Gastrointestinal signals regulating appetite in humans

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Table of contents

Abbreviations

AgRP	agouti-related peptide
ANOVA	analysis of variance
Apo A-IV	apolipoprotein A-IV
ARC	arcuate nucleus
AUC	area under the curve
BBB	blood brain barrier
BMI	body mass index
CCK	cholecystokinin
CNS	central nervous system
DPP-IV	dipeptidyl peptidase IV
EDTA	ethylenediaminetetraacetic acid
FFA	free fatty acids
GERD	gastroesophageal reflux disease
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
GRP	gastrin-releasing peptide
ICV	intracerebroventricular
ID	intraduodenal
i.v.	intravenous
LHA	lateral hypothalamus area
LOX	loxiglumide
MDP	minimal distending pressure level
MG	monoglycerides
NPY	neuropeptide Y
NTS	nucleus tractus solitarius
Ob	obese
PFA	periformal area
PP	pancreatic polypeptide
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus
PYY	peptide tyrosine-tyrosine
RIA	radioimmunoassay

s.c.	subcutaneous
SE/SEM	standard error of the mean
SNS	sympathetic nervous system
TG	triglycerides
US	United States
VAS	visual analogue scale
VLDL	very low density lipoprotein
WHO	World Health Organisation

Summary

Obesity and its associated complications are a significant health problem in industrialized countries. This fact has generated great interest in the role of the gut in the regulation of food intake in the past three decades. Especially after the discoveries of gastrin, secretin and cholecystokinin (CCK), it was generally accepted that digestive processes are mainly regulated by hormones, but it has become apparent that there is a complex interplay between neural and hormonal pathways. Several systems seem to be involved in the regulation of bodyweight; one of them is primarily concerned with short-term regulation of food intake. The control circuits include central and peripheral signals. Much insight has been gained into physiological processes of satiety peptides like CCK, glucagon-like peptide-1 (GLP-1) and peptide tyrosine-tyrosine (PYY) in the past few years. The informations depicting their mechanism of action and potential interactions between different physiological signals involved in the short-term regulation of satiety are still limited in humans. The main interest of this thesis focuses on the further characterization of some of those signals, especially on the investigation in potential interactions between individual satiety factors.

The study set-up applied in the projects was standardized and is similar to experimental conditions used in prior studies. Here are some examples: nearly in every study, volunteers received an intraduodenal (ID) perfusion. To investigate a potential interaction between the stomach and the small intestine, a preload was given or the stomach was distended by a balloon for a short period of time before the test meal was offered. After the preload or the distension of the stomach, a standard meal was presented to the subjects, and they were invited to eat and drink as much as they wished for 60 min. During the study subjects scored their subjective feelings for hunger and fullness for the duration of each experiment using a visual analogue scale (VAS) and blood was drawn in regular intervals. The study was finished 60 min after meal start.

The stomach has an obvious role in the regulation of eating behavior, but until recently it was still unclear, if and to what extent pure mechanical distension of the fundus and the antrum can influence food intake. Therefore the first project was

designed to further understand the role of a) the gastric fundus and b) the gastric antrum in triggering satiation in healthy male volunteers. From previous study results it was anticipated that distension of the distal stomach could play a role in the generation of satiation.

In the first part of our study the fundus was distended by a balloon with increasing volumes. When the fundus was distended, no effect on food intake was observed, but a short-lasting effect on feelings of hunger and fullness. Gastric distension only seems to trigger satiety as long as mechanoreceptors in the stomach are stimulated; the short-lasting effect could indicate that the signals are transmitted via afferent nerves to the central nervous system (CNS).

The second part of this study was designed to examine the effect of antrum balloon distension on subsequent food intake; in addition, it was of interest to test whether ID fat could intensify the effect of distal gastric distension. Neither gastric distension alone nor in combination with ID fat reduced the subsequent calorie intake; also no effect was observed on feelings of hunger or fullness. ID fat does not seem to intensify gastric satiety signals induced by pure mechanical distension of the stomach.

Neither fundus nor antrum distension altered CCK and PYY plasma concentrations. This fact implies that signals induced by pure mechanical gastric distension are not mediated by circulating CCK or PYY. During ID fat perfusion, CCK and PYY plasma concentrations were significantly increased. The increase of CCK after ID fat confirmed previous study results. However, this study was one of the first which could show an increase of PYY after ID fat in humans.

Due to these study results pure mechanical gastric distension of the fundus or the antrum is presumably not a sufficient satiety signal to influence subsequent food intake.

An interaction effect on food intake resulting from an intestinal and a gastric satiety signal was already explored for CCK, but not for GLP-1. It was therefore of interest to find out whether an interaction exists between a preload and intravenous (i.v.) GLP-1. In the second project GLP-1 was given i.v. in a dose which mimics physiological GLP-1 plasma concentrations; the dose reduced calorie intake confirming previous study results. One major observation of this study was the demonstration that a protein-containing preload together with i.v. GLP-1 enhanced the satiety-inducing

effects of GLP-1 compared to a water preload plus infusion of GLP-1. This result provides strong evidence that GLP-1 interacts with gastric signals to modulate food intake and satiety in humans.
We inferred that GLP-1 is an important satiety factor which interacts with other satiety signals in order to control food intake and satiety. However, it still remains unclear whether the satiety effects of GLP-1 are directly mediated through peripheral or central receptors or indirectly by releasing other satiety peptides.

The following study was designed to further understand the potential interaction between protein and fat in regulating food intake in humans. It is known that ID lipid infusions and protein given as an oral preload reduce food intake in humans and from previous study results we inferred that ID fat interacts with gastric signals to regulate food intake. In the third project we were interested in exploring the potential interaction of the stomach and the small intestine; we also wanted to see whether GLP-1 and PYY are associated with this interaction.
ID fat perfusion alone reduced the amount of food eaten and the total calorie intake, but the reduction did not reach statistical significance. Although the design of the present study was similar to previous studies with respect to fat dose, experimental design and duration of fat perfusion, the variability of the individual responses to ID fat was greater than in previous studies and the reduction of food intake did not reach statistical significance. Due to these results it can be speculated that certain individuals have a reduced sensation to ID fat. The effects of ID fat on food intake do not seem to be mediated by changes in plasma GLP-1 or PYY levels, but they are largely dependent on CCK release, which is in agreement with previous findings.
When subjects consumed an oral protein preload, calorie intake was significantly reduced. The increase of premeal plasma concentrations of GLP-1 and PYY did not differ compared to placebo. Therefore the inhibitory effect of oral protein on eating behavior is not mediated by changes in circulating plasma hormone levels.
The simultaneous administration of an oral protein preload and ID fat did not show a synergistic reduction in calorie consumption, rejecting the hypothesis that oral protein and ID fat exert a positive synergistic effect.

The fourth project examined the physiological and the pharmacological role of PYY in regulating eating behavior. Due to human study results it was supposed that

PYY (3-36) is a potent physiological regulator of satiety with a potential for therapeutic application. Because the physiological role of PYY in humans has not been investigated in detail, we first wanted to define a range of physiological PYY plasma levels after two meals differing in their calorie content. The results showed that only large meals are able to stimulate the release of larger amounts of PYY, whereas a low-calorie meal has minimal effects on postprandial hormone plasma levels.

In the second part of the study we wanted to examine the effects of graded doses of i.v. PYY (3-36) on eating behavior in healthy human subjects. We found a dose-dependent satiety effect of i.v. applied PYY (3-36). These results support the hypothesis that exogenously administrated PYY (3-36) is able to suppress food intake in humans. However, when the postprandial physiological levels of PYY after the high calorie meal are compared to those obtained after peripheral administration, it can be suggested that the significant satiety effect of PYY (3-36) is only seen at plasma concentrations higher than those after a large meal. The smallest administered dose of PYY (3-36) did not significantly reduce food intake and showed PYY plasma levels about in the same range as the physiological ones. Due to these results we infer that the PYY satiety effect seen with the middle and the highest dose of exogenous PYY (3-36) was rather a pharmacological than a physiological effect. It seems to be unlikely that PYY is a major physiological satiety factor, but still more information is necessary.

Dose-dependent side effects of PYY (3-36) like nausea and vomiting could be observed. PYY (3-36) seems to have a narrow therapeutic window, which could limit its therapeutic potential.

In summary it was shown in the present thesis that 1) pure gastric balloon distension of the fundus and the antrum is not a sufficient satiety signal to influence subsequent food intake; 2) the effect of gastric distension on eating behavior is not amplified by ID fat; 3) GLP-1 is an important satiety factor and seems to interact with gastric signals to modulate food intake and satiety in humans; 4) the satiating effect of protein as an oral preload cannot be amplified by ID fat; 5) the release of physiological PYY is dependent on the calorie content of a meal; 6) exogenous PYY (3-36) reduces food intake in a dose-dependent manner; 7) the effect of

exogenous PYY (3-36) on food intake seems to be a pharmacological rather than a physiological effect; 8) PYY (3-36) has a narrow therapeutic window.

Chapter 1: Aims and structure of the thesis

The growing prevalence of obesity is one of the most important reasons why research focuses on the control of eating behaviour in humans. Over the last 30 years many progresses in this field of research have been made.

The aim of the present thesis was to further investigate the role of specific nutrients and the action of certain satiety peptides on food intake in humans. Furthermore, the potential interactions between different satiety factors was explored to better understand the regulation of appetite in healthy humans.

In *chapter 2* the most important facts about obesity are summarised. This chapter includes facts about the epidemiology, the symptomatology, possible causes and the treatment of obesity.

Chapter 3 gives a general overview on the control of food intake in humans. The term satiation is explained, the development of research on satiation and the central, the short-term and the long-term regulation of food intake are discussed.

C*hapter 4* deals with signals such as gastric distension and gastrointestinal (GI) hormones which are involved in the regulation of food intake and satiety feelings.

The role of fat and protein in the process of satiation is explained in *chapter 5*.

Chapter 6 addresses the experimental measurement of food intake and human eating behaviour. It also discusses the basic principle of the study set-up and the validity of visual analogue scales used in the present thesis.

Chapter 7 contains all the projects in detail which were performed for the present thesis.

The most important findings of the projects described in the previous chapter are discussed in *chapter 8* and an outlook on what could be done in future research is given.

Chapter 2: Obesity

The World Health Organisation (WHO) has classified obesity as an epidemic. Obesity and its associated pathologic characteristics are major causes of illness and death worldwide. At its simplest level obesity can be defined as an imbalance between the energy that is ingested and the energy that is expended. The causes for obesity are however, much more complicated, because an interplay of different factors is acting. During the past 30 years, obesity as a disorder has dramatically increased in countries having an abundance of high calorie food products and low tendency to exercise. Numerous diseases arise from being overweight and obese with limitations for the quality of life and a lower life expectancy. In the United States (US) obesity accounts for about 300`000 deaths per year, and at current rates of increase it will displace smoking as the primary cause of preventable death (24).

2.1. Epidemiology of obesity

The body mass index (BMI) is the most widely used measure for classifying the different degrees of being overweight or obese, and is calculated by dividing the weight in kg by the height in m^2. The most important definitions of overweight and obesity by BMI with the risk of co-morbidities are shown in *Table 2.1*.

Table 2.1: Definitions of body weight disorders in adults by BMI (19) and corresponding risk of co-morbidities (18)

BMI (kg/m2)	Classification	Risk of co-morbidities
18-25 (women) 19-25 (men)	Normal weight	Average
25-30	Overweight	Increased
30-50	Obesity Classes I-III	Moderate-very severe
50-60	Obesity Class IV (superobese)	Very severe
>60	Obesity Class V (super-super obese)	Very severe

Data collected from 1999 to 2002 in the United States estimate that among adults aged at least 20 years, 35% are overweight (BMI 25-30 kg m^{-2}), 30% are obese (BMI ≥ 30 kg m^{-2}), and 5% are extremely obese. In *Figure 2.1* the increase in prevalence

of obesity among US adults between 1991 and 2001 is illustrated (23). Among children aged 6 through 19 years in 1999-2002, one in six was overweight. Increased prevalence of excessive weight was noted among all age, gender and racial/ethnic groups. (2, 16). In Europe nearly every third person is overweight. In industrial countries almost 5% of the total medical costs are spent for the treatment of obesity or its consequences, that means about 2-3 billion (Mia) Swiss Francs per year.

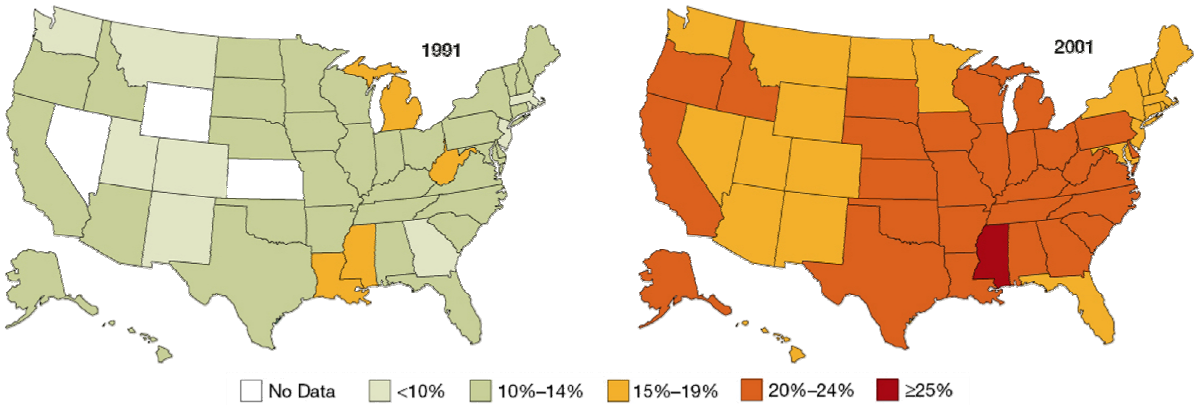

Figure 2.1: Prevalence of obesity among US adults between 1991 and 2001; by Mokdad et al. (23).

2.2. Symptomatology of obesity

Morbidity from complicating disorders, as well as overall mortality, has been shown to be closely associated with the degree of obesity (26, 32, 35). Depending on the particular medical complication examined, the risk may increase linearly (e.g. hypercholesterolemia) or exponentially (e.g. diabetes) with increasing BMI *(Figure 2.2)*.

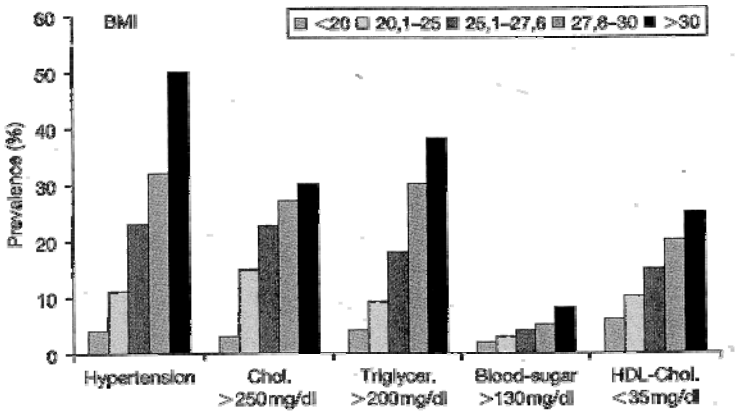

Figure 2.2: Relationship between body weight and cardiovascular risk factors (25)

More than 40 distinct disorders are caused, exacerbated, or made more likely by obesity, e.g. Diabetes mellitus type II; cardiovascular diseases like hypertriglyceridemia, hypercholesterolemia, hypertension, atherosclerosis, but also carcinoma etc. *Table 2.2* shows possible medical complications of obesity (19).

Table 2.2: Medical complications of obesity

A Metabolic
Diabetes mellitus, type II
Hypertriglyeridemia
Hypercholesterolemia
Hypertension
Fatty liver disease
Pancreatis
Central sleep apnea
Reproductive dysfunction
B Anatomic/structural
Obstructive sleep apnea
Gastroesophageal reflux disease (GERD)
GERD-associated asthma
Deep venous thrombosis
Pulmonary embolism
Decubitus ulcers
C Degenerative
Atherosclerotic cardiovascular disease
Complications of diabetes (neurologic, ophthalmologic, renal)
Hear failure
Degenerative joint disease
Vertebral disc disease
D Neoplastic
Carcinoma (breast, ovarian, endometrial, prostate, colorectal, gallbladder, pancreatic, esophageal, renal)
E Psychological
Anxiety disorders
Depression
Binge eating disorder

2.3. What causes obesity?

Despite the rapid increase in knowledge about the physiological mechanisms regulating body weight and energy balance, the causes of human obesity remain poorly understood. Human obesity appears to result from a combination of genetic, developmental, environmental, and psychological influences (22), as can be seen in *Figure 2.3* It is likely that different defects or groups of defects in the cortical (including psychological), hypothalamic, GI, endocrine, and metabolic components of the weight regulatory system are responsible for the development of obesity.

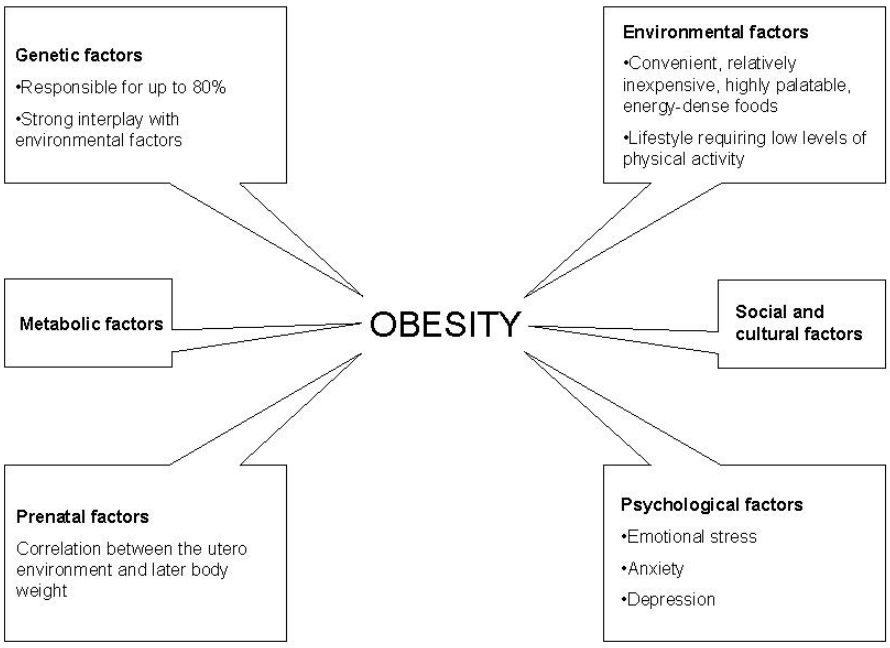

Figure 2.3: Influences on the development of human obesity

2.3.1. Genetic factors

Several lines of epidemiological evidence suggest that genetic factors account for up to 80% of a person`s predisposition to develop obesity (22). Evidence for a strong genetic contribution to human obesity comes from a variety of sources including twin and family studies, and animal studies (1, 22). However, the dramatically escalating rate of obesity documented in recent years has occurred in a relatively constant gene pool. Therefore together with a genetic predisposition abetting environmental factors seem to play a dominant role.

Most of the previously existing mutations in mouse obesity genes have now been cloned, and several homologous mutations have been discovered as rare causes of human obesity. As an example, in 1994 Zhang et al (37) discovered a naturally occurring mutant, the obese (ob/ob) mouse *(Figure 2.4)*. They discovered that this mouse has a mutation in the gene encoding for leptin, so that it is unable to produce any of this protein. As a result, these animals are stimulated to excessive food intake and profound energy conservation, leading to severe obesity. Although a few human individuals with severe obesity have been shown to lack leptin, nearly all obese individuals exhibit an excess of circulating leptin in direct proportion to their BMI (13).

Thus human obesity appears to result from functional resistance to the effects of leptin, much as type II diabetes results, in part, from resistance to the physiological effects of insulin. Not surprisingly, early clinical trials have shown little effect of leptin in substantially decreasing body weight in the large majority of obese individuals. The precise mechanism of leptin resistance in human obesity are currently unknown (10, 11). The high plasma levels of leptin in obese only reflect one phenotypical character of obesity, but the causes are unknown.

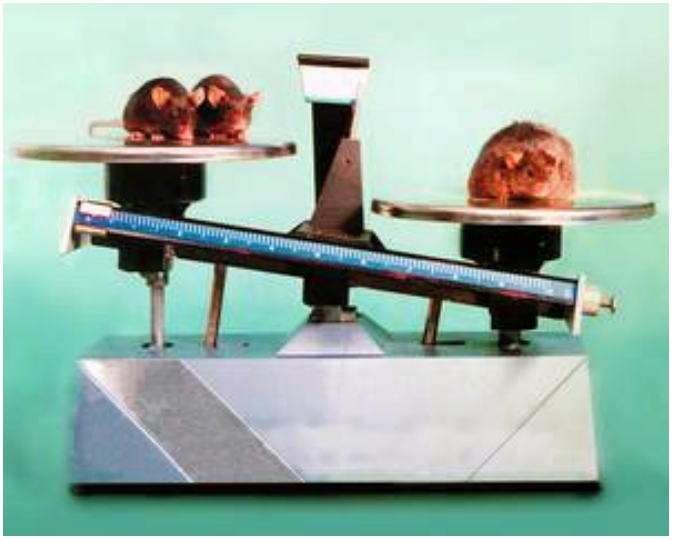

Figure 2.4: Obese mouse with leptin deficiency

2.3.2. Eating behaviour

Although genetic mechanisms strongly influence the regulation of body weight, the important role of the environment has been clearly demonstrated. During the past generation, the prevalence of obesity has doubled in the adult population and more than doubled in children and adolescents. Because our genes have not changed substantially during the past two decades, these dramatic changes underscore the influence of environmental factors on the regulation of body weight and their importance to the development of obesity. Several environmental factors have been proposed to explain the recent rise in the prevalence of obesity (31, 35). One way in which the current environment promotes obesity is by providing more frequent opportunities for the consumption of large quantities of food. A variety of highly palatable, inexpensive foods is available nearly everywhere. Changing patterns of

food consumption (e.g. irregular meals, snacks, rapid eating, “super sizing” of menus) and composition could also be potential environmental contributors to obesity.

2.3.3. Physical activity

Low levels of physical activity are associated with an increased risk of obesity (17), and our current environment tends to discourage physical activity. Work and leisure time activities are less and less likely to require physical exertion. Increased physical activity is, however, perhaps the single best correlate of long-term weight control (25).

2.4. Treatment of obesity

2.4.1. Treatment options of obesity

There is a number of strategies of correcting the imbalance between energy intake and energy expenditure. Many different diets have been recommended over the past 150 years, but none of them seems to have a lasting effect. Exercise can be an obvious way to increase energy expenditure, but for many people, exercise adds little extra weight loss to that produced by a diet program. Medications and surgical interventions (e.g. laparoscopic gastric banding or gastric bypass) should only be considered for clinically overweight individuals.

2.4.2. Pharmacological treatment of obesity

Current antiobesity drugs include appetite suppressants that act on the central nervous system (serotonin, neuropeptide Y (NPY) and adrenergic receptor ligands), and orlistat, which blocks the pancreatic lipase (5, 20, 34, 36). Most of those available drugs are only approved for short-term use and their efficacy is limited. To date only orlistat and sibutramine are approved for long-term treatment of obesity (25). *Table 2.3* shows a few substances which represent the different pharmacological groups of the current treatment of obesity.

Table 2.3: Pharmacological treatment of obesity

Drug name	Brand name (CH)	Pharmacological action	Side effects/ comments
Orlistat	Xenical®	Pancreatic lipase inhibitor	Oily stool, flatulence
Sibutramine	Reductil®	Norepinephrine-serotonin reuptake inhibitor	Raises blood pressure
Phentermine	Adipex®	Sympathomimetic drugs	CNS, cardiovascular, GI Only for short-term use

2.4.3. Potential future drugs for the treatment of obesity

The increasing knowledge of the molecular basis of weight regulation has led to the identification of a large number of potential targets for antiobesity drugs (5, 21).

Table 2.4 shows an overview of potential future peptides for the treatment of obesity. The physiological effects of those gut peptides are described in *chapter 4*.

Table 2.4: Antiobesity peptides as potential future drugs and their physiological effects

Peptide	Food intake	Gastric emptying	Glucose
Leptin	↓		
GLP-1	↓	↓	↓
Exendin-4 (GLP-1 analogue)	↓	↓	↓
CCK	↓	↓	
PYY (3-36)	↓		

The initial hopes that leptin would provide a therapeutic breakthrough for the treatment of human obesity have not materialised because of a suggested leptin resistance in obese persons (34).

In several human studies, GLP-1 infusions 1) reduced fasting and postprandial glucose levels, 2) delayed gastric emptying, and 3) induced a reduction in calorie consumption (7, 12, 14, 15, 27). Because GLP-1 has only a very short half life (8) and a sustained response requires therefore a continuous i.v. infusion, a great interest was generated in isolating a more stable GLP-1 which is resistant to enzymatic degradation (29). Exendin-4 is a 39-amino acid peptide isolated from Gila monster (type of lizard) salivary gland, which acts as an agonist of the GLP-1 receptor (8, 29, 30). The pharmacological findings of exendin-4 are consistent with

those previously found with GLP-1. The preliminary results suggest that exendin-4 may have a role in the treatment of obese patients with type 2 diabetes (8, 9).

CCK reduces food intake in human beings and in experimental animals (5). It is possible that CCK agonists might be useful in the treatment of obesity. Peptide analogues have been developed, but clinical data have not yet been published.

PYY (3-36) reduces food intake in several studies, in animals and humans when administered systemically (3, 4) although not all studies have documented an anorectic effect (33). While obese individuals are known to have leptin resistance, they do not appear to be resistant to the anorectic effects of PYY (3). Limiting factors for therapeutic use of PYY (3-36) could be the short half life and the narrow therapeutic range (6).

None of the mentioned molecules can be taken orally, as they are peptides. Therefore it would be of special interest to find a non-invasive application-form for these peptides to substitute for the daily injections. A US pharmaceutical company has already finished three Phase I trials with PYY (3-36) in form of an intranasal application (28).

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Chapter 3: Control of food intake

The obesity epidemic continues unabated, bringing with it a series of serious complications such as diabetes, coronary heart disease, and cancer *(see chapter 2)*. The WHO has described obesity as the greatest threat to human health, and therefore it has never been so important to understand the control of appetite. The regulation of energy homeostasis is complex and integrates neurobiology, endocrinology and metabolism.

The present thesis mainly concentrates on endocrinology and deals with factors which influence the termination of a meal (satiation). The following introduction gives a general review on the process of satiation.

3.1. The meaning of satiation

Appetite as well as satiating processes can be considered as an interplay between biological, behavioural and environmental influences. All living organisms require food for growth and maintenance of tissues. Stability of body weight and body composition is controlled by regulatory systems, which on one hand strongly defend against undernutrition and on the other hand protect against overnutrition. To better understand the biological and physiological processes that stimulate and inhibit food consumption, a number of general terms need to be defined. The biological drive that impels individuals to search for food is hunger. The feeling of hunger is an important component in determining what, how much, and when to eat (3). When food consumption reduces hunger, physiological processes are stimulated to inhibit further eating; this process of feeling full and consequently terminating food consumption during the course of eating is termed satiation (intrameal satiety). Satiation develops during a meal and tends to bring the period of eating to an end. Satiation, therefore, reduces hunger and limits the amount of energy consumed during that meal (3). Satiety (intermeal satiety), on the other hand, develops after food has been ingested. It is the state of satiety that delays the onset of the next meal and may reduce food consumption at the next eating occasion (3).

Appetite and satiation are complex phenomenons arising from a sequence of interactions among peripheral and central mechanisms. The GI tract contains chemo- and mechanoreceptors that transmit the information about its nutrient content to the brain, mainly via peripheral nerves (vagus afferent fibres) (17), but also via the blood through receptors within the brain itself (34). Signals of appetite or satiation may arise from peripheral or central mechanisms. The overall process of food intake control is governed by a series of complex mechanisms. Not only are the macronutrient composition, the size, and the caloric density of the meals important, but also their organoleptic properties (sight, smell, taste and texture) play a role in the termination of satiation. The amount of energy ingested over 24 hours depends on the size of individual meals and the frequency with which meals are ingested.

3.2. Development of research on satiation

The understanding of the physiological regulation of food intake has advanced rapidly, because new and more refined techniques and measurement have been developed. Advances in the physiology of human feeding have arisen from two major routes. First, the role of organ function has been investigated by studying how food intake is affected by pharmacological agents available for human use, and by improvements in the techniques available for direct measurement and manipulation of organ function. Second, advances in the techniques of measurement of the feeding process itself have led to a better understanding of the underlying factors and of the impact of external factors (e.g. diet composition) (8).

The conviction that the GI tract controls appetite for food is rooted in antiquity. Perhaps, the earliest account of satiation was mentioned by Plato. The situation has changed markedly in the last two centuries, during which time satiation has received quite a lot of attention. Much of this attention has been focused on the role of the GI tract. Compelling experimental evidence for GI involvement in satiation has been shown in the mid- to late 1800s. Human case reports indicated that people with gastric or intestinal fistulas remained hungry when most of the food they ate drained from the upper GI tract (21). In 1895, Shumova-Simonovskia and Pavlov reported that dogs with experimental esophageal fistulas ate continuously, suggesting that

stimuli for satiation were absent or attenuated (21). In 1902, only a few years later, Bayliss and Starling coined the term “hormone” when they isolated secretin from duodenal mucosa and observed that it stimulated pancreatic exocrine secretion. Some of the earliest experimental attempts to link the GI tract with control of food intake were undertaken in 1912 by W.B. Cannon and his student Washburn. Cannon and Washburn recorded and correlated the strength of gastric contractions with conscious sensations that Cannon termed “hunger pangs” *(Figure 3.1)*. While Cannon`s efforts focused on the role of the GI tract in the sensation of hunger, the desire to seek and eat food, he was acutely aware of the process of satiation. Due to his experiments Cannon stated that satiation must, at least in part, derive from GI signals. Investigations of food intake by modern behavioural scientists still apply the basic principles illustrated by Cannon over 90 years ago, coupling objective measures of gastric function with reports of sensations (e.g. fullness, discomfort) and the additional behavioural measure of food intake.

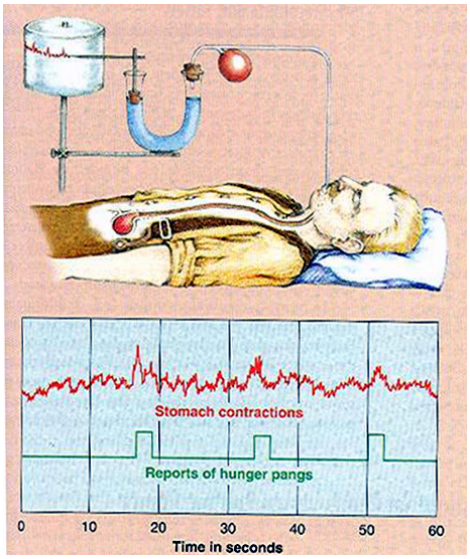

Figure 3.1: Experiment by W.B. Cannon in 1912

In the following decades, especially after the discoveries of gastrin and CCK, it was generally accepted that digestive processes were mainly regulated by hormones. However, it was not until the 1970s that significant progress in GI endocrinology occurred, with over 40 novel hormones being discovered (30). During the following years the important finding came up that many GI hormones are also expressed in

the CNS and that many of them are relevant signals which transfer information between the GI tract and the brain (30). Looking back it seems to be strange that investigators have tended for decades to adopt an “all-or-nothing” approach to explain the regulation of the various digestive functions. Today it is accepted that digestive processes are regulated through an interplay of multiple neural and hormonal pathways (6).

3.3. Central signals involved in the regulation of satiation

Although much of the research on the control of food intake has been carried out in animals, parallels of these models could be shown in humans. Metabolism or energy balance is primarily regulated by the CNS, which uses a wide range of humoral and neural signals to sense the metabolic status and control energy intake. Appetite control is dependent on the peripheral physiology and the signals from metabolic processes which are transmitted to the brain. The general mechanism for appetite control involves the intake of food followed by the release of peptides from the GI tract, which then act as hormones or trigger visceral feedback signals to the brain. Hormones such as leptin, insulin or ghrelin pass through the blood-brain barrier (BBB) via the arcuate nucleus (ARC) to other hypothalamic areas which control energy balance, like the paraventricular nucleus (PVN). The visceral feedback signals include gastric distension or the release of CCK and are transmitted by afferent fibers of the vagal nerve via the nucleus tractus solitarius (NTS) to hypothalamic nuclei. *Figure 3.2* shows the central anatomical sites involved in energy homeostasis in the lateral view and the transmission of peripheral signals to the brain (27).

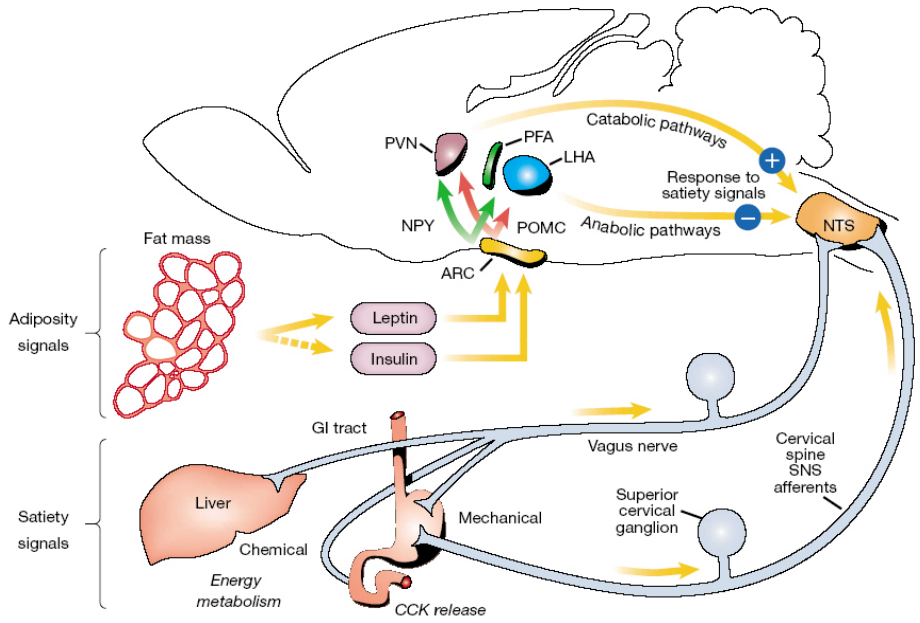

Figure 3.2: Pathways by which satiety and adiposity signals interact with central anatomical sites involved in energy homeostasis; by Schwartz et al (27). AgRP, agouti-related peptide; ARC, arcuate nucleus; LHA, lateral hypothalamus area; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; PFA, periformal area; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; SNS, sympathetic nervous system

The ARC occupies almost half of the length of the hypothalamus and contains several functionally different populations of neurons. These include one that expresses the appetite-stimulating neuropeptides NPY (14, 19) and agouti-related peptide (AgRP). NPY is a 36 amino acid neuropeptide and a member of the pancreatic polypeptide (PP) family. Axons from these neurons in the ARC project to several other hypothalamic nuclei, including the PVN, a key brain area mediating a potent effect of NPY to stimulate feeding (29). NPY is one of the most potent appetite-stimulating agents known. A single intracerebroventricular (ICV) injection acutely stimulates feeding in rodents (11). Daily injection of NPY into the hypothalamic PVN not only causes sustained hyperphagia and weight gain (14, 18, 29), but also metabolic actions that favour fat deposition.

Although there are many peripheral signals that can contribute to feeding behaviour and body weight regulation, it is important to recognize that so-called short-term and long-term signals regulate food intake and energy balance through different, but also interacting mechanisms (10).

3.4. Short-term regulation of food intake

In the present thesis the focus is above all laid on short-term signals triggering satiation. More in detail, signals which are stimulated in the GI tract. Particularly, the influence of fat and protein as short-term satiation signals will be addressed *(see Chapter 5)*.

In adult animals and humans, body weight tends to remain within a relatively narrow range, despite large day-to-day fluctuations in the amount of food consumed. Some peripheral so-called short-term signals, e.g. nutrients and GI hormones, are meal-related and act primarily as determinants of satiation to limit the size of a single meal. These short-term signals have a different function than the long-term regulators of energy homeostasis that are activated in proportion to both body adipose stores and to the amount of energy consumed over a more prolonged period of time. Short-term signals are not primary determinants of body adiposity, since they can be overridden by long-term regulatory signals. Food intake and energy expenditure are influenced over the short term by input from a variety of situational and meal-related factors such as physiological, metabolic and GI signals (resulting e.g. from gastric distension, or the release of peptides from the GI tract in response to nutrient ingestion), emotional factors, palatability and nutrient content of food (31). *Figure 3.3* shows the origin of short-term satiety signals.

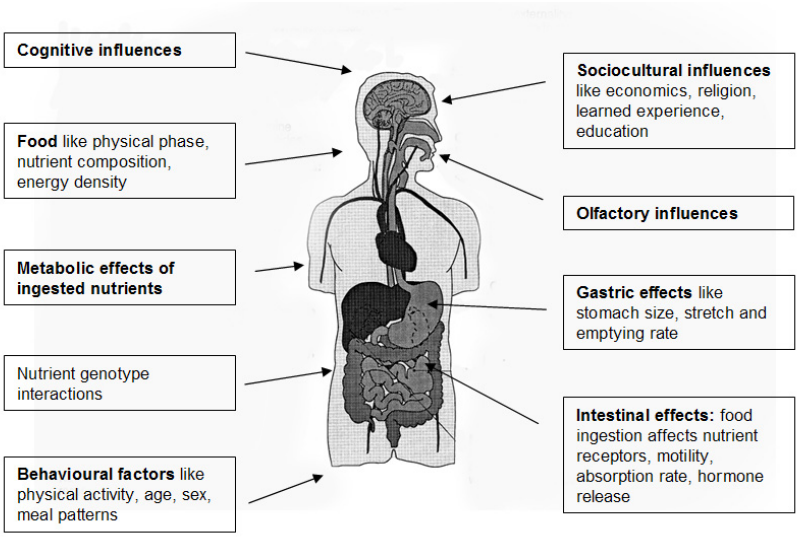

Figure 3.3: Peripheral and central physiological signals which influence the human appetite system

Satiation factors are molecules responsible for short-term feeding control that provide information to the brain to inhibit feeding. They lead to meal termination, acting centrally via peripheral nerves or via the circulatory system (34). The most important peptides produced by the digestive tract are described in chapter “Gastrointestinal signals triggering satiation”. *Figure 3.4* (10) is an overview on short-term signals regulating food intake.

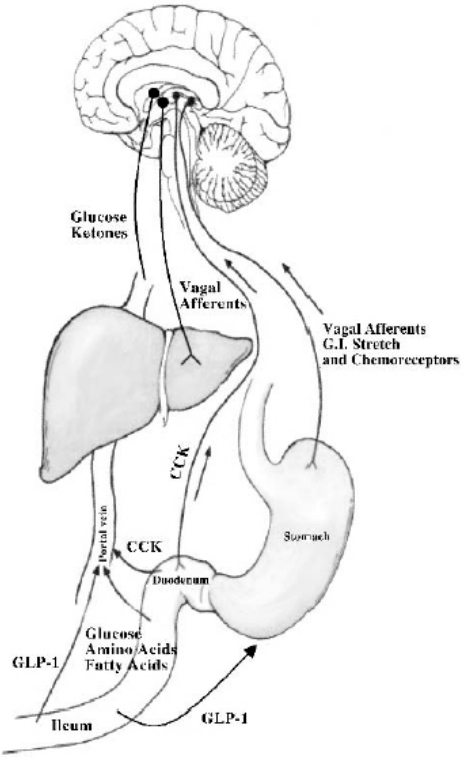

Figure 3.4: Short-term signals regulating food intake; by Havel (10)

3.5. Long-term regulation of food intake

Long-term hormonal signals (see overview in *Figure 3.5*) not only influence signalling by central effector pathways to favour a particular change in energy balance, but they also modulate the sensitivity of the brain to afferent inputs generated in response to short-term factors. During weight-loss for example, a reduced concentration of long-term adiposity signals is proposed to 1) diminish the efficacy of satiety-inducing inputs, 2) suppress catabolic effector pathways, and 3) activate anabolic effector pathways. These responses to weight loss are proposed to stimulate feeding and to reduce energy expenditure, thereby ensuring the recovery of depleted fuel stores (13). The hormones insulin and leptin are the two most important long-term regulators of food intake and energy balance. Both insulin and leptin are secreted in proportion to body adiposity and act in the CNS to inhibit food intake and to increase energy expenditure, most likely by activating the sympathetic nervous system. In contrast with short-term inputs, insulin and leptin exert effects in the CNS that are slow in onset and offset (hours to days), with an effect that is sustained over long intervals (22).

3.5.1. Insulin

The active form of insulin consists of two amino acid sequences, an α-chain with 21 amino acids and a β-chain with 30 amino acids, and has a molecular weight of 6-kDa. Insulin was first proposed by Woods and colleagues (33) in the early 1970s to be a long-term regulator of food intake, energy balance, and body adiposity. Since that time, much additional evidence has been generated in support of this hypothesis (23, 32). Insulin is secreted from islet β cells of the endocrine pancreas and is a well-characterized adiposity signal. It circulates at levels proportional to fat mass and its concentrations are increased after food ingestion, specifically by glucose and amino acids. Insulin reaches the CNS via receptor-mediated transport across the BBB. CNS administration of insulin to rodents, either into the third ventricle or directly into the hypothalamus, causes a reduction in food intake (2). Insulin, like leptin, has been shown to inhibit arcuate nucleus NPY expression and NPY stimulation of food intake (25, 26). There is much evidence that insulin acts as a satiety factor on the brain in a manner similar to leptin.

3.5.2. Leptin

The ob-gene product leptin is a 164-kDa protein. Leptin is mainly produced in adipose tissue and is secreted into the bloodstream. It circulates at concentrations proportional to body fat mass in rodents (7, 15) and humans (4, 5, 12, 15, 16, 28). Leptin can rapidly cross the BBB and appears to be transported into the CNS by a saturable receptor-mediated process (1). Consistent with the hypothesis that changes in fat mass are transmitted to the CNS by changes in leptin concentrations, plasma leptin concentrations decrease after weight loss (4, 15) and are strongly correlated with leptin concentrations in cerebrospinal fluid (24). Leptin secretion in adults displays a prominent circadian rhythm and is not affected by individual meals (28). In obese, serum leptin is elevated (15).

Central and peripheral administration of leptin in rodents causes a profound decrease in food intake and weight loss (9). However, it is the central ICV route that is the more potent, suggesting that leptin`s actions are mediated mainly through the hypothalamus (20).

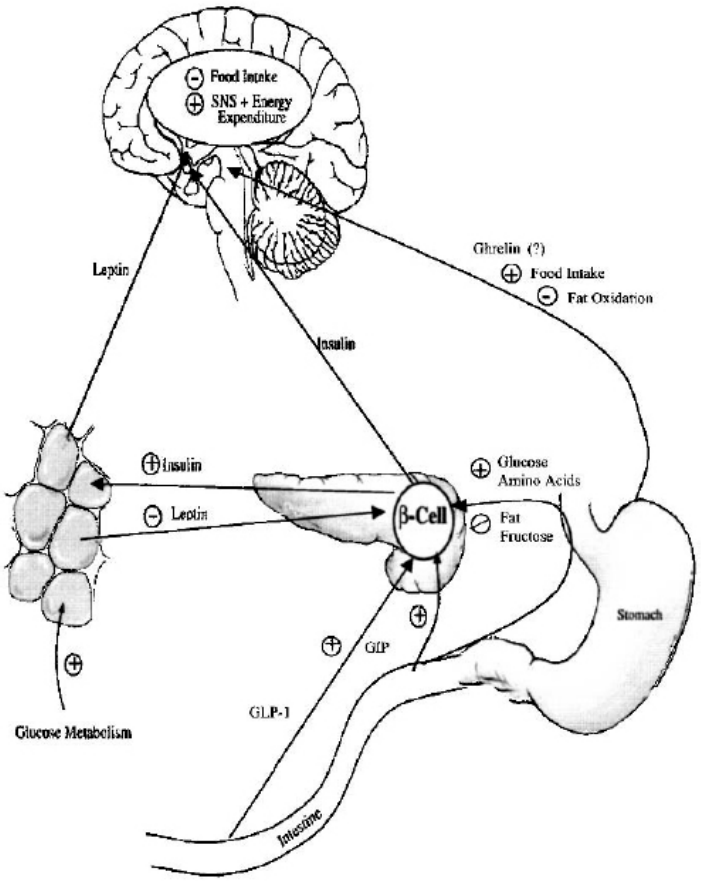

Figure 3.5: Long-term signals regulating food intake; by Havel (10)

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Chapter 4: Gastrointestinal signals triggering satiation

GI factors, which are involved in the short-term regulation of food intake and trigger satiation, include gastric distension, exposure of small-intestine receptors to nutrients, and GI hormones. *Figure 4.1* shows the anatomy of the human GI tract with some of the organs, where peripheral satiety signals are generated.

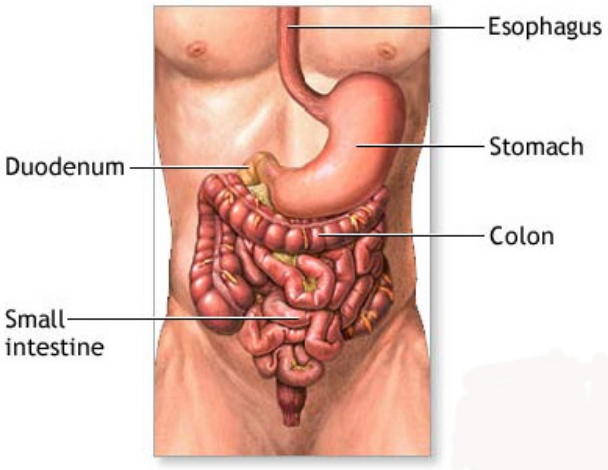

Figure 4.1: Anatomy of the human GI tract

4.1. Stomach distension

It is obvious that the stomach (*Figure 4.2*) has an important role in the regulation of food intake, yet the mechanisms are only partly understood. Best appreciated are mechanisms related to mechanoreceptors involved in the reservoir and propulsive functions of the stomach, where distension is the adequate stimulus influencing all types of motility. The stomach is extensively innervated by afferent and efferent fibers of the vagus nerve.

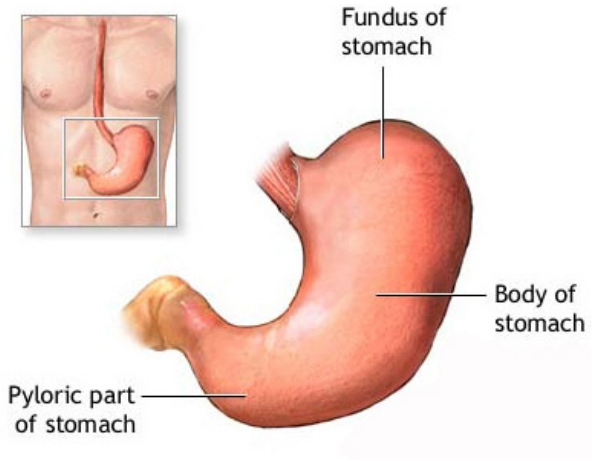

Figure 4.2: Anatomy of the stomach

4.1.1. Stomach and satiation

Animal studies support the concept that the stomach is involved in the termination of a meal (104). In rats, stomach distension decreases the vagal firing rate and there is evidence that vagotomy blocks the satiating effect of stomach distension (119) supporting the hypothesis that the vagus plays an important role in peripheral signaling of satiety. In humans gastric distension causes a feeling of satiety (12, 15, 67) and reduces food intake in young and obese subjects (37, 40). The role of stomach distension in satiety and food intake in humans is supported by a series of studies of Geliebter et al (38-40) and Geliebter (37). In one of the earliest of these studies, Geliebter showed that a gastric balloon with a volume $>$ 400 ml reduced food intake. Intragastric balloons may reduce food intake, but the effect is very short lasting (123). In animals (97) and humans (129) the stomach can sense both nutrient quality and quantity; this information is used to alter the rate of gastric emptying and the amount of food ingested.

4.1.2. Site of gastric distension and satiation

Mechanical properties and neural innervation vary in different regions of the stomach (66), and it is uncertain whether the site of gastric distension (fundus or antrum) is important in mediating satiation (72, 125). In young subjects, distension of the proximal stomach with the use of a barostat *(Figures 4.3)* increases the perception of fullness (28, 57), but effects on food intake have not been evaluated. Observations in young subjects using ultrasound-technique established that the perception of postprandial fullness is closely related to antral content or area and not to the content

of the proximal stomach (68, 72, 125). In several studies of Sturm et al (131, 132) healthy young volunteers consumed oral preloads before a subsequent meal. By measuring antral area with an ultrasound machine, energy intake and satiety were both inversely related to antral area. Hence, antral rather than proximal gastric distension might be the dominant intragastric mechanism in the induction of appetite-related sensations. It remains unclear, if and to what extent the mechanical induced afferent signals of gastric fundus and antrum distension can alter food intake.

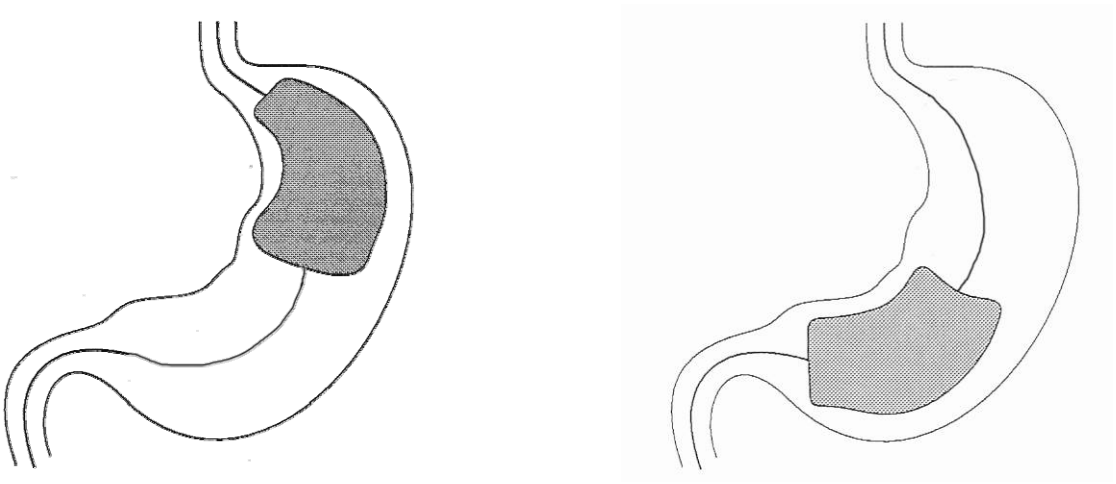

Figure 4.3: Distension of the fundus or the antrum by a balloon of the barostat

4.1.3. Gastric chemoreceptors

Although less well characterised, gastric chemoreceptors have a fundamental role in all aspects of gastric physiology involved in appetite regulation. For example, noncaloric liquid saline empties exponentially from the stomach, whereas nutrients empty rapidly during the first few minutes and afterwards at a steady linear rate until the stomach is completely empty. There is evidence that the pylorus detects the energy content of the food: a fixed number of calories empties into the duodenum per unit of time, regardless of the composition of the food (96). On the other hand, a study of our own group led to the suggestion that the stomach could sense the nutrient composition of an oral preload. Preloads with differing contents (protein vs. carbohydrate vs. water) had variable effects on appetite perception and food intake (21, 95).

4.1.4. Gastric hormones and appetite regulation

As far as gastric hormones are concerned, gastrin-releasing peptide (GRP) and somatostatin are released from the antrum of the stomach. These two peptides have been shown to have satiating effects in laboratory animals and humans (48, 88). It is not clear whether those effects are systemic or local. A few years ago leptin has been found in rodent and human gastric mucosa (6), although it is not clear whether this has any implications for food intake. On the other hand, ghrelin is abundantly synthesized in the fundus of the human stomach (5) and is one of the first hormones that has a stimulating effect on appetite (18). It seems to work both in the short-term as well as in the long-term regulation of food intake (18).

Under normal conditions, meal termination likely results from a combination of gastric and postgastric signals.

4.2. Gut peptides

The distal intestine plays an important role in the control of GI function. The anatomy of the intestine is shown in *Figure 4.4*.

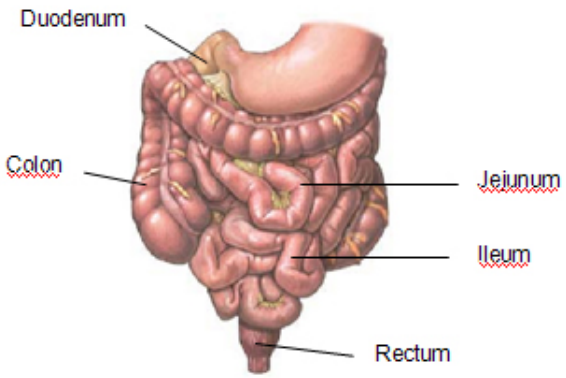

Figure 4.4: Anatomy of the small and the large intestine

In 1973 Gibbs et al (42) reported that intraperitoneal injection of the intestinal peptide hormone CCK reduces meal size in rats, marking the start of the “peptide revolution” in the study of the control of food intake. To date, many peptide hormones released by the gut have been shown to decrease food intake. Many of those peptides are

ubiquitous and multifunctional with considerable redundancy and overlap. The ones which are relevant for the present thesis are described in the following chapter. *Figure 4.5* shows hormones, which are produced by the gut and which are known to alter food intake and body weight.

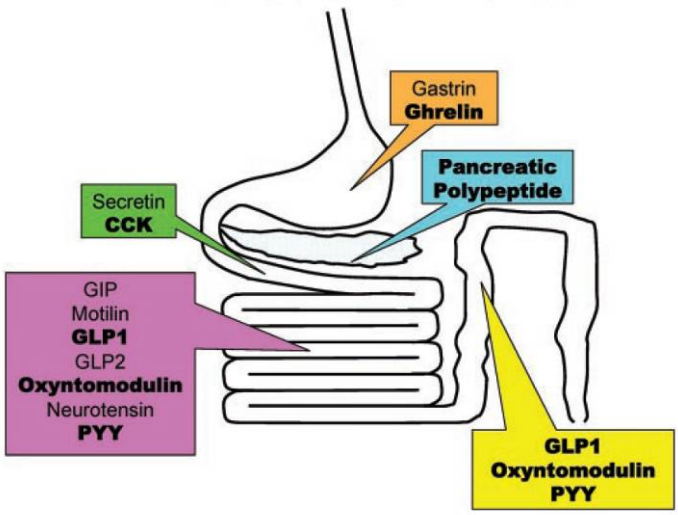

Figure 4.5: Hormones produced by the gut. Those shown in bold are known to alter food intake and body weight; by Druce et al. (22).

There are several criteria in classical endocrinology to establish a physiological endocrine action of a given molecule (*Table 4.1*). Many of the following described gut-peptides have not been established yet and require further research.

Table 4.1: Criteria for the identification of physiological effects of a hormone or neurotransmitter on eating; by Geary (36).

Secretion	Hormone secretion is associated with a change in eating
Receptors	Binding receptors for the hormone are expressed at its site of action
Physiological dose	i.v. infusions of the hormone reproduce the physiological patterns of the endogenous hormone according secretion and effect on eating
Removal and replacement	1) Removal of the hormone or the receptors mediating the eating effect should prevent this effect ("Knockout") 2) When the hormone has been eliminated, i.v. replacement of this hormone at physiological doses should normalize the effect
Antagonism	i.v. infusion of a selective and potent antagonist to the hormone should prevent the effect of the endogenous or exogenously given hormone and evoke the opposite effect

4.2.1. Cholecystokinin (CCK)

4.2.1.1. Physiology of CCK

CCK is found in the brain and the GI tract and has a number of regulatory functions, both centrally and peripherally (103). In the brain, CCK acts as a neurotransmitter; it is present in high but variable concentrations. In the periphery, CCK is widely distributed throughout the GI tract, but is most concentrated in the endocrine I-cells of the duodenum and the jejunum (13, 128) and in enteric nerves (128). CCK has a number of physiological GI functions, including triggering satiation, inhibition of gastric emptying, stimulation of pancreatic secretion, gallbladder contraction and intestinal motility.

CCK exists in several bioactive molecular forms ranging from 8 to 58 amino acid residues. All forms of CCK have retained the bioactive C-terminal portion, and the molecular forms CCK-8, -33 and –58 have all been shown to have biological effects in association with food intake (58, 85, 141).

The receptors for CCK have been subdivided into CCK-A for “alimentary type” (new nomenclature: CCK1) and CCK-B for “brain type” (CCK2) (93, 143). CCK-A receptors were found mainly in the periphery, but also in some areas of the brain. In the GI tract CCK-A receptors have been found in the pancreas, gallbladder, lower esophageal sphincter, stomach, ileum and colon (140, 142). CCK-B receptors have been identified mainly in the brain, but also in the stomach.

4.2.1.2. Nutrients and CCK release

After food intake, CCK is released into the bloodstream from the endocrine I-cells of the duodenum and the jejunum. CCK is released in the blood as a function of the presence of fat (long-chain free fatty acids) or protein (amino acids) in the duodenum, where CCK has an effect on receptors of the nervus vagus (20). The nervus vagus transports the signal to the nucleus tractus solitarius in the brainstem and from there to the CNS (54). CCK levels gradually increase to a maximum 10-45 minutes following the start of a meal, then gradually decline, but remain elevated up to 3-5 hours after eating (85).

4.2.1.3. CCK and satiation

The most widely investigated gut hormone in relation to appetite is CCK. In their classic paper in 1973, Gibbs, Young and Smith (42) demonstrated the ability of exogenously administered CCK to inhibit food intake in rats. From these results the feeding inhibitory action of exogenously administered CCK was proposed to mimick a physiological hormonal action of the endogenous peptide. This initial report has stimulated over 30 years of research aimed at demonstrating a physiological role for CCK in the control of food intake and at understanding the mechanisms of action through which CCK produces satiety.

Studies in humans have repeatedly shown that exogenous CCK inhibits food intake (7, 79). The first report of the appetite-suppressing effect of CCK in humans is a study by Kissileff et al (79) showing that exogenous, peripheral (i.v.) administration of high nonphysiological doses of CCK suppressed food intake in a test meal in humans by 19%. Since that study, many studies to examine the effect of CCK on appetite in humans have been conducted (7, 11, 14, 18, 49, 86, 87, 89, 92, 106, 107, 118).

In 1985 Welch and colleagues (144) observed in humans that infusion of lipids into the distal small intestine reduced food intake. They suggested that fat in the small intestine acts as a signal for short-term control of food intake, most likely via release of endogenous CCK. An i.v. infusion of a similar lipid emulsion had no effect on eating. Further experiments by Welch et al with lipid perfusion to the small intestine caused a decrease in food consumption, early satiety and a delay in gastric emptying (145, 146) These effects were paralleled by an increase in plasma CCK concentrations. Those observations formed the basis for the hypothesis that fat acts as a pre-absorptive signal to regulate food intake and that this effect is mediated by the release of endogenous CCK. CCK release depends on adequate fat hydrolysis (63); finally, only free fatty acids $>$ 12 carbons are able to stimulate CCK release (94, 98). Along the same lines of investigations, our group recently reported that long chain fatty acids are crucial for the secretion of CCK (94).

On the basis of several studies in animals and humans it has been proposed that the increased gastric distension induced by slowing of gastric emptying may be one of the mechanisms by which CCK reduces food intake. The effect of CCK on food intake was shown to be an interaction of various factors: the satiating effect of a CCK-8 infusion in healthy volunteers was enhanced by gastric distension with liquid oral preloads or gastric balloon distension (78, 95, 100, 106, 107). Along these lines

of investigations results of a study conducted by our research group (49) led to the conclusion that the synergistic interaction between CCK-8 and an oral preload could be mediated by peripheral CCK-A receptors.

4.2.1.4. Antagonists of CCK

The availability of potent and selective CCK-A receptor antagonists has made it possible to further characterise the effect of endogenous CCK on satiety in humans. To test the hypothesis that CCK is mediating the effects of ID long chain fatty acids via endogenous CCK release and through CCK-A receptors, another series of studies with loxiglumide (LOX) was performed by our group (11). LOX is a potent and selective CCK-A receptor antagonist (60-62, 102). ID perfusion of long chain fatty acids significantly reduced food consumption and calorie intake. Concomitant i.v. infusion of the CCK-A receptor antagonist LOX completely abolished the satiation effects of ID long chain fatty acids (11).

4.2.2. Peptide tyrosine-tyrosine (PYY) (3-36)

4.2.2.1. Physiology of PYY

Peptide tyrosine-tyrosine or PYY is a 36-amino-acid GI hormone and belongs to the NPY family together with NPY and PP. The L-cells are the major source of PYY, with highest levels found in the rectum, followed by the ileum and colon (2, 25). The same cells synthesize and release GLP-1. There are two main endogenous forms of PYY: PYY (1-36) and PYY (3-36) (44). PYY is secreted as PYY (1-36) and is then degraded to PYY (3-36) by dipeptidyl peptidase IV (DPP-IV) (43, 101). Receptors that mediate the effects of PYY include Y1, Y2, Y3, Y4, and Y5 (8). PYY (3-36) is a Y2 receptor agonist in the hypothalamus and inhibits the release of NPY. PYY plasma levels are low in obesity, suggesting that PYY could be involved in the pathogenesis of this disease (9).

4.2.2.2. Nutrients and PYY release

After a meal, PYY (3-36) is the major circulating form (43). Following ingestion of food, plasma levels increase within 15 min, reach a peak at approximately 90 min, and then remain elevated for up to 6 hours (2). These levels are influenced not only

by calorie intake but also by meal composition. Higher levels are seen following fatty meals compared with meals containing high protein or carbohydrate (90). The increase of plasma PYY occurs even before nutrients have reached the distal parts of the GI tract, where PYY is produced. This suggests a neuronal (probably through the vagus nerve) (32) or endocrine mechanism.

4.2.2.3. PYY and satiation

The effects of PYY (3-36) on appetite have only recently been discovered (9, 10). Peripheral administration of PYY (3-36) has been shown to acutely inhibit food intake in rodents by several groups (10, 16, 53). Batterham et al (10) have shown that chronic peripheral injections of PYY (3-36) reduce food intake and body weight gain in rodents. Furthermore, direct intra-arcuate injections of the peptide decreases food intake in rodents (10), which leads to the suggestion that peripheral PYY (3-36) inhibits food intake by acting through Y2 receptors in the hypothalamic ARC. However, the results of pharmacological experiments regarding PYY (3-36) as a satiety signal are controversial. First, the results of Batterham et al (10) on food intake after exogenous administration of PYY (3-36) in rodents, could not been replicated by Tschöp et al (136). Second, several reports indicate that PYY is an orexigenic peptide (17, 51, 52, 71, 105, 108), with feeding stimulatory properties superior to those of NPY. This is particularly the case when PYY is administered directly into the cerebral ventricles (51). In contrast, the anorectic effects of PYY (3-36) (administered peripherally), which have already been shown in rodents, have been effectively reproduced in humans. When given intravenously to both normal weight and obese volunteers, PYY (3-36) reduced food intake by more than 30% (9, 10). Because of the controversial study results and the limited human data, it has to be proofed in further studies, if PYY (3-36) really is a physiological satiety factor.

4.2.3. Glucagon-like peptide-1 (GLP-1)

4.2.3.1. Physiology of GLP-1

The proglucagon gene encodes two glucagon-like peptides, GLP-1 and GLP-2, that exhibit approximately 50% amino acid identity to pancreatic glucagon (23, 24, 76).

GLP-1 is released from enteroendocrine L-cells from the distal gut in response to food intake (46, 59, 113). The GLP-1 receptor is known to exist as a single receptor type in the brain, lung, stomach, skeletal muscle and adipose tissue (3, 82, 127, 139). Physiologically GLP-1 regulates postprandial blood glucose levels by enhancing insulin secretion and suppressing glucagon secretion after a meal (81, 117); furthermore it inhibits gastric emptying (110, 147). Like PYY (3-36), GLP-1 is believed to play an important role as one of the hormones of the “ileal brake mechanism” (24, 64), an endocrine feedback mechanism that is activated by the presence of nutrients in the ileal lumen. This feedback mechanism results in inhibition of gastric emptying (91), decreased intestinal motility and transit (130), decreased pancreatic secretion (83) and inhibition of food intake (144). There is evidence that the effects of GLP-1 on gastric functions are mediated via the vagus nerve, both in animals and humans (70, 148, 149).

Following an initial nutrient-stimulated rise in circulating levels of GLP-1, the levels of the bioactive form of this peptide fall rapidly, largely due to renal clearance and degradation by DPP-IV (19, 77). The biologically active form of GLP-1, GLP-1 (7-36 amide), is degraded by the peptidase to the inactive form GLP-1 (9-36) (77). GLP-1 has a short half-life of a few minutes (55). The plasma increase of GLP-1 has been shown to be attenuated after a meal in obese persons (111, 120, 121).

4.2.3.2. Nutrients and GLP-1 release

GLP-1 is mainly stimulated by carbohydrates (65, 122) and to a lesser degree by other macronutrients. Oral glucose is a stimulus for GLP-1 release, whereas intravenously applied glucose has no effect on endogenous GLP-1.

Feinle and coworkers (27, 29) have suggested that GLP-1 plasma levels rise after ID fat, but these findings could not be confirmed in the present thesis. Others have not found a substantial increase in plasma GLP-1 release after intestinal fat (122).

4.2.3.3. GLP-1 and satiation

GLP-1 has been proposed as playing a physiological regulatory role in controlling appetite and energy intake in humans (30, 50) as well as in animals (134, 138).

Several reports have demonstrated that an intracerebroventricular injection of GLP-1 in rats inhibits food intake (126, 138). Central administration of the GLP-1 agonist exendin-4 increases feeding in rats (138). No effect was seen after intraperitoneal

injections of GLP-1 in rats (138) suggesting a central mode of action for GLP-1. However, this loss of effect after peripheral application could also be explained by the rapid degradation of the peptide, particularly in rats (77).

In humans, studies have shown that GLP-1 increases satiety and decreases food intake in normal weight (30, 31, 50), diabetic (47, 135) and obese subjects (109, 112). In these studies, GLP-1 was administered by either i.v. or subcutaneous (s.c.) infusions. Two possible explanations for the effect of peripherally secreted or injected GLP-1 on central nervous regulation of food intake in humans are shown in *Figure 4.6*.

Taken these findings together, the data indicate that GLP-1 acts as a physiological regulator of food intake. However, since GLP-1 is produced both in the periphery and in hypothalamic neurons, the extent to which GLP-1 from each of these sources participates in the physiological regulation of feeding behaviour is unclear.

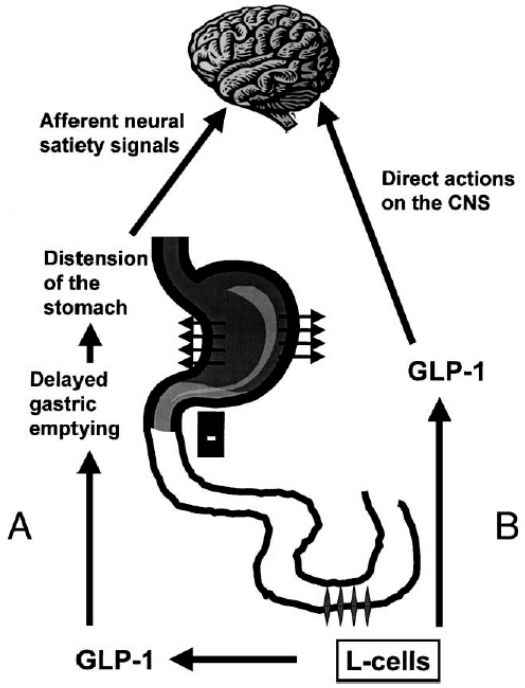

Figure 4.6: Two possible explanations for the effect of peripherally secreted or injected GLP-1 on central nervous regulation of feeding: A) indirect effects on fullness and satiety via distension of the stomach induced by delayed gastric emptying; B) direct effects on GLP-1 receptors in the CNS with afferent projections to the hypothalamus; by Meier et al (99).

4.2.4. Apolipoprotein A-IV (Apo A-IV)

4.2.4.1. Physiology of Apo A-IV

The role of apo A-IV was discovered about 27 years ago (133), but its physiological role was not firmly established until recently. Apo A-IV is a 46kDa glycoprotein secreted only by the small intestine in humans (4, 45). Its synthesis is higher in the jejunum than in the ileum (1). Apo A-IV is a major component of intestinal triacylglycerol-rich lipoproteins (chylomicrons, very low density lipoproteins (VLDL)) (45, 69, 114, 133). In response to a lipid-containing meal, apo A-IV is secreted into intestinal lymph on chylomicrons (45, 69, 114, 133). With regard to the stimulation of intestinal apo A-IV by dietary lipid, several lines of evidence support the hypothesis that assembly and transport of chylomicrons is necessary for the apo A-IV response to dietary lipid. This evidence is supported by studies in rats in which intestinal synthesis and lymphatic secretion of apo A-IV in response to intestinal infusion of fatty acids differing in chain length (and therefore different routes of transport from the intestine) was examined. Infusion of long-chain fatty acids, which are transported via the lymph on chylomicrons, stimulates synthesis and output of apo A-IV, whereas medium- and short-chain fatty acids, primarily transported as free fatty acids in the portal vein, elicited a negligible apo A-IV response (74).

During plasma passage and metabolism of chylomicrons, apo A-IV dissociates from this lipoprotein. About 25% is then found circulating in the density range of high density lipoproteins; the rest is found in the lipoprotein-free fraction of plasma (41, 84).

Studies in rats could show that ileally infused lipid elicits an increase in proximal jejunal apo A-IV synthesis independent of the presence of jejunal lipid. These results suggest the existence of a signal, arising from the distal gut, capable of stimulating synthesis of apo A-IV in the proximal gut (75). It could be demonstrated both in humans and rodents that apo A-IV synthesis and secretion by the small intestine are also stimulated by PYY (137) (*Figure 4.7*).

The stimulation of intestinal synthesis and the secretion of apo A-IV by lipid absorption are rapid (between 15 and 30 minutes). This was associated with significant stimulation of lymphatic output and plasma levels of apo A-IV by 30 minutes after the gastric lipid load (124). Thus apo A-IV likely plays a role in the short-term regulation of food intake. Apo A-IV is also a physiological modulator of

upper GI function: it inhibits gastric acid secretion (115) and gastric emptying (116) in rats.

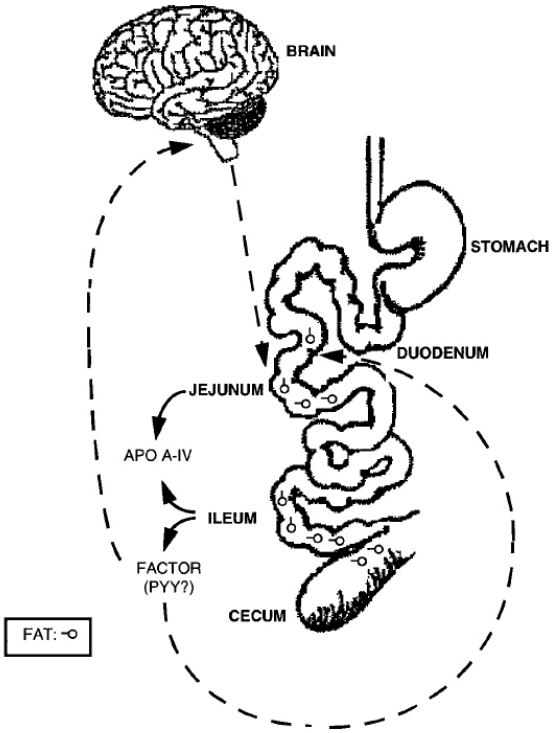

Figure 4.7: Proposed pathway for control of apo A-IV by intestinal lipid. Fat in the proximal intestine stimulates synthesis and secretion of apo A-IV in a proximal-distal gradient in the intestine, depending upon the total lipid load. Fat in the distal gut (ileum, cecum) also stimulates apo A-IV, both in the ileum and in the proximal jejunum. This latter effect is independent of the presence of jejunal lipid and is presumably mediated by a signal released in response to the presence of lipid in the distal intestine. This signal may be PYY, although other gut hormones have not been ruled out; by Kalogeris et al (75).

4.2.4.2. Nutrients and Apo A-IV release

In rats it has been demonstrated that apo A-IV production by the small intestine is stimulated by active lipid absorption (4, 56, 73). Hayashi et al (56) demonstrated that the stimulation of apo A-IV production by lipid feeding is associated with the formation of chylomicrons. In vivo studies (33-35) have provided evidence that apo A-IV may be involved in the inhibition of food intake after the ingestion of fat.

4.2.4.3. Apo A-IV and satiation

I.v. infusion of apo A-IV at doses which reproduce plasma levels seen after a lipid meal (33) has been shown to depress feeding in animals (33, 34, 124). In a study of Fujimoto et al (35) cerebroventricular administration of apo A-IV decreased feeding in

rats in a dose-dependent manner. Because available evidence suggests that de novo synthesis of apo A-IV in the brain is unlikely (26), it has been proposed (33, 35) that apo A-IV released by the intestine may traverse the BBB and act in the CNS to influence feeding behaviour. Although further work is necessary to clarify the precise role of apo A-IV in the control of food intake, the available evidence suggests that it may act via the CNS.

4.3. Summary

It has become apparent, that redundant systems are active in the regulation of food intake. For example both CCK and GLP-1 are able to inhibit food intake. CCK-A receptor or GLP-1 receptor knockout mice do not affect the nutrient status of the animals (80, 138), supporting the hypothesis of redundant systems. These systems may replace either peptide when one is absent. However, still little is known about those redundant systems, as well as about interactions between different satiety signals.

In *Figure 4.8* and *Table 4.2* the pathways of the most important satiety signals and their origin and effect on food intake are summarized.

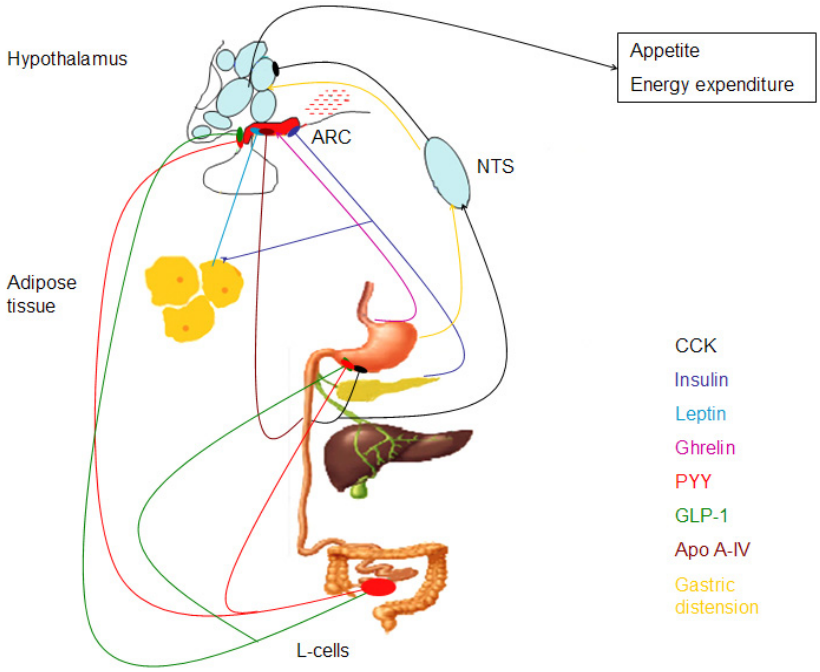

Figure 4.8: Peripheral satiety signals. This figure shows the source and the connections to the CNS of peripheral satiety signals. Fat induced satiety signals are mediated through CCK via peripheral CCK-1 receptors. CCK also inhibits gastric emptying. Insulin and leptin regulate energy homeostasis and body weight. Ghrelin is released from the stomach and induces feelings of hunger via the ARC. PYY and GLP-1 are both released from the L-cells, inhibit gastric emptying and induce satiety. Apo A-IV is secreted from the small intestine and also inhibits food intake. Gastric distension amplifies satiety signals via the NTS.

Table 4.2: Origin and effects on food intake of GI satiety peptides

Peptide	**Cell Type**	**Physiological effect on food intake**	**Exogenous effect on food intake (peripherally)**	**Exogenous effect on food intake (centrally)**
CCK	I (small intestine)	↓	↓	↓
GLP-1	L (small/large intestine)	↓	↓	↓
PYY(3-36)	L (small/large intestine)	↓ (?)	↓	↓/↑
Apo A-IV	Villus epithelia (small intestine)	↓	↓	↓

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Chapter 5: The role of fat and protein in the process of satiation

As mentioned before powerful satiety signals arise from the GI tract during the consumption of a meal. The release of GI peptides is above all triggered by orosensory stimulation, gastric distension, and most important, by the stimulation of specific chemoreceptors activated by nutrients in the lumen of the small intestine (21, 22, 53-56). The impact of individual macronutrients on satiety is typically measured in experimental studies by using an oral preload design and/or GI perfusion of nutrients. In several studies it has been shown that the effects of oral preloads and ID perfusions on satiation vary between nutrient classes (i.e. carbohydrates, fat, protein). The impact of possible interactions between different nutrients on food intake have hardly been explored.

5.1. Effect of different macronutrients on satiation

Foods, and more specifically macronutrients, with the same caloric content exert different effects on satiation and satiety independent of their caloric value (7, 28). In other words, not all calories are treated equally by the body. In a review of Stubbs et al of the energy density of foods (calories/g) (47), they noted that under normal circumstances in which fat contributes disproportionately to energy density, protein, carbohydrate, and fat exert hierarchical effects on satiety in the order of protein > carbohydrate > fat; further studies have confirmed these findings (8, 29). Although most research has suggested that protein has the most potent action on satiety (8, 15, 29, 36), there is less clear consensus regarding the relative satiety values of carbohydrates and fats. The relative satiety values of carbohydrates and fats tend to vary depending on whether the macronutrients are studied in isolation or in foods (9) and the way they are administered. Studies using meal preloads, for example, often yield very different results when compared with studies in which macronutrients are infused directly into the gut. Research into the relative satiating efficiency of fat and carbohydrate reflects the relative abilities of different nutrients to suppress hunger, induce fullness and decrease subsequent food intake. Data supporting the hypothesis that carbohydrate has a greater physiological satiating efficiency than fat were derived from experiments using meal preloads (6, 43, 45, 51).

Other studies based on a meal preload design have failed to show any differences in the satiating efficiency of carbohydrate and fat (17, 19, 20, 44, 52). Controversial results are also observed when orosensory stimulation is eliminated by direct perfusion of nutrients into the GI tract. When fat and carbohydrate were administered as infusions directly into the stomach (11, 46) or into the small intestine (12), some data failed to demonstrate any differences in the short-term effects of fat and carbohydrate on hunger, fullness, and energy intake from a test meal. Other studies showed that nonobese, young men ate less after ID lipid infusions than after equienergetic carbohydrate infusions (1, 13, 14). Thus, to find out the relative satiating efficiency of fat and carbohydrate using oral preloads and direct GI infusions have produced inconsistent data. Cecil et al (12) hypothesised that reported differences in the relative satiating effects of fat and carbohydrate may be related to their differential effects on the influence of orosensory mechanisms when given as an oral preload. Other explanations could be due to differences in experimental methodology or to interindividual different sensitivities of the responses to nutrients.

5.2. ID fat and food intake

Administration of fat offers inconsistent effects on food intake in humans (6, 25, 31, 42, 53) and rats. In rats infusion of fat into the small intestine (26, 41, 49, 57-60) or stomach (23, 24, 34, 35) decreases food intake rapidly. It has been suggested that satiety from fat is due to a preabsorptive signal, because infusion of lipids into the small intestine is associated with suppression of food intake to a much greater extent than when fat is given i.v. (53-56). Two decades ago it was observed that lipids perfused to the small intestine induced a cascade of events related to the regulation of appetite and satiety: Welch et al (53, 56) could show that perfusion of corn oil to the jejunum of healthy volunteers induced early satiety and reduced energy intake; furthermore jejunal infusions of corn oil decreased hunger feelings. On the basis of their work, Welch and coworkers suggested that the effects were caused by CCK release (56). Studies from our laboratory (37, 38) have confirmed these findings as we could show that a fat perfusion to the duodenum significantly reduced food intake compared to an ID saline perfusion. In the same study, it could also be shown that the inhibition of food intake in response to intestinal lipid is mediated by CCK. The

suggested preabsorptive site of action for the inhibitory effect of fats on short-term food intake in humans is supported by the following evidence: 1) i.v. infusion of fat has no effect on food intake (53, 56); and 2) when fats are infused into the small intestine of rats, food intake is reduced within 10 min of the start of the infusions, but radiolabeled fat does not appear in the blood until 30 min after the start of the infusions. In later studies it was documented that inhibition of fat hydrolysis through the specific lipase inhibitor orlistat abolishes the fat-induced satiety signals from the small intestine (37, 38). It was then shown that only long-chain fatty acids are able to reduce food intake and stimulate early satiety *(Figure 5.1)*; medium-chain fatty acids on the other hand had no effect (37). The satiating effect of long-chain fatty acids is mediated by CCK, which binds at CCK-A receptors (5, 38). CCK slows down gastric emptying, thereby prolonging postprandial gastric distension. In general nutrients in the small intestine also delay the transit of food through the gut, and, therefore the time of absorption.

Nevertheless research in humans has shown conflicting results. Several studies which have shown a decrease in food intake in humans after intestinal lipid infusions, have administered infusions at a supraphysiological rate. Infusions of fat emulsions (50% corn oil) into the ileum and the jejunum at a rate of 4.9 kcal/min for a total of 75 min reduced food intake from a solid meal presented 30 min after the start of the infusion (18, 53, 56). However, when lipid infusions were administered into the small intestine at a physiological rate (2 kcal/min) over a period of 420 min, they failed to show a reduction of food intake from a self-selection, buffet-style meal presented 10 min after the infusion had finished (10).

It is likely that the controversial results of the effects of intestinal nutrient infusion on food intake shown in human studies are produced by differences in the methodological design. The timing of the test meal in relation to the infusion, the amount of energy delivered, the perfusion rate, the duration of perfusion and the nature of the test meal are all critical factors in measuring the effects of infusions on food intake (10). Effects of ID infusions on reduction of food intake are likely to be a combination of the number of kcal infused, the duration of exposure of any one segment of duodenum to nutrients, and the length of intestine exposed to nutrients (26, 32, 33, 41).

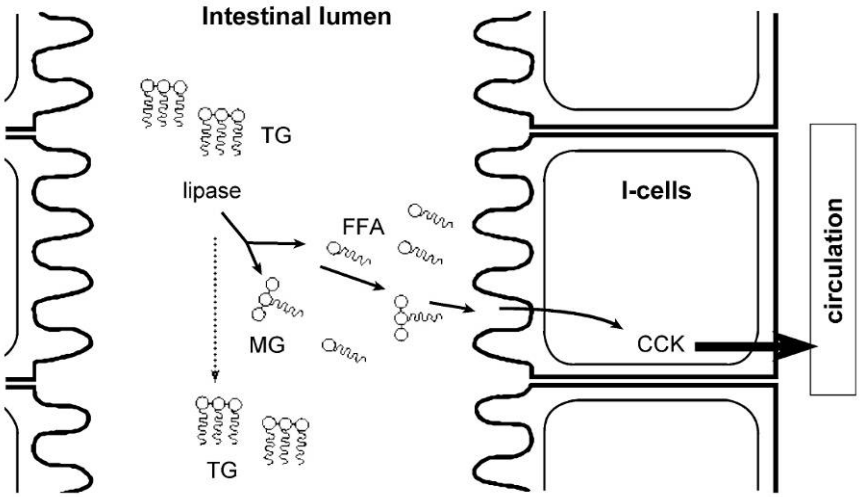

Figure 5.1: Triglycerides from an ingested fatty meal are hydrolysed by the lipase to monoglycerides and free fatty acids. The generation of long-chain free fatty acids is a crucial step for the stimulation and the release of CCK. FFA, free fatty acids; MG, monoglycerides; TG, triglycerides;. by Beglinger et al. (4).

5.3. Protein and food intake

Compared to the macronutrients carbohydrate and fat quite little is known about the effect of protein on eating behavior in humans. However, several short-term studies have been done to examine the satiating effect of oral protein preloads in healthy human volunteers (16, 27, 30, 36, 39, 40, 43, 48, 50, 52). Thus evidence suggests that protein is more satiating than carbohydrate and fat. These various studies compared a variety of nutrient preloads, from ingested whole foods to modified preloads. Subjects felt less hungry and food intake was decreased after the consumption of high-protein compared to high-carbohydrate preloads (3, 24, 40, 50). Protein may differ in its effects on appetite depending on the protein source and therefore variation in digestion and absorption (27). Ballinger et al. examined the effect of amino acids given intraduodenally (2). Subjects consumed significantly less calories and CCK plasma levels were increased after the perfusion of L-phenylalanine compared to placebo.

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Chapter 6: Experimental measurement of food intake and human eating behaviour

An understanding of the factors that control appetite and eating behaviour is often confounded by differences in experimental methodology. The study of appetite control asks for an establishment of a legitimate set of measurements which can be used to measure human eating behaviour under diverse experimental circumstances. One of the dilemmas of researchers in the field of food intake concerns the relationship between “precision” and “naturalness”. On one hand the measurement of eating behaviour should be as accurate and precise as possible. This means that the measurements will be conducted under scientifically controlled conditions in a laboratory environment. Studies of this kind have a high validity and the capacity to establish good cause-effect relationships. However, the problem with those studies is external validity or the power of generalisation. On the other hand, food intake studies under “natural” conditions, i.e. that people are allowed to eat freely, have a high external validity and appear to have real meaning. Unfortunately, the precision of measurement is lower. Errors occur in recording what people are eating and it is much more difficult to establish the effects of experimental manipulations. Another important advantage of human food intake studies in a laboratory environment is the statistical power. Observed effects of manipulations on variables of eating behaviour under controlled laboratory investigations may achieve adequate statistical power with a group of 16 or 20 subjects. Within less controlled environments, a similar degree of statistical power would only be achieved with perhaps 200 or even 2000 subjects (8). However, ideally the best method would optimise both forms of validity, but for the moment researchers usually have to make some compromise between precision and naturalness in the design of studies (8).

6.1. Measurement of food intake

Observing eating behaviour is a strategy which has its roots in the study of animal behaviour. The aim is to quantify eating behaviour components. Automated apparatuses, which deliver and monitor the food intake of animals, are known for a long time. For humans that kind of machine was described about 40 years ago, when

the food intake of a patient with a carcinoma of the lip was detected. A tubing with a mouthpiece was connected to a reservoir containing a liquid diet. When the patient pressed a button, a pump was activated and a fixed amount of food was delivered through the mouthpiece. The activation of the pump was recorded and allowed the record of liquid food intake (6). The idea of this technique was further enhanced and has been used to develop intragastric feeding (via a naso-gastric tube) (11).
An alternative method to monitoring the liquid food intake is to measure the changes in the weight of food as it is being eaten. This is achieved by the continuous weighing of the subject`s plate (or other vessel) with a concealed electronic balance on which the plate rests (12). The advantage of this method is, that liquid as well as solid food can be recorded.
Another alternative record of food intake is to prepare a standardised test meal and offer food in small fixed portions and in excess like in the conducted studies of the present thesis. The strength of this technique lies in collecting data concerning manipulations of food deprivation, preloading and comparisons of lean and obese subjects (18, 19).

The test meal applied in this thesis *(Table 6.1)* consisted of a) orange juice; b) ham sandwiches (73 g white bread, 10 g butter and 25 g ham); c) chocolate pudding; and d) coffee with cream and sweetener (cream and sweetener for the coffee were optional). The order of food intake had to follow the above schedule. To reduce the participant`s awareness of the amount of food eaten, food was presented in small samples and in excess.

Table 6.1: Composition of the test meal with corresponding nutritive values

Nutrients	Carbohydrates (g)	Protein (g)	Fat (g)	Energy (kcal)
Orange juice (1l)	100	<1	0	430
Ham sandwich (108 g)	39	5	10	298
White bread (73 g)	39	6.6	0.8	194
Ham (25 g)	<1	5	1.1	31
Butter (10 g)	0.05	0.05	8.2	73
Chocolate pudding (30 g)	6	1.1	1.2	40
Coffee (113 ml)				20
Coffee	-	-	-	0
Cream (12 g)	0.5	0.5	1.8	20
Assugrin		-	-	0

6.2. Measurement of eating behaviour

A number of systems has been devised to ask subjects specific questions relating to aspects of their motivation to eat. One of the most productive and popular systems is the use of VAS, which have become particularly popular in pain (2, 15) and appetite research (1, 7, 14). VAS for assessing feelings of hunger and satiation are routinely used in studies of human eating behaviour. Silverstone and Stunkard (17) were among the first who published results obtained with this method. Basically, VAS consist of a horizontal 100 or 150 mm line anchored at one end with a description such as “not hungry (or full)” and at the other end with a description such as “as hungry as I have ever felt” or “extremely full” (7).

VAS used in the present thesis have been designed and described by Drewe et al (3) and Welch et al (22). In brief, a score of *zero* for hunger indicated that the subject was not hungry at all, *two* indicated “slightly hungry”, *five* indicated “moderately hungry”, *eight* indicated “very hungry”, and *10* indicated “absolutely ravenous”. The score for fullness was similar.

Such VAS have the advantage of being simple and quick to use and easy to interpret. They are presented in a standardized format that can be compared under a variety of different experimental manipulations. Hunger and fullness VAS are best-measured in within-subject, repeated-measures designs, because the amount of

perceived hunger or fullness will differ between individuals in a given situation and will differ within an individual in different situations (21).
If subjective ratings of hunger and satiation are applied, analyzed and interpreted appropriately, they have been shown to be reproducible, sensitive to exposures of food components, and predictive of food intake (4, 21). They are therefore an invaluable instrument for food intake research. VAS can be useful as an internal control in order to evaluate whether volunteers eat till their individual point of satiation. However, the results obtained from VAS are neither objective nor strictly quantitative and offers the most valuable information when combined with other aspects of feeding behaviour. It is important to recognize that subjectively rated motivation to eat is not an inevitable outcome of underlying physiological processes. Rather it is the subject`s own interpretation of their own sensations, which are, among other factors, influenced by underlying physiological processes.

6.3. The study set-up

The study set-up applied in this thesis is similar to the experimental conditions chosen by Welch et al (23). Welch et al could observe that an infusion of a lipid emulsion into the jejunum or the ileum reduced food intake. The small intestine is an important source of satiety signals and it has been well documented that infusion of different nutrients into the small intestine is associated with suppression of food intake in humans to a much greater extent than when nutrients are given intravenously (13, 22). It is possible that direct infusion of nutrients into the duodenum bypasses several suprapyloric mechanisms that reduce the satiating effect of lipid. These mechanisms include a nutrient-specific effect on the rate of gastric emptying. Ingestion of fat delays gastric emptying, largely because of feedback signals arising from the contact of nutrients with small-intestinal receptors (9, 10, 16, 20). On the other hand the technique of direct infusion of fat into the small intestine eliminates the effects of orosensory factors and of the stomach so that the isolated direct effect of nutrients on appetite, feeding behaviour and gastrointestinal mechanisms can be examined (5). Caution must nevertheless be exerted when extrapolating findings to the normal feeding condition.

Much of the current understanding of the regulatory processes involved in human eating behaviour comes from preload studies. Many studies have been conducted to examine the effects of a food preload with a defined nutrient composition, energy content and/or volume on subsequent food intake. In the present thesis the preload strategy has been used to investigate the satiating power of protein and to examine possible interactions between signals arising from the stomach and signals caused by nutrients in the small intestine. It is very difficult, however, to manipulate protein within a preload without large effects on the orosensory properties of the preload, as well as different direct sensory effects on appetite. Another important weak point of those preload-studies is the fact that the preload-macronutrient concentrations often exceed those in the usual diet. Preload-studies do not reflect free-living conditions where foods consumed are of mixed nutrient composition. Thus, while studies suggest the existence of a macronutrient satiating effect, they have not determined whether this is also present when people are free to eat.

The study-design *(Figure 6.1)* of the present thesis used for food intake studies is briefly explained below. The detailed study set-up is explained in *chapter 7*. 20 or less healthy male volunteers were included for a given protocol. The single treatments, separated by at least 7 days, were randomly performed in each subject under different conditions. Nearly in every of the performed food intake studies volunteers received an ID perfusion. To investigate a potential interaction between the stomach and the gut a preload was given or the stomach was distended for a short period of time before the test meal. After the preload was ingested or the distension of the stomach was done, a standard meal was presented to the subjects, and they were invited to eat and drink as much as they wished for 60 min. During the study subjects scored their subjective feelings for hunger and fullness for the duration of each experiment using a VAS. Blood was drawn in regular intervals for hormone analyses. The study was finished 60 min after meal start.

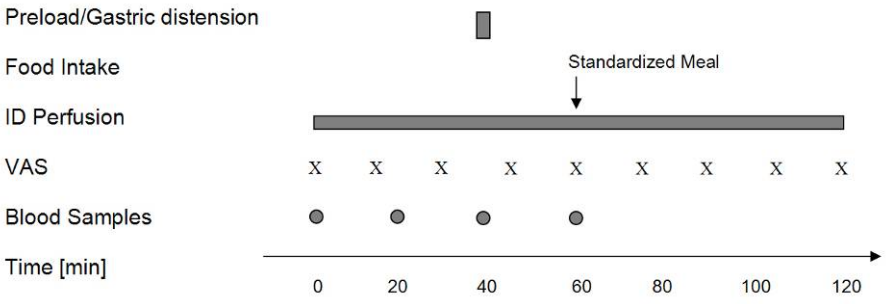

Figure 6.1: Study Set-Up

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Chapter 7: Projects

Project 1

Chapter 7.1. Effect of gastric distension on food intake and feelings of satiety in humans

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Running title: Satiety in response to gastric distension

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Abstract

The factors that regulate food intake and satiation are complex; it has been suggested that signals arising from the small intestine and the stomach play an important role. It is still unknown, to what extent pure mechanical distension of the gastric fundus and antrum can alter food intake. Our aim was therefore to investigate whether gastric fundus and antrum distension can trigger satiation in healthy humans. Two sequential, randomized, double-blind, 4-period cross over designed studies were performed in 24 healthy male volunteers: 1) Twelve subjects underwent four intragastric balloon distension experiments of the fundus (0, 400, 600, 800 ml) before a standard meal intake; 2) Twelve subjects underwent one of the following four treatments: 0 ml balloon distension of the gastric antrum plus intraduodenal (ID) saline or ID fat and 300 ml gastric distension plus ID saline or ID fat. Shortly after the distension period, subjects were free to eat and drink as much as they wished. Neither gastric fundus nor antrum distension showed a reduction in calorie intake. Distending the fundus affected the mean Visual Analogue Scale (VAS) in the premeal period: subjects experienced a reduced degree of hunger and a concomitant feeling of fullness, but the effect was short-lasting and was only apparent with a volume of 600 ml or even 800 ml. Cholecystokinin (CCK) and peptide YY (PYY) were not altered by gastric distension. Neither pure mechanical distension of the fundus nor the antrum seems to be a major trigger of satiation.

Key words: eating behavior, balloon distension

Introduction

The factors that regulate food intake and satiation are complex. Food intake is regulated by chemical and mechanical factors acting in concert to produce sensations of satiety. The stomach has an obvious role in the regulation of food intake, yet its importance of triggering satiety and the mechanisms involved are only partly understood. Animal studies support the concept that the stomach is involved in the termination of a meal (29). In rats, stomach distension decreases the vagal firing rate and there is evidence that vagotomy blocks the satiating effect of stomach distension (31) supporting the hypothesis that the vagus plays an important role in peripheral signaling of satiety. Also, in animals (27) and humans (37) the stomach can sense both nutrient quality and quantity; this information is used to alter the rate of gastric emptying and the amount of food ingested. Finally, gastric distension causes a feeling of satiety in humans (4, 5, 18) and an unpleasant feeling of fullness can occur with balloon distension. Intragastric balloons may reduce food intake in obese subjects, but only with a short-lasting effect (32).

The site of gastric distension (fundus or antrum) may also be important in regulating satiation (20, 36). For example, in a study by Jones et al (20) the perception of postprandial fullness after ingestion of a glucose drink was much more strongly related to the antral area and content than the content of the proximal or total stomach. Similarly, after ingestion of a liquid preload, Sturm et al (38) found a close relationship between food intake at a subsequent meal and antral area in both healthy young and older subjects. All these data support the concept of an important role for the distal stomach in the generation of “appetite-related” sensations and satiation.

It remains unclear, if and to what extent the mechanical induced afferent signals of gastric fundus and antrum distension can alter food intake. Hence, this study was designed to further understand the role of pure mechanical distension of the gastric fundus and the antrum in the regulation of food intake in healthy subjects. The studies were performed sequentially. When the results of fundic distension were analyzed, the design of the antral distension part was modified. By perfusing fat to the small intestine, we felt that a potential effect of antral distension could be enhanced. An interaction between intraduodenal (ID) fat and gastric distension (induced by an oral preload) has previously been documented (26). Furthermore, ID

fat slows gastric emptying (16) and reduces hunger feelings and subsequent food intake (7, 23-25). The gastric antrum was therefore distended to stimulate gastric distension; in combination with ID fat, the gastrointestinal (GI) satiety hormones cholecystokinin (CCK) and peptide YY (PYY) should be released.

Methods

Overview

Two experimental series were sequentially performed.

First, a randomized, double-blind, four-period, Latin square design was carried out in 12 healthy, paid, male volunteers. Each participant underwent tests on four experimental days, separated by at least 1 week. On each experimental day, subjects swallowed a barostat assembly which was positioned in the fundus of the stomach. The balloon of the barostat was then inflated for a total of 10 min. The volumes of 0 (control), 400, 600 and 800 ml were applied separately on a single experimental day. When the balloon had been deflated and the tube taken out, subjects were invited to eat and drink as much as they wished for 60 min.

The design of the second series was similar: 12 healthy male subjects were studied in a randomized, double-blind, four-period crossover fashion. Each participant underwent four tests separated by at least 1 week. On each experimental day, volunteers swallowed a barostat assembly. The tip of the tube was positioned in the duodenum and the balloon of the barostat was positioned in the gastric antrum. A continuous ID perfusion of either fat or saline (control experiment together with 0 ml distending volume) was given for the next 90 min. 70 min after starting the respective ID perfusion, the balloon of the barostat was either inflated with the volume of 0 or 300 ml for 20 min. When the balloon had been deflated and the tube had been taken out, subjects were invited to eat and drink as much as they wished for 60 min.

Subjects

Each subject gave written informed consent for the study. The protocol was approved by the Human Ethics Committee of the University Hospital in Basel. Before

acceptance, each participant was required to complete a medical interview and received a full physical examination. Inclusion criteria were:

1) BMI within 15% of desirable weight for height
2) Age between 18-45 years
3) Non-smokers
4) No active medical problems
5) Taking no medication
6) No allergies including food allergies
7) No history of GI disorders or weight problems

Experimental procedure

Part one: Effect of increasing gastric volumes induced by balloon distension of the fundus on food intake

Four treatments, separated by at least seven days, were performed in 12 healthy male subjects in a randomized order. On each study day, subjects ate a liquid breakfast before 8 am. At noon, after insertion of a catheter into a forearm vein for blood drawings, the experiment started with a baseline period of 60 min. Subjects swallowed a barostat assembly which was positioned in the fundus of the stomach. After placement of the barostat assembly, the minimal distending pressure was assessed during the following 10 min. After a recovery period of 30 min the balloon of the barostat was inflated. Starting from the minimal distending pressure level (MDP), one of the following volumes was applied on a single experimental day: 0 (control), 400, 600 and 800 ml. The order of volumes was randomized; furthermore, the computer, which was connected to the barostat unit, was controlled by a person who was not involved in the experiment. The investigator and the subject were unaware of the respective treatment thereby making it possible to perform treatments in a double-blind manner. The barostat balloon was inflated for a total of 10 min; then the balloon was deflated and the tube was taken out. Ten minutes later, subjects were invited to eat and drink as much as they wished for 60 min. The study design is shown in *Figure 7.1.1*.

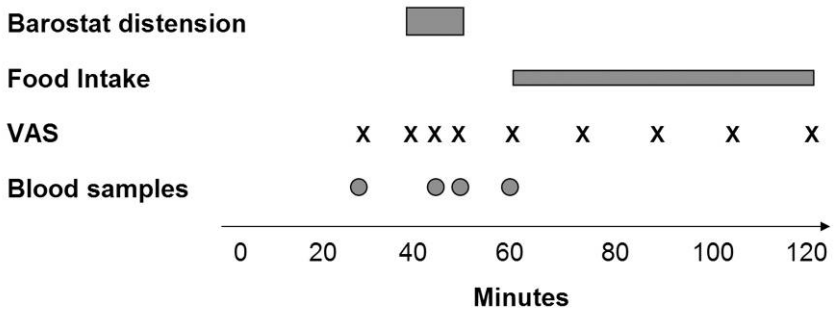

Figure 7.1.1: Experimental design of study part one. VAS, Visual Analogue Scale.

The meal consisted of a) orange juice, b) ham sandwiches (60 g white bread; 10 g butter and 25 g ham), c) chocolate pudding, and d) coffee with cream and sugar (coffee could be sweetened if desired; therefore cream and sugar were optional). The composition of the test meal with its corresponding nutritive values is listed in *Table 7.1.1*. The order of food intake had to follow the above schedule. To reduce the participants` awareness of the amount of food eaten, food was presented in small samples and in excess. The amount of food eaten and the volume of fluid drunk were quantified for each subject. From these observations, the total calorie intake could be calculated. Blood was taken at regular intervals for plasma CCK determinations in EDTA-coated tubes (6μmol/l) containing aprotinin (500 KIU/ml blood). Plasma samples were kept frozen at –20°C until analysis. Subjects scored their subjective feelings of hunger and fullness in regular intervals for the duration of each experiment using a VAS from 1 to 100 and indicated their scores on a ruler. The scale and scores have previously been designed and described in detail by Welch et al (40).

Table 7.1.1: Composition of test meal with corresponding nutritive values.

Nutrients	**Carbohydrates (g)**	**Protein (g)**	**Fat (g)**	**Energy (kcal)**
Orange juice (100ml)	10	<1	0	43
Ham sandwich (100g)	36	5	9	274
Chocolate pudding (100g)	20	4	4	132

Part two: Effect of ID fat perfusion combined with balloon distension of the antrum on food intake

The procedures in this part were similar to part one except for the site of distension and the ID perfusion. Four treatments, separated by at least 7 days, were randomly performed in each subject. The barostat had an opening at the tip of the tube, through which the fat could be perfused. The tip of the tube was inserted into the duodenum and the balloon of the barostat was positioned in the gastric antrum. After placement, the position of the tube was located fluoroscopically. To prevent further progression of the tube during the experiment, a small balloon (60 ml), which was located 3-3.5 cm distally from the barostat-bag, was inflated in the duodenum.

At noon, after insertion of a catheter into a forearm vein for blood drawings, the MDP was defined. Then the experiment was started with a continuous ID perfusion. The treatments were identical in design except for the ID perfusions and the distending volumes.

The first treatment consisted of an ID perfusion of saline for the duration of 90 min. Seventy minutes after starting the perfusion, the bag was inflated with 0 ml (control experiment) above the MDP level. After twenty min, the bag was deflated and the tube was taken out. After removing the barostat tube, subjects were invited to eat and drink as much as they wished. The standard meal has already been described in part one. The second treatment was similar. ID saline was given, but the bag was inflated with 300 ml above the MDP level. In the third and fourth experiment ID fat (corn seed oil) was perfused instead of saline, combined with inflation of the bag with either 0 ml or 300 ml above the MDP level. A perfusion rate of 0.5 ml/min for a total of 90 min (load 41 g of fat; total energy content: 371 kcal) was chosen derived from previous experiments (8, 30, 40). The study design is shown in *Figure 7.1.2*.

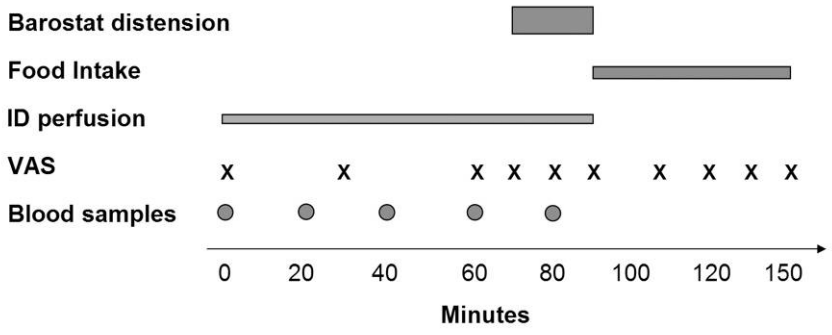

Figure 7.1.2: Experimental design of study part two. ID, intraduodenal; VAS, Visual Analogue Scale.

The ID fat perfusion solution was filled in a black syringe which made it indistinguishable in appearance from the control solution (saline), and the person in charge of the experiments was unaware of the respective treatment, thereby making it possible to deliver treatments in a double-blind fashion. Blood was taken at regular intervals for plasma CCK and PYY determinations in EDTA-coated tubes (6μmol/l) containing aprotinin (500 KIU/ml blood). Plasma samples were kept frozen at –20°C until analysis.

After the start of the perfusion, subjects scored their subjective feelings for hunger and fullness at regular intervals for the duration of each experiment using a VAS from 1 through 10 and indicated their scores on a questionnaire. The scale and scores have previously been designed and described in detail (8, 40). In brief, a score of *zero* for hunger indicated that the subject was not hungry at all, *two* indicated “slightly hungry”, *five* indicated “moderately hungry”, *eight* indicated “very hungry”, and *10* indicated “absolutely ravenous”. The score for fullness was similar. The study was finished 60 min after meal start.

Barostat

The barostat consisted of a strain gauge linked by an electronic relay to an air injection-aspiration system. Both the strain gauge and the injection system were connected by means of a polyvinyl tube to an ultra-thin polyethylene bag (First part: 1100 ml capacity, 20 cm maximum diameter; second part: 500-570 ml capacity and 12 cm maximum diameter). In the first part a double-lumen polyvinyl tube was used, in the second part the tube had seven lumen. The barostat measures the volume or the pressure within this flaccid, air filled bag maintained at a constant pre-selected level by the electronic feedback mechanism (2, 3). A dial in the electronic system allows selection of the desired volume level or pressure level to be maintained within the bag. The barostat can be used to induce gastric distension, continuously recording the resulting intragastric bag volume and pressure.

The bag of the barostat, finely folded, was introduced through the mouth into the stomach. After placement of the barostat assembly, the participant was placed in a 30° recumbent position and asked to relax comfortably. To unfold the gastric bag, one lumen of the connecting tube was connected to a pressure transducer and the bag was slowly inflated through the other lumen of the tube with the respective

volume in ml of air. Thereafter, the bag was completely deflated and connected to the barostat.

Using the pressure-selection dial of the barostat, intrabag pressure was gradually increased by 2 mm Hg stepwise increments every 3 min, to designate the MDP defined as the pressure that first provides intrabag volume variation induced by respiratory motion (34, 35). After deflation of the barostat bag to the MDP for 30 min, the intrabag volume was increased to the pre-determined volume level. The patient as well as the investigator were unaware of the distending volume levels.

Biochemical analysis

Plasma immunoreactive CCK concentrations were measured by a sensitive radioimmunoassay (RIA) based on an antiserum against CCK-8. It has a negligible cross-reactivity to gastrin. Plasma samples were extracted with ethanol. The detection limit of the assay was 0.3 pmol/l plasma using CCK-8 as a standard. Details of the assay have already been described (14). Total PYY concentrations were measured by a sensitive RIA based on an antiserum against PYY 1-36 and 3-36. The lowest level of PYY which could be detected by this assay was 10pg/ml when using a 100µl sample size. There is no crossreactivity between the antiserum and other members of the pancreatic polypeptide (PP) family.

Statistical analysis

The amount of food eaten (g) and the amount of fluid drunk (ml), including the corresponding energy intake (kcal), were compared between the treatments by analysis of variance (ANOVA). In case of significance ANOVA was followed by multiple paired t-tests with Bonferroni correction. For part two Plasma CCK and PYY data were evaluated by calculating area under the plasma concentration/time curve (AUC). AUC was calculated by linear trapezoidal rule from T 0 to 80 min for CCK and PYY. CCK and PYY data were analyzed by ANOVA. If significant differences were detected, ANOVA was followed by a paired t-test with Bonferroni correction or, in case of non-normal distributed data, non-parametric ANOVA (Friedman-test) was followed by Dunn correction. Differences in scores for hunger and fullness were obtained by subtracting the feelings at 45 respectively 90 min from the baseline-value. The differences between the treatments were compared using the same statistical procedures described above.

Results

Food Intake

Part one

The amount of food eaten, the amount of fluid consumed and the corresponding calorie intake were not significantly affected by increasing volumes of gastric balloon distension of the fundus compared to the control treatment (0 ml distending volume). Data are shown in *Table 7.1.2*.

Table 7.1.2: Effect of balloon distension of the fundus (0, 400, 600, 800 ml) on eating behavior in 12 healthy male subjects.

Treatment	Food Intake (g)	Calories (kcal)	Volume drunk (ml)
a) 0 ml	775 ± 49	2337 ± 117	777 ± 101
b) 400 ml	805 ± 58	2241 ± 119	727 ± 89
c) 600 ml	773 ± 53	2251 ± 121	696 ± 91
d) 800 ml	787 ± 73	2230 ± 146	744 ± 88

Data are means ± SE.

Part two

The amount of food eaten and the corresponding calorie intake were not significantly affected by gastric balloon distension of the antrum with 300 ml above the MDP level compared to the control treatment (ID saline plus 0 ml distending volume). ID fat combined with distension of the antrum with 0 or 300 ml above the MDP level decreased the amount of food eaten and the corresponding calorie intake compared to the control experiment, but the reductions did not reach statistical significance. Fluid intake was neither affected by ID saline or fat plus gastric balloon distension with 300 ml above the MDP level compared to the control treatment. Data are shown in *Table 7.1.3*.

Table 7.1.3: Effect of ID saline or ID fat together with a distension of the antrum with either 0 or 300 ml on eating behavior in 12 healthy male subjects.

Treatment	Food Intake (g)	Calories (kcal)	Volume drunk (ml)
a) Saline/0 ml	446 ± 47	1213 ± 96	484 ± 63
b) Saline/300 ml	446 ± 53	1217 ± 105	484 ± 56
c) Fat/0 ml	355 ± 36	1033 ± 98	449 ± 57
d) Fat/300 ml	371 ± 44	1038 ± 120	475 ± 69

Data are means ± SE.

Eating behavior

Part one

Increasing gastric volumes affected the mean VAS *(Figures 7.1.3a and 7.1.3b)* in the premeal period: subjects experienced a reduced degree of hunger and a concomitant feeling of fullness, but the effect was short-lasting. When we compared baseline scores with the 45 min values, the difference reached statistical significance for hunger ($p < 0.01$) as well as for fullness ($p < 0.01$) for 600 ml balloon distension, but only for fullness with 800 ml balloon distension ($p < 0.01$).

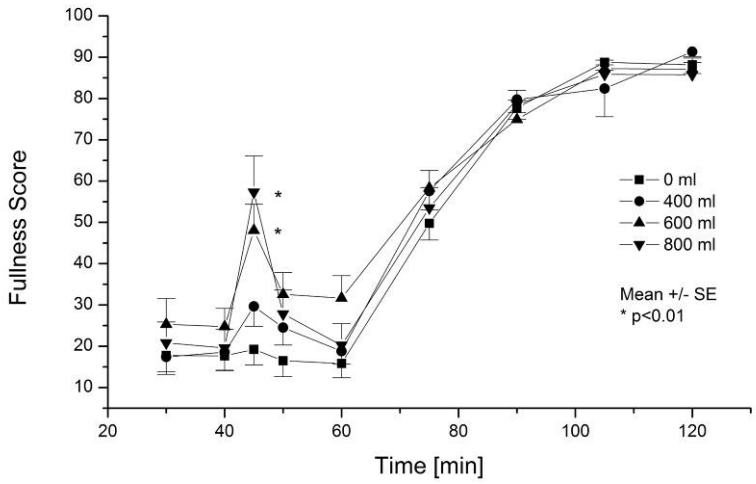

Figure 7.1.3a: Subject sensations for fullness experienced by 12 healthy male subjects before and after food ingestion during gastric fundus distension with increasing volumes (0, 400, 600, 800 ml). Ten minutes before food consumption the gastric fundus was distended for ten minutes. Results are expressed as means ± SE.
* indicates $p<0.01$, all vs. control (0 ml). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

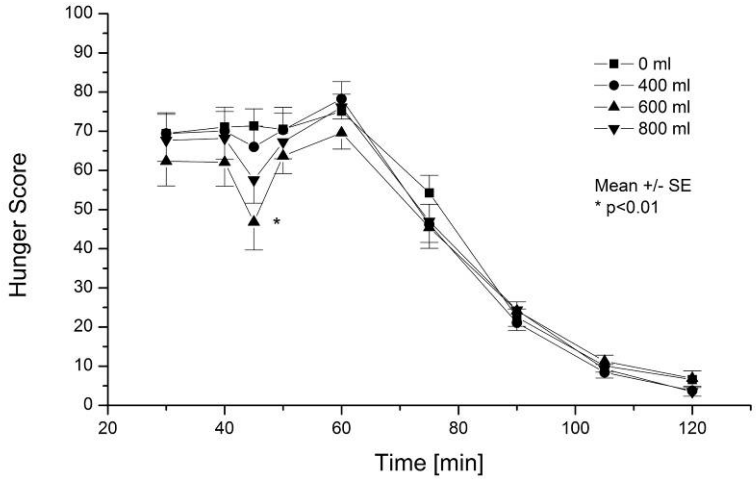

Figure 7.1.3b: Subject sensations for hunger experienced by 12 healthy male subjects before and after food ingestion during gastric fundus distension with increasing volumes (0, 400, 600, 800 ml). Ten minutes before food consumption the gastric fundus was distended for ten minutes. Results are expressed as means ± SE.
* indicates $p<0.01$, all vs. control (0 ml). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

Part two

ID fat in combination with 300 ml balloon distension influenced the mean VAS *(Figures 7.1.4a and 7.1.4b)*. Subjects experienced a reduced degree of hunger and a concomitant increased feeling of fullness in the premeal period, but the difference did not reach statistical significance. When we compared baseline scores with 90 min values, the difference did neither reach statistical significance, although subjects felt less hungry and fuller with ID fat plus 300 ml distending volume (data not shown). Antrum distension alone did not have any effect on feelings of hunger or fullness. These data indicate that antrum distension alone does not influence feelings of hunger or fullness detected with VAS.

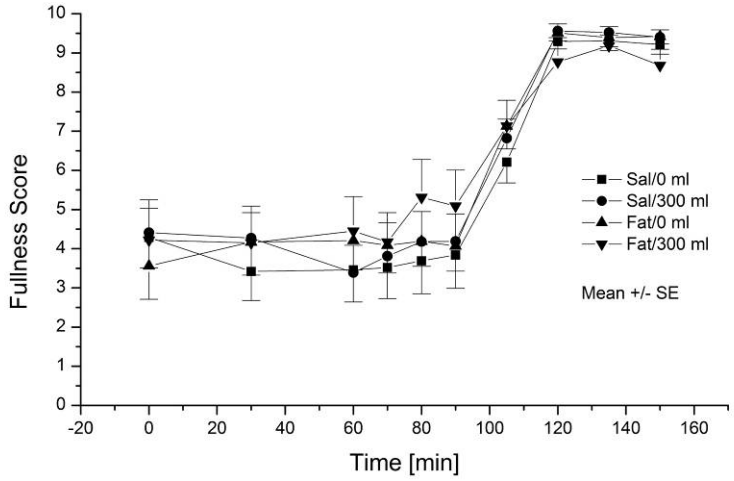

Figure 7.1.4a: Subject sensations for fullness experienced by 12 healthy male subjects before and after food ingestion during gastric antrum distension (0/300 ml) with either ID saline or fat perfusion. Shortly before food consumption the gastric antrum was distended for twenty minutes. Results are expressed as means ± SE.

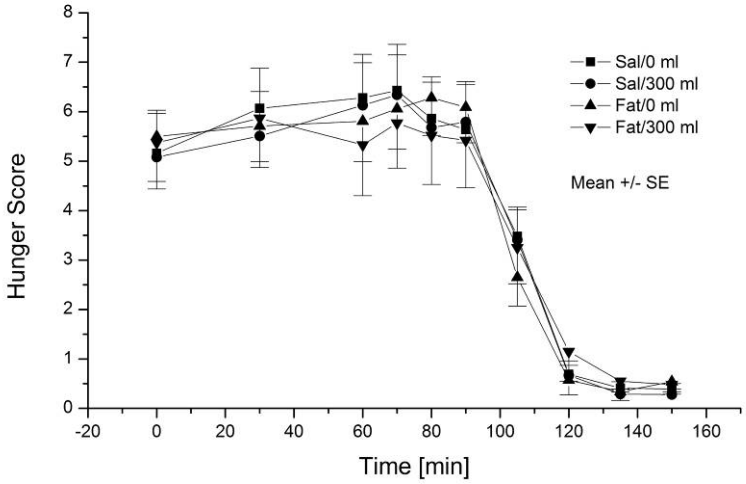

Figure 7.1.4b: Subject sensations for hunger experienced by 12 healthy male subjects before and after food ingestion during gastric antrum distension (0/300 ml) with either ID saline or fat perfusion. Shortly before food consumption the gastric antrum was distended for twenty minutes Results are expressed as means ± SE.

Plasma Hormones

Part one

During gastric fundus distension CCK plasma hormone responses remained unchanged in the premeal period (data not shown), independently of the distending volume.

Part two

During the control treatment (ID saline plus 0 ml distending volume), plasma hormone responses (CCK, PYY) remained stable in the premeal period. The distension of the antrum with 300 ml above the MDP level did not stimulate plasma CCK and PYY concentrations. During ID fat, a significant increase in plasma CCK ($p < 0.01$) was obtained compared to the control experiment. PYY levels significantly increased when ID fat was given compared to the control experiment (pooled data; $p < 0.01$). The PYY data of ID fat and of the control experiment were pooled for both distending volumes. The data are shown in *Figures 7.1.5a and 7.1.5b.*

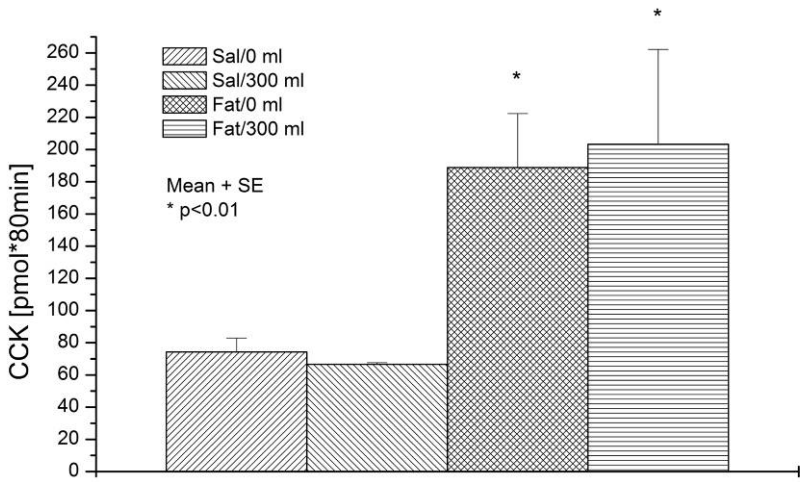

Figure 7.1.5a: Area under plasma concentration/time curve (AUC) plasma CCK responses to gastric antrum distension (0/300 ml) together with ID perfusion of saline (Sal) or fat. Results are expressed as means + SE. * = $p<0.01$. Significant difference between control (saline/0 ml) and ID fat (with and without gastric distension). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

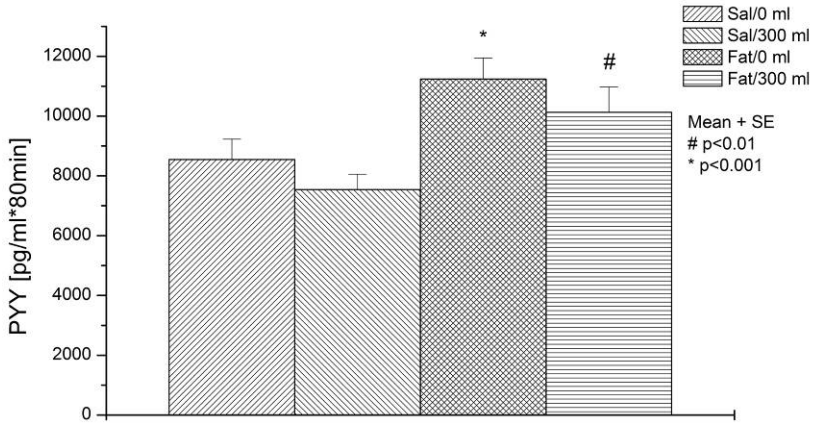

Figure 7.1.5b: Area under plasma concentration/time curve (AUC) plasma PYY responses to gastric antrum distension (0/300 ml) together with ID perfusion of saline (Sal) or fat. Results are expressed as means + SE. * = $p<0.001$. Significant difference between ID saline plus 300 ml distending volume and ID fat. # = $p<0.01$. Significant difference between ID saline plus 300 ml distending volume and and ID fat with 300 ml distending volume. Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

Discussion

The classical approach for studying inhibitory controls of food intake involve the manipulation of specific organs associated with regulation of eating. In the present study we have examined the effect of both gastric fundus and antrum distensions by balloon inflation on food intake and appetite sensations in healthy male subjects. The results can be summarized as follows: 1) Gastric fundus distension with increasing volumes (400, 600, 800 ml) did not lead to a reduction in food intake compared to the control treatment; 2) Hunger ratings fell significantly with a distending volume of 600 ml and fullness ratings rose significantly with both 600 and 800 ml distending volumes of the fundus, but the change in ratings was only short-lasting; 3) Gastric antrum distension alone did not lead to a reduction in food intake compared to the control treatment; 4) ID fat in combination with antrum distension reduced food intake, but non-significantly; 5) Gastric antrum distension alone did not change hunger or fullness ratings, but in combination with ID fat subjects experienced a reduced degree of hunger and a concomitant increased feeling of fullness; 6) CCK and PYY plasma levels were not altered by gastric antrum distension, but they showed a significant increase after ID fat in the premeal period.

Gastric distension is considered to be an important factor in the regulation of food intake and in triggering satiety in animals and humans. The role of stomach distension on satiety and food intake has been described in a series of studies by Geliebter et al (10-13). In two of his earlier studies, Geliebter et al (10, 13) examined the effects of various levels of gastric distension on food intake. The stomach was distended with a balloon, by filling it with different volumes (0-800 ml) of water. Food intake decreased significantly with increasing balloon volumes, but only when the volume was equal or greater than 400 ml. Further evidence for a role of gastric distension on appetite is derived from studies by Melton et al (28) and Cecil et al (6). Melton et al (28) showed in 4 subjects a positive correlation between gastric pressure rise due to balloon inflation and fullness ratings. Cecil et al (6) discovered in a study with 9 subjects that intragastric infusion of tomato soup suppressed appetite in contrast to ID infusions of soup. Rolls and Roe (33) showed that by increasing the volume, but not the energy content, of gastrically infused food, hunger ratings and food intake in 29 obese and 25 nonobese women were reduced. In summary, there is substantial evidence for a direct, inverse relationship between gastric distension and appetite. However, the mechanisms of action and the importance of the site of gastric distension are still unclear. Therefore our main interest was the investigation of a potential reduction of energy intake induced by gastric antrum distension compared to the distension of the fundus.

The present study is above all based on the ideas of Geliebter et al (10, 13) with two main differences. First, the position of the balloon was not precisely defined, in those studies, we therefore chose the more specific barostat method to place the balloon of the tube fluoroscopically either in the fundus or the antrum. Second, Geliebter et al (10, 13) removed the balloon only after the subjects had finished the meal, whereas in our study the tube was already taken out before volunteers began to eat. We were interested to test pure mechanical distension of the fundus or antrum on subsequent food intake.

Differences in the properties and functions between the proximal and distal stomach have previously been recognised. Mechanical properties and neural innervation vary in different regions of the stomach (17, 21) and it is uncertain whether the site of gastric distension is important in mediating appetite related sensations. In young subjects, distension of the proximal stomach (with the use of a barostat) increased the perception of fullness (9, 15), but effects on food intake have not been evaluated.

Observations suggest that antral distension, rather than the overall rate of gastric emptying or the content of the proximal stomach, is a major determinant of satiety (19, 20, 36, 39). The mechanisms also remain to be defined. The perception of fullness is likely, at least in part, to reflect the activation of gastric stretch receptors by gastric distension (39). Possibly there are regional differences in the sensitivities of gastric fundus and antral mechanoreceptors (22).

In this study neither increasing volumes of gastric distension of the fundus (400, 600, 800 ml) nor distension of the antrum with 300 ml reduced the amount of food eaten and the corresponding calorie intake. When the fundus was distended, 600, respectively 800 ml were necessary to reduce feelings of hunger and increase feelings of fullness, but the effect was only short-lasting. The volume of 300 ml for distending the antrum was apparently too small to significantly influence hunger and fullness ratings. As soon as the bag of the barostat was deflated and the tube taken out, the feelings of hunger and fullness reached basic ratings again. The fact that pure mechanical distension had only a short-lasting effect on hunger and fullness ratings, explains why total calorie intake after gastric distension was not reduced compared to the control treatment. Therefore, mechanical distension of the stomach only seems to play a role in triggering satiety as long as mechanoreceptors are stimulated. The short-lasting effect of pure mechanical distension on feelings of hunger and fullness could indicate that impulses from mechanoreceptors of the stomach are transmitted via neural pathways.

In the second part of the study we aimed to evaluate whether satiety signals from the distal stomach can be intensified by ID fat. ID fat in combination with distension of the antrum non-significantly reduced the amount of food eaten and the corresponding calorie intake. On the basis of these results ID fat does not seem to intensify gastric satiety signals.

CCK as satiety peptide was measured in both parts of the present study. In the study with gastric antrum distension we additionally measured the release of PYY. Both peptides have been shown to modulate short-term control of food intake during a test meal intake. Gastric distension of either the fundus or the antrum did not alter CCK plasma levels compared to the control treatment. We observed a significant increase in the premeal period both in plasma CCK and PYY after ID fat. With the increase of CCK after ID fat we could confirm previous observations (26). Indeed, the present study is one of the first studies in humans investigating the effect of ID fat on the

secretion of PYY. PYY is characteristically released in proportion to both the calorie content of a meal and its energy source composition. Increasing ingested amounts of an identical meal lead to proportionally increased plasma levels of PYY (1). With isocaloric meals consisting exclusively of either fat, carbohydrates or proteins, the highest levels of plasma PYY were detected after the fat meal followed by the carbohydrate meal and very little with the protein meal (1). On the other hand gastric distension of the antrum did not have any influence on the release of CCK and PYY. These data imply that signals elicited by pure mechanical gastric distension are not mediated by CCK or PYY.

To summarize the findings of this study, we have observed that neither mechanical distension of the fundus nor the antrum reduced the amount of food eaten and the corresponding calorie intake. The alteration of feelings of hunger and fullness was dependent on the gastric distending volume and was only short-lasting. ID fat and gastric distension of the antrum do not seem to exhibit synergistic effects on food intake. Pure mechanical distension of the gastric fundus and antrum induced by barostat controlled balloon inflation do not seem to be a sufficient signal to promote satiety. Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety.

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Project 2

Chapter 7.2. Effects of a preload on reduction of food intake by GLP-1 in healthy subjects

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Running title: Effects of a preload and GLP-1 on food intake

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Abstract

Background / Aims: Glucagon-like peptide-1 (GLP-1) inhibits food intake in animals and humans. Whether GLP-1 interacts with other satiety signals to modulate food intake is unknown. We investigated therefore in healthy volunteers the potential interactions of GLP-1 with signals from the stomach in regulating food intake. ***Methods:*** Three sequential, double-blind, crossover studies were performed in male subjects: I) fourteen subjects underwent three experiments (preloads) 20 min before meal intake; II) twelve volunteers received intravenous (iv) GLP-1 (0.9 pmol/kg/min) or saline; III) fifteen subjects received iv GLP-1 or saline (control) together with a preload of either 400 ml water or 400 ml protein shake. The effect of these treatments on food intake and feelings of hunger was quantified. Subjects were free to eat and drink as much as they wished. ***Results:*** GLP-1 induced a reduction in food and calorie intake ($p<0.005$) compared to controls. If combined with a protein preload, the inhibitory effect of GLP-1 on food intake was markedly increased ($p<0.001$). Furthermore, a decrease in hunger feelings and an increase in satiety feelings was documented. ***Conclusion:*** GLP-1 interacts with signals from the stomach to modulate energy intake in humans.

Key words: eating behaviour, gastric signals, GLP-1

Introduction

The pro-glucagon-derived peptide glucagon-like peptide-1(7-36) amide (GLP-1) is a gastrointestinal hormone that is released in response to food intake from the distal small intestine (1, 2, 9). Its biological effects include a glucose-dependent insulinotropic action and inhibition of gastric emptying (18, 19). The last effect can be interpreted as being part of the “ileal break mechanism”, an endocrine feedback loop that is activated by nutrients in the ileum (10). This feedback loop inhibits upper gastrointestinal digestive functions (gastric acid secretion, gastric emptying, exocrine pancreatic secretion), but also affects appetite and food ingestion (3, 5, 7).

A series of remarkable discoveries and the emergence of obesity as major health problem have stimulated research efforts into how the body controls appetite and food intake. The close relationship between the gastrointestinal endocrine system and the brain in regulating food intake and satiety requires a co-ordinated interplay, in which circulating hormones convey information about food consumption and appetite to brain centers that control eating. The regulatory circuits are complex and several pathways act in parallel. However, little is known about the interactions of various physiological satiety signals that control food intake. The interaction effect on food intake resulting from an intestinal satiety signal and a gastric signal has previously been explored for hormone cholecystokinin (CCK), but not for GLP-1. This interaction was reported as a greater reduction in food ingestion in healthy volunteers when CCK8 was given together with a large soup preload (13). Previous work from our laboratory has extended these observations by demonstrating that the appetite-suppressing effect of a carbohydrate preload together with CCK8 infusion was mediated by CCK-1 receptors (6, 11). Similar interactions are likely to occur with a variety of satiety signals; it has, however, not been tested whether similar interactions occur with GLP-1. It was therefore of interest to determine whether an interaction exists between a preload and intravenous GLP-1, and to evaluate how important the preload was for the effectiveness of GLP-1 to reduce food intake. Hence the present study was designed to further understand the role of GLP-1 in regulating food intake in healthy male subjects.

Three consecutive experimental series were performed. In the first series we tested the effect of different nutrient preloads on subsequent food consumption in order to test whether gastric distension alone was able to modulate food intake. In the second

series, we determined the effect of intravenous GLP-1 in comparison to saline for initiating feedback inhibition of food intake. In the third series, we tested the hypothesis that the feedback response on food intake initiated by GLP-1 is modulated by a nutrient preload.

Experimental procedure

Part I: Effect of different preloads with variable nutrient composition on food intake
Three treatments, separated by at least seven days, were performed in 14 healthy male subjects in a randomised order. On each study day, subjects ate a liquid breakfast before 8 am. At noon, the experiment started with a baseline period of 60 min. Forty minutes later, a preload of 400 ml was given. After an additional 20 min, subjects were invited to eat and drink as much as they wished (for details, see *Figure 7.2.1*). The three treatments were similar but differed with respect to the composition of the preload. The following preloads were tested: 400 ml water, 400 ml of protein shake, and 400 ml of carbohydrate shake. The protein shake was made of 47.4 g milk protein, 5.3 g chicken protein, 0.05% aspartam and 0.3% vanilla flavour and mixed with water to a total volume of 400 ml; total energy content 200 kcal. The carbohydrate shake was made of 100 g whey, 100 g banana and 16 g sugar mixed with water to a total amount of 400 ml (total energy content: 200 kcal). During each study, blood was drawn in regular intervals for glucose and hormone (CCK, GLP-1) determinations.

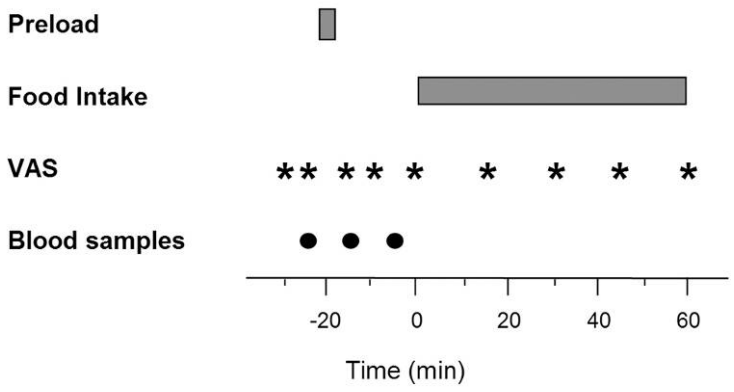

Figure 7.2.1: Experimental design of part I.

The meal consisted of a) orange juice; b) ham sandwiches (60 g wheat bread; 10 g butter, and 25 g ham); c) chocolate pudding; and d) coffee with cream and sugar (coffee could be sweetened if desired; therefore cream and sugar were optional). The order of food intake had to follow the above schedule. To reduce the participant's awareness of the amount of food eaten, food was presented in small samples and in excess. The amount of food eaten, volume and fluid drunk, and the time for each subject to complete a meal were quantified. From these observations the total calorie intake was calculated. Subjects scored their subjective feelings of hunger and fullness in regular intervals for the duration of each single experiment using a visual analogue scale from 0 to 100 and indicated their scores on a ruler. The scale and scores have previously been designed and described in detail (22).

Part II: Effect of GLP-1 on food intake

16 healthy male subjects participated in this part. The study was designed as a randomised, double-blind, two-period crossover trial. On each test day, subjects arrived in the research unit towards 11 am after fasting overnight. At 11 am, two Teflon catheters were inserted into the anticubital veins of each arm, one for infusion, the other one for blood drawing. At 11:30 am, a pre-study blood sample was taken and the infusion was started (either GLP-1 at a dose of 0.9 pmol/kg/min or saline as placebo) and continued for the next two hours. This dose was chosen from previous experiments (5, 7). Infusions were delivered by ambulatory pumps. Sixty minutes after the start of the respective infusion, the test meal described in part I was presented, and the participants were invited to eat and drink as much as they liked. Beginning with the infusions, participants scored their subjective feelings of hunger and fullness in 15 min intervals throughout the experiments using the VAS described before. In the pre-meal period and after eating, blood samples were taken in regular intervals (0, 20, 40, 60, 80, 100, 120 min) for glucose and hormone determinations.

Part III: Effect of GLP-1 in combination with a preload on food intake

Three treatments, separated by at least seven days, were performed in each of 15 subjects. At 11 am, experiments were prepared as in part II. At 11:30 am, a pre-study blood sample was taken and the test started with a continuous infusion. The treatments were identical in design except for the intravenous infusion and the preloads (see *Figure 7.2.2*).

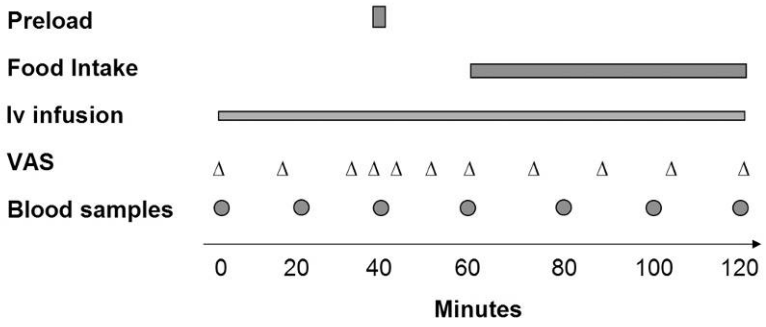

Figure 7.2.2: Experimental design of part III.

The first treatment consisted of an intravenous infusion of GLP-1 (0.9 pmol/kg/min) for the duration of the experiment. Forty minutes after starting the infusion, a preload of 400 ml water was given. After an additional 20 min, subjects were invited to eat and drink as much as they wished. The second treatment was similar: an intravenous GLP-1 (0.9 pmol/kg/min) infusion was given throughout the test, but 400 ml of protein shake was given instead of water. The third experiment used intravenous saline (placebo) throughout the entire study instead of GLP-1 combined with water as a preload. A protein shake was chosen for this experimental series, because the glucose (1.4 g carbohydrates) and fat (0.9 g fat) contents of this protein preload were very low to avoid stimulation of endogenous GLP-1; total energy content 200 kcal. During each study, blood was drawn in regular intervals for glucose and hormone determinations.

Infusions

For the GLP-1 infusions, recombinant human GLP-1 (7-36) amide was used, a kind gift of Bionebraska, Omaha, USA. The peptide was dissolved in 0.9% saline solution containing 0.5% human serum albumin and prepared under aseptic conditions by the University of Basel Hospital Pharmacy. Aliquots of 50 µg/ 5 ml were stored at –20°C. Infusion solutions were prepared by diluting appropriate amounts of GLP-1 with 0.9% saline containing 0.1 % human serum albumin. Control solutions contained albumin in saline alone. The solutions were prepared by a person who was not involved in the study. The physician in charge of the study was therefore not aware of the respective treatment thereby making it possible to conduct treatments in a double-blind fashion.

Laboratory analysis

Blood was drawn through an indwelling anticubital cannula into EDTA (6µmol/l) and aprotinine (1000kIU/ml). After centrifugation, plasma samples were kept frozen at –20°C until analysis. Plasma glucose was analysed by the hexokinase method. GLP-1 was measured as previously described (5, 7).

Statistical analysis

The amount of food eaten and the amount of fluid drunk, the corresponding energy intake as well as the eating rate, were compared between the different treatments by analysis of variance (ANOVA). The eating rate was calculated by adding the number of sandwich and chocolate mousse units per time. For significant differences, multiple paired tests with Bonferroni's correction were performed. The same statistical procedure was used to analyse the results of plasma hormone concentrations using area under the curve analysis. Scores for hunger and fullness were compared by comparing area under the curve analysis; in addition scores were compared by calculating the delta score from baseline (0 min) to values observed under gastric distension induced by drinking a preload (45 min) using the Wilcoxon signed ranks test. Differences were considered significant if p was < 0.05.

Results

Part I

The effects of the different preloads on food parameters are given in *Table 7.2.1*. Compared to 400 ml water ingestion, a 400 ml protein or a 400 ml carbohydrate preload reduced the total calorie intake by 10% and 9%, respectively (non-significantly). Food and fluid consumption were both slightly reduced with both oral nutrient-based preloads, but the respective reductions did not reach statistical significance.

Table 7.2.1: Effect of different preloads on food parameters in 14 healthy male subjects.

	400 ml water (control)	**400 ml CH-shake**	**400 ml protein shake**
Calorie intake (kcal)	1968 ± 120	1802 ± 91	1773 ± 125
Amount of food (g)	654 ± 42	616 ± 33	621 ± 47
Amount of fluid (ml)	713 ± 36	645 ± 48	633 ± 50

Data are means ± SE.

Hunger and fullness sensations (VAS) were found to be reduced shortly after the consumption of the two nutrient-based preloads, but the difference did not reach statistical significance compared to water ingestion (data not shown).

Part II

Intravenous infusion of synthetic GLP-1 (0.9 pmol/kg/min) reduced the amount of food eaten ($p<0.01$) and the amount of calories consumed ($p< 0.005$); the amount of fluid consumption was not significantly affected. The maximal reduction in food consumption with GLP-1 amounted to 17% resulting in a decrease in calorie intake of 16% *(Table 7.2.2)*.

Table 7.2.2: Effect of intravenous GLP-1 (0.9 pmol/kg/min) or saline on food parameters in 16 healthy male subjects.

	Saline	**IV GLP-1**
Calorie intake (kcal)	1875 ± 68*	1597 ± 73
Amount of food (g)	644 ± 24†	532 ± 29
Amount of fluid (ml)	785 ± 47	698 ± 53

Data are means ± SE. * = $p \leq 0.005$, † = $p \leq 0.01$, all vs. control (IV saline).

None of the participants reported any abdominal discomfort or side effects during infusion of GLP-1. Furthermore, when questioned at the end of each experiment, none of them experienced or reported any adverse reaction.

Subjects felt less hungry and fuller with GLP-1 in the pre-meal period, but the difference did not reach statistical significance.

Figure 7.2.3 depicts blood glucose concentrations. With GLP-1, blood glucose levels were significantly lower ($AUC_{glucose}$ = 316 $\pm$ 6 mmol/l x 60 min with GLP-1 vs 402 $\pm$ 10 mmol/l x 60 min with saline; $p < 0.0001$).

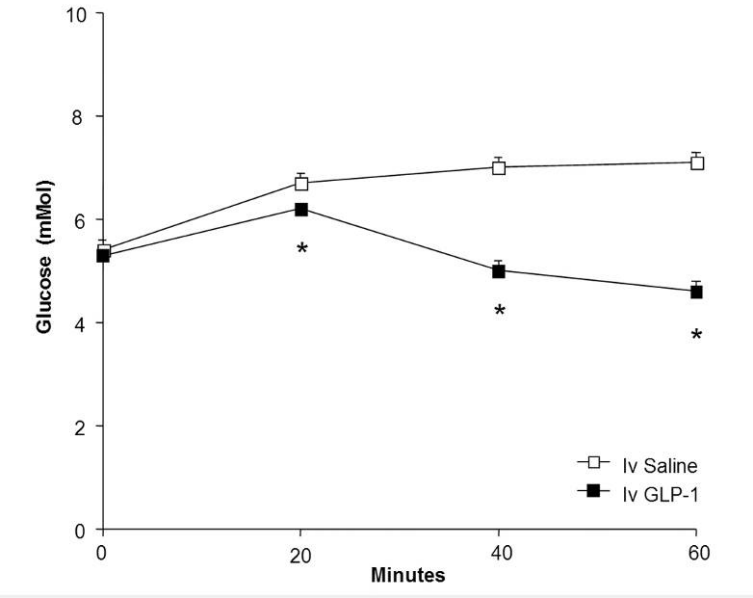

Figure 7.2.3: Plasma glucose concentrations in response to intravenous GLP-1 or saline (control) in 12 healthy male volunteers. Data are means ± SE. * = $p < 0.05$ vs. control (i.v. saline).

Part III

The amount of food eaten and the corresponding calorie intake were both significantly ($p < 0.005$) reduced after the application of intravenous GLP-1 together with a preload (PL) of water *(Table 7.2.3)*. The reduction amounted to 18 % for food eaten and 13 % for calorie intake, respectively, in comparison to the control treatment (intravenous saline plus PL water). The combination of intravenous GLP-1 plus protein shake as PL resulted in the strongest reduction ($p < 0.001$) in the amount of food consumed (31 %) with consequently reduced calorie intake (25 %). Therefore, calorie and food intake were reduced on both treatment days with intravenous GLP-1; the effect was most striking after the combination GLP-1 plus protein shake. Fluid intake was not affected by any treatment *(Table 7.2.3)*.

Table 7.2.3: Effect of intravenous (IV) GLP-1 or saline together with a preload (PL) of water or protein shake on food parameters in 12 healthy male subjects.

	IV Saline Plus PL Water	**IV GLP-1 Plus PL Protein**	**IV GLP-1 Plus PL Water**
Calorie intake (kcal)	1773 ± 761,3	1326 ± 672	1552 ± 70
Amount of food (g)	622 ± 251,3	430 ± 272	509 ± 33
Amount of fluid (ml)	581 ± 42	598 ± 45	564 ± 28

Data are means ± SE.
1) = $p \leq 0.005$ IV GLP-1 plus PL protein versus control (IV saline plus PL water) analysed by analysis of variance (ANOVA) followed by multiple paired t-tests with Bonferroni's correction.
2) = $p \leq 0.05$ IV GLP-1 plus PL protein versus IV GLP-1 plus PL water.
3) = $p \leq 0.05$ control (IV saline plus PL water) versus IV GLP-1 plus water.

As expected, hunger ratings fell and fullness ratings rose significantly ($p < 0.05$) after the subjects had drunk the protein shake preload together with intravenous GLP-1. The administration of GLP-1 plus water preload did not significantly affect hunger and fullness feelings in comparison to the control treatment (intravenous saline plus water preload) *(Figures 7.2.4a and 7.2.4b)*.

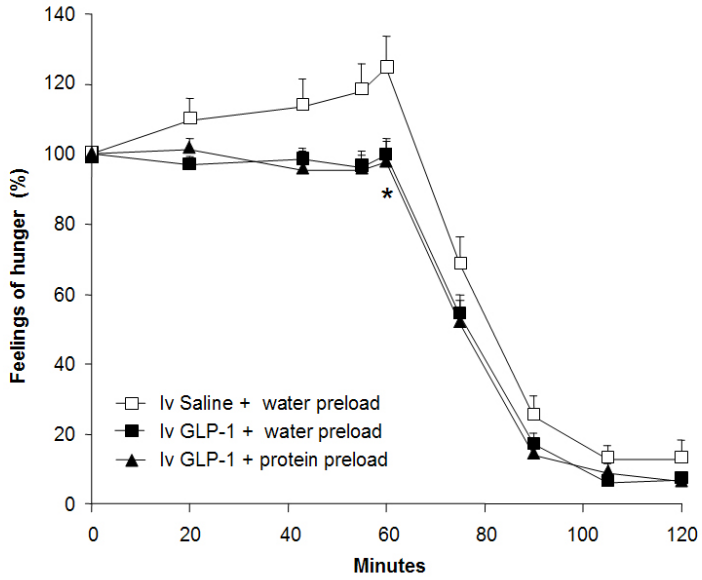

Figure 7.2.4a: Feelings of hunger in response to GLP-1 plus protein preload vs GLP-1 plus water preload or intravenous saline plus water preload in 12 healthy male volunteers. Data are means ± SE. * indicates significant difference of hunger ratings at experimental time 0 and 20 min between IV GLP-1 plus PL protein and IV saline plus PL water ($p < 0.05$).

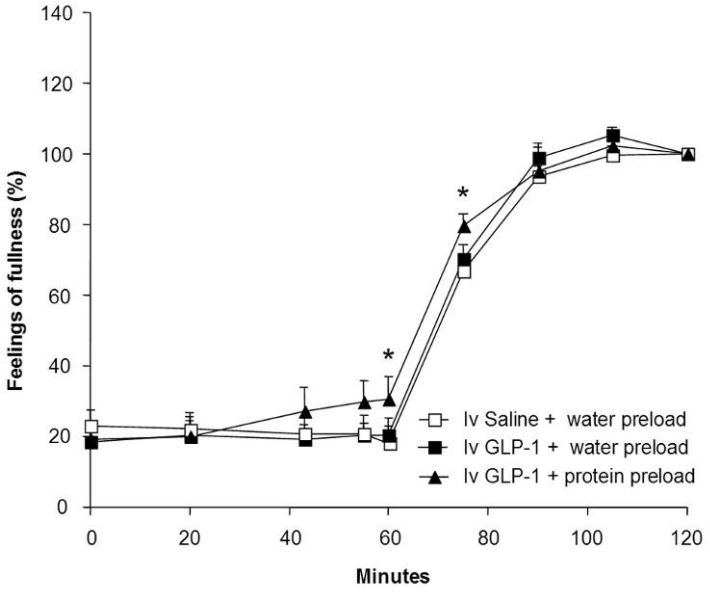

Figure 7.2.4b: Feelings of fullness in response to GLP-1 plus protein preload vs GLP-1 plus water preload or intravenous saline plus water preload in 12 healthy male volunteers. Data are means ± SE. * indicates significant difference of fullness ratings at experimental time 0 and 20 min between IV GLP-1 plus PL protein and IV saline plus PL water ($p < 0.05$).

Fasting prestudy glucose concentrations were similar in the different experiments; with both GLP-1 infusions, a significant ($p < 0.001$) decrease in glucose concentrations was seen thereby documenting the biological potency of the peptide *(Figure 7.2.5)*.

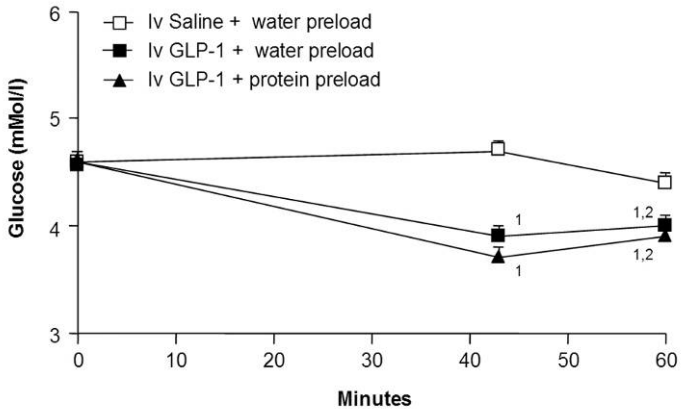

Figure 7.2.5: Pre-meal glucose concentrations, expressed as means ± SE (n=12). IV, intravenous; PL, preload.
1: indicates significant difference of treatment in comparison to control
($p< 0.0001$).
2: indicates significant difference of treatment at experimental time –5 min compared to fasting plasma glucose ($p<0.0001$).

Discussion

The role of exogenous GLP-1 in the induction of meal-ending satiety has been previously studied in healthy human subjects, in patients with diabetes mellitus type 2 and in patients with obesity (3-5, 7, 14, 16, 17). The potential interactions of GLP-1 with other satiety signals has, however, not been investigated in detail yet.

Such interactions have previously been described for CCK: Muurahainen et al. (12, 13) have provided compelling evidence that the satiety-inducing effects of CCK can be enhanced by giving a soup as a preload shortly before meal intake. Our own group has confirmed these data by documenting that exogenous and endogenous CCK interacts with a nutrient preload to modulate satiety in humans through activation of CCK-1 receptors (6, 11).

In the present study we have used the same approach to document an interaction between exogenous GLP-1 infusion with gastric signals on food intake. The results can be summarised as follows:
1) Exogenous GLP-1 in a dose which mimics physiological GLP-1 plasma concentrations induced a reduction in food and calorie intake confirming previous reports (5, 21).
2) Exogenous GLP-1 together with a 400 ml preload of protein shake enhanced the satiety-inducing effects of GLP-1 compared to controls.
3) GLP-1 infusion together with a water preload induced a significant reduction in food intake compared to the control treatment (saline infusion plus water preload).

The results provide strong evidence for the hypothesis that GLP-1 interacts with gastric signals to modulate food intake and satiety in humans. There are several possible explanations for these observations, and we will consider them with their relative strengths and weaknesses.

Preload may release endogenous GLP-1. In the first series of the present study, we have determined the GLP-1 releasing effects of different preloads (carbohydrate, protein, non-nutrient). The data reveal that the protein preload used in the present study does not stimulate endogenous GLP-1 release. This is not surprising, as the main components of the preload, mainly proteins, are not particularly good secretagogues for GLP-1 release (8). It is therefore highly unlikely that GLP-1 release from endogenous stores in response to the preload was able to produce the observed interaction.
GLP-1 inhibits gastric emptying. Gastric emptying is a major determinant in the regulation of food intake (20). Exogenous administration of GLP-1 has been shown to retard gastric emptying of liquids and solids (15, 19). A second possible explanation for our findings is therefore that GLP-1 slowed gastric emptying of the protein-rich preload. The stomach could therefore be fuller after the protein-rich preload with GLP-1 infusion compared to the treatment with water preload plus GLP-1 or with water plus saline infusion. After either of these latter two combinations (water preload with concomitant GLP-1 or water preload with saline infusion) the stomach could be relatively empty. The greater fullness of the stomach in response to the protein shake plus GLP-1 could have triggered an inhibitory signal. Increased gastric fullness

induced by slowing down gastric emptying could be the principal mechanism of action of GLP-1 on food intake. Previous studies with GLP-1 infusion have shown, however, that GLP-1 can reduce hunger and stimulate fullness feelings in the fasting state suggesting that at least some of the effects are independent of gastric emptying (21). Additional experiments are therefore required to fully understand this mechanism.

GLP-1 amplifies gastric signals. One major observation of this study is the demonstration that a protein-containing preload together with intravenous GLP-1 produced an augmented effect on food intake compared to a water preload plus infusion of GLP-1. These findings suggest that GLP-1 amplified signals from the stomach that would stimulate satiety. Without this amplification, it is difficult to understand how a 113 g difference in food consumption (resulting in a 221 kcal difference in energy consumption) between GLP-1 with water preload in comparison to saline plus water preload could be explained in the present study. Detailed comparisons on the correlation between the inhibition of gastric emptying and the inhibition of food intake with GLP-1 infusion have not been done so far. At this stage it is therefore not possible to decide whether a satiety action of GLP-1 exists independent of its effect on gastric emptying; the experimental evidence discussed before supports, however, such a role.

Several key issues remain unclear. Does GLP-1 inhibit food intake directly in the gastrointestinal tract by binding to peripheral receptors or does it act through central receptors? Does it act indirectly by releasing other satiety factors? Here we have observed that the combination of protein preload plus GLP-1 infusion did not stimulate significant amounts of endogenous CCK (data not shown). This is in keeping with previous observations from our laboratory (21) where we could document that similar doses of GLP-1 did neither stimulate CCK nor leptin release before meal ingestion making it unlikely that the latter two satiety peptides are the mediators of this response.

Facilitation of satiety. Hunger and satiety as an integration of neural signals brings us to another possible explanation for the observed effects, namely that consumption of the protein preload brought the subjects closer to satiety. If we accept the notion that GLP-1 promotes satiety, it would be more effective when satiety had already been partially achieved. Indeed, the hunger and satiety ratings were significantly affected by the combination protein preload plus GLP-1 but not with GLP-1 infusion and water

preload. The idea that the preload affects the response to GLP-1 by enhancing satiety (decreasing hunger) is supported by the correlation between hunger scores and intake. When the plasma GLP-1 concentrations of this study are compared with digestive effects of GLP-1 (stimulation of insulin secretion, inhibition of gastric emptying), they can be termed physiological. The protein shake itself did not stimulate GLP-1 release. Therefore it is unlikely that circulating levels of GLP-1 mediated these effects.

Taken together, we have seen in the present study that a protein-rich preload together with GLP-1 infusion induces an enhanced inhibition of food intake compared to GLP-1 infusion plus water preload. Under all treatments with GLP-1 infusion, food intake was reduced compared to saline controls. These results suggest that GLP-1 interacts with nutrient-based signals from the stomach. The results furthermore indicate that hunger ratings are more sensitive predictors of intake when the stomach is relatively full than when it is almost empty. We infer that GLP-1 is an important satiety factor interacting with other regulatory circuits to control food intake and satiety.

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Project 3

Chapter 7.3. Effect of a protein preload on food intake and satiety feelings in response to duodenal fat perfusions in healthy male subjects

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Abstract

The control of food intake and satiety requires a coordinated interplay. Oral protein and duodenal fat inhibit food intake and induce satiety, but their interactive potential is unclear. Our aim was therefore to investigate the interactions between an oral protein preload and intraduodenal (ID) fat on food intake and satiety feelings. Twenty healthy male volunteers, were studied in a randomized, double-blind, 4-period crossover design. On each study day, subjects underwent one of the following treatments: a) water preload plus ID saline perfusion; b) water preload plus ID fat perfusion; c) protein preload plus ID saline perfusion; d) protein preload plus ID fat perfusion. Subjects were free to eat and drink as much as they wished. An oral protein preload significantly reduced caloric intake (19%, $p < 0.01$). The simultaneous administration of an oral protein preload and ID fat did not result in a positive synergistic effect with respect to caloric consumption, rejecting the initial hypothesis that the two nutrients exert a positive synergistic effect on food intake. An oral protein preload but not ID fat altered the feelings of hunger and fullness. These data indicate that the satiety effect of an oral protein preload is not amplified by ID fat: indeed, the effect of a protein preload does not seem to be mediated by cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) or peptide YY (PYY). Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety.

Key words: eating behavior; gastrointestinal satiety signals

Introduction

Obesity and its associated complications are a significant health problem in industrialized countries. By current estimates, one-third of US adults are obese and another third are overweight (13). Similar trends can be seen worldwide and there is no sign that this trend is abating. Obesity is a major risk factor for various diseases such as type II diabetes, cardiovascular diseases, stroke and several types of cancer (breast cancer, colon cancer) (18). Several systems seem to be involved in the regulation of bodyweight; one of them is primarily concerned with short-term regulation of food intake, i.e. how often and how much is eaten on a given day. Over the past years numerous components of this regulatory network have been identified and the gastrointestinal (GI) tract has been found to be a major player. The close relationship between the GI system and the brain in regulating food intake and satiety requires a coordinated interplay. However, little is known about the interaction between different physiological signals and processes that control food intake and satiety in humans.

On the basis of animal experiments, it is assumed that food intake is suppressed by stimulation of specific receptors within the GI tract. Inspired by this hypothesis, Welch *et al.* observed some 20 years ago that a lipid emulsion infused into the ileum reduced food intake in healthy volunteers, but eating habits were not influenced by an intravenous administration of a similar fatty emulsion (23). In follow-up studies, we and others extended these observations by documenting a satiating effect of duodenal fat perfusions with the following key elements: 1) decreased food consumption, 2) decreased feelings of hunger, and 3) increased plasma cholecystokinin (CCK) release (17). Additional effects of duodenal lipid infusion include early fullness and a delay in gastric emptying (6). Other gastrointestinal peptides that have been associated with nutrient stimulated inhibition of food intake include glucagon-like peptide-1 (GLP-1) and peptide YY (PYY3-36) (3, 11). Nevertheless, the interactions between different macronutrients in the regulation of appetite and food intake have hardly been explored. In general, protein as an oral preload is considered to be the most satiating component, followed by fat (8). In humans, the interactions between protein and fat have not been investigated.

The present study is thus designed to further understand the potential interaction between protein and fat in regulating food intake in humans. We were particularly

interested to see whether GLP-1 and PYY are associated with this interaction. The aim of this study was to better understand the regulation of food intake in humans by exploring the interaction of the stomach and the small intestine. An oral protein preload was given to stimulate gastric signals, together with intraduodenal (ID) fat perfusion, which should trigger intestinal signals (CCK, GLP-1, PYY).

Methods

Overview

A randomized, double-blind, four-period, Latin square design was carried out in 20 healthy, paid male volunteers. Each participant underwent tests on four experimental days, separated by at least 1 week. On each experimental day, the intake of a standardized meal with related variables was measured. A continuous ID perfusion of either fat or saline (control) was given throughout the entire experiment. Forty minutes after starting the respective ID perfusion, an oral preload of either 400 ml of water or 400 ml of protein shake was given. After an additional 20 min, subjects were invited to eat and drink as much as they wished. The experimental design is shown in *Figure 7.3.1*.

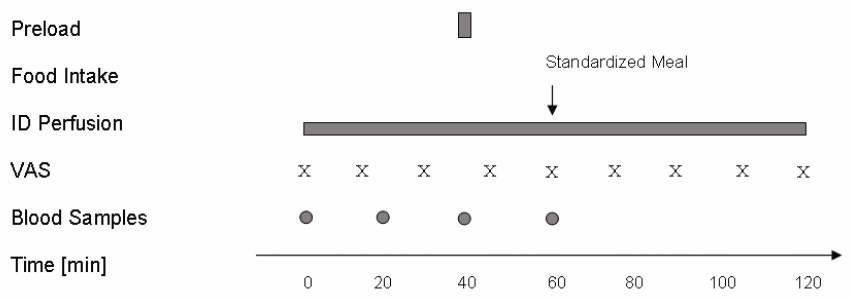

Figure 7.3.1: Experimental design of study. ID, intraduodenal; VAS, Visual Analogue Scale.

Subjects

Each subject gave written informed consent for the study. The protocol was approved by the Human Ethics Committee of the University Hospital in Basel. Before

acceptance, each participant was required to complete a medical interview and received a full physical examination. Inclusion criteria were:

8) BMI within 15% of desirable weight for height
9) Age between 18-45 years
10) Non-smokers
11) No active medical problems
12) Taking no medication
13) No allergies including food allergies
14) No history of GI disorders or weight problems

Twenty male subjects completed the study (mean age 26.7 $\pm$ 4.9 years, range 21-43 years; BMI 22.2 $\pm$ 1.3 kg/ m^2 , range 20.1-24.5 kg/ m^2).

Experimental procedure

Four treatments, separated by at least 7 days, were randomly performed in each subject. Shortly before each experiment, a radiopaque polyvinyl feeding tube (external diameter: 8 French) with an opening at the tip of the tube was inserted through the nose into the duodenum. This procedure allowed subjects to eat and drink with a minimum amount of discomfort from the tube. After placement, the position of the tube was located fluoroscopically and the tip of the tube was positioned 100 cm distally to the teeth. It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment.

On the day of the experiment, each subject ate a light breakfast (if this was his normal habit), but no snacks were allowed after 8 AM. At noon, after insertion of a catheter into a forearm vein for phlebotomy, the experiment was started with a first continuous perfusion. The treatments were identical in design except for the ID perfusions and the oral preloads.

The first treatment consisted of an ID perfusion of saline for the duration of the experiment. Forty minutes after starting the perfusion, an preload of 400 ml of water was given orally. After an additional twenty min, subjects were invited to eat and drink as much as they wished. The second treatment was similar: ID saline was given throughout the whole experiment, but 400 ml of an oral protein shake was given instead of water. The third and fourth experiments used ID fat (corn seed oil) throughout the entire experiment instead of saline, combined with either water or

protein shake as respective preloads. A perfusion rate of 0.375 ml/min for a total of 120 min (load 41 g of fat; total energy content: 371 kcal) was chosen from previous experiments (5, 23).

The preload used in this study was based on experiments performed by Matzinger *et al.* (16). The shake was made of protein mixed with water to equal a total of 400 ml. The shake contained the following nutrients: 52.7 g milk protein, 0.29 g carbohydrates, 0.58 g fat, 0.05% aspartam and 1.3% vanilla flavor (total energy content: 218 kcal).

Twenty minutes after the preload, a standard meal was presented to the subjects, who were then invited to eat and drink as much as they wished for 60 min. The meal consisted of 1) orange juice, 2) ham sandwiches (72 g wheat bread, 10 g butter, and 25 g ham) and 3) chocolate pudding. The composition of the test meal with its corresponding nutritive values is listed in *Table 7.3.1*. Non-sparkling water could be taken during the meal as a non-caloric beverage. The order of food intake had to follow the above schedule. To reduce the participants` awareness of the amount of food eaten, food was presented in small samples and in excess. The ID fat perfusion solution was filled in a black syringe which made it indistinguishable in appearance from the control solution (saline), and the person in charge of the experiments was unaware of the respective treatment, thereby making it possible to deliver treatments in a double-blind fashion. The amount of food eaten, the volume of fluid imbibed, and the time for each subject to complete the meal were quantified. From these observations, the total calorie intake could be calculated. Before, during, and after the preload, blood was drawn at 20 min intervals for plasma CCK, GLP-1 and PYY determinations in EDTA-coated tubes (6 μmol/l) containing aprotinin (500 KIU/ml blood). Plasma samples were kept frozen at –20°C until analysis.

After the start of the perfusion, subjects scored their subjective feelings for hunger and fullness at 15 min intervals for the duration of each experiment. A visual analog scale (VAS) that ranged from 1 through 10 indicated their respective scores on a questionnaire. The scale and scores have previously been designed and described in detail by Drewe *et al.* (5) and Welch *et al.* (23). In brief, a score of *zero* for hunger indicated that the subject was not hungry at all, *two* indicated “slightly hungry”, *five* indicated “moderately hungry”, *eight* indicated “very hungry”, and *10* indicated “absolutely ravenous”. The score for fullness was similar. The study was finished 60 min after meal start.

Table 7.3.1: Composition of test meal with corresponding nutritive values

Nutrients	Carbohydrates (g)	Protein (g)	Fat (g)	Energy (kcal)
Orange juice (100ml)	10	<1	0	43
Ham sandwich (100g)	36	5	9	274
Chocolate pudding (100g)	20	4	4	132

Biochemical analysis

Plasma immunoreactive CCK concentrations were measured by a sensitive radioimmunoassay (RIA) based on an antiserum against CCK-8. It has a negligible cross-reactivity to gastrin. Plasma samples were extracted with ethanol. The detection limit of the assay was 0.3 pmol/l plasma using CCK-8 as a standard. Details of the assay have already been described (10). GLP-1 (bioactive form) immunoreactivity was measured as previously described (9). The antiserum is specific for GLP-1 and does not cross-react with any other members of the glucagon family of peptides. The detection limit of the assay was 3 pmol/l. Before the RIA, plasma samples were extracted with ethanol.

Total PYY concentrations were measured by a sensitive RIA based on an antiserum against PYY 1-36 and 3-36. The lowest level of PYY which could be detected by this assay was 10 pg/ml when using a 100 µl sample size. There is no cross-reactivity between the antiserum and other members of the glucagon family of peptides.

Statistical analysis

The power calculations of this study are based on previous studies. The expected reduction of food intake (kcal) by ID fat was assumed to be 12% compared to the control treatment (water preload and ID saline), whereas the expected reduction of food intake in response to a protein preload was assumed to be 20%. Accepting a significance level of 95% and a power of 80% the required sample size had to be at least 18 subjects.

The amount of food eaten (g) and the amount of fluid drunk (ml), including the corresponding energy intake (kcal), were compared between the treatments by analysis of variance (ANOVA). In case of significance, ANOVA was followed by

multiple paired t-tests with Bonferroni correction. Plasma hormone data were evaluated by calculating the area under the plasma concentration/time curve (AUC). AUC was calculated by a linear trapezoidal rule from T 0 to 80 min. Hormone data were analyzed by ANOVA. If significant differences were detected, ANOVA was followed by a paired t-test with Bonferroni correction. Differences in scores for hunger and fullness were obtained by subtracting the feelings at 60 min from the baseline value. The differences between the treatments were compared using the same statistical procedures described above.

Results

All subjects tolerated the study procedures well. None of the volunteers experienced any side effects such as nausea.

Food Intake

The amount of food eaten and the corresponding caloric intake were both reduced after perfusion of fat into the duodenum *(Table 7.3.2)*. Indeed, when compared to the control treatment, 15 of 20 subjects ate less and consumed fewer calories with fat perfusion, but these effects did not statistically differ from controls. When ID fat was given with a water preload, the reduction in the amount of food eaten was 13%, resulting in an 11% reduction in caloric intake compared to the control experiment (ID saline and water preload). Fluid intake was not affected by ID fat, but eating time was reduced by 12%. An oral protein shake given in combination with ID saline perfusion significantly reduced the amount of food eaten (20%), with a corresponding 19% reduced caloric intake compared to the control experiment ($p < 0.01$ and $p < 0.01$, respectively). Fluid intake was not significantly affected, but eating time was reduced ($p < 0.05$). Finally, the administration of ID fat plus an oral protein shake preload resulted in the strongest reduction in the amount of food consumed (29%) and reduced caloric intake (27%). The reduction in food intake and caloric consumption was, however, neither significantly different from the combination protein shake preload plus saline ID perfusion nor from the combination water preload plus ID fat. Fluid intake was lowest with the combination of a protein preload plus ID fat, but the difference was only significant in comparison to the control treatment (water preload

with ID saline) ($p < 0.01$). The decrease in food and fluid intake after a protein preload and ID fat was accompanied by a significantly reduced eating time (*Table 7.3.2*, $p < 0.01$).

Table 7.3.2: Effect of ID saline or ID fat together with a preload of either water or a protein shake on eating behavior in 20 healthy male subjects.

Treatment: Preload/ID perfusion	**Food Intake (g)**	**Calories (kcal)**	**Eating time (min)**	**Volume imbibed (ml)**
a) Water/saline	470 ± 27	1243±74	26±2	361±44
b) Water/fat	408 ± 30	1100±78	23±2	310±40
c) Protein/saline	376±39†	1013±112†	21±2*	332±50
d) Protein/fat	334±34‡	906±102‡	19±2†	263±31†

Data are means ± SE. ID, intraduodenal. *= p<0.05, †= p<0.01, ‡= p<0.001, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

To further analyze potential interactions, the following contrasts were calculated: water/saline – protein/saline – (water/fat – protein/fat). The data presented in *Table 7.3.3* clearly indicate that the disparity between water/ID saline and protein shake plus ID saline on the one hand, and that between water/ID fat and protein shake plus ID fat on the other, showed no significant difference either for the amount of food eaten and the resulting caloric intake or for the amount of fluid imbibed.

Table 7.3.3: Effect of treatments on food parameters.

Parameter	**Food Intake (g)**	**Calories (kcal)**	**Volume imbibed (ml)**
Results	20 ± 36	35 ± 81	-18 ± 41
t-value	0.55	0.43	0.44
p-value	0.59	0.67	0.67

Data are means ± SE. Intrasubject differences between treatments were calculated by the formula: water/ID saline – protein/ID saline – (water/ID fat – protein/ID fat). Differences were analyzed by the paired t-test.

We also analyzed whether the potential interactions between the oral protein preload and ID fat were additive or positive/negative synergistic: the measured value from the combined treatment (the delta between protein preload plus ID fat and control) was compared to the calculated value of both treatments alone (the sum of the deltas between protein preload and control and ID fat and control). The data are presented in *Table 7.3.4*.

Table 7.3.4: Comparison of the mean (± SE) difference in calorie intake from the combined treatment of protein preload and ID fat with the calculated values of both treatments when given alone.

Parameter	Combined treatment	Treatments given alone
Calories [kcal]	337 ± 74	372 ± 94

Data are mean ± SE. The difference between the measured value from the combined treatment and the calculated value of both treatments alone was calculated by the formula: (water/ID saline – protein/ID fat) – [(water/ID saline – protein/ID saline) + (water/ID saline – water/ID fat)].

Eating behavior

The protein preload significantly influenced the mean VAS *(Figures 7.3.2a and 7.3.2b)*. Subjects experienced a reduced degree of hunger and a concomitant increased feeling of fullness in the premeal period with administration of the protein preload.

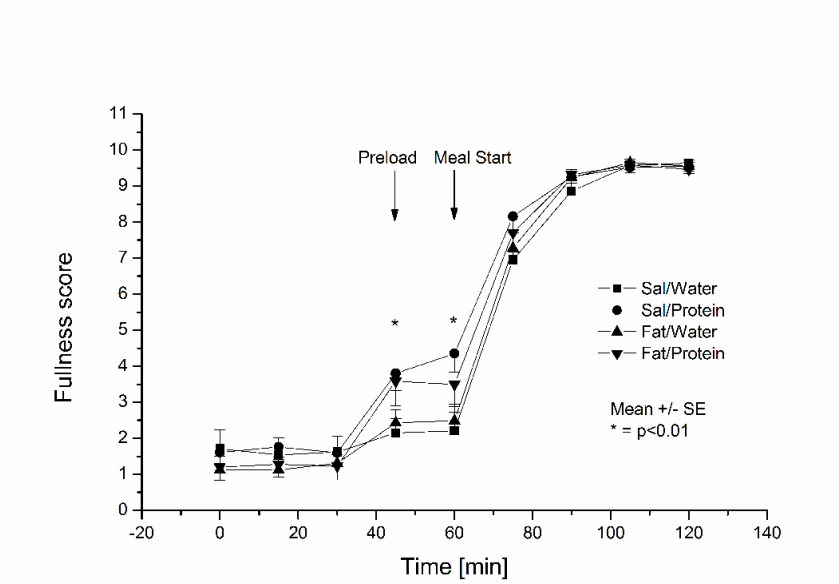

Figure 7.3.2a: Subjective sensations for fullness experienced by 20 healthy male subjects before and after food ingestion during ID perfusion of saline (Sal) or fat. Twenty minutes before food consumption volunteers received a preload of either water or protein (400 ml). Results are expressed as means +/- SE. * = $p<0.01$, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

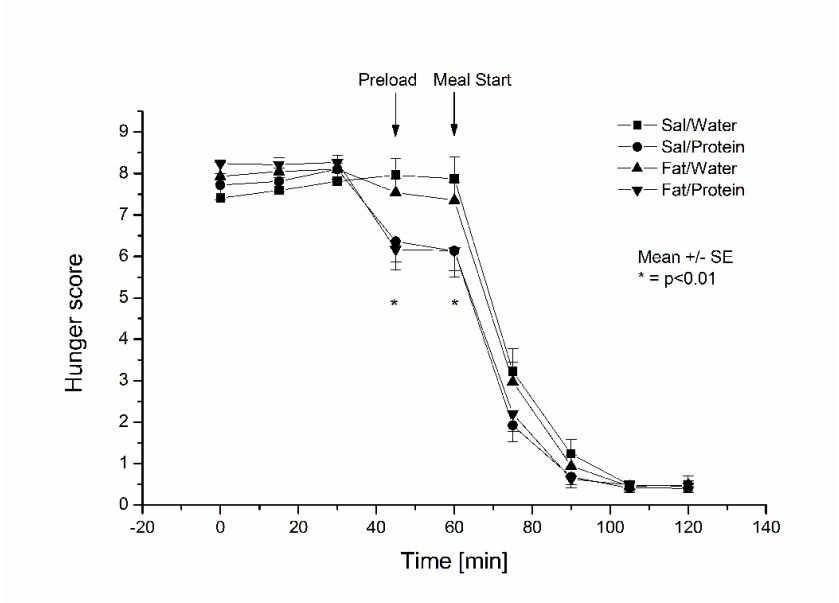

Figure 7.3.2b: Subjective sensations for hunger experienced by 20 healthy male subjects before and after food ingestion during ID perfusion of saline (Sal) or fat. Twenty minutes before food consumption volunteers received a preload of either water or protein (400 ml). Results are expressed as means +/- SE. * = $p<0.01$, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

When we compared baseline scores with 60 min values, the difference reached statistical significance *(Table 7.3.5)*. Subjects felt less hungry and fuller with the protein preload compared to ID saline or fat. Fat perfusion alone to the duodenum had no significant effect; furthermore, the combination of a protein preload plus ID fat was not more effective than protein preload plus ID saline. These data indicate that the protein preload was largely responsible for the observations.

Table 7.3.5: Baseline and 60 min scores after ID saline or fat with a preload of water or protein in 20 healthy male subjects.

Fullness	**Water/saline**	**Water/fat**	**Protein/saline†**	**Protein/fat***
Baseline	1.6 ± 0.4	1.1 ± 0.3	1.7 ± 0.3	1.2 ± 0.3
60min	2.2 ± 0.5	2.5 ± 0.4	4.4 ± 0.5	3.5 ± 0.6

Hunger	**Water/saline**	**Water/fat**	**Protein/saline***	**Protein/fat‡**
Baseline	7.5 ± 0.5	8.0 ± 0.3	7.8 ± 0.3	8.2 ± 0.3
60min	7.9 ± 0.5	7.4 ± 0.4	6.1 ± 0.5	6.1 ± 0.6

Data are mean ± SE. *p < 0.05, †p < 0.01, ‡p < 0.001, all vs. control (water/saline). Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

Plasma Hormones

During the control treatment (water preload plus ID saline), plasma hormone responses (CCK, PYY and GLP-1) remained stable in the premeal period (*Figures 7.3.3a and 7.3.3b*, data for CCK not shown).

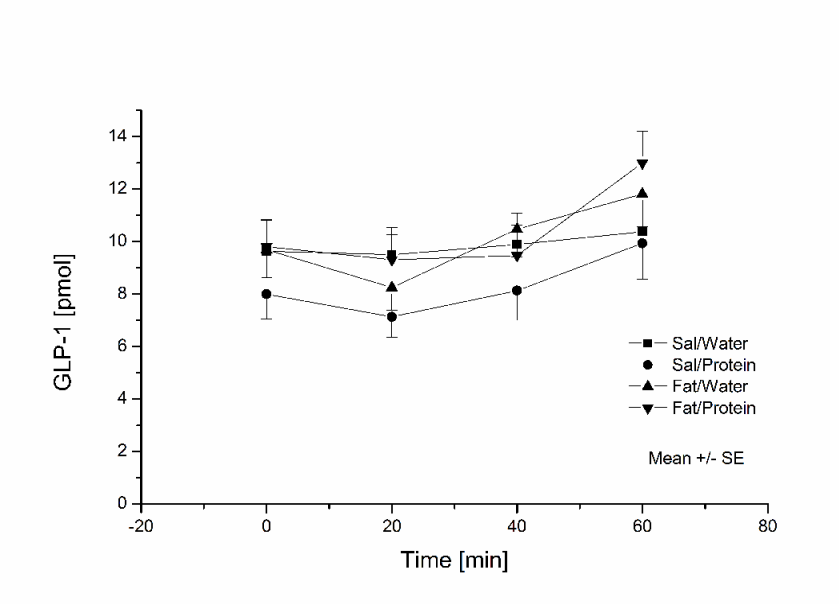

Figure 7.3.3a: GLP-1 plasma responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means +/- SE.

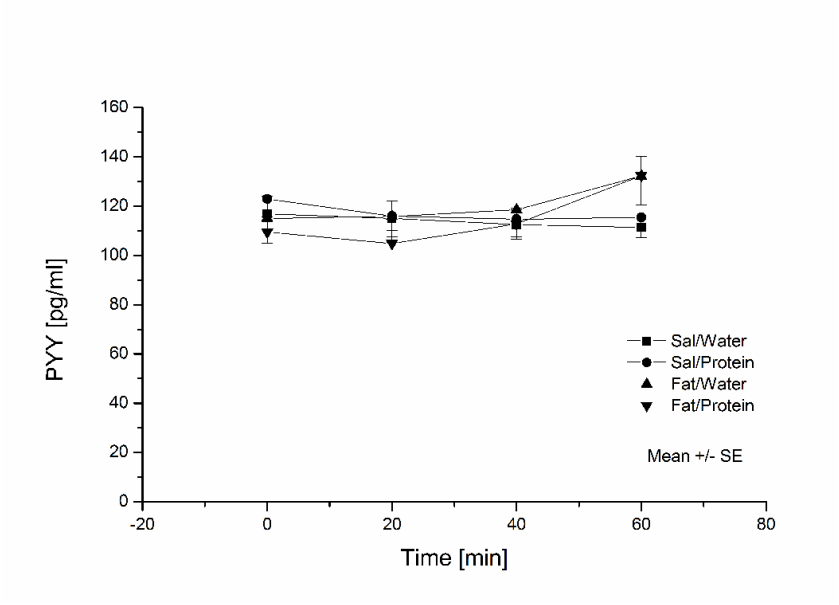

Figure 7.3.3b: PYY plasma responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means +/- SE.

The protein preload (400 ml) did not stimulate plasma CCK, PYY or GLP-1 concentrations. With the fat ID perfusion, PYY and GLP-1 concentrations slightly increased, but not significantly *(Figures 7.3.3a and 7.3.3b)*. However, the ID fat perfusion did evoke a significant increase ($p < 0.05$) in plasma CCK levels *(Figure 7.3.4)*.

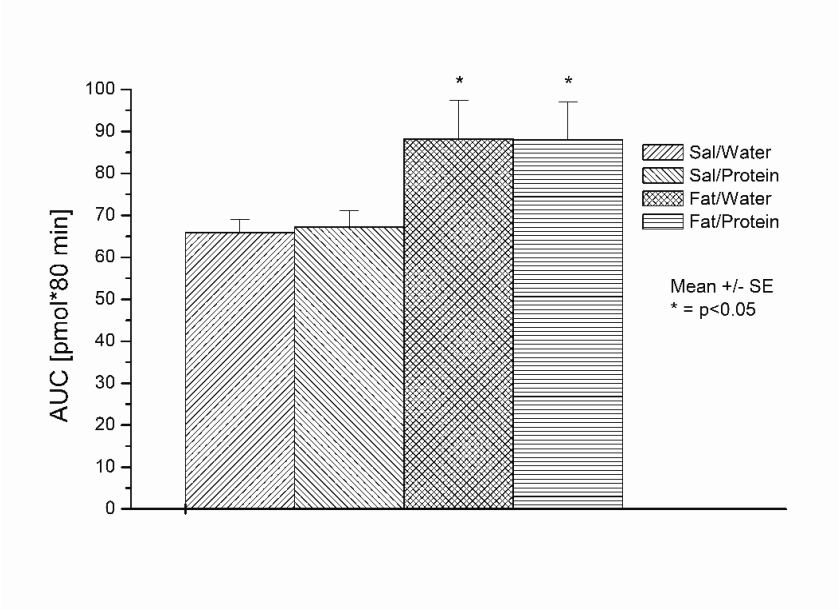

Figure 7.3.4: Area under plasma concentration/time curve (AUC) plasma CCK responses to ID perfusion of saline (Sal) or fat together with a preload of water or protein shake. Results are expressed as means + SE. * = $p<0.05$. Significant difference between control (water/saline) and ID fat plus water preload. Significant difference between control and ID fat plus protein preload and between ID saline plus protein preload and ID fat plus protein preload. Analyzed by ANOVA followed by multiple paired t-tests with Bonferroni correction.

Discussion

In the present study we have examined the interactions evoked by an oral protein preload with duodenal fat perfusion on food intake and appetite sensations in healthy male subjects.

The role of ID fat in initiating short-term satiation was first extensively explored in animals. On the basis of these observations, it was assumed that food intake is suppressed by stimulation of specific receptors within the GI tract. Inspired by this hypothesis, Welch *et al. (23)* observed that the infusion of a lipid emulsion into the ileum reduced food intake in healthy volunteers. Studies from our laboratory (15) have confirmed these findings as we could show that a fat perfusion to the

duodenum significantly reduced food intake compared to an ID saline perfusion. In the same study, it could also be shown that the inhibition of food intake in response to intestinal lipid was mediated by CCK. In the present study, ID fat perfusion alone also reduced the amount of food eaten (13% compared to placebo) and the total caloric intake (11% compared to placebo), but the reduction did not reach statistical significance. Although the design of the present study was similar to previous studies with respect to fat dose, experimental design and duration of fat perfusion, the variability of the individual responses to ID fat was greater than in previous studies and the reduction of food intake did not reach statistical significance. Fifteen volunteers ate less when ID fat was perfused (water as preload) compared to the control treatment, but the five remaining volunteers ate less under placebo conditions compared to a water preload and ID fat perfusion. Due to these results it can be speculated that certain individuals have a reduced sensation to ID fat.

It is well-established that, among all macronutrients, protein is more satiating than carbohydrate or fat as oral preloads (12). Several short-term studies have been done to examine the satiating effect of oral protein preloads in healthy human volunteers (4, 12, 19, 20, 22). These various studies compared a variety of nutrient preloads and examined the effect of amino acids given intraduodenally (2), but none has examined the interaction between an oral protein preload and ID fat. Our main interest was the investigation of potential interactions between gastric satiety signals induced by the protein shake and satiety signals induced by ID fat. Both macronutrients, when given alone, can reduce food intake and trigger satiety, but do they exert additive or synergistic effects when combined? We have previously seen that a nutrient-based preload interacts with ID fat (17), whereas gastric distension induced by a non-nutrient based distension with barostat did not produce such an effect (unpublished data). When we investigated the interaction of an oral carbohydrate-based preload in combination with ID fat, a synergistic inhibitory effect was observed. From these results we inferred that ID fat interacts with gastric signals to regulate food intake. The results of the present study illustrate that an oral protein preload with ID fat or ID saline reduced the amount of food eaten and the total caloric intake to a similar extent compared to the control treatment. There was no statistically significant difference between the two experimental conditions protein preload/ID saline and protein preload/ID fat. Further analyzing the data, the measured value from the combined treatment (the delta between protein preload plus ID fat and control) did

not differ from the calculated value of both treatments alone (the sum of the deltas between protein preload and control and ID fat and control). This result implies that the simultaneous administration of an oral protein preload and ID fat resulted in no synergistic reduction in caloric consumption, thereby rejecting the hypothesis that the two nutrients exert a positive synergistic effect on food intake. The potential interaction between an oral protein preload and ID fat seems to be additive. These observations have been made with one single dose of ID fat and oral protein. Different doses of ID fat and/or different amounts of a protein preload could show differing results with respect to potential interactions.

Two observations were unexpected: 1) the fact that the reduction of food intake caused by ID fat did not reach statistical significance and 2) that the interaction between an oral protein preload and ID fat seems to be additive. There are several possible explanations for these unexpected observations, and we will consider them with their relative limitations. One potential limitation is the time interval between the preload and the test meal. Gastric emptying is a major determinant in the regulation of food intake (7, 8, 17). Perfusion of fat to the small intestine has been shown to retard gastric emptying (7, 8). The rate of gastric emptying of the oral preload could be relevant, if the satiety effects induced by the preload are mediated by intestinal rather than gastric mechanisms. The stomach would therefore be fuller after the protein-rich preload with fat perfusion compared to the treatment with water preload plus ID saline perfusion. On the other hand, if the oral protein preload activates intestinal mechanisms rather than gastric signals, a delay in gastric emptying would retard activation of the intestinal mechanisms. Our initial hypothesis was based on the assumption that the oral preload would stimulate gastric signals, which would be synergistic to the intestinal mechanisms induced by fat. The results clearly illustrate that this is not the case. Another potential problem is the difference in taste between the oral protein preload and the water preload. Orosensory differences can affect eating behavior. This could have been avoided by a direct intragastric infusion of the preload, but at the expense of additional discomfort for the volunteers as this would have required a second tube.

ID fat stimulates the secretion of a number of gastrointestinal hormones, some of which are associated with the regulation of food intake. In the present study we measured the increase in plasma concentrations of the satiety peptides, CCK, GLP-1 and PYY. All three peptides have been shown to modulate short-term control of food

intake during a test meal intake. Here we observed a significant increase in plasma CCK after ID fat, confirming previous observations. On the other hand, no significant changes in plasma GLP-1 or plasma PYY concentrations occurred in the premeal period. GLP-1 is mainly stimulated by carbohydrates (14, 21) and less so by other macronutrients.

Feinle and coworkers (6, 7) have suggested that GLP-1 plasma levels rise after ID fat, but these findings could not be confirmed in the present study: a small, but non-significant, increase in GLP-1 secretion could be seen after 60 min of duodenal fat perfusion. It is conceivable that the 60 min perfusion of fat used in the present study was too short to induce a significant increase in GLP-1 release. On the other hand, the release of PYY is clearly dependent on the caloric load and on duodenal fat. The amount of fat delivered to the small intestine seems to be a crucial factor, as small loads of calories or fat are not associated with significant changes in plasma PYY concentrations (1). In the present study we could not detect any significant change in plasma PYY in response to the small duodenal fat load given. This finding concurs with previous observations investigating PYY responses to various types of nutrients (1). Indeed, the present study is one of the first attempts in humans investigating the effect of intraduodenal fat administration on the secretion of PYY. PYY is characteristically released in proportion to both the caloric content of a meal and its energy source composition. Increasing ingested amounts of an identical meal lead to proportionally increased plasma levels of PYY (1). With isocaloric meals consisting exclusively of either fat, carbohydrates, or proteins, the highest levels of plasma PYY were detected after the fat meal, followed by the carbohydrate meal, while very little PYY was noted with the protein meal (1). Under the present experimental conditions, neither ID fat perfusion nor the protein meal were able to stimulate significant amounts of PYY, indicating that the caloric load of both macronutrients was too small to induce PYY release. Data from our laboratory suggest that mixed meals with less than 500 kcalories do not stimulate PYY secretion (C Beglinger and G Gamboni, unpublished observations). Taken together, the plasma hormone data imply that the inhibitory effects of the oral protein preload on food intake and appetite sensation are not mediated by changes in circulating plasma hormone concentrations. Furthermore, the effects of duodenal fat are not mediated by changes in plasma GLP-1 and/or plasma PYY levels, but largely dependent on CCK release. These observations are in agreement with our previous findings: the reduction of food intake

induced by ID fat plus a liquid shake was reversed by administration of a CCK-A receptor antagonist (15, 17) suggesting that CCK is indeed the mediator of this effect. To summarize the findings of the present study, we have observed that an oral protein preload significantly reduced caloric intake. The protein preload and ID fat in combination resulted in no additive reduction in calorie consumption, which means that protein and ID fat do not exhibit synergistic effects on food intake. As a consequence, the satiety effects of an oral protein preload are not amplified by ID fat. A protein preload triggers GI signals to induce satiety, but this effect does not seem to be mediated by CCK, GLP-1 or PYY. Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety.

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Project 4

Chapter 7.4. Effect of PYY3-36 on food intake in humans

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Running title: PYY and human satiety

Submitted to: Gastroenterology

Abstract

Background and Aims*:** Studies in animals and humans suggest a role for peptide YY (PYY_{3-36}) in regulating satiety. The physiological role of PYY_{3-36} has, however, not been investigated in detail. ***Methods: The present study was therefore designed to examine the PYY release in response to two meals differing in their calorie content and to relate the plasma levels to those obtained after exogenous infusion. In a second step, the effect of graded intravenous doses (0, 0.2, 0.4 and 0.8 pmol/kg per minute) of synthetic human PYY_{3-36} on food intake was investigated in healthy male volunteers in a double-blind, placebo-controlled fashion. ***Results:*** Plasma PYY concentrations rose in response to food intake reflecting the size of the calorie load. Graded PYY_{3-36} infusions resulted in a dose-dependent reduction in food intake (maximal inhibition 35%, $p < 0.001$ vs control) and a similar reduction in calorie intake (32%; $p < 0.001$). Fluid ingestion was also reduced by PYY (18% reduction, $p < 0.01$). Nausea and fullness were the most common side effects produced by PYY, especially at the highest dose. Furthermore, subjects experienced less hunger and early fullness in the pre-meal period during PYY_{3-36} infusion at the highest dose ($p < 0.05$). ***Conclusions:*** This study demonstrates that intravenous infusions of PYY_{3-36} decreases spontaneous food intake; the inhibition is, however, only significant at pharmacological plasma concentrations. Whether PYY_{3-36} has a physiological role in the regulation of satiety in humans remains to be defined.

Introduction

The World Health Organization has classified obesity as an epidemic. Obesity and its associated pathologic features are major causes of illness and death worldwide. In the United States, obesity accounts for about 300'000 deaths annually, and at current rates of increase it will supplant smoking as the primary cause of preventable death (1, 18). To date there have been few effective treatments for obesity, although surgery has been shown effective in certain patient populations.

How do we decide when and how much to eat? Important advances have been made in the past 10 years in our understanding of the peripheral signals that regulate appetite and energy homeostasis (21-24). Several peptides synthesized and secreted within the gastrointestinal tract are known to modulate eating behavior: cholecystokinin, GLP-1, ghrelin and PYY . These peptides respond to nutrients within the gut and interact with specific receptors to modulate appetite (22).

Peptide tyrosine-tyrosine (PYY) is one of these gut-derived hormones. Like proglucagon-derived peptides, PYY is synthesized and released from endocrine L-cells from the distal gut in response to food consumption (2, 3). Fat is a strong stimulus for PYY release, whereas intravenously applied lipids have no effect on circulating PYY concentrations. PYY is converted into PYY_{3-36} by the enzyme dipeptidyl peptidase IV (4). Receptors that mediate the effects of PYY belong to the NPY receptor family and include Y1, Y2, Y3, Y4, and Y5 (4). Once PYY_{3-36} is formed, it binds with high affinity to the Y2 receptor. Recently, the effect of the truncated form of PYY, PYY_{3-36}, on appetite and food intake has been reported (5, 6). Intravenous infusion of a single dose of PYY_{3-36} reduced appetite and food consumption by > 30% in lean and obese subjects (5). The authors also reported that PYY_{3-36}, when injected into rodents, dampened appetite for 12 hours or more (6). The animal results were recently questioned, as several groups were unable to reproduce these effects (11). A dose response curve to increasing amounts of PYY_{3-36} on food intake in humans has, however, not been investigated before. To more fully characterize the potential appetite reducing effects of PYY_{3-36}, the present study was designed to investigate the effects of graded intravenous infusions of synthetic human PYY_{3-36} on food intake, meal duration, satiety and fullness feelings in healthy male subjects. The plasma concentrations achieved after exogenous infusion were then compared to

levels obtained after meal intake in order to test whether the appetite inhibiting effects occurred at physiological plasma concentrations.

Materials and Methods

Subjects

28 healthy subjects aged 23.6 ± 0.5 years participated in the study. The weight of all subjects was within normal range considering their age, sex, and height.

Each subject gave written informed consent for the study. The protocols were approved by the Human Ethical Research Committee of the University Hospital of Basel. Before acceptance, each participant was required to complete a medical interview, receive a full physical examination, and participate in an initial laboratory screening. No one was taking any medication or had a history of food allergies or dietary restrictions.

Part I: Plasma Peptide YY release after meal intake

Fasting and postprandial blood samples were taken from 12 healthy subjects aged 20-25 years. On different days and in random order subjects had blood samples taken after two different test meal stimuli: a light lunch (500 kcal) or a large lunch (1500 kcal); the meals were identical in their composition, but differed in their calorie content: orange juice as an appetizer (430 kcal per l); ham sandwiches (68 g bread, 10 or 20 g butter, 25 g ham; 284 or 357 kcal per sandwich) and chocolate pudding (133 kcal per 100 g). In each case the subjects had fasted for at least 6 hours before sampling.

Part II: Dose response curve to PYY_{3-36}

Four treatments, separated by at least 7 days, were performed in 16 male subjects. The treatments were identical in design *(Figure 7.4.1)* except for the intravenous infusion (isotonic saline as placebo control or one dose of PYY_{3-36}); the order of the experiments was randomized. An identical standard meal was presented to the subjects on each occasion. The meal consisted of a) orange juice as an appetizer (430 kcal per l); ham sandwiches (68 g bread, 10 g butter, 25 g ham; 284 kcal per sandwich) and more orange juice and chocolate pudding (133 kcal per 100 g); coffee

with cream and sugar (coffee could be sweetened if desired; therefore both cream and sugar were optional: 12 g cream = 20 kcal, 4.5 sugar = 18 kcal). No additional food or fluid was allowed during the study. At the end of the experiment, the amount of food eaten and the amount of fluid ingested was measured by absolute weight from which total calorie intake (food and fluid intake) was calculated.

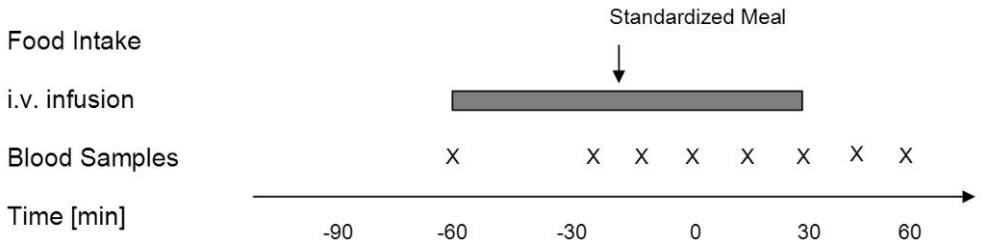

Figure 7.4.1: Daily time course of procedures for studies with exogenous PYY_{3-36}.

Each subject was free to eat and drink as much as he wished, but the order of food intake had to follow the above schedule. To reduce participant's awareness of the amount of food being provided, food was served in excess.

On the day of the experiment, each subject ate breakfast if this was his normal custom, but no snacks were allowed after 8.00 am. At 12.00 noon, an intravenous infusion of saline or one dose of synthetic PYY_{3-36} (0.2, 0.4 or 0.8 pmol / kg per min, dissolved in isotonic saline containing 0.1% human serum albumin) was started and continued for the duration of each test. Infusions were delivered by ambulatory infusion pumps through a teflon catheter inserted into a forearm vein. Participants were able to sit, eat, stand, and walk comfortably while receiving infusions. At 60 minutes after the start of the respective infusion, the test meal was presented and each participant was invited to eat and drink as much as he liked.

Beginning at 12.00 noon, the subjects scored their subjective feelings of hunger and fullness at 15 minute intervals throughout the experiments using a visual analogue scale of 0-10 and indicated the scores on a questionnaire. The scales and scores were designed as previously described (12-14). For example, a score of 0 for hunger indicated the subject was not hungry at all, 2 indicated slightly hungry, 5 indicated moderately hungry, 8 indicated very hungry, and 10 indicated absolutely ravenous.

The quantity of food eaten and volume of fluid drunk was measured. The time for each subject to complete his meal was also measured. From these observations, the average rate of food and fluid intake as well as the calorie intake could be calculated. In the pre-meal period and after eating, blood was drawn in regular intervals in ethylenediaminetetraacetic acid (EDTA) tubes containing aprotinine (500 KIU / ml blood) for hormone determinations. Adverse effects were assessed by the attending physician through close observation of each subject; in addition, each participant was questioned after each experiment and after he had completed all tests whether he had experienced any adverse effects.

Infusions

The PYY_{3-36} infusions were prepared from a freeze-dried synthetic powder, PYY_{3-36}, purchased from Bachem (Bubendorf, Switzerland). The peptide was dissolved in isotonic saline containing 0.5% human serum albumin, and prepared under aseptic conditions by the University of Basel Hospital Pharmacy. Aliquots of 50 µg/5 ml were stored at -20° C. Infusion solutions were prepared by diluting appropriate amounts of PYY_{3-36} with saline containing human serum albumin 0.1%. Control solutions contained albumin in saline alone; they were indistinguishable in appearance from PYY_{3-36} infusions.

The person in charge of the experiments was unaware of the respective treatment thereby making it possible to deliver treatments in a double-blind fashion.

Plasma Hormone Determinations

Specimen Collection and Storage: Samples were collected on ice with tubes containing aprotinin at a final concentration of 500 KIU/ml of blood; they were processed as quickly as possible and kept on ice to retard the breakdown of PYY.

Radioimmunoassay of PYY: PYY was measured with a commercially available kit (Linco Research Inc. St. Charles, Missouri, USA). The antibody, raised in guinea pigs, displays 100% cross-reactivity with human PYY1-36 and human PYY3-36, but no cross-reactivity with human pancreatic polypeptide, NPY and unrelated peptides such as leptin and ghrelin. ^{125}I-PYY was used as a label; the labeled peptide was purified by HPLC (specific activity 302 µCi/µg). The lowest level of PYY that can be detected by this assay is 10 pg/ml when using a 100 µl plasma sample size.

Ghrelin was measured with a commercially available kit (Linco Research Inc. St. Charles, Missouri, USA).

Statistical Analysis

Data are presented as mean ± SEM unless stated otherwise. The amount of food eaten and the amount of fluid drunk, the corresponding energy intake, and the duration of meal consumption were compared between the four treatments by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SPSS software package. In the event of significant differences, ANOVA was followed by the Dunnett multi-comparison test for pair-wise comparisons. The same statistical procedure was used to analyse the results of PYY_{3-36} induced changes in plasma hormone concentrations using area under the curve (AUC) analysis. Scores for hunger and fullness were compared at the different time points before and after the meal between the different treatment using multiple paired t-tests with Bonferroni correction.

Results

Peptide YY in plasma after meal intake

The mean fasting plasma PYY concentrations were 126 ± 6 pg/ml in 12 healthy subjects. After ingestion of the light lunch, a small, albeit significant ($p<0.05$) increase in plasma PYY concentration was observed *(Figure 7.4.2)*.

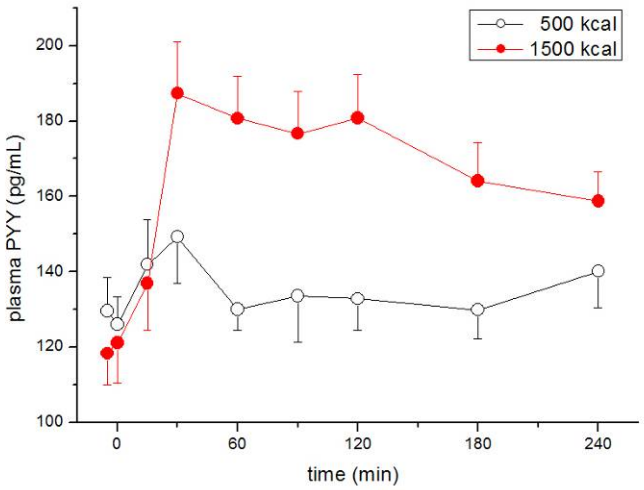

Figure 7.4.2: Plasma PYY concentrations after ingestion of a light lunch (500 kcal) or a large lunch (1500 kcal) in 12 healthy subjects. Data are mean ± SEM.

After ingestion of the large lunch, a marked and sustained increase in PYY levels was seen; the size of the postprandial PYY response clearly reflected the calorie load of the meal. The AUC of the postprandial PYY responses to the two meals is depicted in *Figure 7.4.3*.

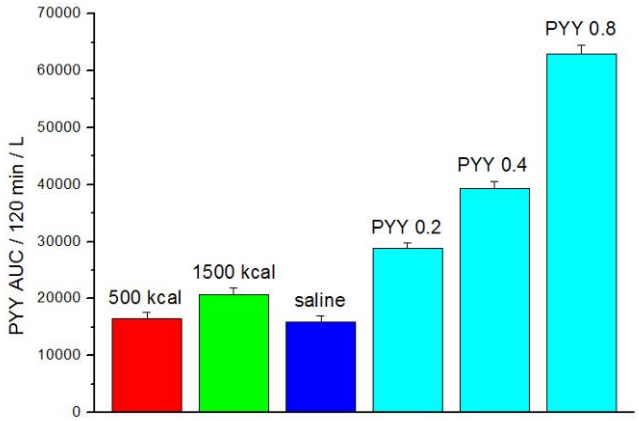

Figure 7.4.3: AUC over 120min of PYY measured in the plasma (pg/ml) in response to two different meals or to graded doses of intravenous PYY_{3-36} or placebo. Data are mean ± SEM.

Effect of graded infusion of PYY_{3-36} on food intake

Intravenous infusion of graded doses of synthetic human PYY_{3-36} dose-dependently reduced the amount of food eaten and the amount of calorie consumption ($p < 0.001$

and p < 0.01 respectively, *Table 7.4.1*). The maximal reduction in food consumption with the highest dose of PYY_{3-36} (0.8 pmol/kg per min) amounted to 35 % resulting in a decrease in calorie intake of 32 % (p < 0.001; *Table 7.4.1*). Fluid ingestion was also reduced by PYY_{3-36} (18% reduction, p < 0.01). Meal duration during PYY_{3-36} infusions were also dose-dependently decreased compared to those with saline infusion and reached statistical significance at the highest dose (p < 0.05).

Table 7.4.1: Effect of graded doses of human PYY_{3-36} or saline (control) on eating behaviour in 16 healthy male subjects (data are mean ± SEM). PYY_{3-36} doses are given in pmol/kg per minute.

Treatment	Control	PYY3-36 0.2 pmol/kg/min^{-1}	PYY3-36 0.4 pmol/kg/min^{-1}	PYY3-36 0.8 pmol/kg/min^{-1}
Food quantity (g)	587 ± 36	531.0 ± 35*	516 ± 40*	384 ± 34***
Calorie intake (kcal)	1627 ± 97	1520 ± 95	1451 ± 101*	1107 ± 84***
Meal duration (min)	38 ± 3	35 ± 3	34 ± 3*	30 ± 3*
Fluid intake (ml)	708 ± 57	748 ± 52	689 ± 48*	584 ± 45**

* p < 0.05, ** p < 0.01, *** p < 0.001 vs control.

Effect of PYY_{3-36} on eating behaviour

No statistical differences were observed for hunger and fullness scores with the two lower doses of treatment, neither in the premeal period nor after meal intake *(Figure 7.4.4)*. The highest dose of PYY reduced hunger feelings in the premeal period (change in hunger scores form baseline (-60 min) to begin of meal intake (0 min): 1.6 ± 0.3 for PYY vs 0.7 ± 0.3 for saline control (p<0.05).

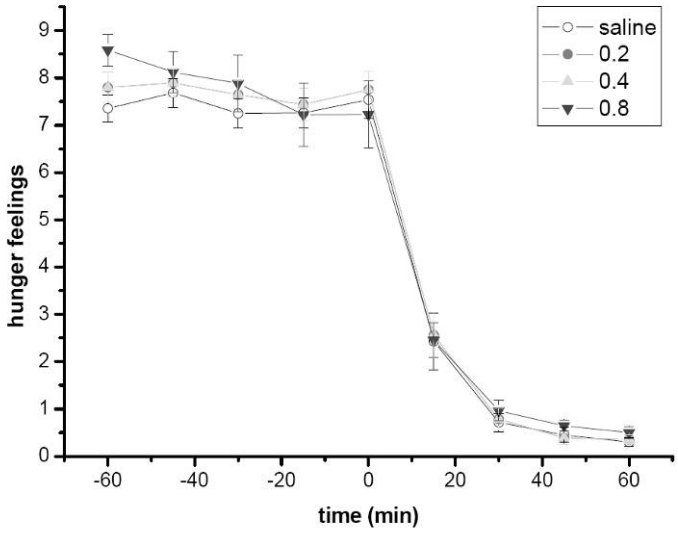

Figure 7.4.4: Subjective sensations of hunger experienced by healthy male subjects before and after food intake during intravenous infusion of saline (control) or one dose (0.2, 0.4 or 0.8 pmol/kg per minute) of human PYY_{3-36}. Results are expressed as mean ± SEM. N=16.

Effect of PYY_{3-36} on hormone levels

Graded doses of exogenous PYY_{3-36} produced dose-dependent increases in plasma PYY_{3-36} concentrations *(Figure 7.4.5)*.

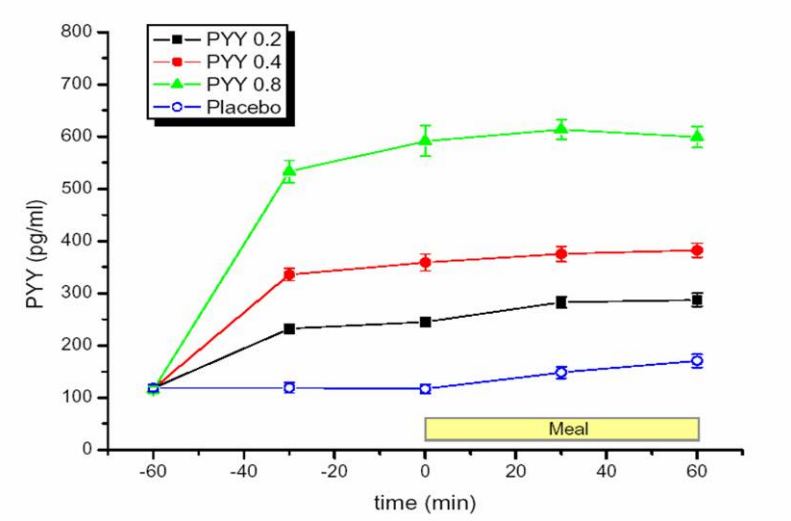

Figure 7.4.5: Plasma PYY (pg/ml) in response to graded doses of intravenous PYY_{3-36} or placebo. Data are mean ± SEM.

An excellent correlation (R=0.999, p< 0.001) was obtained between the infused dose and the measured plasma concentrations *(Figure 7.4.6)*. The PYY concentrations observed after the lowest dose of peptide infusion produced plasma levels within the postprandial range; they can therefore be considered physiological concentrations. The higher two doses of exogenous PYY_{3-36} produced plasma levels which are clearly above the postprandial range; we infer from these data that they are pharmacological rather than physiological plasma concentrations.

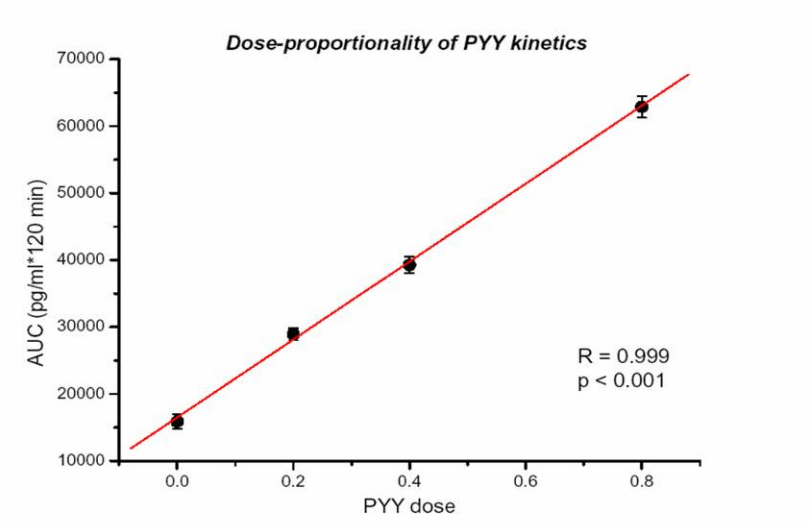

Figure 7.4.6: Correlation between graded doses of exogenous human PYY_{3-36} and plasma PYY concentrations (expressed as AUC). Data are mean ± SEM.

Ghrelin levels increased throughout the pre-meal period on the day the subjects received saline and then fell postprandially 30 min after the meal began *(Figure 7.4.7)*. The lower two doses of PYY_{3-36} did not significantly change fasting and postprandial ghrelin concentrations (data not shown). The highest dose of PYY infusion, however, significantly ($p<0.05$) decreased ghrelin levels during the premeal period and reduced the early increase after the begin of meal intake (see *Figure 7.4.7*). The area under the curve for ghrelin was 97677 ± 4637 pmol per 120 min/ml on the day subjects received saline and 84587 ± 5136 pmol per 120 min/ml on the day they received the highest dose of PYY_{3-36} ($P<0.05$).

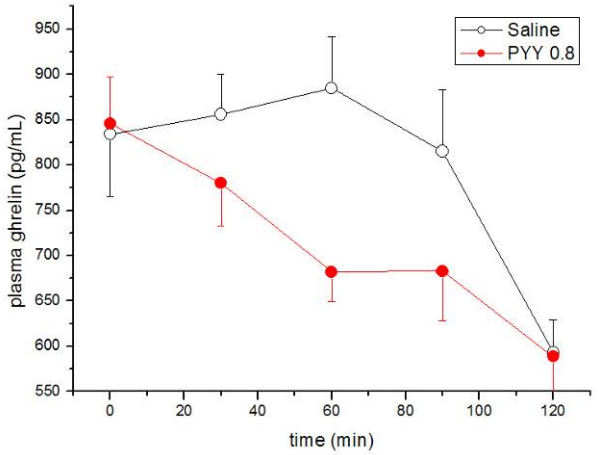

Figure 7.4.7: Ghrelin concentrations measured in plasma (ng/ml) in response to graded doses of intravenous PYY_{3-36} or placebo. Data are mean ± SEM.

Adverse events

The most common adverse events after intravenous PYY_{3-36} were nausea, abdominal discomfort and sweating. The adverse events were clearly dose-dependent and largely occurred with the highest dose of PYY_{3-36}. In one subject, the infusion had to be stopped prematurely (highest dose of PYY_{3-36}) because of vomiting. The plasma concentrations leading to nausea were in the order of >300 pg/ml (total PYY). This indicates a relatively narrow therapeutic range. All adverse events disappeared spontaneously within a few minutes without any specific treatment or after stopping the infusion.

Discussion

In animals, expression of Y2 receptors has been found in the hypothalamus, the medulla, pons, but not in the cortex (7, 9, 15). Furthermore, PYY immunoreactivity has been reported in the CNS, in the hypothalamus, the medulla and pons (9, 15, 20). With the presence of Y2 receptors at sites where administration of exogenous PYY_{3-36} appears to cause satiety, one is faced with attempting to determine if the satiety effect of PYY_{3-36} is physiological, and if so, whether it is a major satiety factor. Recent data obtained in rodents and humans have provided experimental evidence that PYY_{3-36} can function as a mediator of food-induced satiety. Intra-arcuate injection of PYY_{3-36} reduced food intake in mice. In addition, the effects of PYY_{3-36} were abolished in $Y2^{-/-}$ mice. Intraperitoneal PYY_{3-36} reduced dark phase and fasting-induced feeding in rodents (6). Repeated PYY_{3-36} administration reduced food intake and body weight gain. Finally, similar anorectic effects were seen in humans (4-6). These findings prompted the authors to suggest that PYY_{3-36} is a potent physiological regulator of satiety with a potential for therapeutic application. However, other laboratories were unable to reproduce the results in laboratory animals causing a controversy on the biological importance of PYY_{3-36} as a physiological satiety factor (11). A set of criteria has been defined in classical endocrinology for establishing a physiological endocrine action of a given molecule (10). According to these criteria, a physiological action of PYY_{3-36} has not yet been established and urgently requires further research.

The purpose of this study was therefore twofold: 1) hormone secretion: first, we studied the effect of two different calorie loads of an identical meal on PYY secretion in order to define the range of postprandial plasma PYY concentrations; 2) second, we constructed a dose response curve and examined the effect of graded doses of intravenous synthetic human PYY_{3-36} on eating behaviour and satiety in healthy male subjects in order to define the physiological dose of the peptide that reproduces the secretion pattern of the endogenous peptide that is associated with changes in food intake. In the following we will analyze our results according to these criteria.

Secretion. PYY is synthesized and released by the L-cells in the distal small intestine (2). Although the specific stimuli for PYY secretion are unknown, the increases in plasma concentration of PYY after meals and the low concentrations in the fasting state are consistent with a satiety-inducing action. The circadian pattern of PYY secretion has, however, not been studied in humans in detail yet. The results of the present study confirm that only large meals are able to stimulate the release of larger amounts of PYY into the circulation, whereas a 500 kcal meal has minimal effects on postprandial hormone concentrations (2). Graded intravenous infusions of PYY_{3-36} increased plasma PYY concentrations 2-5 fold over fasting levels, indicating that only the lowest dose mimicked physiological PYY levels, whereas the upper two doses produced plasma levels which were clearly out of the physiological range.

Physiological dose. The results of the study clearly illustrate that dose-dependent satiety effects can be induced by peripherally infused PYY_{3-36} in human subjects; the results support the hypothesis that exogenously administered PYY_{3-36} can suppress food intake in man. The lack of a specific PYY_{3-36} receptor antagonist that could be given to humans prevents us for the moment from deciding whether the effects produced by the exogenous administration of PYY_{3-36} (as used in this study) are true physiological effects. Comparison of the plasma concentrations seen after exogenous infusion to the levels seen after a 1500 kcal meal suggests, however, that the significant satiety effects of PYY_{3-36} were only seen at plasma concentrations, which were above those of a high calorie meal. Indeed, the pharmacological nature of the upper two doses is indicated by these observations, as a significant inhibitory effect on food parameters was only observed under these experimental conditions. Thus more work is required to determine if PYY_{3-36} meets the criterion for a fully coupled physiological hormonal effect. We infer that the results of this study represent a pharmacological rather than a physiological effect of PYY_{3-36}. The early

reports form Batterham and coworkers (5, 6) used only one dose of peptide to induce satiation in healthy subjects and in obese people; the plasma concentrations observed in these experiments suggest that a pharmacological dose of PYY_{3-36} was infused. More important, the inhibition of feeding induced with these pharmacological doses was accompanied with subjective side effects in the present study, whereas the physiological dose (0.2 pmol/kg per min) was insufficient to reduce meal size or calorie intake. The results therefore indicate that under these experimental conditions PYY_{3-36} satiation does not meet the criterion for a physiological hormonal effect.

Mechanism of action. The mechanism by which PYY_{3-36} inhibits food intake is not clear and could possibly be due to different actions. Is the effect directly mediated by binding to peripheral or central receptors or is it mediated through stimulation of other satiety factors? The question cannot be answered at the present time as a demonstration of a direct action of PYY_{3-36} would require experiments with a selective PYY_{3-36} receptor antagonist specifically blocking endogenous PYY_{3-36}. Is the effect peripheral or mediated by central receptors? Does PYY_{3-36} act as a hormone and does it cross the blood-brain barrier? A direct central mechanism of PYY_{3-36} rather than a peripheral effect is derived from an experimental model of the blood brain barrier (16, 17, 19). These data suggest that PYY_{3-36} is selectively transported through the blood brain barrier.

Does PYY_{3-36} inhibit food intake by stimulating the release of other peptides which are known to be involved in the regulation? In the present study we have measured the effect of PYY_{3-36} on plasma ghrelin concentrations. The results presented in this study confirm that ghrelin levels are decreased in response to high doses of PYY_{3-36} in the premeal period (5). Plasma levels of ghrelin rise before a meal ingestion and administration of ghrelin increases food intake in humans suggesting that ghrelin has a role in the regulation of meal initiation (8, 25). The suppression of ghrelin levels seen with high doses of PYY suggest an interaction between these two regulatory circuits. Whether this interaction is a pharmacological effect or a true physiological action remains to be determined.

In conclusion, we have shown that graded doses of human PYY_{3-36} reduce intake of food in nonobese, healthy male subjects. The effect is a pharmacological rather than a physiological action of the peptide. The mechanism of action has to be clarified. Further investigation is needed to define a potential physiologic role of PYY_{3-36} in the control of human food intake. Whether the peptide can emerge as a powerful

antiobesity drug remains to be seen; the present results suggest that the therapeutic window is rather narrow.

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Chapter 8: Discussion and Outlook

In the past three decades much insight has been gained into physiological processes that regulate food intake and appetite. Within this control circuit satiety peptides like CCK, GLP-1 and PYY have received a lot of attention. The information with respect to their mechanism of action and potential interactions between different physiological signals involved in the short-term regulation of satiety are still limited. The main interest of this thesis is focused on a further characterization of some of those signals; we were especially interested in the investigation of potential interactions between individual satiety factors.

Previous study results had suggested that distension of the distal stomach could play a role in the generation of satiation (6, 9, 10). Therefore the **first project (chapter 7.1.)** was designed to further understand the role of the gastric fundus and the antrum in triggering satiation in healthy male volunteers. In contrast to previous studies with a similar background but with serious methodological concerns, we chose a different approach (3, 4). The balloon was precisely positioned under fluoroscopic control and the barostat method was chosen to distend either the fundus or the antrum.
When the fundus was distended, food intake was not reduced compared to the control treatment and the effect on satiety feelings was only short-lasting. This fact can explain that the subsequent food intake was not influenced by the balloon distension. Gastric distension seems to trigger satiety as long as mechanoreceptors in the stomach are stimulated; the short-lasting effect could indicate that the signals are transmitted via vagal afferent nerves to the CNS.
The second part of this study was designed to examine the effect of antrum balloon distension (0, 300 ml) on subsequent food intake; in addition it was investigated whether ID fat could intensify the effect of distal gastric distension. Calorie intake was not changed neither with gastric distension alone nor in combination with ID fat. Feelings of hunger or fullness were unaltered compared to the control treatment, these results show that the volume of 300 ml was obviously too small to significantly influence satiety feelings. ID fat does not seem to intensify gastric satiety signals induced by pure mechanical distension of the stomach.

Finally, the satiety hormones CCK and PYY were measured. Neither fundus nor antrum distension altered CCK, respectively PYY plasma concentrations compared to placebo. This fact implies that signals induced by pure mechanical gastric distension are not mediated by CCK or PYY. During ID fat perfusion CCK and PYY plasma concentrations were significantly increased. The increase of CCK after ID fat confirmed previous study results (7). However, this study was one of the first which could show an increase of PYY after ID fat in humans.
Based on these study results we infer that pure mechanical gastric distension of the fundus or the antrum is not a sufficient satiety signal to influence subsequent food intake.

An interaction effect on food intake resulting from an intestinal and a gastric satiety signal has previously been explored for CCK, but not for GLP-1. It was therefore of interest to find out whether an interaction exists between a preload and i.v. GLP-1.
In the **second project (chapter 7.2.)** GLP-1 was given i.v. in a dose which mimics physiological GLP-1 plasma concentrations and reduced calorie intake confirming previous study results (5, 12). One major observation of this study was the demonstration that a protein-containing preload together with i.v. GLP-1 enhanced the satiety-inducing effects of GLP-1 compared to a water preload plus infusion of GLP-1. This result provides strong evidence that GLP-1 interacts with gastric signals to modulate food intake and satiety in humans.
We inferred that GLP-1 is an important satiety factor which interacts with other satiety signals in order to control food intake and satiety. However, it still remains unclear whether the satiety effects of GLP-1 are directly mediated through peripheral or central receptors or indirectly by releasing other satiety peptides.

The **third project (chapter 7.3.)** was designed to further understand the potential interaction between protein and fat in regulating food intake in humans. From previous study results (7) we inferred that ID fat interacts with gastric signals to regulate food intake. In our study we were above all interested in exploring the interaction of the stomach and the small intestine and secondly we wanted to see whether GLP-1 and PYY are associated with this interaction.
ID fat perfusion alone reduced the amount of food eaten and the total calorie intake, but the reduction did not reach statistical significance. Although the design of the

present study was similar to previous studies with respect to fat dose, experimental design and duration of fat perfusion, the variability of the individual responses to ID fat was greater than in previous studies and the reduction of food intake did not reach statistical significance. Due to these results it can be speculated that certain individuals have a reduced sensation to ID fat. The effects of ID fat on food intake do not seem to be mediated by changes in plasma GLP-1 or PYY levels, but they are largely dependent on CCK release, which is in agreement with previous findings (7). When subjects consumed an oral protein preload, calorie intake was significantly reduced compared to the control treatment. The increase of premeal plasma concentrations of GLP-1 and PYY did not differ compared to placebo. Therefore the inhibitory effect of oral protein on eating behavior is not mediated by changes in circulating plasma hormone levels.

The simultaneous administration of an oral protein preload and ID fat did not show a synergistic reduction in calorie consumption, rejecting the hypothesis that oral protein and ID fat exert a positive synergistic effect. There are several explanations for this unexpected result: 1) the observations of this study have been made with one single dose of ID fat and oral protein. Different doses of ID fat and/or different amounts of a protein preload could show differing results with respect to potential interactions; 2) a potential limitation could be the time interval between the preload and the test meal. It was assumed that the satiety effects of the oral protein preload would mainly be mediated by gastric signals. Otherwise, if those effects would be mediated by intestinal signals, the delay in gastric emptying by fat must have been considered; 3) another important limitation of this study design was the difference in taste between the protein and the water preload. Orosensory differences can affect eating behavior. This could have been avoided by a direct intragastric infusion of the preload with a second tube, but an oral administration of the protein preload was chosen to keep the discomfort for the volunteers on a minimal level.

Project 4 (chapter 7.4.) examined the physiological and the pharmacological role of PYY in regulating eating behavior. Due to human study results it was supposed that PYY (3-36) is a potent physiological regulator of satiety (1, 2) with a potential for therapeutic application.

Because the physiological role of PYY in humans has not been investigated in detail, we first wanted to define a range of physiological PYY plasma levels after two meals

differing in their calorie content. The results showed that only large meals are able to stimulate the release of larger amounts of PYY, whereas a low-calorie meal has minimal effects on postprandial hormone plasma levels.

In a second part we wanted to examine the effects of graded doses of i.v. PYY (3-36) on eating behavior in healthy human subjects. We found a dose-dependent satiety effect of i.v. applied PYY (3-36). These results support the hypothesis that exogenously administered PYY (3-36) is able to suppress food intake in humans. However, when the postprandial physiological levels of PYY after the high calorie meal are compared to those obtained after peripheral administration, it can be seen that the significant satiety effect of PYY (3-36) is only seen at plasma concentrations higher than those after a large meal. The smallest administered dose of PYY (3-36) did not significantly reduce food intake and showed PYY plasma levels in the same range as the meal stimulated concentrations. Due to these results we infer that the PYY satiety effects seen with the middle and the highest dose of exogenous PYY (3-36) is rather a pharmacological than a physiological effect. Due to methodological difficulties these suggestions are supported by the fact that total PYY and not only PYY (3-36) was measured; the difference between physiological PYY plasma concentrations and those obtained after exogenous administration of PYY (3-36) would therefore be even greater. It seems to be unlikely that PYY is a major physiological satiety factor, but still more information is necessary.

Dose-dependent side effects of PYY (3-36) like nausea and vomiting could be observed after the middle and especially after the highest dose. PYY (3-36) seems to have a narrow therapeutic range, which could limit its therapeutic potential.

Interactions between different satiety signals seem to be important in triggering satiety, but the mechanisms are difficult to detect and more research in this field is necessary.

The development of more specific receptor antagonists of satiety peptides would be needed to further examine their mechanisms of action and their physiological role in the short-term regulation of food intake. A selective PYY (3-36) receptor antagonist could be useful to further analyze the mechanism by which PYY (3-36) inhibits food intake and it could help to answer the question whether PYY really is a physiological satiety factor.

It has become apparent, that redundant systems are active in the regulation of food intake. That means that a peptide may be replaced by another. However, not enough information is available with respect to these redundant systems. An additional problem is the role of interactions between different satiety signals, which have hardly been explored. One group has recently examined possible synergistic interactions between exendin-4 and PYY in mice and their findings suggest that administration of low doses of exendin-4 together with PYY (3-36) may increase the suppression of food intake (11). Further research in humans will be necessary.

Another chance of future research lies in the field of therapeutic treatment of obesity. Nastech Pharmaceutical Company, to give an example, has developed a nasal spray with PYY (3-36) and has already initiated a phase I trial (8). However, one problem could be the narrow therapeutic window of PYY (3-36). It still has to be proved that PYY (3-36) really has the potential for therapeutic application.

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Curriculum vitae

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Publications

Original articles

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Abstracts

Oesch S, Degen L, and Beglinger C. Protein induced satiety is not altered by fat in healthy human subjects. Digestive Disease Week (DDW), May 2004, New Orleans, USA (Poster presentation)

Oesch S, Degen L, and Beglinger C. Protein induced satiety is not altered by fat in healthy human subjects. Jahrestagung der Schweizerischen Gesellschaft für Gastroenterologie und Hepatologie (SGGH), September 2004, Montreux, Switzerland (Poster presentation and Poster-Prize)