP-1 afgifte was vastgesteld, werd deze gecombineerd met een standaard ontbijt. De combinatie van galactose en guar gum met een ontbijt leidde tot een significant hogere GLP-1 afgifte dan het ontbijt in combinatie met water. Uit de analyse van het effect van galactose en guar gum met ontbijt op GLP-1 afgifte apart voor mannen en vrouwen bleek dat vrouwen significant hogere GLP-1 afgiften als reactie op de voedselinname lieten zien dan mannen. Lichaamsvet was de meest bepalende parameter voor het verschil tussen mannen en vrouwen en bleek bij vrouwen significant gerelateerd te zijn aan de toename in GLP-1 afgifte.
In dit verband is het hormoon leptine relevant, dat geproduceerd wordt in relatie tot de hoeveelheid lichaamsvet. Hiervan is al eerder aangetoond dat het de GLP-1 afgifte stimuleert. Het effect van leptine zou dus een verklaring voor het geslachts-gerelateerde verschil in GLP-1 afgifte kunnen zijn. Tevens werd in dit experiment een verband gevonden tussen de GLP-1-afgifte en verzadiging op 60 en 90 minuten na de maaltijd. Dit effect was onafhankelijk van geslacht aanwezig wanneer het ontbijt met water werd gecombineerd.
GLP-1 receptor stimulatie wordt verondersteld een rol te spelen in de fysiologische aanpassing aan stress, met name door verhoging van de bloeddruk en de hartslag. Als alternatief voor nutriënten werd GLP-1 afgifte onderzocht na stimulatie met een fysiologische stressor, te weten fysieke activiteit. De meeste experimenten die effecten van stress, activiteit en het zenuwstelsel op GLP-1 onderzoeken, hebben dit in cel-lijnen of dier-experimenten gedaan. In dit proefschrift werd aangetoond dat fysieke activiteit de GLP-1 afgifte in normaal-gewichtige en in overgewichtige/obese individuen na een bescheiden gewichtsverlies bevordert. Dit is een indicatie dat lichaamsgewicht een rol speelt bij GLP-1 gevoeligheid.
Omdat gewichtsstatus een rol blijkt te spelen bij de GLP-1 afgifte is het onderzoek in dit proefschrift tevens op de rol van lichaamsgewicht gericht. Uit vergelijking van de GLP-1 afgifte bij de overgewichtige/obese groep met die van de normaal-gewichtige groep, bleek dat er geen verschil was bij een sterke trigger zoals galactose met guar gum in combinatie met een ontbijt, maar wel bij een normale trigger, zoals wanneer het ontbijt met water gecombineerd werd. De overgewichtige groep had dan een lagere GLP-1 afgifte. Dit leidt tot de conclusie, dat gewichtsstatus belangrijk is in relatie tot de trigger die ter stimulatie van de peptide gebruikt wordt.
Om de invloed van gewichtsstatus op GLP-1 verder te verhelderen, is een experiment met gewichtsverlies en een aansluitende gewichtsbehoud fase uitgevoerd. Er wordt verondersteld dat GLP-1 afgifte gradueel verbetert tijdens gewichtsverlies wat inderdaad ook bleek uit het beschreven experiment met fysieke activiteit.
Een zeer laag energetisch dieet werd gedurende zes weken aan de proefpersonen aangeboden, om gewichtsverlies te induceren. Onverwacht bleek de GLP-1 afgifte na gewichtverlies gedaald te zijn en bleek deze ook niet meer door de combinatie van

galactose, guar gum en ontbijt gestimuleerd te kunnen worden. Na een daarop volgende gewichtsbehoud fase van drie maanden waren de GLP-1 concentraties terug op een niveau vergelijkbaar met de uitgangswaarden van voor het gewichtsverlies, terwijl de proefpersonen niet significant waren aangekomen. Verzadiging en GLP-1 afgifte waren zowel voor gewichtsverlies, na gewichtsverlies of na gewichtsbehoud in deze groep niet gerelateerd.

Concluderend kan gesteld worden dat galactose met guar gum alleen zowel als in combinatie met een ontbijt effectief was in het stimuleren van de GLP-1 afgifte in normaal-gewichtige, in overgewichtige en obese individuen.

Normaal-gewichtige individuen gaven echter een significant verhoogde GLP-1 afgifte ten opzichte van de overgewichtige en obese individuen te zien wanneer enkel een ontbijt met water werd aangeboden. Dit kan als een indicatie gezien worden voor een verlaagde gevoeligheid voor GLP-1 afgifte in overgewichtige en obese individuen, afhankelijk van de trigger die gebruikt is. Het stimulus- afhankelijke verschil in GLP-1 afgifte bij de overgewichtigen en obesen werd niet weerspiegeld in subjectieve verzadigingsgevoelens. Dit leidde tot de conclusie dat een verstoorde perceptie van de fysiologische feedback van verzadiging meer bijdraagt aan het ontstaan van obesitas, dan een verstoorde fysiologische feedback zelf.

Naast de werkzame nutriënten combinatie die in dit proefschrift voor het stimuleren van GLP-1 vastgesteld is, is ook fysieke activiteit een effectieve manier voor het stimuleren van GLP-1 gebleken. Omdat dit afhankelijk was van gewichtsstatus kan worden geconcludeerd dat verlaagde GLP-1 afgifte in overgewichtige en obese individuen reversibel zou kunnen zijn en eerder een gevolg dan een oorzaak van overgewicht en obesitas is.

Dit was echter niet van toepassing op een toestand van negatieve energie balans, omdat de GLP-1 afgifte na gewichtsverlies significant verlaagd was ten opzichte van voor het gewichtsverlies en ook door de effectieve nutriëntencombinatie niet meer stimuleerbaar bleek te zijn. Na een gewichtbehoud fase was de GLP-1 afgifte weer vergelijkbaar met de afgifte van voor het gewichtsverlies, wat tot de conclusie leidt dat het effect van gewichtsverlies op GLP-1 afgifte en concentraties van voorbijgaande aard is.

Zusammenfassung

Zusammenfassung

Während der letzten Jahrzehnte hat die Prävalenz der Adipositas dramatisch zugenommen. Gewichtszunahme kann zum einen Resultat übermäßiger Energiezufuhr und/oder zum anderen unzureichenden Energieverbrauchs sein. Die akute, aber auch die Energieaufnahme über einen längeren Zeitraum, sowie die Energiebalance sind durch verschiedene Mechanismen geregelt, die in ständiger Interaktion miteinander stehen. Zu diesen Mechanismen gehören zum Beispiel neurale und endokrinologische Signale, die Informationen über Hunger und Sättigung vermitteln. Zu den endokrinogischen Signalen gehören unter anderem Hormone, sowie Peptide YY 3-36 oder Glucagon-like Peptide 1 (GLP-1), die von sogenannten L-Zellen im Dünndarm abgegeben werden, sobald dieser mit Nährstoffen in Berührung kommt. Diese Hormone haben sowohl periphäre als auch zentrale Effekte. Die zentralen Effekte können lokalisiert werden in Gerhirnarealen, von denen bekannt ist, das sie in die Regulation von Hunger und Sättigung involviert sind.
Von GLP-1 nimmt man an, daß sowohl basale Konzentrationen wie auch die Abgabe nach einer Mahlzeit, bei übergewichtigen und adipösen Menschen niedriger sind als bei normalgewichtigen Personen. Dies wird spekulativ als eine mögliche Ursache für die Enstehung von Übergewicht diskutiert.
GLP-1 wird gesehen als einer der Hormone, die subjektiv Sättigung vermitteln. Bisher haben Studien, die eine deutliche Beziehung zwischen gastrointestinalen Hormonen und Sättigung nachweisen konnten jedoch leider vorwiegend mit exogener Hormonapplikation in supra-physiologischer Dosierung operiert. Die Studien, die in dieser Arbeit beschrieben sind, konzentrieren sich auf die endogene Stimulation eines Hormones, nämlich GLP-1 in normal- und übergewichtien Versuchspersonen. Der Versuch der endogenen Stimulation von Peptidhormonen ist besonders interessant, durch 'Compliance'-Probleme, die für den Patieten mit der Injektionen von Peptidhormonen verbunden sind. Im weiteren Verlauf wurde untersucht, ob Geschlechtsunterschiede bestehen hinsichtlich der Abgabe von GLP-1 und welchen Einfluß Gewichtsabnahme, sowie eine anschließende Gewichtsstabilisationsphase auf GLP-1 Spiegel haben. In allen Experimenten wurde ausserdem untersucht, ob GLP-1 in Beziehung mit dem subjektiven Erleben von Sättigung steht und wenn ja, wie dies möglicherweise durch Gewichtsstatus beeinflußt ist. Neben einer Funktion in der Regulation von Hunger und Sättigung wird GLP-1 auch eine Rolle als Incretin zugesprochen. Incretine stimulieren die Insulinabgabe und verbessern die Glukosetoleranz. Darum wird GLP-1 augenblicklich auch als ein Target Peptid für die Behandlung von Typ 2 Diabetes diskutiert und untersucht.
Es konnte bereits gezeigt werden daß Kohlenhydrate, besonders Glukose, besonders potente GLP-1 Stimulatoren sind. Entsprechend der Rolle die GLP-1 für die Behandlung von Diabetes und Übergewicht potentiell spielen kann, erscheint es nötig andere Kohlehydrate, mit niedrigerem glycämischen Index in ihrem Potential für die Stimulation der GLP-1 Abgabe zu testen. Im Besonderen setzen sich die hier beschriebenen Studien mit dem Effect von Galaktose auf die GLP-1 Abgabe auseinander. Galaktose wurde dabei

kombiniert mit Guar Gum, einem Ballaststoff, von dem man annimmt, daß er zu einem verlängerten Kontakt von Kohlehydraten mit Rezeptoren des Dünndarmes beiträgt und so zu einer vermehrten Abgabe gastrointestinaler Hormone führt. In Kombination mit Guar Gum stimulierte Galaktose GLP-1 in ähnlichen Konzentrationen wie Glukose in Kombination mit Guar Gum. Unabhängig von der getesteten Nährstoffkombination konnte eine Beziehung zwischen GLP-1 und Sättigung 30 und 120 Minuten nach Nahrungsaufnahme gefunden werden.

Nachdem die Kombination von Galaktose und Guar Gum sich als effektiv herausgestellt hatten, wurde diese Mixtur mit einem Standard-Frühstück kombiniert. Die Supplementierung des Frühstücks durch die Mixtur führte zu einer Verbesserung der GLP-1 Abgabe, die größer war, als aufgrund der zusätzlich aufgenommenen Energie erwartet worden war. Dies ergab sich aus einem Vergleich mit dem Effekt des Frühstücks in Kombination mit Wasser anstelle der Galaktose - Guar Gum Mixtur. Anschließend wurden die Ergebnisse getrennt für Männer und Frauen analysiert. Die Ergebnisse zeigten, daß beinahe ausschließlich die Frauen zu dem Effekt der Mahlzeit in Kombination mit Galaktose und Guar Gum auf die GLP-1 Abgabe beitrugen, verglichen mit der Kombination des Frühstücks mit Wasser. Körperfett war die am stärksten diskriminierende Variable zwischen den Männern und Frauen des Experiments und signifikant korreliert mit der GLP-1 Abgabe für die Frauen. Leptin ist ein Hormon, das in direkter Proportion zum Körperfett, das in den Adipozyten gespeichert ist, abgegeben wird. Durch Tier- und Humanexperimente ist nachgewiesen, daß Leptin die Abgabe von GLP-1 stimuliert. Diese Ergebnisse machen die unterschiedlichen Leptinkonzentrationen von Männern und Frauen zu einem potentiellen Erklärungsansatz für geschlechtsspezifisch Unterschiede in der GLP-1 Abgabe.

Eine Beziehung zwischen GLP-1 und Sättigung konnte 60 und 90 Minuten nach Nahrungsaufnahme gefunden werden. Dieser war jedoch geschlechtsunabhängig und nur present, wenn des Frühstück mit Wasser kombiniert war, nicht nach Supplementation mit Galaktose und Guar Gum.

GLP-1 Rezeptor Stimulation spielt eine Rolle bei der physiologischen Anpassung and einen Stressor, durch Blutdruck und Herzrate zu erhöhen. Alternativ zu den Nährstoffen, die in den beschrieben Experimenten getested wurden, wurde ausserdem Aktivität als ein pysiologischer Stressor angewendet und in seinem Potential für die GLP- 1 Stimulation untersucht. Arbeiten, die bisher den Zusammenhang von Streß, Nervensystem und GLP-1 dokumentiert haben, wurden in tiertexperimentellen- oder Zellstudien durchgeführt. Die hier beschriebene Studie zeigt, daß die GLP-1 Abgabe durch Aktivität stimuliert wird. Dieser Effekt war jedoch Gewichts-Status abhängig und konnte in schlanken Versuchspersonen, aber auch in übergewichtigen Versuchspersonen, jedoch nur nach mäßiger Gewichtsreduktion, gefunden werden. Diese Ergebnis suggeriert eine Rolle von Gewicht und Gewichts-Status für GLP-1 Sensitivität. In den folgenden Experimenten wurde darum diese Rolle weiter untersucht. Der Vergleich einer normalgewichtigen Gruppe mit einer überewictigen Gruppe ergab keinen Unterschied, weder in basalen noch in stimulierten GLP-1 Konzentrationen zwischen diesen beiden Gruppen, wenn das Standard Frühstück in Kombination mit

Galaktose und Guar Gum konsumiert wurde. Ein signifikanter Unterschied zwischen den Gruppen konnte jedoch festgestellt werden, wenn Galaktose und Guar Gum durch Wasser ersetzt wurden.
Dieses Ergebnis zeigt, daß Gewichtsstatus möglicherweise nur in Abhängigkeit vom trigger, der verwendet wird, eine Rolle spielt. Um den Einfluß von Gewichtsstatus weiter zu kären wurde im Folgenden ein Gewichtsreduktionsexperiment durchgefuhrt mit einer anschließenden Gewichtsstabilisationsphase. Da andere Arbeiten bereits eine graduelle Verbesserung der GLP-1 Abgabe mit zunehmender Gewichtsabnahme suggerierten, waren unsere Ergebnisse überraschend in dem Sinne, daß nach Gewichtsreduktion die GLP-1 Abgabe nicht nur niedriger war als vor der Gewichtsreduktion, sondern auch nicht mehr stimulierbar durch die bewährte Nährstffkombination. Nach einer anschließenden Gewichtsstabilisationsphase jedoch waren GLP-1 Konzentrationen ähnlich den Ausgangswerten vor Gewichtsreduktion, obwohl die untersuchte Gruppe nicht signifikant an Gewicht zugenommen hatte. Ein Hinweis auf eine verbesserte GLP-1 Sensitivität.

Zusammenfassend kann festgehalten werden, daß Galaktose in Verbindung mit Guar Gum, sowie kombiniert mit einem Standard Frühstück die GLP-1 Abgabe in schlanken, aber auch in übergewichtigen Versuchspersonen effizient stimuliert. Ein Unteschied zwischen den beiden Gruppen mit unterschiedlichem Gewichts-Status wurde nur gefunden, wenn Galaktose und Guar Gum durch Wasser ersetzt wurden. Ein Ergebnis, das, abhängig vom Trigger, auf eine leicht erniedrigte GLP-1 Sensitivität bei Übergewichtigen hindeutet. Der Stimulus-abhängige Unterschied in der GLP-1 Abgabe bei den beiden untersuchten Gruppen konnte nicht zurückgefunden worden in der subjektiven Sättigung. Dieses Ergebnis weist darauf hin, daß möglicherweise nicht das physiologische feedback, sondern die Perzeption dieses Feedbacks verstört ist in der Gruppe der Übergewichtigen, was einen Beitrag zu der Entstehung von Adipositas beitragen könnte. Neben einer effizienten Nährstoffkombination wurde auch Aktivität als effizient für die GLP-1 Stimulation gefunden. Dies war jedoch abhängig von Gewichts-Status, was zu der Schlußfolgerung führt, das das erniedrigte GLP-1 Niveau bei Adipösen reversibel ist und eher ein Effekt als eine Ursache der Adipositas darstellt.
In eienem Zustand der negativen Energiebalnce haben sich GLP-1 Konzentrationen als erniedrigt und nicht mehr stimulierbar herausgestellt. Ein Befund der sich jedoch als vorübergehend herausgestellt hat nach Gewichtsstabilisation.

Dankwoord

Dankwoord

"If adventures do not befall a young lady in her own village she must seek them abroad"
(Jane Austen)

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Publications

Publications

Full papers

Adam, T.C.M. & Westerterp-Plantenga. Nutrient stimulated GLP-1 release in normal-weight men and women. Hormone and Metabolic Research (In Press)

Adam, T.C.M. & Westerterp-Plantenga. Activity induced GLP-1 release in lean and obese subjects. Physiology & Behavior (In Press)

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Curriculum vitae

Curriculum vitae

Tanja Cornelia Maria Adam was born on 11 January 1974 in Andernach, Germany. She completed secondary school in Bad Neuenahr Ahrweiler in 1993.
In the same year she started her academic education at the Trier University, Germany, where she studied Psychology. As part of her training she completed a four months internship at the Psychosomatic Clinic Roseneck in Germany and a three months internship at the Columbia University, New York at the Obesity Research Center. She graduated in 2000.
In the same year she started her Ph.D. research "Glucagon-like Peptide 1(GLP-1) stimulation in relation to body weight" at the Department of Human Biology, Maastricht University. Her research was mainly focused on finding different ways to endogenously stimulate GLP-1 release in subjects with different weight status, as well as the effects on satiety and changes in body weight. The research performed during this period is described in this thesis. In November 2004 she started her postdoctoral fellowship at the Department of Human Biology, Maastricht University, which will partly take place at the Department of Health Sciences at the University of California, San Francisco.

.....Wohlan denn, Herz,
nimm Abschied und gesunde!

(*Herman Hesse, "Stufen"*)