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Signals Arising from the Gastrointestinal Tract that Affect Food Intake

Doctoral dissertation

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ABSTRACT

Excess energy intake, in relation to expenditure, leads to weight gain and eventually to obesity. Obesity is rapidly increasing in Western countries and is associated with an increased risk of several diseases and a significantly higher mortality rate. Control of food intake consists of complex interplay between the peripheral body and the central nervous system. The gastrointestinal tract secretes several peptide hormones that participate in the control of feeding behaviour. The release of these peptides is controlled by energy status and nutrients. In addition to food intake, gastrointestinal peptides control digestion and gastrointestinal motility. This study investigated the effects of food components on the release of gastrointestinal peptides and their effects on food intake.

Our everyday diet is rich in plant lectins, proteins with carbohydrate binding capacity. In this thesis we showed that human gallbladder contraction was effectively stimulated by red kidney bean lectin phytohaemagglutinin (PHA). Intraduodenal administration of PHA induced gallbladder contraction via a cholinergic mechanism, without affecting peripheral plasma cholecystokinin (CCK) levels.

Dietary fibre is considered as a key element in a healthy diet. Postprandial glucose and insulin lowering effects of fibre have been shown to be dependent on its viscosity. In this thesis we demonstrate that viscosity also affects other postprandial gastrointestinal peptide responses and satiety. Interestingly, low-viscous oat bran beverage evoked higher satiety scores compared to otherwise similar but high-viscous beverage. This was in accordance with significantly increased releases of the hormones CCK, peptide YY (PYY) and glucagon like peptide 1 (GLP-1) as well as the more efficiently suppressed ghrelin levels after the consumption of the low-viscous beverage compared to high-viscous one. Our results indicate that the rheological properties of dietary fibre significantly affect the postprandial responses and satiety.

Spices, besides giving flavour to food, also induce thermogenesis and may affect satiety. Pungent ingredients of wasabi, mustard oil and garlic are known to activate TRPA1 (transient receptor potential ankyrin 1) channels. We found TRPA1 expression in mouse and human duodenum and revealed that activation of these channels caused a robust increase in CCK secretion from the mouse intestinal neuroendocrine cell line STC-1 suggesting that these spices may improve digestion and affect satiety by stimulating CCK release.

PYY, secreted from the ileum and colon, has been proposed to be a satiety peptide because it has been shown to inhibit food intake in humans and in rodents. We performed proctocolectomy in rats in order to create a surgical knock-out model for PYY. Colectomized rats were implanted with minipumps delivering PYY(3-36) or saline. In contrast to our expectations, colectomy increased rather than decreased plasma PYY levels. In spite of significantly different plasma PYY levels between the treatment groups, no difference in daily food intake or body weight gain was observed.

In conclusion, this thesis shows that components in our food significantly affect gastrointestinal functions and peptide secretion. Thus by modulating the meal composition we may be able to affect food intake and satiety.

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ABBREVIATIONS

α-MSH	alpha-melanocyte stimulating hormone
AgRP	agouti-related peptide
AITC	allyl isothiocyanate
BBB	blood-brain barrier
BMI	body mass index
CART	cocaine and amphetamine-related transcript
CB-1	cannabinoid receptor 1
CCK	cholecystokinin
CCK-RP	CCK-releasing peptides
CGRP	calcitonin-gene related peptide
CNS	central nervous system
DBI	diazepam binding inhibitor
DPP-4	dipeptidyl peptidase 4
DVC	dorsal vagal complex
FFA	free fatty acid
GHS-R1a	growth hormone secretagogue receptor type 1a
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide 1
GLP-1R	GLP-1 receptor
IBS	irritable bowel syndrome
LCRF	luminal CCK releasing factor
MC3/4	melanocortin receptors 3 and 4
MCH-1	melanin-concentrating hormone receptor 1
NPY	neuropeptide Y
NTS	nucleus tractus solitarius
OGTT	oral glucose tolerance test
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
POMC	pro-opiomelanocortin
PYY	peptide YY
RR	ruthenium red
TPN	total parenteral nutrition
TRP	transient receptor potential
TRPA	transient receptor potential ankyrin
TRPV	transient receptor potential vanilloid receptor
VAS	visual analogue scales

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their corresponding Roman numerals:

I   **Purhonen, A.K.**, Herzig, K.H., Gabius, H.J., André, S., Ketterer, S., Matzinger, D., Beglinger, C., and Hildebrand, P. (2008). Duodenal phytohaemagglutinin (red kidney bean lectin) stimulates gallbladder contraction in man. Acta Physiol (Oxf) *In press*.

II   Juvonen, K., **Purhonen, A.K.,** Salmenkallio-Marttila, M., Lähteenmäki, L., Laaksonen, D., Herzig, K.H., Uusitupa, M., Poutanen, K., and Karhunen, L. (2008). Effect of viscosity of oat bran on gastrointestinal responses in healthy humans. *Manuscript*.

III   **Purhonen, A.K.**, Louhivuori, L.M., Kiehne, K., Åkerman, K.E.O., and Herzig, K.H. (2008). TRPA1 channel activation induces cholecystokinin release via extracellular calcium. FEBS Lett *582,* 229-232.

IV   Babu, M.,* **Purhonen, A.K.,*** Bansiewicz, T., Mäkelä, K., Walkowiak, J., Miettinen, P., and Herzig, K.H. (2005). Effect of total colectomy and PYY infusion on food intake and body weight in rats. Regul Pept *131,* 29-33.
     * Equal contribution

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1 INTRODUCTION

From the days of undernutrition, we have come to the time where the increasing prevalence of obesity is a significant medical and social problem in most of the Western countries. In Finland, 60 % of men and 40 % of women were overweight (body mass index, BMI $\geq$ 25 kg/m^2) and 15 % of men and 14 % of women were obese reaching the BMI of $\geq$ 30 kg/m^2 in 2005 (Helakorpi et al., 2005). In the United States between 1980 and 2004 the prevalence of obesity has increased from 15 % to 33 % among adults and alarmingly, from 6 % to 19 % amongst children (Ogden et al., 2007). Obesity is clearly a risk factor for the development of type 2 diabetes, cardiovascular disease, dyslipidemias, fatty liver, Alzheimer's disease, vascular dementia and some cancers thus causing potential health risk for the individual as well as economical burden for the social health care system. In developing countries adapted to a Western lifestyle the rates of obesity have tripled suggesting that soon in the near future, obesity will be a real global epidemic (Hossain et al., 2007).

Overconsumption of energy in relation to expenditure leads to obesity. Decreased physical activity and increased energy intake both contribute to the gain of excess body weight. In modern Western society with its sedentary lifestyle, it may be easier to reduce daily energy intake together with increasing expenditure than control body weight by solely increasing physical activity. Increased food intake has recently been suggested as the main cause of increased body weight (Jeffery and Harnack, 2007). However, regulation of feeding behaviour is complex and still incompletely understood. In addition to its essential purpose to satisfy the energy needs of the body, ingestive behaviour is also associated with social, cultural and environmental factors which may hinder the homeostatic control systems to appropriately control the energy balance.

The sophisticated control of feeding behaviour consists of a complex crosstalk between the brain and peripheral body to control food intake in order to meet the energy needs of the body. The central nervous system (CNS) receives information about the peripheral energy status via a variety of circulating and neural signals arising from the gastrointestinal organs and adipose tissue. This information is interpreted in the CNS and implicated as induction of either orexigenic or anorexigenic behaviour. For example, in the fasting state ghrelin as a hunger signal is secreted from the stomach and induces food intake. During a meal, nutrients in the gastrointestinal lumen elicit an array of postprandial signals that contribute to the perception of satiation and lead to termination of the meal. These postprandial signals are affected by the macronutrient composition, calorie content and structural properties of the ingested food. Within the CNS, specialized areas including hypothalamus and brain stem are responsive to the peripheral signals and integrate the afferent information with the inputs from higher cortical areas thus being key centres in the appetite control.

Our knowledge about this very multimodal and complex regulatory network is still limited and thus more intensive research is needed to better understand the physiological mechanisms behind the control of energy homeostasis. In the growing prevalence of obesity, development of healthier foods which

produce higher satiety might be one asset to combat the epidemic on the population level. This study aimed at investigating peptides released from the gastrointestinal tract in response to nutrients, their secretion and significance in the control of food intake.

2 REVIEW OF THE LITERATURE

2.1 CONTROL OF FOOD INTAKE – GUT-BRAIN AXIS

The central nervous system receives information about the whole body energy balance from the peripheral tissues through metabolic, neural and endocrine signals and accordingly regulates energy homeostasis. Considering the fact that digestion and nutrient absorption take place in the gastrointestinal tract whereas sensation of hunger or satiety arise in the CNS, a tight reciprocal communication is required between these organs. Thus gastrointestinal hormones and neural signals are key components in the control of food intake.

In the cephalic phase of digestion, priming of the gastrointestinal tract for food to optimize digestion and absorption is triggered by sight and smell even before the food is physically present in the oropharyngeal cavity. Efferent signalling from the CNS towards the gastrointestinal tract occurs mainly via the vagus nerve stimulating gastric, duodenal, pancreatic and biliary secretion. During a meal, ingested food interacts with both the stomach and intestine, eliciting an array of gastrointestinal peptides as well as an arousal of neural signals that function to coordinate and optimize the digestive process and eventually cause the feeling of satiation leading to the termination of the meal. These signals are conveyed to the CNS via circulation or via the afferent fibres of the vagus nerve. Peripheral signals arising from the gastrointestinal tract are mostly short-term meal-related signals affecting the daily intake in contrast to, for example, adipose tissue derived signals such as leptin that reflects more the long-term energy balance. In addition, the endocrine pancreas secretes peptide hormones such as insulin, which participate in the control of food intake. All these signals are integrated and interpreted in the CNS, forming the basis for the central metabolic regulation and maintaining a stable body weight despite of day-to-day fluctuations in the energy intake (Figure 1).

2.1.1 Stomach

The stomach acts as a reservoir of ingested food and is responsible for mixing and grinding of solid foods as well as modulating the emptying of the chyme into the duodenum. Regulation of gastric emptying ensures optimal digestion of ingested foodstuffs and the absorption of the liberated nutrients in the intestine. Nutrient composition and solidity of the meal affect gastric emptying patterns. Chyme entering the duodenum affects the gastric emptying via the release of peptide hormones that act as part of the negative feedback mechanism, which prevents an overflow of nutrients into the duodenum and thus maldigestion. Indeed, satiety peptides like peptide YY (PYY) and cholecystokinin (CCK) inhibit gastric emptying among their other functions.

Gastric cells are able to sense nutrients and control, for example, gastrin secretion accordingly yet the satiation signals arising from the stomach are mainly evoked by mechanical distention and not the composition of the ingesta. The stomach is densely innervated by sensory vagal and splanchnic nerves

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sensitive to tension, stretch and volume that relay this information to the CNS. Early studies with pyloric occlusion in rats suggested that gastric distention alone is sufficient to terminate ingestion but the amount of food required for this exceeds the amounts eaten in a typical meal. However, in the normal situation, contents of the stomach enter the duodenum already during the meal stimulating intestinal peptide release and neural signalling, which, together with gastric distention, may contribute to the satiation (Ritter, 2004).

In addition, the stomach secretes substances that participate in the control of food intake via paracrine and / or endocrine mechanisms. Ghrelin released by the endocrine cells of the oxyntic mucosa is the only known orexigenic hormone in humans (see 2.2.1). In addition, gastric leptin may affect short-term food intake via activation of vagal afferents (Peters et al., 2006) in contrast to adipose tissue derived leptin which is secreted in proportion to the fat mass and thus controls long-term energy balance.

2.1.2 Intestine

In the intestine, nutrient composition is the important factor driving satiation signal release from enteroendocrine cells. Enteroendocrine cells, embedded among the absorptive enterocytes on the mucosal villi of the small-intestinal wall, are able to sense the nutritive and non-nutritive properties of luminal contents by their apical surface enriched with microvilli and in response to food these cells release peptides from their basolateral side. The released peptides may enter circulation, acting like hormones and reach the CNS and other distant targets, or act locally by activating nerve fibres (e.g. enteric myenteric neurons or vagal afferent and spinal afferent fibres).

The intestinal enteroendocrine cell population consists of different subtypes of cells with distinct expression patterns of gastrointestinal peptides. For example, I-type cells release CCK, K-cells gastric inhibitory polypeptide (GIP) and L-type cells glucagon-like peptide 1 (GLP-1) and PYY. Different cell types are also differently distributed along the intestinal tract. I-cells are present in the duodenal and jejunal mucosa whereas majority of L-cells are located in the distal ileum and colon. Furthermore, postprandial peptide release is influenced by the macronutrient composition of the ingested meal. As different peptides vary in their actions, the composition of meals will affect the gastrointestinal responses and thereby influence the digestive processes and satiation.

Nutrient status regulates the responsiveness of the gastrointestinal tract to signals by regulating the expression levels of peptide receptors in the vagus nerve and intestine. For example, fasting upregulates the expression of orexigenic cannabinoid receptor 1 (CB-1) and melanin-concentrating hormone receptor 1 (MCH-1) in the nodose ganglia of rats thus potentiating the effect of their orexigenic agonists on food intake and this upregulation is reversed by refeeding or administration of CCK in the fasted state (Burdyga et al., 2004; Burdyga et al., 2006a). Furthermore, orexin receptors are upregulated during the fed state and mediate orexin-A stimulated bicarbonate secretion from the rat duodenum whereas in the fasted state the receptors are downregulated and the stimulation abolished (Bengtsson et al., 2007).
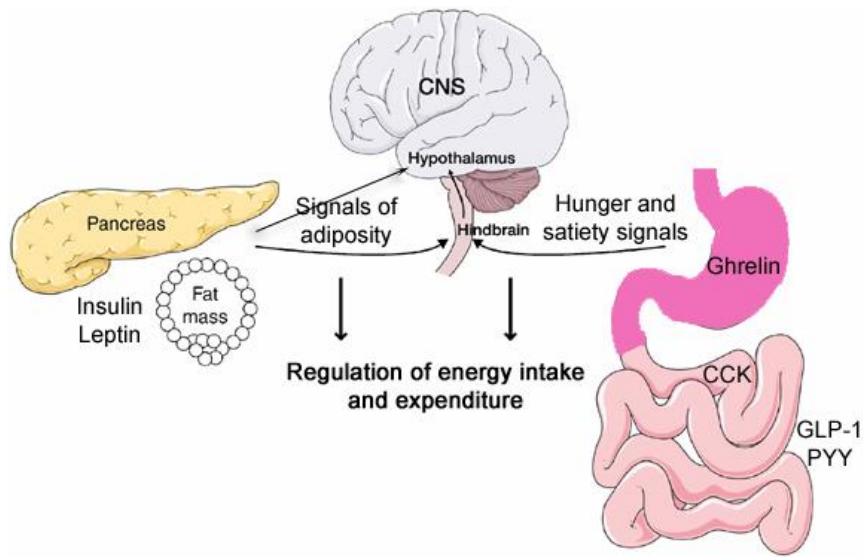
Figure 1. Signals from the adipose tissue and pancreas (e.g. leptin and insulin) reflect the state of body adiposity whereas hunger signals (ghrelin) and meal related satiety peptides (e.g. CCK, GLP-1 and PYY) from the gastrointestinal tract participate mainly in the short-term control of energy balance and signal to CNS via circulation and vagus nerve. Modified from Aronne and Thornton-Jones, 2007.

2.1.3 Central circuits controlling food intake

Peripheral signals elicited by food and energy status have to be conveyed to and interpreted in the CNS to be mirrored to appropriate behavioural responses. Classical lesion experiments in rats showed that bilateral lesion of the lateral hypothalamus caused anorexia whereas damage of the ventromedial hypothalamus induced overeating and obesity. These studies lead to oversimplification of the lateral hypothalamus as "hunger centre" and the ventromedial hypothalamus as "satiety centre". Undisputively, the hypothalamus with its complex connections between its nuclei and with other brain areas is a key site in the regulation of energy balance and food intake.

The hypothalamus receives neural, metabolic and endocrine signals from the peripheral body. The median eminence of the hypothalamus, adjacent to the arcuate nucleus, lacks a functional blood-brain barrier (BBB) and thus circulating peripheral substances reach neurons of the arcuate nucleus, which express receptors for several peripheral signals.

The arcuate nucleus contains two major neuronal subpopulations that are crucial in the control of food intake, one expressing orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the other one anorexigenic peptides cocaine and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC). Activation of NPY/AgRP neurons increases food intake and weight gain via NPY's stimulatory action on Y receptors (specifically subtypes Y1 and Y5) in second order neurons and inhibition of melanocortin receptors 3 and 4 (MC3/4) mediated by AgRP. In contrast, POMC/CART

neurons secrete alpha-melanocyte stimulating hormone (α-MSH), which is an agonist of melanocortin receptors. Thus activation of POMC/CART neurons inhibits food intake, promotes energy expenditure and weight loss (Figure 2).

Fasting upregulates NPY as well as AgRP and downregulates POMC expression. Leptin, the first discovered peptide inhibiting feeding, secreted from the adipose tissue exerts its effects by inhibiting the activity of NPY/AgRP neurons and stimulating POMC/CART neurons. Ghrelin, the orexigenic signal from the gastric mucosa stimulates NPY/AgRP neurons thus promoting appetite. Satiety signals such as PYY(3-36) and GLP-1 on the other hand inhibit these orexigenic neurons and decrease food intake. The arcuate nucleus neurons project to second order neurons in the paraventricular nucleus, lateral hypothalamus and other hypothalamic nuclei, which further signal to higher brain areas and mediate the effects on appetite and energy balance (for review, see Coll et al., 2007).

The brainstem in the hindbrain is another key appetite regulation centre in the CNS. This was demonstrated in chronically decerebrated rats which still become satiated suggesting that gastrointestinal feedback signals to the caudal brainstem are sufficient for the control of food intake (Grill and Norgren, 1978; Grill and Smith, 1988). Afferent fibres of the vagus nerve project into the brainstem dorsal vagal complex (DVC), which consists of dorsal motor nucleus, area postrema and the sensory nucleus of the tractus solitarius (NTS). Circulating signals reach brainstem structures like area postrema which lacks a complete BBB. The brainstem and hypothalamus are extensively and reciprocally connected providing another route by which gastrointestinal signals can affect the activity of neurons in the arcuate nucleus (Figure 2). Furthermore, mounting evidence has shown that the descending connections from the hypothalamus to the brainstem are important for example in the mechanism via which leptin reduces meal size (Morton et al., 2005).
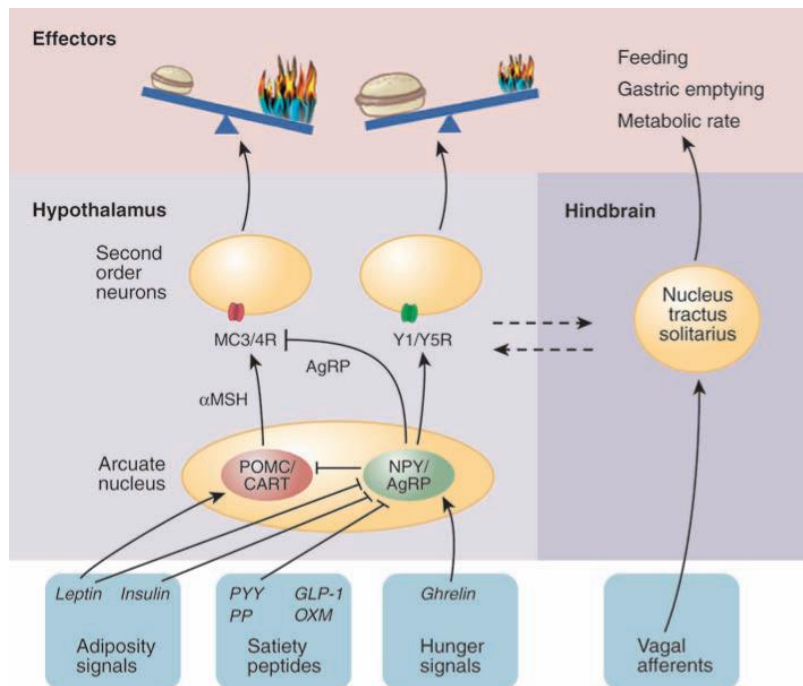
Figure 2. A simplified model of the regulation of energy balance. Peripheral signals (e.g. leptin, insulin, PYY, ghrelin) can access the hypothalamic arcuate nucleus via relaxed BBB and act on orexigenic NPY/AgRP and anorexigenic POMC/CART neurons expressing their receptors. NPY/AgRP neurons inhibit POMC/CART neurons via local axon collaterals in the arcuate nucleus. NPY/AgRP and POMC/CART neurons release neuromodulator peptides and project to second order neurons expressing Y receptors (stimulated by NPY) or melanocortin receptors (inhibited by AgRP and stimulated by α-MSH). Afferent vagus nerve projects to nucleus tractus solitarius in the brainstem. Tight reciprocal connections exist between hypothalamus and hindbrain. These signals together with inputs from higher cortical areas regulate energy balance. Arrow means stimulatory connection, blunted arrow inhibitory connection, dashed arrow indirect pathway. From Badman and Flier, 2005.

2.2 GASTROINTESTINAL SIGNALS CONTROLLING FOOD INTAKE

The gastrointestinal tract secretes several peptides that have been implicated in the regulation of energy balance. In this work four of these, namely ghrelin secreted from gastric mucosa, CCK from duodenum and jejunum as well as PYY and GLP-1 from the distal small intestine and colon have been studied. In addition to these, oxyntomodulin, a cleavage product of proglucagon (see 2.2.4) is known to be secreted in response to food and reduce gastric motility and secretion as well as diminish food intake (Cohen et al., 2003; Dakin et al., 2004; Wynne et al., 2005). Pancreatic polypeptide and amylin secreted from the endocrine pancreas have also been implicated in the control of food intake. Pancreatic polypeptide reduces food intake and may delay gastric emptying and increase energy expenditure (Batterham et al., 2003; Malaisse-Lagae et al., 1977). Amylin released from the beta cells, together with insulin, participates in the control of glucose homeostasis and can also suppress food intake, at least at high levels (Morley and Flood, 1991; Roth et al., 2007).

2.2.1 Ghrelin

As an exception among the peptides secreted from the gastrointestinal tract, the gastric mucosa secretes an orexigenic 28-amino acid hormone ghrelin (Kojima et al., 1999) that stimulates food intake when injected peripherally or centrally into animals (Wren et al., 2000) and peripherally into humans (Wren et al., 2001). The stomach produces approximately 70 % of the circulating ghrelin, the small intestine being responsible for the rest (Ariyasu et al., 2001; Jeon et al., 2004). Ghrelin undergoes a post-translational modification in which the serine-3 residue is acylated with a medium chain fatty acid, most commonly octanoic acid, by a recently characterized acyltransferase (Yang et al., 2008). Octanoylated ghrelin can exert various biological actions by binding to the growth hormone secretagogue receptor type 1a (GHS-R1a), including stimulation of growth hormone secretion from the pituitary gland. GHS-R1a is expressed in the brain and peripheral tissues, especially in the hypothalamus, pituitary, stomach, intestine, pancreas, thymus, gonads, thyroid and heart. However, the role of ghrelin in the regulation of energy homeostasis is generally viewed as its most important function. Non-acylated ghrelin circulates in the plasma as well, but its physiological activities are currently unclear. Some have reported that it stimulates food intake although less potently than acylated ghrelin (Toshinai et al., 2006), whereas others have found opposite, anorexigenic effects (Asakawa et al., 2005; Chen et al., 2005), and some no effect (Neary et al., 2006). Recently it was reported that also another secreted peptide, obestatin, is produced from preproghrelin after proteolytic cleavage, and inhibits food intake (Zhang et al., 2005). However, these results could not be reproduced and currently the physiological significance of obestatin is unclear (Nogueiras et al., 2007).

Plasma ghrelin levels are high prior to meals and decline shortly after food intake (Cummings et al., 2001) indicating a role for this peptide hormone in hunger and meal initiation. Ghrelin crosses the BBB (Banks et al., 2002) and stimulates food intake by acting in the brain on several important weight and

energy homeostasis centres, including the hypothalamus, hindbrain and mesolimbic reward centres. GHS-R1a is expressed in the afferent vagus nerve and the nodose ganglion neurons in both humans and rats (Burdyga et al., 2006b) and ghrelin decreases vagal afferent activity in rats (Date et al., 2002). Based on total vagotomy experiments in rodents and humans a role for vagus nerve in ghrelin stimulated feeding has been suggested (Asakawa et al., 2001; Date et al., 2002; le Roux et al., 2005). In contrast, cutting only the subdiaphragmatic vagal afferents did not affect the feeding stimulatory effect of intraperitoneal ghrelin, suggesting that vagal afferents are not necessary for the peripheral ghrelin induced stimulation of feeding in rats (Arnold et al., 2006).

Exogenous ghrelin has been shown to increase animals' motivation to seek out food and initiate feeding thus increasing the number of eating bouts rather than the amount of food ingested during a single meal (Faulconbridge et al., 2003). In addition to meal-to-meal fluctuations of ghrelin levels and the short-term regulation of ingestive behaviour, plasma ghrelin levels respond also in a compensatory fashion to alterations in body adiposity. In obese individuals, plasma ghrelin levels are decreased (Tschop et al., 2001), but after weight loss the levels increase (Cummings et al., 2002; Leidy et al., 2004). Conversely, weight gain decreases circulating ghrelin levels (Williams and Cummings, 2005; Williams et al., 2006) indicating that ghrelin has a role in the long-term body weight regulation as well.

2.2.1.1 Regulation of ghrelin release

Ghrelin levels are elevated before meals but the mechanisms regulating ghrelin secretion and the decrease of plasma levels postprandially are not very well known. As many other premeal conditioned responses such as salivation, gastric motility and acid secretion and insulin secretion are mediated via the autonomic nervous system, this might apply to ghrelin surges as well. Sympathetic tone is activated during fasting and indeed, stimulation of sympathetic nerves increased plasma ghrelin levels in rats (Mundinger et al., 2006). In contrast, studies in humans have suggested that the parasympathetic nervous system stimulates ghrelin secretion because muscarinic receptor blockers such as atropine (Maier et al., 2004) and pirenzepine (Broglio et al., 2004) decrease whereas the acetylcholine esterase inhibitor pyridostigmine increase (Broglio et al., 2004) ghrelin levels in fasting humans. Vagotomy does not abolish the postprandial ghrelin suppression in rats (Williams et al., 2003).

The enteroendocrine cells of the gastric oxyntic mucosa that secrete ghrelin (X/A-like cells) are not directly in contact with the gastric lumen, but their basolateral membrane is adjacent to the bloodstream (Date et al., 2000; Sakata et al., 2002). The postprandial ghrelin suppression does not require luminal exposure to nutrients in the stomach or duodenum. Instead, signals further in the intestine and postabsorptive plasma insulin, intestinal osmolarity and enteric nervous system seem to contribute, whereas gastric distention, vagal nerve and GLP-1 are not required (Murdolo et al., 2003; Williams et al., 2003; Williams et al., 2003). However, interactions with other gastrointestinal peptides are possible, as exogenous (Brennan et al., 2007) and endogenous (Degen et al., 2007) CCK and intravenously administered PYY(3-36) (Batterham et al., 2003) suppress ghrelin secretion. An inverse connection

between plasma ghrelin levels and GLP-1 or GLP-1 receptor agonist exendin-4 (Djurhuus et al., 2002; Hagemann et al., 2007; Perez-Tilve et al., 2007) has been shown.

Insulin and leptin have been suggested as regulators of plasma ghrelin levels. Fasting plasma ghrelin levels correlate negatively with fasting insulin and leptin levels (Tshop et al., 2001). Negative correlation between fasting ghrelin concentration and insulin resistance has been demonstrated (Ikezaki et al., 2002; Cummings et al., 2002; Poykko et al., 2003). However, it seems that in the regulation of meal-related ghrelin suppression insulin may not play a significant role since supraphysiological insulin levels are required to achieve suppression of ghrelin levels in response to glucose infusion (Schaller et al., 2003). The same may apply to leptin, because administration of neither physiological nor pharmacological doses of leptin influenced ghrelin levels (Chan et al., 2004).

Since recognized as a hunger hormone, prolonged suppression of postprandial ghrelin levels would be expected to correlate with increased satiety. Indeed, ghrelin levels are suppressed in relation to the amount of ingested calories if all the other components of the meal are kept the same (Callahan et al., 2004). Macronutrients differ in their effectivity to suppress postprandial ghrelin levels. Carbohydrates have been shown to be effective in suppressing postprandial ghrelin concentration (Monteleone et al., 2003; Tannous dit El Khoury,D. et al., 2006). When subjects were followed for six hours after carbohydrate, lipid or protein beverage ingestion, ghrelin levels were most markedly suppressed after the carbohydrate drink when only the first three hours were analysed. However, the ghrelin curve after carbohydrate beverage showed a biphasic nature and the levels increased above fasting levels at four hours, whereas after protein and lipid drinks they stayed below baseline until the end of the study. The most effective suppression was observed after the protein beverage when the whole study period of six hours was analysed (Foster-Schubert et al., 2008). Also other studies have reported more prolonged ghrelin suppression after protein than carbohydrate meals (Al Awar et al., 2005; Blom et al., 2006; Bowen et al., 2006a; Bowen et al., 2006b; Bowen et al., 2006b) yet also controversial results have been published with no change (Greenman et al., 2004) or even an increase (Erdmann et al., 2003; Erdmann et al., 2004). Regarding high protein diets used in the weight control, the prolonged suppression of ghrelin by protein would be consistent.

The effect of fibre on postprandial ghrelin is not fully understood due to a limited number of studies as well as a wide range of fibres with different physical and chemical properties. Increased meal fibre content has been shown both to decrease postprandial ghrelin concentration as well as to inhibit the decrease. Consumption of a small amount (4 g) of non-caloric soluble psyllium fibre with water was as effective in suppressing postprandial plasma ghrelin concentrations in healthy subjects as the 585-kcal mixed meal (Nedvidkova et al., 2003). In contrast, no decrease in ghrelin was reported after intake of a non-caloric liquid containing 21 g of guar gum (Erdmann et al., 2003). A soluble arabinoxylan fibre (6 g) enriched breakfast induced a shorter postprandial decrease in ghrelin when compared to a control breakfast (Mohlig et al., 2005). In contrast, arabinoxylan supplementation for 6 weeks increased total but not acylated ghrelin levels in subjects with impaired glucose tolerance (Garcia et al., 2007). Enrichment of bread with 10 g of insoluble wheat fibre blunted the decrease in postprandial ghrelin concentration,

whereas the same amount of insoluble oat fibre did not differ from the control bread (Weickert et al., 2006). Addition of insoluble carob fibre (5, 10 or 20 g) to a liquid meal decreased acylated ghrelin without dose-dependent effects but failed to affect total or nonacylated plasma ghrelin in comparison to a non-fibre meal (Gruendel et al., 2006).

The effect of dietary fat on postprandial release of ghrelin is also unclear. Intravenous lipid infusion seems not to affect fasting ghrelin concentrations (Mohlig et al., 2002; Murray et al., 2006). After oral ingestion of a high-fat meal, ghrelin concentrations have been shown to decrease (Greenman et al., 2004; Monteleone et al., 2003) or to increase (Erdmann et al., 2004). If decreased, the decrease has been characterized by a slower return to baseline than after a high-carbohydrate meal (Otto et al., 2006; Romon et al., 2006) or beverage (Foster-Schubert et al., 2008). Fat-induced suppression of ghrelin is dependent on fat digestion (Feinle-Bisset et al., 2005) and on free fatty acid (FFA) chain length. Intraduodenal infusion of FFA with 12 carbon atom length chains (C12, lauric acid) markedly suppressed plasma ghrelin compared with a FFA with 10 carbons (C10, decanoic acid) that had no effect (Feltrin et al., 2006). Similarly, in a recent study long-chain FFA (C18, sodium oleate) inhibited ghrelin whereas medium-chain FFA (C8, sodium caprylate) was ineffective (Degen et al., 2007).

In conclusion, both increased and decreased ghrelin concentrations have been reported after meals with a greater satiety effect, such as those high in fibre or protein. Thus, the contribution of postprandial ghrelin suppression on satiety still remains unclear.

2.2.2 CCK

CCK is the first known satiety hormone and was identified in 1928 by Ivy and Oldberg who observed that extracts of swine intestinal mucosa stimulated gallbladder contraction in dogs (Ivy and Oldberg, 1928). Purification and characterization of CCK peptide was reported by Jorpes and Mutt in the 1960s (Jorpes and Mutt, 1961), and the first reports on its satiating effects were published in 1973 (Gibbs et al., 1973a; Gibbs et al., 1973b) followed by intensive research and a vast number of publications during the following decades until today.

CCK is synthesised as a 115-amino acid prepropeptide, which is then processed by endoproteolytic cleavage into at least six different bioactive C-terminal peptides (CCK-8, CCK-22, CCK-33, CCK-39, CCK-58 and CCK-83, figure 3). The seventh residue (tyrosine) from C-terminus is sulphated and this heptapeptide is required for efficient binding to CCK_1 receptors. The bioactivity of different peptides varies as does their abundance between tissues. The major circulating peptide forms in most mammals are CCK-8, -22, -33 and 58. CCK is also expressed in various areas of the central nervous system acting as a neurotransmitter, and in the brain CCK-8 predominates (for review, see Rehfeld, 2004). The five most C-terminal amino acids of CCK are identical to those of gastrin, which has caused problems in the measurement of plasma levels with antibodies crossreacting with both peptides since gastrin circulates in the plasma at 5-10 times higher concentrations than CCK.

Two receptor subtypes bind CCK peptides. The CCK_1 receptor is expressed in the gastrointestinal tract, pancreas, vagal afferents and enteric neurons but also in the CNS areas including NTS, area postrema and dorsal medial hypothalamus and requires the sulphated tyrosine moiety in the C-terminal tyrosine residue for high affinity binding. CCK_2 receptors are expressed widely in the brain but also in the afferent vagus and the stomach and bind desulphated forms of CCK and gastrin with high affinity.
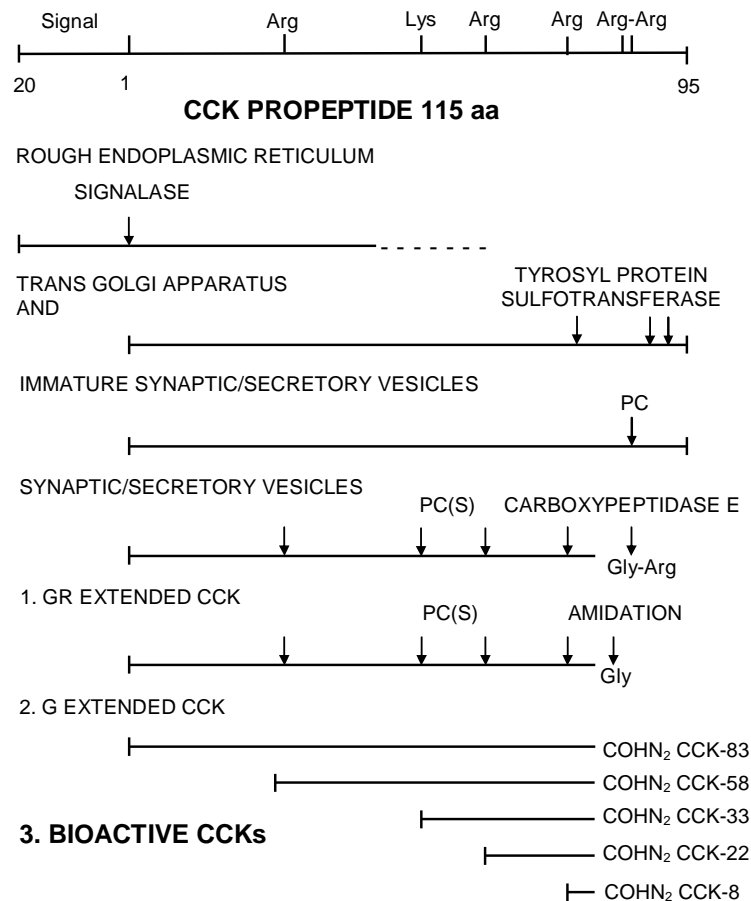
Figure 3. Post-translational processing of CCK from the 115 amino acid propeptide (modified from Rehfeld, 2004). The propeptide is endoproteolytically cleaved mainly on mono-arginine but also on lysine residues (CCK-33). Glycine extended CCK is amidated at the C-terminal glycine by alpha-amidating mono-oxygenase resulting in the bioactive CCKs. In addition, CCK-39 has been described. The processing may differ between tissues. PC = prohormone convertase(s), GR = glycine (G) and arginine (R) extended CCK, G = glycine extended CCK.

2.2.2.1 Regulation of CCK release in the intestine

In the intestine, most of the CCK is localized in the specific enteroendocrine cells called I cells in the duodenal and proximal jejunal mucosa and in enteric nerves as CCK-58, CCK-33, CCK-22 and CCK-8. CCK-containing neurons are present in the myenteric and submucosal plexi, in the muscle layers of the

small intestine and colon, and in the celiac plexus and vagus nerve (Liddle, 1997). Fasting CCK levels are low, but CCK is released into the circulation in response to nutrients in the intestinal lumen. This is a rapid response because elevated plasma CCK levels can be observed already 15 minutes after the start of the meal. CCK-58 is the most abundant circulating peptide, at least in rats and dogs (Eysselein et al., 1987; Eysselein et al., 1990; Reeve et al., 2003), but in humans this remains controversial, since also CCK-33 has been suggested to predominate (Rehfeld et al., 2001). The nutrient composition of the meal regulates CCK release, fat and protein being the most potent stimuli causing about five fold elevation of plasma CCK levels.

Ingested fat, first, has to be hydrolyzed to FFAs and monoglycerides in order to stimulate CCK release (Hildebrand et al., 1998) with the acyl chain length of the FFA being decisive in the CCK response (Egberts et al., 2000). In humans FFAs with more than 11 carbons have been reported to stimulate CCK release (Lal et al., 2004; McLaughlin et al., 1999), whereas a recent study found a smaller but clearly significant increase in plasma CCK levels with decanoic acid of 10 carbon atoms (Feltrin et al., 2004). The difference between these three studies is that McLaughlin and Lal with their coworkers used a vehicle to solubilize the fatty acids (Tween 80), which alone elevated CCK levels and thus possibly masked the milder effect of C10. Feltrin and coworkers used sodium salts of the FFAs, thereby avoiding the use of the vehicle. Recently, it has been shown that long-chain fatty acids may mediate CCK release via binding to G-protein coupled receptor GPR120 (Tanaka et al., 2007), which mediates also the fatty acid induced secretion of GLP-1 (see 2.2.4.1). Interestingly, feeding a high fat diet for 21 days increased the fasting CCK plasma levels compared to controls fed on low fat diet in normal weight men (Little et al., 2008).

Dietary protein effectively stimulates CCK release as well. Proteins are digested in the intestine and absorbed by enterocytes mainly as di- and tripeptides via the protein-coupled transporter PepT1 (Adibi, 1997; Fei et al., 1994). Protein hydrolysates (peptones) seem to produce more pronounced responses than monomeric amino acids (Cuber et al., 1990), although in humans L-phenylalanine alone also releases CCK (Ballinger and Clark, 1994). A direct stimulatory action of peptones on enteroendocrine cells has been suggested (Cordier-Bussat et al., 1997). In addition, the stimulation of CCK release by dietary protein is mediated via endogenous trypsin sensitive duodenal CCK-releasing peptides (CCK-RP). Duodenal trypsin and other proteolytic enzymes inactivate CCK-releasing peptides in the absence of other substrates. The presence of dietary protein in the gut lumen protects CCK-RPs from proteolysis by competing as substrates for the degrading enzymes and thus increases the amount of active CCK-RP in the lumen and stimulating CCK release when proteins are present. At least four CCK-RPs have been suggested (Herzig, 1998). Monitor peptide was isolated from rat pancreatic juice (Iwai et al., 1987), diazepam binding inhibitor (DBI) from rat intestinal mucosa (Herzig et al., 1996) and luminal CCK releasing factor (LCRF) from rat intestinal secretions (Spannagel et al., 1996).

Carbohydrates are less potent stimulants of CCK release than fat and proteins, but in humans intragastric or intraduodenal glucose infusion increases plasma CCK levels (Little et al., 2006; Parker et al.,

2005). The CCK increase after carbohydrates is, however, quite short-lived and the plasma levels return close to baseline within one hour (Bowen et al., 2006b).

Hydrolyzed guar gum fibre (20 g) increased the postprandial CCK response in obese women during a weight loss program without affecting satiety ratings (Heini et al., 1998). In contrast, 15 g of pectin included in a solid meal slowed gastric emptying and increased satiety, but did not modulate CCK responses compared to methylcellulose (Di Lorenzo et al., 1988). Adding oat fibre to low fat solid meal compensated the postprandial CCK response to the level of isocaloric low fibre, high fat meal in women, whereas no difference was observed in men. Furthermore, plasma CCK levels correlated with the subjective satiety measures (Burton-Freeman et al., 2002). In contrast, no difference in total CCK responses were observed after high fibre pasta (total 15.7 g of barley fibre of which 5 g beta-glucan) compared to low fibre pasta although the CCK levels stayed elevated longer after the high fibre pasta (Bourdon et al., 1999). A high fibre meal containing bean flakes evoked a significantly greater CCK response compared to low fibre meal in men although part of the pronounced response may have been caused by the trypsin inhibitor present in beans (Bourdon et al., 2001).

2.2.2.2 Effects of CCK

Plasma CCK levels are elevated postprandially but the majority of the effects of CCK are mediated via activation of extrinsic neural pathways, especially the vagal afferent pathway. Vagal fibres expressing CCK_1 receptors innervate multiple abdominal organs including the stomach and the small intestinal mucosa. Anatomically vagal afferents terminate within the lamina propria of the gastrointestinal mucosa in close apposition to the basolateral membrane of enteroendocrine cells, thus making it possible for CCK and other peptides to act in a paracrine fashion (Berthoud et al., 2004). In addition to CCK_1 receptor expression, the vagal afferent fibres express also several other receptors including CB-1 (Burdyga et al., 2004), MCH-1 (Burdyga et al., 2006a), leptin receptor (Burdyga et al., 2002), GLP-1 receptor (Nakagawa et al., 2004) and GHS-R1a (Burdyga et al., 2006b). CCK downregulates the expression of CB-1 and MCH-1 (Burdyga et al., 2004; Burdyga et al., 2006a) and in addition, the orexigenic hormones orexin-A and ghrelin inhibit vagal afferent discharge in response to CCK (Burdyga et al., 2003).

The multiple actions of CCK serve to optimise the digestive processes in the gut. CCK inhibits gastric emptying and food intake, thus limiting the delivery of ingested food to the intestine. It stimulates both gallbladder contraction and pancreatic exocrine secretion, thereby ensuring that digestive enzymes and bile salts are available for digestion.

Inhibition of food intake. Exogenous peripheral CCK administration inhibits food intake by reducing meal size and duration in animals (Gibbs et al., 1973a) and humans (Kissileff et al., 1981). Postprandially released CCK induces satiety mainly by activating vagal afferent fibres expressing CCK_1 receptors in the intestine, which relay the signal to the appetite centres in the brainstem. CCK signalling in the CNS is dependent on the melanocortin receptor MC4R in the brainstem (Fan et al., 2004). In addition, CCK_1 receptors in the CNS (brainstem and hypothalamus) also seem to be able to induce satiation

(Reidelberger et al., 2004). Additionally, central CCK administration suppresses food intake (Blevins et al., 2000). The role of CCK in satiation has been confirmed by experiments conducted in several species. The importance of the endogenous CCK in the control of food intake has been demonstrated with the use of selective CCK_1 receptor antagonists, which lead to increases in the meal size (Beglinger et al., 2001).

The plasma half-life of CCK is only 1-2 minutes and it does not reduce meal size if delivered more than 15 minutes before a meal. Thus, CCK acts as a short-term signal of meal termination. Chronically repeated or continuous administration of CCK does not induce weight loss in rats. Although the meal size is reduced continuously, the long-term 24-hour food intake does not change significantly because the animals compensate by increasing meal frequency (Crawley and Beinfeld, 1983; West et al., 1984). Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which carry a spontaneous mutation in the CCK_1 receptor, eat enlarged meals and gradually develop mild obesity (Moran and Bi, 2006).

However, interactions between CCK and long-term satiety signals leptin and insulin occurs. Satiating effects of CCK are attenuated in rats lacking leptin receptors (Morton et al., 2005). Furthermore, coadministration of CCK with leptin or insulin increases the satiating effects of CCK in mice (Barrachina et al., 1997), and prolonged fasting, which leads to a leptin deficient state, reduces the satiety inducing effects of CCK (McMinn et al., 2000). This could be interpreted as fine tuned mechanism via which signals of body adiposity, such as leptin and insulin, modulate the sensitivity of brain to CCK; when fat is lost, reduced leptin and insulin levels would decrease the sensitivity to CCK and thus an individual would eat larger meals.

Inhibition of gastric emptying. CCK delays gastric emptying in animals (Moran and McHugh, 1982) and humans (Fried et al., 1991a; Fried et al., 1991b; Lal et al., 2004) by inducing the relaxation of the proximal stomach via a vago-vagal reflex (Holzer et al., 1994; Raybould et al., 1987; Raybould and Tache, 1988). In addition, CCK may act directly on smooth muscle cells in the distal stomach and regulate contractions of the antrum (McLaughlin et al., 1999). After intragastric fatty acid administration, C12 fatty acids caused higher plasma CCK levels than C10 fatty acids and the half gastric emptying time was significantly longer after C12 than after vehicle or C10 administration. The subjects tolerated the same amount of distention of the intragastric bag after either fatty acid, but the volume of water tolerated was lower after C12 demonstrating that gastric emptying was indeed inhibited by the stimulated release of endogenous CCK. Furthermore, subjects experienced an enhanced sensation of fullness and satiety (Lal et al., 2004). CCK may inhibit food intake in part by delaying gastric emptying and thus enhance gastric mechanoreceptor stimulation. Gastric distention and exogenous CCK have been shown to synergistically activate vagal afferents (Schwartz et al., 1993) and to inhibit food intake (Kissileff et al., 2003).

Effects on intestinal and colonic motility. CCK immunoreactive neurons innervate the smooth muscle cells of the distal intestine and colon. Exogenous CCK increases intestinal motor activity and shortens the intestinal transit time (Gutierrez et al., 1974). In contrast to increased motility of the intestine, CCK inhibits colonic transit. CCK_1 receptors are expressed in the neurons of the myenteric plexus and to a lower extent also in longitudinal muscle of colon and thus CCK may exert it functions both directly and via

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the neural pathway (Rettenbacher and Reubi, 2001). Exaggerated release of or sensitivity to CCK has been suggested to contribute to the pathogenesis of irritable bowel syndrome (IBS) characterized by constipation and lower abdominal pain (Sjolund et al., 1996). Accordingly, CCK_1 receptor antagonists are being developed for the treatment of IBS (for review, see Varga et al., 2004).

Stimulation of pancreatic exocrine secretion. Initially porcine intestine extracts were shown to stimulate pancreatic exocrine secretion, and the active component was named pancreozymin (Harper and Raper, 1943). However, later it appeared that the isolated peptide was identical to already previously isolated CCK (Jorpes and Mutt, 1961).

CCK stimulates pancreatic enzyme secretion in many species, including humans and rodents. However, the mechanisms seem to differ. In human acinar cells, very low amounts of CCK_1 receptor are expressed and the cells do not secrete enzymes when stimulated with CCK *in vitro* (Ji et al., 2001; Ji et al., 2002). *In vivo*, atropine (a muscarinic acetylcholine receptor antagonist) essentially blocked postprandial pancreatic enzyme secretion (Beglinger et al., 1992). Thus, it seems that in humans CCK released from the intestinal cells activates a vago-vagal reflex that stimulates enzyme secretion from the pancreatic acinar cells via cholinergic M3 muscarinic receptors. In contrast, rat acinar cells are responsive to direct CCK stimulation via CCK_1 receptors and thus endocrine actions, in addition to neural pathways, mediate CCK's stimulatory effect on enzyme secretion (for review, see Wang and Cui, 2007).

Stimulation of gallbladder contraction. As implicated by its name, cholecystokinin induces gallbladder contraction thus releasing bile into the duodenum. Physiological postprandial concentrations of CCK stimulate contraction of the gallbladder (Nitsche et al., 1998). In addition, CCK relaxes the sphincter of Oddi, thereby facilitating bile flow. The gallbladder expresses CCK_1 receptors, but in addition to the direct actions of CCK on the gallbladder smooth muscle, it has been suggested that the contraction is mediated also via neural pathways. Cholinergic mechanisms are involved because atropine, without affecting plasma CCK levels, is able to reduce the contraction of the gallbladder during the duodenal perfusion of a test meal, but does not totally abolish it (Beglinger et al., 1992). Also other studies have demonstrated that gallbladder contraction is reduced, but not totally abrogated by muscarinic antagonists (Hopman et al., 1990; Nelson et al., 1996). In humans gallbladder volume has been reported either to increase (Parkin et al., 1973) or stay the same (Shaffer, 1982) after vagotomy. Specific CCK_1 receptor antagonists, however, remarkably dilate gallbladder volume in the fasting state (Jebbink et al., 1992) and totally block the postprandial gallbladder contraction. This indicates that CCK is the major regulator of the gallbladder response, modulated by the cholinergic system (Beglinger et al., 1992).

2.2.3 PYY

Peptide YY (PYY) was isolated in 1980 from the porcine jejunal mucosa by Tatemoto and coworkers (Tatemoto and Mutt, 1980; Tatemoto et al., 1988). PYY is a 36-amino acid peptide that is produced by the endocrine L-cells, mainly in the distal intestine and colon. It is released into the circulation in response to food intake. The released precursor peptide PYY(1-36) is rapidly metabolized by dipeptidyl

peptidase 4 (DPP-4) to the bio-active form, PYY(3-36). Approximately 60-70 % of the postprandial total plasma PYY consists of PYY(3-36) (Batterham et al., 2006; Grandt et al., 1994). The highest amounts of PYY are found in the rectum, followed by the colon and ileum (Adrian et al., 1985). It is also detected in the pancreas as well as in the hypothalamus and hindbrain regions of the CNS (Ekman et al., 1986; Glavas et al., 2008; Lundberg et al., 1984).

PYY inhibits many gastrointestinal functions, including gastric motility and emptying, gastric acid secretion, jejunal and colonic motility and mouth-to-cecum-transit time. Thus it is one of the major mediators of the ileal brake, which means the inhibition of the upper gastrointestinal motor activity promoting digestive activities to increase nutrient absorption, and is triggered particularly in response to fat (for review, see Van Citters and Lin, 2006). In addition, PYY reduces intestinal blood flow and causes intestinal vasoconstriction (Lundberg et al., 1982). PYY also inhibits pancreatic exocrine secretion and insulin secretion (Boey et al., 2007).

Recently, most of the interest on PYY(3-36) has concentrated on its suggested role as an endogenous satiety factor in both humans and rodents. In humans, a 90 min intravenous infusion of PYY(3-36) reduced energy intake by 36 % acutely after administration, and also reduced the 24 h calorie consumption by a similar proportion despite the fact that PYY plasma levels returned to its pre-stimulation baseline levels (Batterham et al., 2002). PYY(3-36) inhibits food intake in obese subjects as well (Batterham et al., 2003). Recent findings in normal weight subjects (Degen et al., 2005) indicate, however, that the PYY doses required to observe significant satiating effects, which were the same as used by Batterham and coworkers (2002), were pharmacological (the resulting plasma levels were nearly three times higher than those elicited by a large meal) and caused adverse effects such as nausea and abdominal discomfort. The reduction in food intake, with severe adverse effects, was also observed in obese subjects in another study with the same dose (Sloth et al., 2007a). Furthermore, in a larger clinical trial, in which PYY(3-36) was administered intranasally to obese humans for 12 weeks, the tolerated dose did not have any effects on weight loss compared to placebo, and the higher dose was not tolerated (Gantz et al., 2007).

In rats, intraperitoneal administration of PYY(3-36) twice daily for 7 days reduced food intake and decreased body weight gain (Batterham et al., 2002). Peripheral PYY(3-36) also reduced weight gain in normal mice as well as several rodent models of obesity and type 2 diabetes (Pittner et al., 2004). Food intake was reduced acutely in rhesus macaques (Moran et al., 2005), and weight gain attenuated after two weeks treatment (Koegler et al., 2005). However, several independent groups have reported that they were unable to reproduce the effects of PYY(3-36) on energy intake and body weight in rodents (Tschop et al., 2004). Challis and colleagues observed the short-term anorectic effects, but none after seven days administration on either cumulative weight gain or food intake (Challis et al., 2004). Some groups have reported that the anorectic effects of PYY were only seen in rodents that have been carefully acclimatized to handling and experimental conditions (Abbott et al., 2006; Halatchev et al., 2004), which suggests that PYY-induced satiety is highly sensitive to stress. Furthermore, differences may be caused by experimental

protocols or animal strains studied. Therefore, as a conclusion, the anorexigenic effects of PYY seem to be subtle and thus the hopes of PYY as a magic bullet to treat obesity seem to be over optimistic.

PYY belongs to the pancreatic polypeptide family, as does NPY, one of the most potent stimulators of food intake in the brain. Interestingly, in contrast to its satiety inducing effects after peripheral administration, centrally administered PYY(1-36) and PYY(3-36) effectively stimulate food intake (Hagan, 2002; Morley et al., 1985). Peptide forms differ in their receptor binding. Full length PYY activates NPY receptor isoforms Y1, Y2 and Y5, while PYY(3-36) activates Y2 and Y5 receptor subtypes (Cox, 2007). Circulating PYY(3-36) crosses the BBB and is thus able to selectively reach Y2 receptors in the arcuate nucleus of the hypothalamus and induce the anorexic effects by inhibiting NPY/AgRP neurons (Acuna-Goycolea and van den Pol, 2005). In contrast, when delivered intracerebroventricularly, PYY by acting on subtype Y1 and Y5 receptors located in the paraventricular nucleus has an orexigenic effect. Additionally, the involvement of the vagal afferents has been implicated in the mechanism of action of peripheral PYY(3-36), since vagotomy or transection of hindbrain-hypothalamic pathways eliminated its anorectic effects (Abbott et al., 2005; Halatchev and Cone, 2005; Koda et al., 2005).

2.2.3.1 Control of PYY release

Plasma levels of PYY increase within 15 minutes after meal initiation, reaching a peak at approximately 60 minutes, and then remaining elevated up to 6 hours (Adrian et al., 1985). Neural pathways and mediation via other gastrointestinal hormones like CCK may be involved in the initial phase of release, since the plasma levels are increased even before the ingested nutrients reach the terminal intestine (Degen et al., 2007; Fu-Cheng et al., 1997; Lin et al., 2000) followed by the direct stimulation of endocrine cells by nutrients. The plasma PYY concentration is not altered by gastric distension (Oesch et al., 2006), water loading (Pedersen-Bjergaard et al., 1996) or sham-feeding (Soffer and Adrian, 1992).

The postprandial elevation of plasma PYY levels is proportional to the calories consumed. Additionally, it is also affected by the nutrient composition of the meal. Intestinal fat is a potent stimulus of PYY release, and because intravenous lipid infusion has no effect on PYY levels, sensing of the nutrients in the gut lumen is required. Lipids induced the largest increase in postprandial PYY concentration followed by protein and then glucose, with the latter causing only a transient and minor release (Adrian et al., 1985). Other groups have also shown lipids to be more effective than carbohydrates (Essah et al., 2007; MacIntosh et al., 1999), despite one paper that reported that PYY concentration increased after proteins and carbohydrates whilst there was only a slight rise after a fat meal (Pedersen-Bjergaard et al., 1996). Different fats elicit different PYY responses. As in the case of CCK and GLP-1 release, fat hydrolysis and fatty acid chain length appear to determine the response (Degen et al., 2007). Fatty acids with 12 carbons stimulate PYY release, whereas 10-carbon fatty acids did not have any effect (Feltrin et al., 2006). Furthermore, recently it was reported that a high-protein meal was the most potent stimulator of PYY release and satiety, followed by fat and carbohydrate rich meals in humans and in mice (Batterham et al.,

2006). The fact that PYY-null mice are obese and resistant to protein-induced satiety further indicates that PYY might be a major mediator of protein induced satiety (Batterham et al., 2006).

Total PYY fasting levels have been reported to be reduced in obese adults (Batterham et al., 2003) and children (Roth et al., 2005). This suggests that obesity might be a PYY-deficient state that could be cured by therapeutical PYY administration. In contrast, several other studies did not find any difference in the fasting PYY levels between lean and obese subjects (Kim et al., 2005; Korner et al., 2005; Korner et al., 2006; Pfluger et al., 2007; Stock et al., 2005; Vazquez Roque et al., 2006). However, the postprandial PYY response seems to be blunted in obese individuals, so that they have to consume more calories to achieve plasma PYY levels that of lean ones (Batterham et al., 2003; le Roux et al., 2006; Stock et al., 2005). Furthermore, bariatric gastric bypass surgery increases PYY levels and improves postprandial responses (Korner et al., 2005; Korner et al., 2006; Morinigo et al., 2006; Reinehr et al., 2007). Postprandial changes in gastric volume and PYY levels were independent predictors of satiation (Vazquez Roque et al., 2006). Therefore, an association between reduced appetite and exaggerated PYY levels is suggested. Analysis of plasma PYY concentration is performed with immunoassays that cross-react equally with PYY(1-36) and PYY(3-36) and thus it remains to be investigated which molecular form causes the changes in the total PYY levels. This may be of importance since PYY(3-36) is more potent in inducing satiation than PYY(1-36) (Chelikani et al., 2005).

2.2.4 Proglucagon cleavage products

The prohormone proglucagon consists of 180 amino acids and is posttranslationally cleaved in a tissue-specific manner by prohormone convertases 1/3 and 2 to yield active peptides GLP-1, GLP-2, glicentin, oxyntomodulin and glucagon (Figure 4). Proglucagon gene expression is differentially regulated in tissues corresponding to the physiological function of the peptides produced. In the alpha cells of the endocrine pancreas, the proglucagon gene transcription is up-regulated by fasting and hypoglycaemia. Cleavage of the prohormone produces glucagon, which is of key importance in maintaining glucose homeostasis in the fasting state by regulating hepatic glucose production via activation of glycogenolysis and gluconeogenesis and inhibition of glycolysis.

In the intestine, proglucagon expression is downregulated by fasting and stimulated by refeeding and the posttranslational processing of the prohormone yields GLP-1, GLP-2, glicentin and oxyntomodulin that are secreted in response to food intake. GLP-1 is known as a potent incretin and reduces food intake similar to oxyntomodulin. GLP-2 stimulates cell proliferation in intestinal crypts and has also been reported to stimulate glucose transport in the intestine and inhibit food intake. Glicentin is less well known but exerts trophic effects in the rodent small intestine. In the CNS, proglucagon is expressed in caudal brainstem (in the NTS) and hypothalamus, where the peptides produced correspond to the intestinal cleavage products (Baggio and Drucker, 2007).

Proglucagon | S | GRPP | Gluc | IP-1 | GLP-1 | IP-2 | GLP-2 |

Pancreas | GRPP | **Gluc** | IP-1 | **GLP-1** | IP-2 | **MPGF** / **GLP-2** |

Intestine & brain **Glicentin** | GRPP | **Gluc** | IP-1 | **OXM** | **Gluc** | IP-1 | **GLP-1** | IP-2 | **GLP-2** |
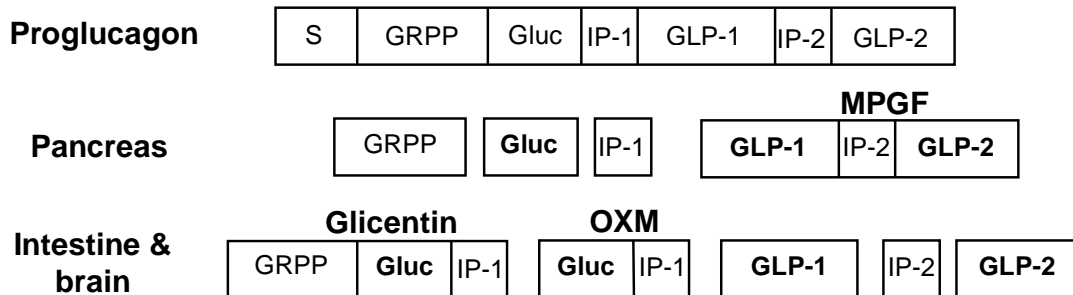
Figure 4. Proglucagon is posttranslationally cleaved in a tissue-specific manner. In the pancreas, glicentin-related polypeptide (GRPP), glucagon (Gluc), intervening peptide-1 (IP-1) and major proglucagon fragment (MPGF) are formed. In the intestine and brain, glicentin, which is further cleaved to oxyntomodulin (OXM), GLP-1, intervening peptide 2 (IP-2) and GLP-2 are produced. No biological activity has been shown for GRPP, MPGF, IP-1 and IP-2 (modified from Baggio and Drucker, 2007).

2.2.4.1 GLP-1

In the endocrine L-cells, primarily in the ileum and colon, proglucagon is cleaved to GLP-1, GLP-2, glicentin and oxyntomodulin that are postprandially co-secreted with PYY(3-36). GLP-1 is secreted as two biologically active peptides, GLP-1(7-36)amide and GLP-1(7-37). The major circulating form in the plasma is GLP-1(7-36)amide. However, most plasma GLP-1 is N-terminally degraded after release within a very short time by DPP-4, which is present on the surface of endothelial cells lining the blood vessels that drain intestinal mucosa. As a result, inactive GLP-1(9-36)amide and GLP-1(9-37) are formed. DPP-4 is also present in many other tissues and a soluble form circulates in the plasma. It has been estimated that only 10-15 % of the intestinally released GLP-1 passes liver and enters the systemic circulation (Holst, 2007).

GLP-1 is released from the L-cells in response to different nutrients. Fasting plasma GLP-1 concentration is low, indicating only a minor basal secretion, but postprandially 2-3 -fold increases in the circulating plasma are measured. Elevated GLP-1 levels are observed already 10 minutes after the meal initiation and continue to stay elevated for 60 to 120 minutes (Herrmann et al., 1995; Karamanlis et al., 2007). The early response may be caused by neural mechanisms originating in the upper small intestine (Berthoud and Morrison, 2008) or partially arise also after the contact of the nutrients with the L-cells located in the upper intestine. However, the later enhanced phase of the response is caused by a direct contact between the nutrients and the mucosa in the lower gut where most of the L-cells are located.

Effects of GLP-1. The incretin effect was demonstrated already in the early 1900s, as it was found that intestinal factors lowered blood glucose levels (Moore et al., 1906), and the term incretin was first introduced in the 1930s (La Barre, 1932). These factors potentiate glucose stimulated insulin secretion and thus oral ingestion of glucose evokes a more pronounced insulin response than the same amount of glucose administered intravenously. It has been estimated that the incretin effect via gastrointestinal hormones is responsible for 50-70 % of the total insulin secreted in response to an oral glucose load. GLP-1 is one of the most potent mediators of the incretin effect. As GLP-1 is secreted rapidly in response to

nutrients, it participates in the control of postprandial glycemia. GLP-1 is able to stimulate insulin secretion only in the presence of glucose and thus is unlikely to induce hypoglycaemia when used as a therapeutic agent in the treatment of type 2 diabetes. In addition to the stimulation of insulin exocytosis, GLP-1 acts synergistically with glucose to promote insulin gene transcription and hormonal biosynthesis. Furthermore, GLP-1 improves the ability of beta-cells to sense glucose levels by upregulating the expression of glucose transporters and glucokinases and thus GLP-1 can restore glucose sensitivity to glucose-resistant beta-cells. GLP-1 also increases beta-cell mass by stimulating beta-cell proliferation and neogenesis as well as inhibiting apoptosis (Farilla et al., 2002). The effects of GLP-1 on the pancreas are mediated partly via neural mechanisms originating from the intestine and liver via activation of vagal afferent nerves, which further in a reflexive manner activate pancreatic vagal efferent fibres (Nakabayashi et al., 1996). The more pronounced GLP-1 responses after larger meals may produce GLP-1 levels that can also act in an endocrine fashion to directly stimulate GLP-1 receptors on the cell membrane of beta cells.

GLP-1 slows down gastric emptying and inhibits gastric acid secretion, being in addition to PYY one of the mediators of ileal brake mechanism. In fact, the inhibitory effect of GLP-1 on gastric acid secretion was shown to be additive to that of PYY which is released in parallel in response to food intake (Wettergren et al., 1997a). Although the GLP-1 receptor (GLP-1R) is expressed in the parietal cells of the stomach, experimental evidence shows that the effects of GLP-1 are mediated by the vagus nerve (Imeryuz et al., 1997; Wettergren et al., 1997b). Endogenous GLP-1 inhibits also antroduodenal motility and mediates the postprandial inhibition of the antral and stimulation of pyloric motility (Schirra et al., 2006). Importantly, the inhibition of gastric emptying and slowing of the transit of nutrients from the stomach to the gut is one of the mechanisms by how GLP-1 lowers postprandial glycemia. Therefore, in spite of its incretin effect, GLP-1 mediated reduction in glycemia is often associated with reduced rather than increased postprandial insulin levels (Meier et al., 2003; Nauck et al., 1997).

Peripheral GLP-1 administration increases satiety and suppresses appetite in both healthy (Flint et al., 1998; Gutzwiller et al., 1999) and diabetic subjects (Toft-Nielsen et al., 1999). In obese subjects, the basal and postprandial GLP-1 concentrations are still under debate. Some have reported lower postprandial GLP-1 levels (Ranganath et al., 1996; Verdich et al., 2001), whereas others did not find any differences (Vilsboll et al., 2003). However, peripheral administration of GLP-1 decreases hunger ratings and reduces energy intake also in obese subjects (Flint et al., 2001; Naslund et al., 1999; Verdich et al., 2001). Subcutaneous preprandial GLP-1 injections for 5 days to obese subjects reduced their energy intake by 15 % and caused a 0.5 kg weight loss (Naslund et al., 2004).

The anorectic effects of GLP-1 are mediated specifically via the GLP-1R which is expressed in the intestine, pancreas and the CNS. The physiological importance of endogenous GLP-1 in the regulation of food intake has been demonstrated by several experiments showing that blocking the GLP-1R with the antagonist exendin(9-39) increases food intake (Meeran et al., 1999). Vagal afferent fibres express GLP-1R, and consequently an intact vagus nerve is required for peripheral GLP-1 induced anorectic effects (Abbott et al., 2005). GLP-1 crosses the blood-brain barrier, but the effects of peripheral GLP-1 on central GLP-

1Rs in the hypothalamus and brainstem are still questionable because it seems unlikely that sufficient amounts of the peptide could be transported from the intestine before degraded by DPP-4. In rodents, GLP-1 reduces food intake also when applied intracerebroventricularly or directly into the paraventricular nucleus (McMahon and Wellman, 1998; Turton et al., 1996).

Due to its beneficial effects on glucose metabolism, several outcomes have been developed to circumvent the rapid degradation of GLP-1 in order to facilitate its therapeutic use in the treatment of type 2 diabetes. The DPP-4 resistant GLP-1 agonist, exendin-4, was originally isolated from the gila monster saliva and is marketed since 2005 in the US and 2006 in the EU as synthetic exenatide (trade name Byetta) for the treatment of type 2 diabetes. Sharing about 50 % of its amino acid sequence with mammalian GLP-1, exendin-4 is protected from DPP-4 and only cleared by glomerular filtration in the kidneys. Its circulating half-life is 60-90 min, and a single subcutaneous injection increases plasma concentrations for 4-6 hours. Exenatide treatment improves glycemic control, fasting plasma glucose and importantly, decreases body weight (Drucker and Nauck, 2006). The weight loss is exceptional considering that usually diabetic medication tends to increase rather than decrease weight. Although 40-50 % of patients experience transient nausea especially at the beginning of the treatment, they chose to continue with the medication. The weight loss is not due to nausea since many patients devoid of side effects also lose weight. Furthermore, once in combination with metformin, hypoglycemic events were not increased. Another GLP-1 analog liraglutide, which is partially DPP-4 resistant, is also on the market.

Orally active DPP-4 inhibitors vildagliptin and sitagliptin have also been developed for the treatment of diabetes. They are effective in stimulating insulin secretion and improve glycemic control, but are generally not associated with deceleration of gastric emptying or weight loss, yet seem to prevent weight gain (Drucker and Nauck, 2006). This may be due to less efficient stabilization of plasma GLP-1 levels compared to GLP-1 agonists.

Regulation of GLP-1 release. Proteins, carbohydrates and fat all stimulate GLP-1 release, yet with different potencies. Proteins seem to be the most effective stimulants. When comparing breakfasts with similar energy density but rich in protein, fat, carbohydrate or alcohol, the greatest GLP-1 response was reported after a protein rich meal followed by carbohydrates, fat and alcohol (Raben et al., 2003). A high-protein dairy product enriched with a whey protein isolate stimulated GLP-1 secretion more than a high-carbohydrate meal (Blom et al., 2006). However, none of the studies observed any differences in subjective sensations of hunger and *ad libitum* energy intake during the subsequent lunch. In contrast, after a longer intervention period of four days on a high protein or adequate protein diet, high protein diet produced lower hunger and higher satiety ratings and elevated GLP-1 responses (Lejeune et al., 2006).

Carbohydrates are also strong stimuli for GLP-1 release, consistent with the role of GLP-1 as incretin, and glucose seems to be the most effective GLP-1 secretagogue (Elliott et al., 1993; Kong et al., 1999). The GLP-1 responses after fat are delayed when compared to carbohydrates (Elliott et al., 1993). The chain length of the fatty acids seems to be important. Intraduodenal administration of fatty acids with 12 carbon atoms (C12, lauric acid) stimulated GLP-1 release, whereas a shorter one (C10, decanoic acid)

did not (Feltrin et al., 2004). Recently, novel G-protein coupled receptors GPR120 and GPR119 have been identified as mediators of fatty acid (Hirasawa et al., 2005) and fatty acid derivative induced GLP-1 release (Chu et al., 2008).

The effects of dietary fibre on GLP-1 release may be influenced by the amount of fibre or in contrast depend on structural food properties. Resistant starch produced a smaller GLP-1 response than digestible starch (Raben et al., 1994b). In contrast, pasta enriched with psyllium fibre did not modify postprandial GLP-1 responses (Frost et al., 2003) nor did a meal enriched with pea fibre (Raben et al., 1994a). Whole-kernel rye bread and whole-meal pasta produced smaller GLP-1 responses than low-fibre wheat bread or rye bread with beta-glucan (Juntunen et al., 2002). Furthermore, wheat bread with low amount of fibre produced similar GLP-1 responses as rye breads with significantly higher amounts of total fibre, although the insulin response after wheat bread was significantly greater, suggesting that merely the amount of fibre does not define the GLP-1 responses (Juntunen et al., 2003). In rats, a diet rich in fibre increased proglucagon expression in the intestine (Reimer and McBurney, 1996). After a standard breakfast the GLP-1 levels were higher in normal weight subjects compared to obese ones. Once galactose and guar gum were ingested prior to breakfast, elevated postprandial GLP-1 responses were observed and the levels did not differ between the groups, yet satiety increased slightly only in normal weight subjects. This suggests that postprandial GLP-1 levels do not correlate with the feelings of satiety in obese individuals (Adam and Westerterp-Plantenga, 2005).

2.2.5 Effects of nutrients on gastrointestinal peptide release

In summary, different macronutrients have different potencies in stimulating or inhibiting gastrointestinal peptide release (Table 1). The type of protein and fibre may affect the responses. Fat hydrolysis to free fatty acids is necessary for CCK, GLP-1 and PYY release and the length of the fatty acid carbon chain affects the response.

Table 1. Effects of macronutrients on the release of gastrointestinal peptides and their effects on food intake. + means stimulatory effect, - means inhibitory effect. The signs in brackets indicate blunted or inhibited increase or decrease. Suppression of ghrelin levels and stimulation of CCK, GLP-1 and PYY secretion is thought to increase satiety.

Peptide	Food intake	Carbohydrate	Fibre	Protein	Fat
Ghrelin	+	-	- / no effect / (-)	- / + / no effect	- / + / no effect
CCK	-	+	+	++	++
GLP-1	-	++	+ / no effect (+)	++	++
PYY	-	+	+ / no effect	++	++

2.3 SELECTED FOOD COMPONENTS IN OUR DAILY NUTRITION

2.3.1 Lectins

Lectins are carbohydrate-binding proteins that were initially discovered from castor beans by Stillmark in 1888. The name "lectin" derived from the Latin word legere, select, describes the ability of lectins to specifically recognize their ligands. Lectins form a heterogenous group of proteins with wide diversity in their ligand specificity. In addition to plants, lectins are found ubiquitously in nature, even in mammals. Lectins are defined by their carbohydrate-binding activity. Lectin proteins are distinct from immunoglobulins, although some animal lectins belong to the Ig superfamily and various lectins are produced by lymphocytes along with antibodies. Lectins have to be also distinguished from enzymes modifying glycoconjugates.

Initially lectins were recognized by their ability to agglutinate erythrocytes, yet this test can result in false positive results and in addition only reveals lectins which have at least two binding sites. Lectins can bind to glycoproteins on the surface of the cells or to soluble extracellular or intercellular glycoproteins. Animal lectins are divided into several subclasses and are important molecules in a wide variety of functions, including cell-cell and cell-matrix interactions, cell migration and routing, cell growth control and recognition of foreign glycoproteins among others.

In plants, the majority of lectins are found in storage tissues like seeds, tubers, bulbs, corns and rhizomes. Also leaves and stems of some plants accumulate storage proteins and lectins. Plant lectins may have defensive roles as some of them are toxic against fungi, insects or higher animals. Lectins are rich in legumes (Leguminosae). Especially seeds, beans, peas, peanuts, lentils, soybean, wheat, potato and rice contain lectins. Since the lectins are widely distributed in plants they are also of physiological relevance as part of our nutrition.

2.3.1.1 PHA

Red kidney bean (*Phaseolus vulgaris*) lectin phytohaemagglutinin (PHA) is composed of two polypeptides, L and E, that form five tetrameric isolectins L_4, L_3E, L_2E_2, LE_3 and E_4 (Leavitt et al., 1977). The L and E polypeptides were named based on their strong mitogenic activity for T-lymphocytes and their ability to agglutinate erythrocytes, respectively. Raw kidney beans are toxic to humans causing diarrhea and abdominal pain, and this toxicity is mainly due to the lectin PHA. However, the toxicity can be abolished by proper cooking of the beans prior to ingestion (Grant et al., 1982). Furthermore, toxic effects depend on the purity of the lectin preparation, and this may also explain the contradictory results obtained in different studies.

PHA is resistant to breakdown by the digestive enzymes of the gut and specifically recognizes and binds to brush-border cells of the intestine. PHA causes disruption of the enterocytes without significant alterations in the absorption capacity in adult rats (Pusztai et al., 1979). Inclusion of red kidney beans or purified PHA in the diet for three weeks induced weight loss with malabsorption in weanling rats (Banwell et al., 1983). Growth retardation was observed in rats fed with different lectins for 10 days as well (Pusztai

et al., 1990). In suckling rats, administration of PHA and attachment to epithelial cells caused a shortening of the intestinal villi, disturbances in the gut morphology and a decrease in macromolecular absorption capacity during the first day. This was followed later by declined binding and endocytosis (Linderoth et al., 2006a).

However, apart from the growth inhibiting effects of lectins, many of them have been reported to be effective mitogens of the gastrointestinal organs. PHA was the most potent mitogen of the lectins tested that led to hyperplasia and hypertrophy of the small intestine (Pusztai et al., 1990). It was found that both intestinal crypt length and the number of enterocytes were increased. In suckling rats, PHA feeding increased crypt cell proliferation, gut growth and functional maturation (Linderoth et al., 2006a). PHA also stimulated the growth of the pancreas and caused the accumulation of polyamines to the organ (Bardocz et al., 1995). Pancreatic growth and digestive enzyme content increased in suckling rats as well (Linderoth et al., 2006a). Since the growth promoting effects were only observed after oral or enteral feeding of lectins, the direct contact with gut epithelia appears to be required.

After 10 days on a PHA containing diet the weight of the rats was approximately 15 % lower compared to pair-fed control animals. However, PHA significantly increased the wet weight of the small bowel and pancreas and dose dependently elevated plasma CCK levels. The effect of PHA on pancreatic but not on small intestinal growth was blocked by CCK_1 receptor antagonist MK 329 (Herzig et al., 1997).

Total parenteral nutrition (TPN) induces atrophy of the gastrointestinal tract in humans and in animals. Rats fed by TPN displayed reduced gastrointestinal epithelia proliferation and lower tissue weights of the gastrointestinal organs compared to orally fed rats. Intragastric administration of lectins for four days was able to fully or partially reverse the atrophy produced by TPN (Jordinson et al., 1999). PHA treatment was the most potent of tested lectins and induced proliferation of gastric fundus, small intestine, pancreas and proximal colon. A stimulatory effect on plasma CCK and enteroglucagon levels was also noted in this study.

2.3.2 Dietary fibre

According to the official definition of dietary fibre given by the American Association of Cereal Chemists, "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and / or blood cholesterol attenuation, and / or blood glucose attenuation" (DeVries, 2003). As implicated by this definition, fibres as part of our daily nutrition have health effects on glucose and lipid metabolism, as well as gut function. Furthermore, the incidence of several common diseases has been shown to have an inverse relationship with dietary fibre intake. These include constipation, diabetes, colorectal cancer, diverticular disease, obesity, gallstones and coronary heart disease amongst others (DeVries, 2003).

There is a rich body of evidence showing that dietary fibre positively affects glucose metabolism by producing lower glucose and insulin responses. Fibre intake has been shown to improve glucose metabolism in type 2 diabetic patients (Chandalia et al., 2000; Jenkins et al., 2002; Jenkins et al., 1976). Fibre intake also lowers serum cholesterol levels (Anderson et al., 2000; Brown et al., 1999; Olson et al., 1997) and improves laxation (for review, see Dikeman and Fahey, 2006).

Dietary fibres can be classified based on their chemical properties to soluble (e.g. guar gum, psyllium fibre, locus bean gum, pectins) and insoluble (e.g. cellulose, wheat bran, soyhulls) fibres. Many, but not all, soluble fibres including beta-glucans, gums and pectins thicken when mixed with fluids, which results in the production of viscous material. Therefore, adding these soluble fibres to food alters its textual properties by increasing viscosity. Another key feature of dietary fibres is their fermentability in the large intestine. Those fibres which are fermentable in the colon produce gases and short chain fatty acids, whereas those which are less fermented are good bulking agents and promote laxation.

Importantly, viscosity is recognized as one of the factors contributing to the beneficial health effects observed after fibre ingestion, and reducing viscosity or using low viscosity fibres inhibits or attenuates these effects. Jenkins and coworkers concluded already in 1978 that viscosity of guar was an important property of fibre in reducing postprandial glucose and insulin concentration (Jenkins et al., 1978). High but not low viscosity guar gum was effective in reducing postprandial insulin responses when included in an oral meal (Leclere et al., 1994). Reducing the viscosity by acid hydrolysis prior to consumption attenuated the glucose and insulin lowering effects of oat gum (Wood et al., 1994). Furthermore, the beneficial effects of fibre on lipid metabolism seem to be dependent on viscosity as well (Gallaher et al., 1993; Wang et al., 1992).

The mechanism by how viscous fibres reduce postprandial glucose and insulin responses has been postulated to be due to slower gastric emptying rate, decreased intestinal absorption and increased intestinal transit time, although the relative importance of these parameters is still unclear (Edwards et al., 1987; Ehrlein and Stockmann, 1998; Leclere et al., 1994; Meyer et al., 1988). Viscous fibres form a gel like matrix as a result of their water holding capacity and may thus thicken the unstirred water layer on the mucosal surface which inhibits digestion processes by decreasing the accessibility of digestive enzymes to nutrients and decreases the diffusion of glucose and nutrients to absorptive epithelium in the intestine (Johnson and Gee, 1981). Viscosity may also hinder nutrient digestion by affecting activity of digestive enzymes (Isaksson et al., 1982), although others have observed increased activities after fibre ingestion (Ikegami et al., 1990). It seems that although the viscosity of the product is dependent on the amount of fibre, the physiological responses may not change proportionally to increasing viscosity and lower doses of viscous polysaccharides included in the diet may be as efficient as high doses (Wood et al., 1994).

Gastric emptying has been shown to be slower after viscous fibre ingestion. Slower gastric emptying and greater satiety was observed in obese subjects after the supplementation of a meal with either 15 g of pectin (Di Lorenzo et al., 1988) or 10.8 g psyllium (Bergmann et al., 1992). However, Marciani and coworkers sophistically demonstrated that a 1000-fold increase in viscosity of a nonnutrient liquid meal

with locust bean gum decreased gastric emptying only modestly suggesting that the stomach adjusts to the viscosity by more effectively diluting the digesta. Despite of the modest decrease in gastric emptying, a more viscous drink induced higher fullness scores (Marciani et al., 2000). Furthermore, they showed in another context that calorie content (nutrient vs. noncaloric meal) was more effective in decreasing gastric emptying than increasing viscosity, but increasing the viscosity of a nutrient meal increased fullness without significantly affecting gastric emptying rate (Marciani et al., 2001). Therefore, the mechanism whereby viscosity of meals induces satiety does not seem to relate exclusively to delayed gastric emptying.

Although correlations between diet fibre content, viscosity and increased satiety have been reported, the effects of dietary fibre on subsequent food intake and weight control are not very well established. Decreased food consumption after higher fibre intake has been observed in both short interventions (Burley et al., 1987; Porikos and Hagamen, 1986; Van de Ven et al., 1994) and after longer periods (Krotkiewski, 1984; Pasman et al., 1997; Ryttig et al., 1989). On the contrary, many studies have reported that dietary fibre has no effect on food intake (Pittler and Ernst, 2001). The variability of the results may be due to several factors, such as the type of the fibre used and the weight of the test subjects. It has also been suggested that fibre supplementation could be most effective in helping the subjects to stick to low calorie diets (Astrup et al., 1990; Pasman et al., 1997).

2.3.3 Spices

Although spices have been used primarily to give flavour to foods, in addition to this they have been shown to affect physiological functions such as thermogenesis and digestion or induce greater satiety. For example capsaicin, the pungent ingredient in red hot pepper, increases thermogenesis. Increased thermogenesis, via the activation of the sympathetic nervous system, may help to dissipate excess energy as heat. Furthermore, a capsaicin preload has been shown to decrease food intake and increase satiety in humans (Westerterp-Plantenga et al., 2005). Spices have also been shown to have beneficial effects on digestion by stimulating digestive enzyme activities, bile flow and bile acid secretion in rats (for review, see Westerterp-Plantenga et al., 2006). Capsaicin activates the transient receptor potential vanilloid receptor 1 (TRPV1), a member of the transient receptor potential (TRP) family. Also other pungent ingredients with thermogenesis stimulating properties, such as piperine from black pepper and 6- and 8-gingerols from ginger, are ligands of TRPV1. However, only a limited number of studies have investigated the involvement of other TRP channel family members activated by spices in the regulation of gastrointestinal functions.

2.3.3.1 TRP channels

The transient receptor potential (TRP) ion channel superfamily consists of seven subfamilies of nonselective cation channels with wide diversity in their activation mechanisms and cation selectivity (for review, see Venkatachalam and Montell, 2007). The common feature of TRP channels is that they are involved in the physiological sensing of the environment by vision, taste, olfaction, hearing, touch,

chemicals, temperature and osmolarity. Their importance is demonstrated by the fact that mutations in at least four TRP channels have been associated with diseases (TRPC6 in focal and segmental glomerulosclerosis of the late-onset type; TRPM6 in hypomagnesemia with secondary hypocalcemia; TRPP1 and TRPP2 in autosomal dominant polycystic kidney disease and TRPML1 in mucolipidosis type IV) and more are suggested (Venkatachalam and Montell, 2007).

TRP channels are mostly permeable to monovalent and divalent cations. Almost all TRP channels (except TRPM4 and TRPM5) are permeable to calcium, an important second messenger in many cellular functions. TRP channels modulate intracellular free calcium concentration either by acting as calcium entry pathways, affecting membrane polarization and calcium entry mediated by other pathways or inducing calcium release from intracellular stores.

TRPA1. TRPA (transient receptor potential ankyrin) channels are a subfamily of the group 1 TRPs, which in humans and in mice consists of only one known variant, the TRPA1 channel (previously ANKTM1) (Jaquemar et al., 1999). TRPA1 is activated by several structurally diverse compounds including the pungent ingredient allyl isothiocyanate (AITC) present in wasabi, horseradish as well as mustard oil, allicin and diallyl disulfide of garlic, cinnamaldehyde of cinnamon, eugenol from clove oil, gingerol from ginger, tetrahydrocannabinol (the psychoactive component of marijuana), acrolein (present in tear gas) and methyl salicylate in winter green oil (Bandell et al., 2004; Bautista et al., 2005; Bautista et al., 2006; Jordt et al., 2004; Macpherson et al., 2005).

TRPA1 is highly expressed in a subset of primary afferent somatosensory neurons of the dorsal root, trigeminal and nodose ganglia, especially in small diameter neurons where it co-localizes with markers of peptidergic nociceptors such as TRPV1, calcitonin gene related peptide (CGRP) and substance P (Bautista et al., 2005; Corey et al., 2004; Nagata et al., 2005; Story et al., 2003). In addition, TRPA1 is expressed in several nonneuronal tissues including the urinary bladder and gastrointestinal tract (Andrade et al., 2006; Penuelas et al., 2007; Stokes et al., 2006).

Extracellular calcium enhances the current rate and magnitude of AITC induced currents (Jordt et al., 2004). TRPA1 activation by highly reactive electrophiles such as AITC is mediated by reversible covalent modification of cysteine and lysine residues in the intracellular N-terminus of the channel (Hinman et al., 2006; Macpherson et al., 2007) indicating that the activator compounds need to diffuse or be transported inside the cell prior to activating TRPA1. In addition, TRPA1 may be indirectly activated via receptor-mediated activation. For example, the inflammatory mediator bradykinin activates TRPA1 after bradykinin 2 (BK2) receptor binding coupled to phospholipase C activation (Bandell et al., 2004). Furthermore, recent studies show that mere increases in the intracellular calcium concentration activate TRPA1, yet the EC_{50} required is rather high (905 nM) (Doerner et al., 2007; Zurborg et al., 2007) indicating that any signal leading to robust increases in the intracellular calcium concentration might activate TRPA1. This is of particular interest because many neurons coexpress TRPA1 with TRPV1 channels and thus calcium influx after TRPV1 activation might also lead to downstream activation of TRPA1. Since TRPA1 channel activation itself increases intracellular calcium levels, a tight negative

feedback is required to avoid cellular calcium overload (Doerner et al., 2007). Indeed, a calcium dependent desinsitization process has been proposed with calcium inducing fast channel closure (Nagata et al., 2005).

TRPA1 has also been implicated in the sensation of pain. As mentioned above, exogenous irritants like AITC in mustard oil and acrolein in tear gas activate TRPA1. More over, the topical application of these substances induces sensory nerve fibre excitation and release of neuropeptides (substance P and CGRP) and other neurotransmitters resulting in a robust hypersensitivity to thermal and mechanical stimuli. The endogenous inflammatory mediator bradykinin activates TRPA1 (Bandell et al., 2004) as does endogenous proalgesic factor (4-hydroxy-2-nonenal, HNE) that is produced by reactive oxygen species after tissue injury (Trevisani et al., 2007) and prostaglandin metabolites with electrophilic carbon structure (Taylor-Clark et al., 2007). Furthermore, inflammation and nerve injury increase TRPA1 expression (Obata et al., 2005). Thus there is a robust body of evidence that TRPA1 acts as a mediator of nociceptive inflammatory pathways and might be a target for the development of novel analgesic and anti-inflammatory medication.

TRPA1 has been proposed to have a role in mechanosensation because of its multiple (14-18) N-terminal ankyrin motif repeats (Figure 5) (Corey et al., 2004; Nagata et al., 2005). Expression of TRPA1 at the tips of the stereocilia in the hair cells of the inner ear suggested a role as the mechanically gated auditory transduction channel which has not been identified so far (Corey et al., 2004). However, both *trpa1* null mice lines have normal auditory responses (Bautista et al., 2006; Kwan et al., 2006).
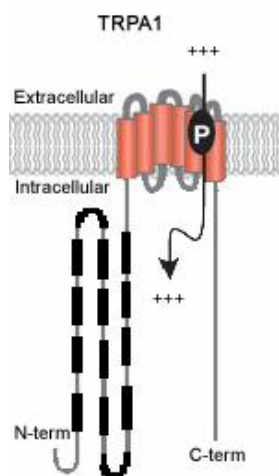
Figure 5. TRPA1 channel is a nonselective cation channel with seven transmembrane segments. It contains several ankyrin domains in its N-terminus (black bars). P indicates cation pore loop. TRPA1 is activated by compounds in wasabi, mustard oil, garlic, and ginger among others. Modified from Venkatachalam and Montell, 2007.

Many TRP channels are activated by temperature changes and thus participate in thermosensation. Members of TRPV family have a role in detection of warm, hot or moderate temperatures whereas TRPM8 is activated by cool temperatures and menthol. TRPA1 was suggested to be activated by noxious cold (17 °C) (Bandell et al., 2004; Story et al., 2003) yet this issue is largely debated (Bautista et al., 2006; Jordt et al., 2004; Nagata et al., 2005). However, nerve injury and inflammation upregulate TRPA1 expression and contribute to cold hyperalgesia (Obata et al., 2005) and antisense knockout of TRPA1 alleviates cold

hyperalgesia in a rat model (Katsura et al., 2006). Two different TRPA1 knock-out mouse lines with different outcomes have been reported. One knock-out line showed no impairment in temperature sensitivity (Bautista et al., 2006) in contrast to the other, which had a defect in paw withdrawal from a cold surface (Kwan et al., 2006). The differences may be explained by slightly different testing procedures, the inbred strain background of the mice lines or the differences in the production of a truncated TRPA1 which might affect other channels yet it seems that the possible role of TRPA1 in the temperature sensation might be very sensitive to confounding factors.

In the gastrointestinal tract, TRPA1 and its functions are so far unknown. A recent publication reported that TRPA1 activation induces contractions in the isolated mouse intestine, especially in the proximal and distal colon suggesting TRPA1 expression in the enteric nervous system and stimulation of digestive processes (Penuelas et al., 2007). TRPA1 expression was also demonstrated in the human gastrointestinal mucosa (Stokes et al., 2006).

3 AIMS OF THE STUDY

The aim of this study was to investigate the release of gastrointestinal hormones in response to different nutritional stimuli and to evaluate their effects on food intake and satiety. The following questions were addressed:

I   Does duodenal administration of red kidney bean lectin PHA affect gallbladder volume or CCK release in humans? (Study I)

II   Do otherwise similar oat bran beverages varying in fibre viscosity differ in their postprandial gastrointestinal hormone responses or effects on satiety in humans? (Study II)

III   Does allyl isothiocyanate, the active compound of the spice wasabi, activate TRPA1 channels in neuroendocrine cells and stimulate CCK release? (Study III)

IV   Does PYY(3-36) affect food intake in colectomized rats (surgical knock-down model)? (Study IV)

4 MATERIALS AND METHODS

4.1 HUMAN STUDIES

4.1.1 Ethical approval

Healthy volunteers participated in the studies. The experiments were approved by the Research Ethics Committee, Hospital District of Northern Savo (II) and by the Ethics Committee of Basel, Switzerland (I) and of the University of Kiel, Germany (III) and were in accordance with the Helsinki Declaration. Each subject provided their verbal and written informed consent before participation in the study.

4.1.2 Intraduodenal PHA administration (I)

4.1.2.1 Preparation of PHA

The complete mixture of PHA isolectins was purified by affinity chromatography with ovomucoid as ligand glycoprotein immobilized on divinyl-sulfone-activated Sepharose 4B and eluted using 150 mM sodium tetraborate, pH 8.0 (Freier et al., 1985; Gabius, 1990; Rüdiger, 1993). Quality control of purity to exclude presence of further proteins except for the lectin fraction was performed by one- and two-dimensional gel electrophoresis, as described (Kohnke-Godt and Gabius, 1989), and activity measurements were carried out by haemagglutination and solid-phase assays using (neo)glycoproteins exposing the cognate complex-type N-glycans (Andre et al., 2004; Andre et al., 2006; Andre et al., 2007). Heat-inactivated PHA was prepared by boiling at 100 °C for 20 min.

4.1.2.2 Study protocol

Five healthy male volunteers (20-29 years) underwent four studies (iv. saline and id. PHA; iv. saline and id. heat-inactivated PHA; iv. atropine and id. PHA; iv. dexloxiglumide and id. PHA) on different days in randomized, single-blind, cross-over manner. Volunteers were taking no medication before the study, and each subject was within 15% of his ideal body weight. Each subject had normal screening physical examination and laboratory test results, including urine analysis, complete blood count, serum chemistries, electrocardiography, and abdominal ultrasonography. Subjects had no history of significant illness or surgery. All studies were conducted in the morning after an overnight (12-hour) fast when an intraduodenal tube was placed under fluoroscopic guidance. After a basal period (30 min) of saline perfusion at a flow rate of 5 ml/min, PHA was perfused in increasing doses of 150 μg, 1.5 mg and 15 mg for 30 min each at a constant flow rate of 5 ml/min. Saline or the specific CCK_1 receptor antagonist dexloxiglumide (Rotta Research Laboratories, Monza, Italy) (5 mg x kg^{-1} x h^{-1}) or the muscarinic acetylcholine receptor antagonist atropine (Streuli Pharma, Uznach, Switzerland) (5 μg x kg^{-1} x h^{-1}) were infused intravenously from time -30 min to 90 min.

Gallbladder contraction was assessed by high-resolution, real-time sonography (ALOKA SSD650, Soma Technology, Cheshire, CT, USA) using a 3.5-MHz probe on a sector scanner. Longitudinal sonograms of the gallbladder were recorded every 10 minutes to calculate the volume based on elliptic sections defined by two diameters (Beglinger et al., 1992). Gallbladder volumes were expressed as % of basal gallbladder volume measured at -20 min. Mean gallbladder volume during each PHA dose was calculated from the three measurement points during 30 minutes and used for statistical analysis. Blood was drawn for determination of plasma CCK concentrations at timepoints -20, -10, -5, 15, 25, 45, 55, 75 and 85 min. As a positive control a 50 ml bolus of a high caloric liquid meal (Ensure plus, Abbott, Abbott Park, IL, USA) was perfused after the last dose of PHA.

4.1.3 Beverages with different viscosity (II)

4.1.3.1 Subjects

Subject baseline characteristics are given in table 2. At the beginning of the study, volunteers were interviewed about their medical history, dietary habits and physical activity. Candidates with any food intolerances or allergies, smokers, who had modified their diet or exercise patterns during the past year to lose weight or were on medication (except oral contraceptives) that would affect appetite were excluded. In addition, all candidates filled in the questionnaires "Three-Factor Eating Questionnaire" (Stunkard and Messick, 1985) and "Bulimic Investigatory Test, Edinbugh (BITE)" (Henderson and Freeman, 1987) to exclude subjects with abnormal eating behaviour.

Table 2. Characteristics of the study subjects in study II.

	Value, mean ± SEM	Range
n (women / men)	20 (16 / 4)	
Age (y)	22.6 ± 0.7	19 - 34
Weight (kg)	62.0 ± 1.8	51.4 - 82.3
Body mass index (kg/m^2)	21.6 ± 0.3	18.9 - 23.5
Systolic blood pressure (mmHg)	114.1 ± 1.9	103.0 - 132.0
Diastolic blood pressure (mmHg)	68.4 ± 1.5	56.0 - 84.0
Oral glucose tolerance test		
Plasma glucose, 0 min (mmol/L)	5.0 ± 0.1	4.5 - 5.5
Plasma glucose, 120 min (mmol/L)	5.0 ± 0.3	2.9 - 6.4
Three-Factor Eating Questionnaire		
- factor 1	10.5 ± 1.1	3 - 19
- factor 2	4.0 ± 0.6	2 - 13
- factor 3	3.1 ± 0.5	0 - 8
Bulimic Investigatory Test		
-sympton subscale	2.6 ± 0.7	0 - 12
-severity subscale	0.7 ± 0.2	0 - 3

4.1.3.2 Test beverages

Two isoenergetic and isovolumic beverages that differed only in relation to viscosity were used as test meals in the study (see table 3). Viscosity of the low-viscous test beverage was eliminated by using 0.2 g of beta-glucanase enzyme (AB Enzymes GmbH, Darmstadt, Germany), which resulted in a marked degradation of the beta-glucan molecules and distinctive distribution of molecular weight fractions compared to the high-viscous test beverage (see table 4). The test beverages were prepared with commercially available ingredients.

Table 3. Energy and macronutrient composition of the test beverages in study II.

	Low-viscous	High-viscous
Portion size (ml)	300[¶]	300[¶]
Energy (kJ / kcal)	1250 / 300	1250 / 300
Energy density (kJ / g)	4.17	4.17
Oat bran concentrate (g)	30[#]	30
Carbohydrates (g / E %)	57.9 / 79.5	57.9 / 79.5
Total dietary fibre (g)	10.2	10.2
- insoluble dietary fibre (g)	5.1	5.1
- soluble dietary fibre (g)	5.1	5.1
Protein (g / E %)	7.8 / 10.7	7.8 / 10.7
Fat (g / E %)	3.3 / 9.8	3.3 / 9.8

[¶] Ingested with 200 ml of water
[#] Low-viscous oat bran, viscosity eliminated using beta-glucanase enzyme

Table 4. Chemical composition of the oat bran beverages in study II.

	Low-viscous	High-viscous
Molecular weight, MW		
> 1 000 000 (%)	5	50
1 000 000 - 100 000 (%)	10	35
< 100 000 (%)	85	15
Viscosity at 20min (mPas)	< 250	> 3000

4.1.3.3 Study design

The study was a single-blind, randomized, within-subject crossover design in which all participants tested both beverages. The study visits were arranged > 2 days apart from each other. The subjects were advised to maintain their habitual diet and exercise routines throughout the study. At each study visit before the actual test the participants were weighed and their overall exercise and alcohol consumption of the previous day were checked by an interview. Heavy exercise was prohibited on the day before the study visit, as was alcohol consumption for 2 days before and smoking in the morning of the test

day. Subjects were requested to use a car or a bus on their way to the laboratory unit to avoid extra physical stress. The use of paracetamol or any analgesic drugs containing paracetamol was forbidden during the study.

At the first visit, all the subjects ingested a standard glucose load (75 g of glucose dissolved in 300 ml water, the oral glucose tolerance test, OGTT), to ascertain normal glucose tolerance. During the subsequent two actual study visits, subjects ingested either a low-viscous oat bran beverage, or a high-viscous oat bran beverage both containing 1500 mg paracetamol for gastric emptying assessment (Naslund et al., 2000; Willems et al., 2001) along with 200 ml of water. The drinks were ingested within two minutes. Three hours after administration of the test beverage an *ad libitum* meal was served which consisted of vegetable soup, oat and rye bread, margarine, cheese, sliced tomato and cucumber, noncaloric juice and tap water.

Blood samples were drawn for the determination of plasma glucose, insulin, ghrelin, CCK, PYY and GLP-1 and serum paracetamol concentrations through an indwelling cannula placed in the forearm vein before and at 15, 30, 45, 60, 90, 120 and 180 min after ingestion of the test beverages. Tubes were prechilled on ice. EDTA-tubes were used for ghrelin, CCK, PYY, GLP-1 and insulin, and centrifuged within 15 minutes, for 15 minutes at 2800 rpm at +4°C and plasma immediately frozen. Fluoride citrate-containing tubes were used for glucose samples and centrifuged for 10 minutes at 3300 rpm at + 4°C. All samples were stored in -70 °C until analyzed.

The subjects rated their appetite sensations at the same time points immediately after the blood samples were drawn. In addition, subjects rated the pleasantness of the test meal immediately before and after ingestion of the test beverage. Corresponding measurements of appetite and pleasantness of the served food components were also used after the *ad libitum* meal.

4.1.3.4 Subjective appetite measurements

Subjective sensations of appetite (hunger, satiety, desire to eat, fullness) and pleasantness were evaluated using Visual Analogue Scales (VAS). Each VAS scale consisted of 100 mm horizontal line verbally anchored at both ends (i.e. 'I am not hungry at all' or 'I have never been hungrier'). The subjects were instructed to draw a vertical line on the horizontal axis corresponding to their sensations that were most appropriate at the time. Distances on the VAS were measured from the left boundary of the axis in millimeters resulting in scores between 0-100.

4.1.3.5 Food intake

Study participants were advised to keep detailed scale-weighed 24-h food records throughout the study to monitor their food intake. These included food records before each study day and half-day records for the remaining day of each experiment to reflect the effects of each test meal on subsequent food intake after the 3 hour study period. Food records were collected every subsequent study visit. The average daily energy and macronutrient intake of the participants from the food records and from the *ad libitum* meal

served after the test beverages were analyzed by using the MICRO-NUTRICA database (version 2.5; Finnish Social Insurance Institution, Turku, Finland).

4.1.4 Human duodenal mucosa samples (III)

Duodenal mucosa for TRPA1 mRNA expression studies was obtained from intestinal endoscopy biopsies from patients undergoing routine diagnostic procedures for various clinical reasons. Samples were snap frozen in liquid nitrogen and stored at - 70 ℃.

4.2 ANIMAL EXPERIMENTS

4.2.1 Ethical approval

The experiments were approved by the Institutional Animal Care and Use Committee of the University of Kuopio and the Provincial Government. Animals were housed in 12 hour dark-light cycle at the animal facility of the University of Kuopio.

4.2.2 Mouse duodenal samples (III)

CD2 male mice were sacrificed by cervical dislocation, proximal 5 cm of the duodenum dissected and the mucosa scraped. The sample was snap frozen in liquid nitrogen and stored at -70 °C until mRNA extraction.

4.2.3 Colectomized rats and PYY(3-36) infusion (IV)

4.2.3.1. Colectomy

Adult male Wistar rats (300-395 g) were fasted for 24-36 h before surgery, during which time their regular drinking water was replaced with water containing 5 % dextrose to reduce the hypermetabolic effects associated with the prolonged postoperative fast required for anastomotic healing. Animals were anesthetized by intramuscular injection of a mixture of ketamine (75 mg/kg body weight) and xylazine hydrochloride (10 mg/kg b.w.). After a 3-4 cm midline laparotomy, the caecum was removed from the abdomen onto saline-soaked sterile gauze. The total proctocolectomy was performed by resecting the colon ligating the mesentery with 4-0 silk (Ethicon, Cincinnati, OH, USA). The intestinal segment was excised from 0.1 cm proximal to the ileocaecal junction. The rectum was resected at the level of the pelvic floor. An ileal J-pouch of 2 cm length was created by duplication of the distal end of the small intestine by single-layer interrupted 6-0 prolene suture (Figure 6). The pouch anal anastomosis was performed by a single layer interrupted 6-0 prolene suture. The sufficiency of the anastomosis was controlled by an injection of 3-5 ml of 0.9 % saline through the anus. After surgery buprenorphine (0,05 mg/kg b.w. sc) was given for two days as pain medication. The animals were allowed to recover from surgery for 2 weeks before being subjected to experimental procedures.
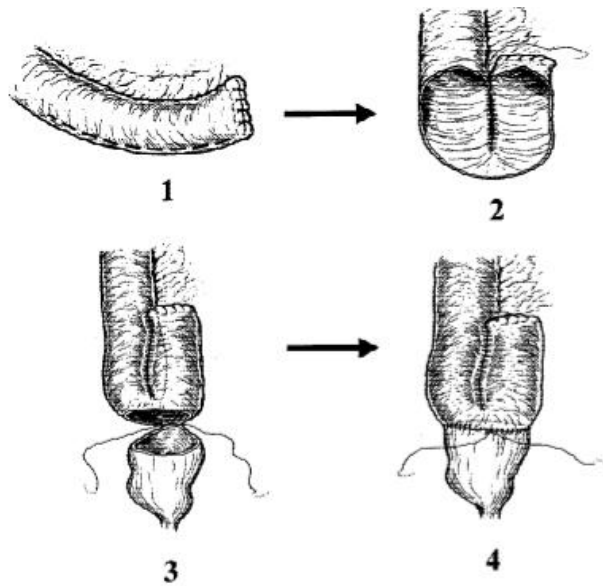
Figure 6. Surgical construction of ileal J pouch after colectomy. Terminal ileum is oversewn (1), incised along its antimesenteric border and the two limbs of the ileal loop are sutured together to form a pouch with the J configuration (2). An anastomosis is performed between the distal end of the pouch and the proximal end of the rectal stump (4). From Shebani et al., 2002.

4.2.3.2 PYY administration

In total, 16 colectomized rats were adapted for 14 days, housed individually and provided with food and water *ad libitum*, before entering the study. Animals were randomized into two experimental groups and Alzet® osmotic mini pumps (Model 2ML2, 14-day delivery; Durect Corp, Cupertino, CA, USA delivering 5 µl/hr) were implanted subcutaneously between the scapulae and the outlet catheter implanted into the jugular vein. The control group of 7 animals received pumps loaded with phosphate-buffered saline (PBS) while in the test group of 9 animals pumps were filled with PYY (3-36) (Phoenix Pharmaceuticals Inc, Belmont, CA, USA) dissolved in PBS delivering 175 µg/kg/day intravenously.

4.2.3.3 Measurement of food intake, body weight and plasma PYY levels

Blood samples were drawn in the fed state prior to colectomy and 7 days as well as 14 days after the beginning of PBS or PYY (3-36) infusion for determination of PYY plasma levels. Blood samples were collected using heparinized micro-pipettes, immediately placed on ice and centrifuged. Plasma was stored at -70 ºC.

Food consumption and body weight was measured daily in the early light phase (8:00-9:00). Cages were carefully monitored for evidence of food spillage or grinding, which was negligible.

4.3 CELL CULTURE

4.3.1 STC-1 cell culture (III)

STC-1 cells are derived from an intestinal endocrine tumor that occurred in a double-transgenic mouse model (Rindi et al., 1990). Cells (passages 32-50) were cultured in DMEM (4.5 g D-glucose/liter)

supplemented with horse serum (15 %), fetal bovine serum (2 %), antibiotics and L-glutamine and maintained in 37 °C incubator (5 % CO_2 / 95 % air).

4.3.2 CCK secretion (III)

STC-1 cells were plated on 6-well plates and cultured for 4-5 days. Cells were washed once with oxygenated HR-buffer (in mM: 130 NaCl, 5 KCl, 1.2 $CaCl_2$, 1 NaH_2PO_4, 1.2 $MgSO_4$, 10 HEPES, 6.7 glucose, 0.4 % bovine serum albumin, pH 7.4) and incubated for 20 minutes with buffer containing stimulants (1 ml / well). Supernatants were collected, spinned to remove cell debris and stored at -20°C until analyzed for CCK concentration.

4.4 ANALYTICAL METHODS

4.4.1 Isolation of mRNA and RT-PCR (III)

Total RNA was extracted using RNeasy Kit (Qiagen, Hilden, Germany). Genomic DNA was digested by deoxyribonuclease I (Qiagen). First-strand cDNA was synthesized from 1 µg of mRNA by using TaqMan Reverse Transcriptase reagents (Applied Biosystems, Warrington, United Kingdom).

Trpa1 gene mRNA was amplified with the following primers: 5'-agtggcaatgtggagcaa-3' & 5'-tctgatccactttgcgta-3'. PCR cocktail consisted of 2.5 µl 10 x PCR-buffer, 1.5 mM $MgCl_2$, 250 µM dNTPs (each), 2 units Dynazyme II polymerase (Finnzymes, Finland), 0.5 µM sense and antisense primers and 5µl of the above described cDNA. The PCR profile performed was (35 cycles): 5 min at 95 °C, 30 sec at 48 °C, 30 sec at 72 °C, 30 sec at 94 °C, final extension 5 min at 72°C. PCR products were separated on 2 % agarose gel, purified (Qiagen, Hilden, Germany) and sequenced (AIVI sequencing service, http://www.uku.fi/aivi/services/sequencing/index.shtml).

4.4.2 Quantitative fluorescence imaging (III)

Coverslips with STC-1 cells were loaded with 4 µM fura-2-acetoxymethyl ester (fura-2-AM) in a buffer (in mM: 137 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose and 20 HEPES, pH 7.4) for 60 min in room temperature. Cells were rinsed with fura-2-AM free medium, placed on the bottom of an RC24-fast exchange chamber (Warner Instruments Inc.) and positioned on top of the microscope. For fura-2 excitation, cells were illuminated with two alternating wavelengths 340 and 380 nm through a dichroic mirror (DM430, Nikon) using a Polychrome IV monochromator (TILL Photonics GmbH, Gräfelfing, Germany). The emission was guided through a 510 nm cut off filter then captured by a cooled 12-bit IMAGO CCD camera and digitized by a computer running the TILLvisION Multi-Color Ratio Imaging System (TILL Photonics GmbH, Gräfelfing, Germany). Ratio images were collected and saved for later analysis. Fluorescence from 340 and 380 nm exposures were imported into the Microcal Software programme (Northampton, MA, USA), and given as absolute intracellular calcium levels $[Ca^{2+}]_i$ or as changes in $[Ca^{2+}]_i$ levels ($\Delta[Ca^{2+}]_i$).

4.4.3 Peptide analysis

4.4.3.1 CCK measurement (I, II, III)

In studies I and II, measurement of plasma CCK levels was performed after sample extraction in C-18 columns (Sep-Pak cartridges, WAT051910, Waters Corporation, Milford, MA, USA). Briefly, columns were activated with 10 ml of 100 % acetonitrile and 10 ml of 100 % methanol and washed with 10 ml of 1% trifluoroacetic acid (TFA). 1 ml (in study I) or 0.9 ml (in study II) of plasma was acidified with an equal volume of 1 % TFA, applied to columns and washed with 10 ml of 1 % TFA. Sample was eluted with 2 ml of 80 % acetonitrile in 1 % TFA, evaporated in SpeedVac concentrator for 3 hours in ambient temperature, frozen and lyophilized. Sample extraction was not performed for cell culture supernatants in study III.

CCK was determined by a radioimmunoassay (Euria-CCK, Euro-Diagnostica, Malmö, Sweden). The antibody recognizes CCK 26-33 sulphate (100 %) and CCK-33 sulphate (134 %), but does not significantly cross react with gastrin-17 sulphate (0.5 %) or with non-sulphated gastrin-17 (< 0.01 %). The inter-assay CV was 22.1 % at 0.55 pmol/l and 13.0 % at 3.3 pmol/l. The intra-assay CV was 5.5 % at 4.4 pmol/l and 2 % at 20.6 pmol/l.

4.4.3.2 PYY measurement (II, IV)

In study II, total plasma PYY was measured with a radioimmunoassay that detects both PYY(1-36) and PYY(3-36) (Linco Research Inc., USA). In these experiments, the intra-assay CV was 11.0 % at 62 pg/ml and 8.0 % at 212 pg/ml.

Rat plasma (IV) was analysed using an enzyme immunoassay for PYY(3-36) (Phoenix Pharmaceuticals Inc, Belmont, CA). The assay recognizes PYY(1-36) and PYY(3-36). The intra-assay variability was < 5% and inter-assay variability < 14%.

4.4.3.3 GLP-1 measurement (II)

Plasma GLP-1 was measured using a radioimmunoassay (Linco Research Inc., USA). The assay measures the active GLP-1, that is both GLP-1(7-36)amide and GLP-1(7-37). The intra-assay CV was 20.6 % and 14.2 % at 8.1 pmol/L and 42.0 pmol/l, respectively.

4.4.3.4 Ghrelin measurement (II)

A radioimmunoassay method was used for the analysis of total plasma ghrelin, that is active octanoyl and inactive des-octanoyl ghrelin (Linco Research Inc., USA). Here, the intra-assay CV was 9.5 % and 8.2 % at 506 pg/ml and 1220 pg/ml, respectively.

4.4.3.5 Glucose, insulin and serum paracetamol measurement (II)

Plasma glucose was analyzed using an enzymatic photometric assay (Konelab 20XTi Clinical Chemistry Analyzer, Thermo Electron Corp, Vantaa, Finland) and plasma insulin using a luminometric

immunoassay (ADVIA Centaur Immunoassay System, Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Serum paracetamol concentration was measured with a fluorescence polarization immunoassay (FPIA) (Abbott, Abbott Park, IL, USA).

4.5 STATISTICAL ANALYSIS

GraphPad Software (GraphPad Software Inc., San Diego, USA) and SPSS for Windows (SPSS version 14.0, Chicago, IL, USA) were used for statistical analysis. Statistical comparisons were performed using analysis of variance (ANOVA) with Tukey's Multiple Comparison Test, student t-test, paired t-test and mixed models analysis of repeated measures when appropriate. Results are expressed as mean ± SEM unless otherwise stated. In study II, repeated measures ANOVA with product and time as within-subject factors and Huynh-Feldt as a correction factor was used to compare the responses after different test beverages by testing for the main effects and product x time interactions. Where a significant interaction or main effect was observed, a post-hoc analysis was performed using a Sidak correction for multiple comparisons.

5 RESULTS

5.1 EFFECTS OF DIETARY PHA ON GALLBLADDER CONTRACTION AND PLASMA CCK LEVELS IN HUMANS

Red kidney bean lectin phytohaemagglutinin (PHA) has been shown to have mitogenic effects on the gastrointestinal tract and stimulate CCK secretion in rats but the effects in humans are not known. PHA was administered intraduodenally to fasted human volunteers in increasing doses (150 µg, 1.5 mg, 15 mg) and gallbladder volume and plasma CCK levels were analysed.

5.1.1 PHA induced gallbladder contraction

The duodenal administration of increasing doses of PHA stimulated gallbladder contraction in a dose-dependent fashion, starting with the lowest dose (150 µg / 30 min). Ultrasonographic measurements demonstrated that the gallbladder contracted: Mean gallbladder volume during the first 30 min of PHA infusion was 83.0 ± 5.8 % of the basal volume ($p < 0.001$) and reached 65.3 ± 9.4 % ($p < 0,001$) with the highest dose. In contrast to native PHA, heat-inactivated PHA at any concentration did not induce gallbladder contraction (mean gallbladder volume during the last 30 min of the experiment was 109.2 ± 7.0 % of basal, $p > 0.05$, not significant).

In order to investigate the possible mechanisms by which PHA induces gallbladder contractions, two distinct antagonists were used. Blocking CCK_1 receptors with dexloxiglumide when PHA was simultaneously infused into the duodenum increased gallbladder volumes over time (208.7 ± 23.7 % of basal volume at the end of the experiment, $p < 0.001$). In addition, the muscarinic receptor antagonist atropine completely inhibited PHA induced gallbladder contraction (104.0 ± 7.0 % of basal volume at the end of the experiment with the highest PHA dose, $p > 0.05$, not significantly different from baseline).

5.1.2 Effect of PHA on plasma CCK levels

Basal plasma CCK levels were approximately 3 pmol/l and were not increased even with the highest dose of native PHA. Furthermore, neither dexloxiglumide, infused simultaneously with intraduodenal PHA, nor heat-inactivated PHA caused any effect on plasma CCK levels. In contrast, a high caloric liquid meal, used as a positive control, stimulated gallbladder contraction and increased plasma CCK concentrations to a typical postprandial range of approximately 10 pmol/l.

5.2 EFFECTS OF FIBRE VISCOSITY ON GASTROINTESTINAL PEPTIDE RELEASE AND APPETITE

Fibres positively affect glucose and insulin metabolism by producing lower postprandial increases of both parameters. This has been shown to depend on the viscosity of the fibre. The effects of fibre

viscosity on postprandial gastrointestinal hormone secretion were studied in healthy humans after ingestion of beverages with soluble oat bran fibre, with or without beta-glucanase treatment to reduce viscosity.

5.2.1 Postprandial glucose and insulin levels

Both oat bran beverages increased plasma glucose and insulin concentration compared to fasting levels. Plasma glucose and insulin responses were significantly attenuated after the high-viscous beverage as compared to the low-viscous one (product x time interaction $p < 0.001$ and $p = 0.008$ for glucose and insulin, respectively).

5.2.2 Postprandial gastrointestinal hormone responses

Plasma ghrelin levels were decreased after both beverages. The decrease was more pronounced after the low-viscous beverage compared to the high-viscous one (product x time $p = 0.009$). The low-viscous beverage elevated plasma CCK, PYY and GLP-1 levels more than the high-viscous beverage. The product x time interaction was statistically significant for CCK (product x time $p = 0.035$) and GLP-1 ($p = 0.037$) and almost reached significance also in case of PYY (product x time $p = 0.051$). The effect of product was significant in all the cases ($p < 0.05$).

5.2.3 Gastric emptying

Gastric emptying, as measured by increasing plasma paracetamol concentrations, was faster after the low-viscous beverage consumption than after the high-viscous one (product x time interaction $p = 0.034$).

5.2.4 Satiety ratings and food intake

The fasting values of the subjective ratings of appetite (hunger, satiety, desire to eat, fullness) showed no significant differences before the ingestion of the test beverages. During the three-hour observation period after the test beverages, the low-viscous beverage produced greater satiety ratings than the high-viscous one (product $p = 0.048$). A similar trend was observed in the feeling of fullness, yet this did not reach statistical significance (product $p = 0.069$). Other parameters did not significantly differ between the treatments.

The energy, macronutrient or dietary fibre consumption on the day preceeding each test day was recorded by food diary and did not differ from the test days. Food intake during the *ad libitum* lunch served three hours after the test beverages was not different between the treatments. Energy consumption during the test day (*ad libitum* lunch and the rest of the day combined) as analyzed by the food diary was significantly higher after the low-viscous (2005 ± 154 kcal) than after the high-viscous beverage (1731 ± 113 kcal) ($p = 0.026$). Macronutrient intake for the rest of the test day was not affected by the tested beverages.

5.3 EFFECTS OF TRPA1 CHANNEL ACTIVATION ON CCK RELEASE

TRPA1 channels have been described in sensory neurons and are activated by multiple plant derived pungent ingredients including allyl isothiocyanate derived from both wasabi and mustard oil. We hypothesized that these ingredients act on TRPA1 channels in the gastrointestinal enteroendocrine cells and stimulate peptide release. As an established model to study the peptide release we utilized the mouse intestinal neuroendocrine cell line STC-1, which is known to secrete CCK, GLP-1 and GIP (Abello et al., 1994; Glassmeier et al., 1998; Kieffer et al., 1995).

5.3.1 Trpa1 gene expression in the mammalian gastrointestinal tract

Trpa1 gene mRNA was detected in native mouse duodenal mucosa, in the mouse intestinal neuroendocrine cell line STC-1 and in human duodenal mucosa biopsies by RT-PCR. The PCR product sequence was confirmed by sequencing.

5.3.2 Intracellular calcium levels and CCK release

The stimulation of STC-1 cells with 100 µM TRPA1 agonist AITC significantly increased CCK secretion by 6.7-fold compared to basal levels ($p < 0.001$). This stimulation was completely blocked by preincubation with 10 µM TRPA1 antagonist ruthenium red (RR).

Since TRPA1 channels are nonselective cation channels permeable to calcium, an important second messenger in exocytosis, the effect of AITC on intracellular calcium levels was investigated. 100 µM AITC caused a significant increase in the intracellular free calcium concentration ($[Ca^{2+}]_i$). The rise in $[Ca^{2+}]_i$ was blocked by 1µM RR ($p < 0.001$).

5.3.3 The effects of extracellular calcium and blockade of L-type calcium channels on TRPA1 mediated CCK release

To evaluate the contribution of extracellular calcium on AITC stimulated CCK release, extracellular calcium was chelated by 2 mM EGTA. In the presence of EGTA, AITC did not stimulate CCK release thus indicating that AITC induced CCK release is dependent on the influx of extracellular calcium.

Activation of L-type calcium channels by the depolarization of the membrane potential has previously been shown to be involved in CCK release from STC-1 cells. STC-1 cells were stimulated with 100 µM AITC in the presence of L-type voltage gated calcium channel blocker 10 µM nifedipine or 5 µM nimodipine. AITC stimulated CCK release was reduced by 36 % in the presence of the nifedipine or nimodipine ($p < 0.001$) suggesting that the main drive for calcium influx and CCK release in response to AITC is mediated via TRPA1 channels.

5.4 THE EFFECT OF PYY(3-36) ON FOOD INTAKE AND BODY WEIGHT IN COLECTOMIZED RATS

PYY(3-36) has been suggested to inhibit food intake in both rodents and humans. Most of the circulating PYY is released from L-cells in the distal small intestine and colon. Therefore, total colectomy was performed in adult rats in order to create a surgical knock-out model of PYY. After a 2-week recovery period, PYY(3-36) or saline was continuously infused intravenously to colectomized animals, with food intake and body weight monitored for two weeks.

5.4.1 Food intake and body weight after PYY(3-36) infusion in colectomized rats

PYY(3-36) did not affect food intake (mean daily food intake 26.22 ± 1.35 g) compared to saline treated control animals (25.63 ± 1.04 g) at any time point during the 14 days experimental period. The weight increases were calculated by subtracting the weight of the rats on the first day of the study from the weight on each subsequent day. Daily body weight gain was not statistically significant between PYY(3-36) (mean 4.68 ± 0.35 g) and saline (5.61 ± 0.48 g) treated animals at any time point during the experiment.

5.4.2 Plasma PYY concentrations

Plasma PYY concentrations before colectomy were similar between the two groups (279.3 ± 60.8 pM in controls; 271.9 ± 17.8 pM in PYY(3-36)-infused animals). In the control group with saline infusion, PYY plasma concentration surprisingly increased from the preoperative basal concentration of 279.3 ± 60.8 pM to 480.3 ± 80.1 pM after 7 days of PBS infusion ($p < 0.0001$). 14 days after the beginning of the saline infusion, the plasma PYY levels were reduced compared to the value recorded at 7 days (480.3 ± 80.1 pM vs. 355.8 ± 54.4 pM, $p < 0.01$). However, significantly elevated levels compared to basal preoperative concentration were still observed (279.3 ± 60.8 pM vs. 355.8 ± 54.4 pM; $p < 0.05$).

In PYY(3-36) infused animals plasma PYY increased from preoperative concentration of 271.9 ± 17.9 pM to 812.6 ± 107.3 pM after 7 days of PYY (3-36) infusion ($p < 0.0001$) and stayed elevated at the same level until the end of the experiment (821 ± 117.7 pM after 14 days infusion, $p < 0.0001$ compared to preoperative as well as $p > 0.05$ compared to 7 days infusion).

6 DISCUSSION

Nutrients and food components in our meals trigger an array of signals in the body regulating the maintenance of energy homeostasis. Nutrients from the ingested food are in direct contact with gut epithelial cells and are therefore able to modulate peptide secretion from enteroendocrine cells which in turn activate neural pathways or modulate the functions of other peripheral organs or the CNS via circulation. In the studies detailed here, some of the food components and properties were investigated in terms of their ability to influence gastrointestinal peptide secretion that ultimately affect digestive processes and food intake.

6.1 RED KIDNEY BEAN LECTIN PHA CONTRACTS THE GALLBLADDER IN HUMANS

Many plant products that are part of our daily nutrition are rich sources of lectins, proteins that are capable of binding to carbohydrate moieties on other molecules. Lectins are especially enriched in seeds, beans, peas, peanuts, lentils, soybean, wheat and rice and tubors (e.g. potatoes). Previously, it has been shown that several plant lectins induce mitogenic effects on pancreas and intestinal epithelial cells and increase plasma CCK levels in animals.

This study shows for the first time that intraduodenal administration of lectin PHA from red kidney beans induces gallbladder contractions in humans. However, in contrast to animal studies (Herzig et al., 1997), no measurable changes in the peripheral plasma CCK levels could be detected in humans in the dosages used. PHA stimulated gallbladder contraction was blocked by both the specific CCK_1 receptor antagonist dexloxiglumide and the muscarinic cholinergic antagonist atropine.

Gallbladder contraction during the cephalic and gastric phase of digestion is mediated via the cholinergic fibres of the vagus nerve (Hopman et al., 1987), yet the highest rate of postprandial gallbladder emptying correlates with the intestinal phase of digestion and elevated peripheral plasma CCK levels. Meal induced gallbladder contraction can be partially but not completely blocked by atropine (Beglinger et al., 1992; Hopman et al., 1990; Nelson et al., 1996) indicating that, in addition to CCK_1 receptors, the cholinergic nervous system is involved in the mediation of gallbladder contraction. Blocking the muscarinic cholinergic receptors by atropine totally abolished the PHA induced gallbladder contraction, thus suggesting that cholinergic nerves are centrally involved in the mechanism.

CCK_1 receptor antagonists have been shown to increase plasma CCK levels in the stimulated state (Degen et al., 2007; Hildebrand et al., 1990; Schmidt et al., 1991). Elevation of plasma CCK levels during CCK_1 receptor antagonist infusion has been suggested to block the autoregulatory negative feedback mechanisms on I-cells during stimulated CCK release. In our study, PHA did not increase plasma CCK levels nor did the concomitant infusion of dexloxiglumide with PHA, suggesting that PHA did not establish a stimulatory state in I-cells. The reliability of the plasma CCK measurement technique was confirmed by the detection of significantly elevated CCK levels after liquid nutrient meal administration.

Dexloxiglumide is a potent and selective CCK_1 receptor antagonist and thus an effective inhibitor of CCK mediated gallbladder contraction. In fact, intravenous dexloxiglumide increases the volume of the gallbladder in the fasted state (Hildebrand et al., 1990; Liddle et al., 1989; Meyer et al., 1989; Niederau et al., 1989). PHA administered simultaneously with dexloxiglumide infusion was no longer able to induce gallbladder contraction. Instead, a maximal increase to 208 % of the basal volume was observed at the end of the experiment. Therefore, our data suggests that PHA might release CCK which could locally activate the CCK_1 receptors on duodenal vagal afferents within the lamina propria of the intestinal wall or on enteric neurons generating entero-cholecystic reflexes (Balemba et al., 2004). Alternatively, PHA might act directly on CCK_1 receptors.

In rats, diets rich in PHA induce the growth of the pancreas and increase plasma CCK levels. The growth promoting effect of PHA on the pancreas is mediated via CCK (Herzig et al., 1997), whereas the stimulation of intestinal hyperplastic growth is probably due to the strong binding of PHA to the brush border membranes (Bardocz et al., 1995) and a direct effect (Otte et al., 2001). It appears that PHA induces growth and functional maturation of the gastrointestinal tract in suckling rats, and this has been shown to be dependent on enteral exposure to PHA, since the parenterally administered compound was without effects on the intestinal organs (Linderoth et al., 2005; Linderoth et al., 2006a; Linderoth et al., 2006b).

The growth-promoting effects of lectins have raised the question whether these plant proteins are beneficial for patients receiving total parenteral nutrition (TPN). Gastrointestinal atrophy during TPN leads to malabsorption and the atrophic epithelial layer predisposes to the penetration of intestinal bacteria into the circulation. The intragastric administration of lectins was able to prevent atrophy in rats fed by TPN, with PHA being most effective in the gastric fundus and in the proximal and mid small intestine (Jordinson et al., 1999).

TPN is one of the risk factors for gallstone formation, possibly because of decreased gallbladder emptying frequencies. Intravenous administration of CCK (Sitzmann et al., 1990) or amino acids (Wu et al., 2000) has been proposed to prevent the formation of gallbladder sludge in adult TPN fed patients, suggesting that a treatment enhancing gallbladder contractions would be beneficial for these patients. We have shown for the first time in the present study that administration of the lectin PHA stimulates gallbladder contraction in healthy humans. Therefore, it would be of utmost interest to evaluate the effects of PHA administration on sludge or gallstone formation in TPN patients or patients with short bowel syndrome (Manji et al., 1989). Interestingly, recent studies proposed that frequent consumption of nuts, which are known to be rich in lectins (Lotan et al., 1975), is correlated with lower incidence of gallstones in a healthy population (Tsai et al., 2004a; Tsai et al., 2004b). Some concerns may rise because extensive amounts of raw red kidney beans cause diarrhea due to lectin PHA. However, toxicity is dependent on the dosage and purity of the PHA preparation used and therefore the adverse effects (e.g. weight loss or intestinal degeneration) demonstrated by some animal studies could be avoided.

6.2 FIBRE VISCOSITY AFFECTS POSTPRANDIAL GASTROINTESTINAL PEPTIDE RESPONSES

Fibre rich diets have many beneficial health effects on the human body. These include the lowering of serum cholesterol levels, glucose and insulin responses as well as improving laxation. Diet procured fibres have also been connected with increased satiety and considered beneficial in weight control, although clear data on this issue is lacking (van Dam and Seidell, 2007). Meal components may modulate satiety and food intake by differentially affecting the postprandial secretion of gastrointestinal peptides, but the effects of fibre viscosity on these responses have not been evaluated before.

The present study demonstrates that the viscosity of an oat bran beverage strongly modifies the short-term postprandial responses, as seen as delayed gastric emptying and attenuated postprandial metabolic and hormonal responses compared to an otherwise identical but low-viscous test product. Our study confirms the previous results showing that glucose and insulin lowering effects of soluble viscous fibres are dependent on the viscosity of the meal (Jenkins et al., 1978; Leclere et al., 1994; Panahi et al., 2007; Wood et al., 1994). The beverage with high-viscous oat bran caused a significantly lower increase in postprandial glucose and insulin levels compared to the low-viscous counterpart drink. Furthermore, the second phase of the glucose curve (i.e. the suppression of glucose levels below the fasting values after 60 minutes), was less pronounced after the high-viscous than after the low-viscous beverage consumption. This may indicate delayed gastric emptying and absorption of glucose from the intestine after the high-viscous drink. However, here, we cannot evaluate whether the beneficial effects of fibre on postprandial glycemia were totally abolished after the reduction of viscosity because our study did not include a control beverage without fibre.

Interestingly, the low-viscous oat bran beverage evoked significantly higher postprandial CCK, PYY and GLP-1 responses compared to the high-viscous one. In addition, the low-viscous fibre beverage induced a more pronounced reduction of postprandial ghrelin levels compared to the high-viscous fibre. The reason for the attenuated peptide responses after the high-viscous beverage might be that the viscosity of the beverage may prevent the close interaction between the nutrients and gastrointestinal mucosa, required for efficient stimulation of enteroendocrine cells and peptide release. Furthermore, digestive processes such as the cleavage of triglycerides to free fatty acids and the stimulation of CCK, PYY and GLP-1 release may have been hindered by viscosity and thus contribute to the markedly lower peptide levels (Isaksson et al., 1982).

Interactions between the gastrointestinal peptides might modulate the secretion of each other. Exogenous (Brennan et al., 2007) and endogenous (Degen et al., 2007) CCK suppresses ghrelin concentration and increases plasma PYY concentration. In addition, intravenous PYY administration suppresses ghrelin secretion (Batterham et al., 2003). These all are in accordance with our observations after the low-viscous fibre beverage. Insulin has been suggested to mediate the suppression of plasma ghrelin levels in normal subjects and in type 1 diabetic patients (Murdolo et al., 2003), although this has not been the case in patients with metabolic syndrome (Heinonen et al., 2007). In our study, the low-viscous beverage consumption caused a more pronounced stimulation of insulin secretion and reduction of

ghrelin levels than the high-viscous drink. Additional factors have been implicated in the suppression of ghrelin secretion. The low-viscous beverage evoked higher GLP-1 responses. An inverse correlation has been suggested between plasma ghrelin levels and GLP-1 or GLP-1 receptor agonists (Djurhuus et al., 2002; Hagemann et al., 2007; Perez-Tilve et al., 2007).

In contrast to expectations, but in accordance with the postprandial peptide responses, the feeling of fullness and satiety evaluated by visual analogue scales was higher after the low-viscous than after the high-viscous beverage. However, no difference between the treatments was observed in the feeling of hunger or in food intake during the *ad libitum* lunch served three hours after the test beverages in spite of the significant increase in circulating satiety hormones recorded after the low-viscous beverage. This may be due to the fact that at three hours most of the differences in peptide responses were attenuated. In addition, other factors cannot be excluded such as a free lunch, which might have encouraged the subjects to consume the habitual amount of food regardless of the actual needs. Interestingly, later during the test day, subjects reported slightly higher energy consumption after the low-viscous than after the high-viscous beverage. This leaves us to speculate whether the high-viscous beverage might have evoked delayed effects on satiety. We did not obtain plasma levels from those time points.

The overall gastric emptying rate after the consumption of the high-viscous beverage was significantly slower compared to the low-viscous one, as has been suggested before for viscous fibres (Marciani et al., 2000; Marciani et al., 2001). Postprandial CCK, GLP-1 and PYY are known to decrease the gastric emptying rate, yet, despite the more pronounced peptide responses after the low-viscous fibre meal gastric emptying was faster compared to the high-viscous fibre meal. Thus, the viscosity of the fibre seems to be a more powerful regulator of gastric emptying than postprandial peptide responses.

Our study has certain limitations. We did not include a test beverage with the same macronutrient composition but without any fibres and therefore can not evaluate the effects of the low-viscous oat bran on glucose and insulin responses or on gastrointestinal peptides. Furthermore, the gender distribution of the subjects is biased towards women because of the difficulty to recruit male volunteers. All the subjects were served the same test meal with the same energy content which may not correspond to equal proportions of their total daily energy requirements.

The current daily dietary reference intake value for fibre is 14 g /1000 calories based on the amount required for risk reduction of coronary heart disease. This means that adequate intake is reached with approximately 25 g total fibre per day in adult women and 38 g per day in men, yet currently the average intake is only about half of this recommendation (Food and Nutrition Board, Institute of Medicine. Dietary, functional and total fibre: dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academy Press, 2002: http://www.nap.edu/openbook.php?record_id=10490&page=R1. February 2008). Therefore, there is a need to incorporate higher amounts of fibre in the food products in the future. This might be challenging because adding fibre also alters the textual properties of the food and this might affect palatability. Reducing the viscosity of the fibre might alleviate this problem, but as this and other studies have shown, reducing

viscosity significantly alters the physiological effects evoked by dietary fibres. Therefore, the effects of fibre viscosity on glycemia but also on other measures like satiety and food intake require more careful investigation in order to achieve better knowledge about the optimal intake and health effects.

6.3 THE PUNGENT INGREDIENT OF WASABI STIMULATES CCK SECRETION VIA ACTIVATON OF TRPA1 CHANNELS

Spices give flavour to foods and drinks but may also affect metabolism, for example, by activating thermogenesis or by stimulating digestive processes (Westerterp-Plantenga et al., 2006). Many plant derived pungent ingredients, including allyl isothiocyanate in wasabi and mustard oil, are known to activate TRPA1 channels. This study shows that TPRA1 channels, which have been previously mainly described in sensory neurons, are also expressed in gastrointestinal tract in humans and in mice.

Furthermore, TRPA1 channel, activated by compounds in ingested food, might also be involved in the neuroendocrine cell signalling and participate in the secretion of gastrointestinal peptides. The mouse neuroendocrine intestinal cell line STC-1 which is known to secrete several gastrointestinal peptides (CCK, GLP-1 and GIP) has been widely used as a model to study the mechanisms of peptide secretion (Glassmeier et al., 1998; McLaughlin et al., 1998). STC-1 cells were shown to endogenously express TRPA1. Activation of TRPA1 channels with AITC showed a robust stimulation of CCK release, which was mediated via extracellular calcium influx. Other pungent ingredients, in addition to AITC in wasabi and mustard oil, activate TRPA1 channels. These include allicin (derived from garlic), cinnamaldelyde (of cinnamon), gingerol (of ginger) and eugenol (of clove oil). Thus a wide range of spices could stimulate CCK secretion via activation of TRPA1 channels.

Recently, also also other reports have shown that TRPA1 is present in the mouse and human gastrointestinal tracts (Penuelas et al., 2007, Stokes et al., 2006). Penuelas and coworkers found that stimulation of TRPA1 channels induced the contraction of the small intestine and colon. In summary, spices via TRPA1 activation and stimulation of CCK secretion might facilitate digestive processes. In the future these findings, so far demonstrated *in vitro* systems, need to be confirmed *in vivo* before further conclusions can be drawn. Furthermore, as CCK is known to induce satiation, it would be interesting to study the effects of these spices on food intake.

6.4 PYY(3-36) DOES NOT AFFECT FOOD INTAKE IN COLECTOMIZED RATS

Recently, gastrointestinal hormones have received new attention in the light of the obesity crisis and the newly rediscovered connections between the peripheral body and the CNS in the regulation of food intake. One of the most discussed peptides during the past years has been PYY after the report of Batterham and coworkers in 2002 showing that PYY, the hormone released from the distal intestine and colon, reduces food intake in humans and in rodents (Batterham et al., 2002). However, many laboratories

were unable to reproduce their results (Tschop et al., 2004). In addition, the doses used have been criticized as unphysiologically high and causing side effects like nausea (Degen et al., 2005). Therefore, we attempted to approach the problem by creating a surgical knock-out model of PYY by performing a total colectomy in rats thus removing the major organ responsible for PYY production in the body. After a 2-week recovery period the rats were implanted with minipumps continuously delivering PYY(3-36) or saline intravenously. However, to our surprise, plasma PYY levels were not reduced at seven days after pump implantation (i.e. 21 days after surgery) in the control group with saline infusion, but were significantly increased compared to presurgery levels. The PYY levels were reduced after 14 days on saline infusion but still remained significantly elevated compared to the preoperational levels.

Indeed, other studies have found elevated PYY levels after colectomy. Abdominal colectomy and ileorectostomy increased basal PYY levels in rats and tissue levels of PYY were higher after colectomy (Vukasin et al., 1992). Additionally, total proctocolectomy and ileal J-pouch increased PYY levels in dogs one year after the surgery (Armstrong et al., 1991), and the number and density of L-cells was increased in the ileal pouch (Imamura et al., 1999). This increase might be due to the fact that ileal and rectal mucosa remaining after colectomy still contain sufficient amounts of PYY secreting cells to compensate for the loss. After colectomy villus length and density increased proximal to the pouch (Willis et al., 2002). This increases the amount of PYY producing cells (Imamura et al., 1999) and therefore might contribute to the elevated systemic PYY levels after colectomy. In contrast, in humans basal PYY levels were decreased after colectomy and ileal J-pouch and anastomosis (Ternent et al., 1998) or remained the same (van Battum et al., 1999). However, in both studies the meal-stimulated PYY release was significantly decreased. The elevated PYY levels observed in our study might have been caused by possible inflammation of the mucosa after surgery. In addition, a small remaining part (1 cm) of rectum could not be surgically removed due to technical reasons (very small lower pelvis) and the remaining PYY cells might have contributed to the PYY production after colectomy.

In spite of the increased PYY levels after colectomy in our study, the colectomized rats with PYY(3-36) infusion had still significantly higher PYY plasma levels compared to the control group with saline infusion at 7 and 14 days after the start of the infusion. Yet, no differences in the food intake or body weight were observed between the groups. Colectomy caused a drop in the body weight of the animals, but before the beginning of the study they had already recovered, as demonstrated by the normal daily food intake and weight gain. In addition, the rats were adapted to handling, thus eliminating the possibility that stress would have hampered the effects of PYY on food intake as suggested by some studies (Abbott et al., 2006; Halatchev et al., 2004).

In our study, the total PYY plasma level of the PYY(3-36) group was 1.7 and 2.3 times higher than that of the control group at the 7 day and 14 day timepoints, respectively. This difference was not effective enough to produce a difference in the food intake or body weight gain. In one human study, a large lunch of 1500 kcal caused approximately a 1.5 fold increase in plasma PYY concentration compared to fasting levels (Degen et al., 2005), and the lowest dose of administered PYY(3-36) that was effective in

reducing calorie intake (0.4 pmol/kg/min) caused approximately a 2.7 fold increase in the fasting state plasma PYY levels.

Some studies, in which robust anorexic effects of PYY(3-36) on food intake have been observed, have reported significantly higher differences between the plasma levels of controls and treated subjects. For example, a 200-fold difference in plasma PYY(3-36) levels between PYY(3-36) infused rats and controls was measured after 3 hours fasting in diet-induced obesity prone rats (approximately 500 g b.w.) after two weeks continuous infusion of PYY(3-36) with a dose of 200 µg/kg/day causing a 13 % drop in food intake and a 3.2 % decrease in body weight gain (Roth et al., 2007). Thus, it remains possible that one of the reasons why we could not detect any difference in the food intake of PYY(3-36) infused and control rats could be that the required pharmacological increases in PYY levels were not achieved.

However, the interest in the use of PYY(3-36) as a therapeutic agent in the treatment of obesity still remains due to several studies that have been able to demonstrate anorexic effects of this hormone. In clinical trials performed so far, this enthusiasm is slightly hampered by the frequently observed side effects (Gantz et al., 2007; Sloth et al., 2007b), and it remains to be seen whether, for example, with escalated dosing these problems could be overcome.

7 SUMMARY

In conclusion, this thesis shows that

I Duodenal administration of red kidney bean lectin PHA stimulates gallbladder contraction, without affecting peripheral plasma CCK levels in humans. This contraction is mediated via cholinergic mechanism and CCK_1 receptors.

II The viscosity of oat bran beverages significantly affects postprandial responses in humans. A high-viscous beverage attenuates short-term glucose, insulin, ghrelin, CCK, GLP-1 and PYY responses, gastric emptying and feelings of satiety and fullness compared to low-viscous beverage.

III AITC, the active pungent ingredient of wasabi and mustard oil stimulates CCK release in STC-1 cells by activating TRPA1 channels, which are also expressed in native human and mouse duodenum.

IV PYY(3-36) infusion, in the concentration used, did not affect food intake or body weight gain in colectomized rats.

8 REFERENCES

Abbott, C.R., Monteiro, M., Small, C.J., Sajedi, A., Smith, K.L., Parkinson, J.R., Ghatei, M.A., and Bloom, S.R. (2005). The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res *1044,* 127-131.

Abbott, C.R., Small, C.J., Sajedi, A., Smith, K.L., Parkinson, J.R., Broadhead, L.L., Ghatei, M.A., and Bloom, S.R. (2006). The importance of acclimatisation and habituation to experimental conditions when investigating the anorectic effects of gastrointestinal hormones in the rat. Int J Obes (Lond) *30,* 288-292.

Abello, J., Ye, F., Bosshard, A., Bernard, C., Cuber, J.C., and Chayvialle, J.A. (1994). Stimulation of glucagon-like peptide-1 secretion by muscarinic agonist in a murine intestinal endocrine cell line. Endocrinology *134,* 2011-2017.

Acuna-Goycolea, C., and van den Pol, A.N. (2005). Peptide YY(3-36) inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. J Neurosci *25,* 10510-10519.

Adam, T.C., and Westerterp-Plantenga, M.S. (2005). Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects. Br J Nutr *93,* 845-851.

Adibi, S.A. (1997). The oligopeptide transporter (Pept-1) in human intestine: biology and function. Gastroenterology *113,* 332-340.

Adrian, T.E., Ferri, G.L., Bacarese-Hamilton, A.J., Fuessl, H.S., Polak, J.M., and Bloom, S.R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology *89,* 1070-1077.

Al Awar, R., Obeid, O., Hwalla, N., and Azar, S. (2005). Postprandial acylated ghrelin status following fat and protein manipulation of meals in healthy young women. Clin Sci (Lond) *109,* 405-411.

Anderson, J.W., Allgood, L.D., Lawrence, A., Altringer, L.A., Jerdack, G.R., Hengehold, D.A., and Morel, J.G. (2000). Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials. Am J Clin Nutr *71,* 472-479.

Andrade, E.L., Ferreira, J., Andre, E., and Calixto, J.B. (2006). Contractile mechanisms coupled to TRPA1 receptor activation in rat urinary bladder. Biochem Pharmacol *72,* 104-114.

Andre, S., Kojima, S., Gundel, G., Russwurm, R., Schratt, X., Unverzagt, C., and Gabius, H.J. (2006). Branching mode in complex-type triantennary N-glycans as regulatory element of their ligand properties. Biochim Biophys Acta *1760,* 768-782.

Andre, S., Kozar, T., Schuberth, R., Unverzagt, C., Kojima, S., and Gabius, H.J. (2007). Substitutions in the N-glycan core as regulators of biorecognition: the case of core-fucose and bisecting GlcNAc moieties. Biochemistry *46,* 6984-6995.

Andre, S., Unverzagt, C., Kojima, S., Frank, M., Seifert, J., Fink, C., Kayser, K., von der Lieth, C.W., and Gabius, H.J. (2004). Determination of modulation of ligand properties of synthetic complex-type biantennary N-glycans by introduction of bisecting GlcNAc in silico, in vitro and in vivo. Eur J Biochem *271,* 118-134.

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S. *et al.* (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab *86,* 4753-4758.

Armstrong, D.N., Ballantyne, G.H., Adrian, T.E., Bilchik, A.J., McMillen, M.A., and Modlin, I.M. (1991). Adaptive increase in peptide YY and enteroglucagon after proctocolectomy and pelvic ileal reservoir construction. Dis Colon Rectum *34,* 119-125.

Arnold, M., Mura, A., Langhans, W., and Geary, N. (2006). Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. J Neurosci *26,* 11052-11060.

Aronne, L.J., and Thornton-Jones, Z.D. (2007). New targets for obesity pharmacotherapy. Clin Pharmacol Ther *81,* 748-752.

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino, S., Fujimiya, M., Niijima, A., Fujino, M.A. *et al.* (2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology *120,* 337-345.

Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., Meguid, M.M., and Kasuga, M. (2005). Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut *54,* 18-24.

Astrup, A., Vrist, E., and Quaade, F. (1990). Dietary fibre added to very low calorie diet reduces hunger and alleviates constipation. Int J Obes *14,* 105-112.

Badman, M.K., and Flier, J.S. (2005). The gut and energy balance: visceral allies in the obesity wars. Science *307,* 1909-1914.

Baggio, L.L., and Drucker, D.J. (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology *132,* 2131-2157.

Balemba, O.B., Salter, M.J., and Mawe, G.M. (2004). Innervation of the extrahepatic biliary tract. Anat Rec A Discov Mol Cell Evol Biol *280,* 836-847.

Ballinger, A.B., and Clark, M.L. (1994). L-phenylalanine releases cholecystokinin (CCK) and is associated with reduced food intake in humans: evidence for a physiological role of CCK in control of eating. Metabolism *43,* 735-738.

Bandell, M., Story, G.M., Hwang, S.W., Viswanath, V., Eid, S.R., Petrus, M.J., Earley, T.J., and Patapoutian, A. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron *41,* 849-857.

Banks, W.A., Tschop, M., Robinson, S.M., and Heiman, M.L. (2002). Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. J Pharmacol Exp Ther *302,* 822-827.

Banwell, J.G., Boldt, D.H., Meyers, J., and Weber, F.L.,Jr. (1983). Phytohemagglutinin derived from red kidney bean (Phaseolus vulgaris): a cause for intestinal malabsorption associated with bacterial overgrowth in the rat. Gastroenterology *84,* 506-515.

Bardocz, S., Grant, G., Ewen, S.W., Duguid, T.J., Brown, D.S., Englyst, K., and Pusztai, A. (1995). Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut *37,* 353-360.

Barrachina, M.D., Martinez, V., Wang, L., Wei, J.Y., and Tache, Y. (1997). Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. Proc Natl Acad Sci U S A *94,* 10455-10460.

Batterham, R.L., Cohen, M.A., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A., and Bloom, S.R. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med *349,* 941-948.

Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A. *et al.* (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. Nature *418,* 650-654.

Batterham, R.L., Heffron, H., Kapoor, S., Chivers, J.E., Chandarana, K., Herzog, H., Le Roux, C.W., Thomas, E.L., Bell, J.D., and Withers, D.J. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab *4,* 223-233.

Batterham, R.L., Le Roux, C.W., Cohen, M.A., Park, A.J., Ellis, S.M., Patterson, M., Frost, G.S., Ghatei, M.A., and Bloom, S.R. (2003). Pancreatic polypeptide reduces appetite and food intake in humans. J Clin Endocrinol Metab *88,* 3989-3992.

Bautista, D.M., Jordt, S.E., Nikai, T., Tsuruda, P.R., Read, A.J., Poblete, J., Yamoah, E.N., Basbaum, A.I., and Julius, D. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell *124,* 1269-1282.

Bautista, D.M., Movahed, P., Hinman, A., Axelsson, H.E., Sterner, O., Hogestatt, E.D., Julius, D., Jordt, S.E., and Zygmunt, P.M. (2005). Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci U S A *102*, 12248-12252.

Beglinger, C., Degen, L., Matzinger, D., D'Amato, M., and Drewe, J. (2001). Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. Am J Physiol Regul Integr Comp Physiol *280*, R1149-1154.

Beglinger, C., Hildebrand, P., Adler, G., Werth, B., Luo, H., Delco, F., and Gyr, K. (1992). Postprandial control of gallbladder contraction and exocrine pancreatic secretion in man. Eur J Clin Invest *22*, 827-834.

Bengtsson, M.W., Makela, K., Sjoblom, M., Uotila, S., Akerman, K.E., Herzig, K.H., and Flemstrom, G. (2007). Food-induced expression of orexin receptors in rat duodenal mucosa regulates the bicarbonate secretory response to orexin-A. Am J Physiol Gastrointest Liver Physiol *293*, G501-509.

Bergmann, J.F., Chassany, O., Petit, A., Triki, R., Caulin, C., and Segrestaa, J.M. (1992). Correlation between echographic gastric emptying and appetite: influence of psyllium. Gut *33*, 1042-1043.

Berthoud, H.R., Blackshaw, L.A., Brookes, S.J., and Grundy, D. (2004). Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract. Neurogastroenterol Motil *16 Suppl 1*, 28-33.

Berthoud, H.R., and Morrison, C. (2008). The brain, appetite, and obesity. Annu Rev Psychol *59*, 55-92.

Blevins, J.E., Stanley, B.G., and Reidelberger, R.D. (2000). Brain regions where cholecystokinin suppresses feeding in rats. Brain Res *860*, 1-10.

Blom, W.A., Lluch, A., Stafleu, A., Vinoy, S., Holst, J.J., Schaafsma, G., and Hendriks, H.F. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response. Am J Clin Nutr *83*, 211-220.

Boey, D., Sainsbury, A., and Herzog, H. (2007). The role of peptide YY in regulating glucose homeostasis. Peptides *28*, 390-395.

Bourdon, I., Olson, B., Backus, R., Richter, B.D., Davis, P.A., and Schneeman, B.O. (2001). Beans, as a source of dietary fiber, increase cholecystokinin and apolipoprotein b48 response to test meals in men. J Nutr *131*, 1485-1490.

Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B., and Schneeman, B.O. (1999). Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. Am J Clin Nutr *69*, 55-63.

Bowen, J., Noakes, M., and Clifton, P.M. (2006a). Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. J Clin Endocrinol Metab *91*, 2913-2919.

Bowen, J., Noakes, M., Trenerry, C., and Clifton, P.M. (2006b). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. J Clin Endocrinol Metab *91*, 1477-1483.

Brennan, I.M., Otto, B., Feltrin, K.L., Meyer, J.H., Horowitz, M., and Feinle-Bisset, C. (2007). Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men. Peptides *28*, 607-611.

Broglio, F., Gottero, C., Van Koetsveld, P., Prodam, F., Destefanis, S., Benso, A., Gauna, C., Hofland, L., Arvat, E., van der Lely, A.J. *et al.* (2004). Acetylcholine regulates ghrelin secretion in humans. J Clin Endocrinol Metab *89*, 2429-2433.

Brown, L., Rosner, B., Willett, W.W., and Sacks, F.M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr *69*, 30-42.

Burdyga, G., Lal, S., Spiller, D., Jiang, W., Thompson, D., Attwood, S., Saeed, S., Grundy, D., Varro, A., Dimaline, R. *et al.* (2003). Localization of orexin-1 receptors to vagal afferent neurons in the rat and humans. Gastroenterology *124*, 129-139.

Burdyga, G., Lal, S., Varro, A., Dimaline, R., Thompson, D.G., and Dockray, G.J. (2004). Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. J Neurosci *24,* 2708-2715.

Burdyga, G., Spiller, D., Morris, R., Lal, S., Thompson, D.G., Saeed, S., Dimaline, R., Varro, A., and Dockray, G.J. (2002). Expression of the leptin receptor in rat and human nodose ganglion neurones. Neuroscience *109,* 339-347.

Burdyga, G., Varro, A., Dimaline, R., Thompson, D.G., and Dockray, G.J. (2006a). Feeding-dependent depression of melanin-concentrating hormone and melanin-concentrating hormone receptor-1 expression in vagal afferent neurones. Neuroscience *137,* 1405-1415.

Burdyga, G., Varro, A., Dimaline, R., Thompson, D.G., and Dockray, G.J. (2006b). Ghrelin receptors in rat and human nodose ganglia: putative role in regulating CB-1 and MCH receptor abundance. Am J Physiol Gastrointest Liver Physiol *290,* G1289-1297.

Burley, V.J., Leeds, A.R., and Blundell, J.E. (1987). The effect of high and low-fibre breakfasts on hunger, satiety and food intake in a subsequent meal. Int J Obes *11 Suppl 1,* 87-93.

Burton-Freeman, B., Davis, P.A., and Schneeman, B.O. (2002). Plasma cholecystokinin is associated with subjective measures of satiety in women. Am J Clin Nutr *76,* 659-667.

Callahan, H.S., Cummings, D.E., Pepe, M.S., Breen, P.A., Matthys, C.C., and Weigle, D.S. (2004). Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. J Clin Endocrinol Metab *89,* 1319-1324.

Challis, B.G., Coll, A.P., Yeo, G.S., Pinnock, S.B., Dickson, S.L., Thresher, R.R., Dixon, J., Zahn, D., Rochford, J.J., White, A. *et al.* (2004). Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3-36). Proc Natl Acad Sci U S A *101,* 4695-4700.

Chan, J.L., Bullen, J., Lee, J.H., Yiannakouris, N., and Mantzoros, C.S. (2004). Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. J Clin Endocrinol Metab *89,* 335-343.

Chandalia, M., Garg, A., Lutjohann, D., von Bergmann, K., Grundy, S.M., and Brinkley, L.J. (2000). Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med *342,* 1392-1398.

Chelikani, P.K., Haver, A.C., and Reidelberger, R.D. (2005). Intravenous infusion of peptide YY(3-36) potently inhibits food intake in rats. Endocrinology *146,* 879-888.

Chen, C.Y., Inui, A., Asakawa, A., Fujino, K., Kato, I., Chen, C.C., Ueno, N., and Fujimiya, M. (2005). Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. Gastroenterology *129,* 8-25.

Chu, Z.L., Carroll, C., Alfonso, J., Gutierrez, V., He, H., Lucman, A., Pedraza, M., Mondala, H., Gao, H., Bagnol, D. *et al.* (2008). A role for intestinal endocrine cell-expressed GPR119 in glycemic control by enhancing GLP-1 and GIP release. Endocrinology 2008 Jan 17 [Epub ahead of print].

Cohen, M.A., Ellis, S.M., Le Roux, C.W., Batterham, R.L., Park, A., Patterson, M., Frost, G.S., Ghatei, M.A., and Bloom, S.R. (2003). Oxyntomodulin suppresses appetite and reduces food intake in humans. J Clin Endocrinol Metab *88,* 4696-4701.

Coll, A.P., Farooqi, I.S., and O'Rahilly, S. (2007). The hormonal control of food intake. Cell *129,* 251-262.

Cordier-Bussat, M., Bernard, C., Haouche, S., Roche, C., Abello, J., Chayvialle, J.A., and Cuber, J.C. (1997). Peptones stimulate cholecystokinin secretion and gene transcription in the intestinal cell line STC-1. Endocrinology *138,* 1137-1144.

Corey, D.P., Garcia-Anoveros, J., Holt, J.R., Kwan, K.Y., Lin, S.Y., Vollrath, M.A., Amalfitano, A., Cheung, E.L., Derfler, B.H., Duggan, A. *et al.* (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature *432,* 723-730.

Cox, H.M. (2007). Peptide YY: a neuroendocrine neighbor of note. Peptides *28,* 345-351.

Crawley, J.N., and Beinfeld, M.C. (1983). Rapid development of tolerance to the behavioural actions of cholecystokinin. Nature *302,* 703-706.

Cuber, J.C., Bernard, G., Fushiki, T., Bernard, C., Yamanishi, R., Sugimoto, E., and Chayvialle, J.A. (1990). Luminal CCK-releasing factors in the isolated vascularly perfused rat duodenojejunum. Am J Physiol *259,* G191-197.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., and Weigle, D.S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes *50,* 1714-1719.

Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P., and Purnell, J.Q. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med *346,* 1623-1630.

Cummings, D.E., Clement, K., Purnell, J.Q., Vaisse, C., Foster, K.E., Frayo, R.S., Schwartz, M.W., Basdevant, A., and Weigle, D.S. (2002). Elevated plasma ghrelin levels in Prader-Willi syndrome. Nat Med *8,* 643–644.

Dakin, C.L., Small, C.J., Batterham, R.L., Neary, N.M., Cohen, M.A., Patterson, M., Ghatei, M.A., and Bloom, S.R. (2004). Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology *145,* 2687-2695.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K., and Nakazato, M. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology *141,* 4255-4261.

Date, Y., Murakami, N., Toshinai, K., Matsukura, S., Niijima, A., Matsuo, H., Kangawa, K., and Nakazato, M. (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology *123,* 1120-1128.

Degen, L., Drewe, J., Piccoli, F., Grani, K., Oesch, S., Bunea, R., D'Amato, M., and Beglinger, C. (2007). Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men. Am J Physiol Regul Integr Comp. Physiol *292,* R1391-1399.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., and Beglinger, C. (2005). Effect of peptide YY3-36 on food intake in humans. Gastroenterology *129,* 1430-1436.

DeVries, J.W. (2003). On defining dietary fibre. Proc Nutr Soc *62,* 37-43.

Di Lorenzo, C., Williams, C.M., Hajnal, F., and Valenzuela, J.E. (1988). Pectin delays gastric emptying and increases satiety in obese subjects. Gastroenterology *95,* 1211-1215.

Dikeman, C.L., and Fahey, G.C. (2006). Viscosity as related to dietary fiber: a review. Crit Rev Food Sci Nutr *46,* 649-663.

Djurhuus, C.B., Hansen, T.K., Gravholt, C., Orskov, L., Hosoda, H., Kangawa, K., Jorgensen, J.O., Holst, J.J., and Schmitz, O. (2002). Circulating levels of ghrelin and GLP-1 are inversely related during glucose ingestion. Horm Metab Res *34,* 411-413.

Doerner, J.F., Gisselmann, G., Hatt, H., and Wetzel, C.H. (2007). Transient receptor potential channel A1 is directly gated by calcium ions. J Biol Chem *282,* 13180-13189.

Drucker, D.J., and Nauck, M.A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet *368,* 1696-1705.

Edwards, C.A., Blackburn, N.A., Craigen, L., Davison, P., Tomlin, J., Sugden, K., Johnson, I.T., and Read, N.W. (1987). Viscosity of food gums determined in vitro related to their hypoglycemic actions. Am J Clin Nutr 46, 72-77.

Egberts, J., Brunke, G., Kiehne, K., Fölsch, U.R., and Herzig, K.H. CCK release by long-chain unsaturated fatty acids from isolated perfused mucosal cells. Digestion 2000; 61: 293 (abstract).

Ehrlein, H., and Stockmann, A. (1998). Absorption of nutrients is only slightly reduced by supplementing enteral formulas with viscous fiber in miniature pigs. J Nutr *128,* 2446-2455.

Ekman, R., Wahlestedt, C., Bottcher, G., Sundler, F., Hakanson, R., and Panula, P. (1986). Peptide YY-like immunoreactivity in the central nervous system of the rat. Regul Pept *16,* 157-168.

Elliott, R.M., Morgan, L.M., Tredger, J.A., Deacon, S., Wright, J., and Marks, V. (1993). Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol *138,* 159-166.

Erdmann, J., Lippl, F., and Schusdziarra, V. (2003). Differential effect of protein and fat on plasma ghrelin levels in man. Regul Pept *116,* 101-107.

Erdmann, J., Topsch, R., Lippl, F., Gussmann, P., and Schusdziarra, V. (2004). Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. J Clin Endocrinol Metab *89,* 3048-3054.

Essah, P.A., Levy, J.R., Sistrun, S.N., Kelly, S.M., and Nestler, J.E. (2007). Effect of macronutrient composition on postprandial peptide YY levels. J Clin Endocrinol Metab *92,* 4052-4055.

Eysselein, V.E., Eberlein, G.A., Hesse, W.H., Schaeffer, M., Grandt, D., Williams, R., Goebell, H., and Reeve, J.R.,Jr. (1990). Molecular variants of cholecystokinin after endogenous stimulation in humans: a time study. Am J Physiol *258,* G951-957.

Eysselein, V.E., Eberlein, G.A., Hesse, W.H., Singer, M.V., Goebell, H., and Reeve, J.R.,Jr. (1987). Cholecystokinin-58 is the major circulating form of cholecystokinin in canine blood. J Biol Chem *262,* 214-217.

Fan, W., Ellacott, K.L., Halatchev, I.G., Takahashi, K., Yu, P., and Cone, R.D. (2004). Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. Nat Neurosci *7,* 335-336.

Farilla, L., Hui, H., Bertolotto, C., Kang, E., Bulotta, A., Di Mario, U., and Perfetti, R. (2002). Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology *143,* 4397-4408.

Faulconbridge, L.F., Cummings, D.E., Kaplan, J.M., and Grill, H.J. (2003). Hyperphagic effects of brainstem ghrelin administration. Diabetes *52,* 2260-2265.

Fei, Y.J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F.H., Romero, M.F., Singh, S.K., Boron, W.F., and Hediger, M.A. (1994). Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature *368,* 563-566.

Feinle-Bisset, C., Patterson, M., Ghatei, M.A., Bloom, S.R., and Horowitz, M. (2005). Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. Am J Physiol Endocrinol Metab *289,* E948-953.

Feltrin, K.L., Little, T.J., Meyer, J.H., Horowitz, M., Smout, A.J., Wishart, J., Pilichiewicz, A.N., Rades, T., Chapman, I.M., and Feinle-Bisset, C. (2004). Effects of intraduodenal fatty acids on appetite, antropyloroduodenal motility, and plasma CCK and GLP-1 in humans vary with their chain length. Am J Physiol Regul Integr Comp Physiol *287,* R524-533.

Feltrin, K.L., Patterson, M., Ghatei, M.A., Bloom, S.R., Meyer, J.H., Horowitz, M., and Feinle-Bisset, C. (2006). Effect of fatty acid chain length on suppression of ghrelin and stimulation of PYY, GLP-2 and PP secretion in healthy men. Peptides *27,* 1638-1643.

Flint, A., Raben, A., Astrup, A., and Holst, J.J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest *101,* 515-520.

Flint, A., Raben, A., Ersboll, A.K., Holst, J.J., and Astrup, A. (2001). The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. Int J Obes Relat Metab Disord *25,* 781-792.

Foster-Schubert, K.E., Overduin, J., Prudom, C.E., Liu, J., Callahan, H.S., Gaylinn, B.D., Thorner, M.O., and Cummings, D.E. (2008). Acyl and Total Ghrelin Are Suppressed Strongly by Ingested Proteins,

Weakly by Lipids, and Biphasically by Carbohydrates. J Clin Endocrinol Metab 2008 Jan 15 [Epub ahead of print].

Freier, T., Fleischmann, G., and Rudiger, H. (1985). Affinity chromatography on immobilized hog gastric mucin and ovomucoid. A general method for isolation of lectins. Biol Chem Hoppe Seyler *366,* 1023-1028.

Fried, M., Erlacher, U., Schwizer, W., Lochner, C., Koerfer, J., Beglinger, C., Jansen, J.B., Lamers, C.B., Harder, F., and Bischof-Delaloye, A. (1991a). Role of cholecystokinin in the regulation of gastric emptying and pancreatic enzyme secretion in humans. Studies with the cholecystokinin-receptor antagonist loxiglumide. Gastroenterology *101,* 503-511.

Fried, M., Schwizer, W., Beglinger, C., Keller, U., Jansen, J.B., and Lamers, C.B. (1991b). Physiological role of cholecystokinin on postprandial insulin secretion and gastric meal emptying in man. Studies with the cholecystokinin receptor antagonist loxiglumide. Diabetologia *34,* 721-726.

Frost, G.S., Brynes, A.E., Dhillo, W.S., Bloom, S.R., and McBurney, M.I. (2003). The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. Eur J Clin Nutr *57,* 293-298.

Fu-Cheng, X., Anini, Y., Chariot, J., Castex, N., Galmiche, J.P., and Roze, C. (1997). Mechanisms of peptide YY release induced by an intraduodenal meal in rats: neural regulation by proximal gut. Pflugers Arch *433,* 571-579.

Gabius, H.J. (1990). Influence of type of linkage and spacer on the interaction of beta-galactoside-binding proteins with immobilized affinity ligands. Anal Biochem *189,* 91-94.

Gallaher, D.D., Hassel, C.A., Lee, K.J., and Gallaher, C.M. (1993). Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. J Nutr *123,* 244-252.

Gantz, I., Erondu, N., Mallick, M., Musser, B., Krishna, R., Tanaka, W.K., Snyder, K., Stevens, C., Stroh, M.A., Zhu, H. *et al.* (2007). Efficacy and safety of intranasal peptide YY3-36 for weight reduction in obese adults. J Clin Endocrinol Metab *92,* 1754-1757.

Garcia, A.L., Otto, B., Reich, S.C., Weickert, M.O., Steiniger, J., Machowetz, A., Rudovich, N.N., Mohlig, M., Katz, N., Speth, M. *et al.* (2007). Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance. Eur J Clin Nutr *61,* 334-341.

Gibbs, J., Young, R.C., and Smith, G.P. (1973a). Cholecystokinin decreases food intake in rats. J Comp Physiol Psychol *84,* 488-495.

Gibbs, J., Young, R.C., and Smith, G.P. (1973b). Cholecystokinin elicits satiety in rats with open gastric fistulas. Nature *245,* 323-325.

Glassmeier, G., Herzig, K.H., Hopfner, M., Lemmer, K., Jansen, A., and Scherubl, H. (1998). Expression of functional GABAA receptors in cholecystokinin-secreting gut neuroendocrine murine STC-1 cells. J Physiol *510,* 805-814.

Glavas, M.M., Grayson, B.E., Allen, S.E., Copp, D.R., Smith, M.S., Cowley, M.A., and Grove, K.L. (2008). Characterization of brainstem peptide YY (PYY) neurons. J Comp Neurol *506,* 194-210.

Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V.E., and Reeve, J.R.,Jr. (1994). Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. Regul Pept *51,* 151-159.

Grant, G., More, L.J., McKenzie, N.H., and Pusztai, A. (1982). The effect of heating on the haemagglutinating activity and nutritional properties of bean (Phaseolus vulgaris) seeds. J Sci Food Agric *33,* 1324-1326.

Greenman, Y., Golani, N., Gilad, S., Yaron, M., Limor, R., and Stern, N. (2004). Ghrelin secretion is modulated in a nutrient- and gender-specific manner. Clin Endocrinol (Oxf) *60,* 382-388.

Grill, H.J., and Norgren, R. (1978). Chronically decerebrate rats demonstrate satiation but not bait shyness. Science *201,* 267-269.

Grill, H.J., and Smith, G.P. (1988). Cholecystokinin decreases sucrose intake in chronic decerebrate rats. Am J Physiol *254,* R853-856.

Gruendel, S., Garcia, A.L., Otto, B., Mueller, C., Steiniger, J., Weickert, M.O., Speth, M., Katz, N., and Koebnick, C. (2006). Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. J Nutr *136,* 1533-1538.

Gutierrez, J.G., Chey, W.Y., and Dinoso, V.P. (1974). Actions of cholecystokinin and secretin on the motor activity of the small intestine in man. Gastroenterology *67,* 35-41.

Gutzwiller, J.P., Goke, B., Drewe, J., Hildebrand, P., Ketterer, S., Handschin, D., Winterhalder, R., Conen, D., and Beglinger, C. (1999). Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut *44,* 81-86.

Hagan, M.M. (2002). Peptide YY: a key mediator of orexigenic behavior. Peptides *23,* 377-382.

Hagemann, D., Holst, J.J., Gethmann, A., Banasch, M., Schmidt, W.E., and Meier, J.J. (2007). Glucagon-like peptide 1 (GLP-1) suppresses ghrelin levels in humans via increased insulin secretion. Regul Pept *143,* 64-68.

Halatchev, I.G., and Cone, R.D. (2005). Peripheral administration of PYY(3-36) produces conditioned taste aversion in mice. Cell Metab *1,* 159-168.

Halatchev, I.G., Ellacott, K.L., Fan, W., and Cone, R.D. (2004). Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. Endocrinology *145,* 2585-2590.

Harper, A.A., and Raper, H.S. (1943). Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J Physiol *102,* 115-125.

Heini, A.F., Lara-Castro, C., Schneider, H., Kirk, K.A., Considine, R.V., and Weinsier, R.L. (1998). Effect of hydrolyzed guar fiber on fasting and postprandial satiety and satiety hormones: a double-blind, placebo-controlled trial during controlled weight loss. Int J Obes Relat Metab Disord *22,* 906-909.

Heinonen, M.V., Karhunen, L.J., Chabot, E.D., Toppinen, L.K., Juntunen, K.S., Laaksonen, D.E., Siloaho, M., Liukkonen, K.H., Herzig, K.H., Niskanen, L.K. *et al.* (2007). Plasma ghrelin levels after two high-carbohydrate meals producing different insulin responses in patients with metabolic syndrome. Regul Pept 138, 118-125.

Helakorpi S., Patja K., Prattala R., and Uutela A. (2005). Health behaviour and health among the Finnish adult population, spring 2005. Publications of the National Public Health Institute. B. No 18.

Henderson, M., and Freeman, C.P. (1987). A self-rating scale for bulimia. The 'BITE'. Br J Psychiatry *150,* 18-24.

Herrmann, C., Goke, R., Richter, G., Fehmann, H.C., Arnold, R., and Goke, B. (1995). Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion *56,* 117-126.

Herzig, K.H. (1998). Cholecystokinin- and secretin-releasing peptides in the intestine--a new regulatory interendocrine mechanism in the gastrointestinal tract. Regul Pept *73,* 89-94.

Herzig, K.H., Bardocz, S., Grant, G., Nustede, R., Folsch, U.R., and Pusztai, A. (1997). Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. Gut *41,* 333-338.

Herzig, K.H., Schon, I., Tatemoto, K., Ohe, Y., Li, Y., Folsch, U.R., and Owyang, C. (1996). Diazepam binding inhibitor is a potent cholecystokinin-releasing peptide in the intestine. Proc Natl Acad Sci U S A *93,* 7927-7932.

Hildebrand, P., Beglinger, C., Gyr, K., Jansen, J.B., Rovati, L.C., Zuercher, M., Lamers, C.B., Setnikar, I., and Stalder, G.A. (1990). Effects of a cholecystokinin receptor antagonist on intestinal phase of pancreatic and biliary responses in man. J Clin Invest *85,* 640-646.

Hildebrand, P., Petrig, C., Burckhardt, B., Ketterer, S., Lengsfeld, H., Fleury, A., Hadvary, P., and Beglinger, C. (1998). Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. Gastroenterology *114,* 123-129.

Hinman, A., Chuang, H.H., Bautista, D.M., and Julius, D. (2006). TRP channel activation by reversible covalent modification. Proc Natl Acad Sci U S A *103,* 19564-19568.

Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S., and Tsujimoto, G. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med *11,* 90-94.

Holst, J.J. (2007). The physiology of glucagon-like peptide 1. Physiol Rev *87,* 1409-1439.

Holzer, H.H., Turkelson, C.M., Solomon, T.E., and Raybould, H.E. (1994). Intestinal lipid inhibits gastric emptying via CCK and a vagal capsaicin-sensitive afferent pathway in rats. Am J Physiol *267,* G625-629.

Hopman, W.P., Jansen, J.B., Rosenbusch, G., and Lamers, C.B. (1990). Role of cholecystokinin and the cholinergic system in intestinal stimulation of gallbladder contraction in man. Hepatology *11,* 261-265.

Hopman, W.P., Jansen, J.B., Rosenbusch, G., and Lamers, C.B. (1987). Cephalic stimulation of gallbladder contraction in humans: role of cholecystokinin and the cholinergic system. Digestion *38,* 197-203.

Hossain, P., Kawar, B., and El Nahas, M. (2007). Obesity and diabetes in the developing world--a growing challenge. N Engl J Med *356,* 213-215.

Ikegami, S., Tsuchihashi, F., Harada, H., Tsuchihashi, N., Nishide, E., and Innami, S. (1990). Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. J Nutr *120,* 353-360.

Ikezaki, A., Hosoda, H., Ito, K., Iwama, S., Miura, N., Matsuoka, H., Kondo, C., Kojima, M., Kangawa, K., and Sugihara, S. (2002). Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes *51,* 3408–3411.

Imamura, M., Nakajima, H., Mikami, Y., and Yamauchi, H. (1999). Morphological and immunohistochemical changes in intestinal mucosa and PYY release following total colectomy with ileal pouch-anal anastomosis in dogs. Dig Dis Sci *44,* 1000-1007.

Imeryuz, N., Yegen, B.C., Bozkurt, A., Coskun, T., Villanueva-Penacarrillo, M.L., and Ulusoy, N.B. (1997). Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. Am J Physiol *273,* G920-927.

Isaksson, G., Lundquist, I., and Ihse, I. (1982). Effect of dietary fiber on pancreatic enzyme activity in vitro. Gastroenterology *82,* 918-924.

Ivy, A.C., and Oldberg, E. (1928). A hormone mechanism for gallbladder contraction and evacuation. Am J Physiol *86,* 599-613.

Iwai, K., Fukuoka, S., Fushiki, T., Tsujikawa, M., Hirose, M., Tsunasawa, S., and Sakiyama, F. (1987). Purification and sequencing of a trypsin-sensitive cholecystokinin-releasing peptide from rat pancreatic juice. Its homology with pancreatic secretory trypsin inhibitor. J Biol Chem *262,* 8956-8959.

Jaquemar, D., Schenker, T., and Trueb, B. (1999). An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. J Biol Chem *274,* 7325-7333.

Jebbink, M.C., Masclee, A.A., van der Kleij, F.G., Schipper, J., Rovati, L.C., Jansen, J.B., and Lamers, C.B. (1992). Effect of loxiglumide and atropine on erythromycin-induced reduction in gallbladder volume in human subjects. Hepatology *16,* 937-942.

Jeffery, R.W., and Harnack, L.J. (2007). Evidence implicating eating as a primary driver for the obesity epidemic. Diabetes *56,* 2673-2676.

Jenkins, A.L., Jenkins, D.J., Zdravkovic, U., Wursch, P., and Vuksan, V. (2002). Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. Eur J Clin Nutr *56,* 622-628.

Jenkins, D.J., Goff, D.V., Leeds, A.R., Alberti, K.G., Wolever, T.M., Gassull, M.A., and Hockaday, T.D. (1976). Unabsorbable carbohydrates and diabetes: Decreased post-prandial hyperglycaemia. Lancet *2,* 172-174.

Jenkins, D.J., Wolever, T.M., Leeds, A.R., Gassull, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., and Alberti, K.G. (1978). Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Br Med J *1,* 1392-1394.

Jeon, T.Y., Lee, S., Kim, H.H., Kim, Y.J., Son, H.C., Kim, D.H., and Sim, M.S. (2004). Changes in plasma ghrelin concentration immediately after gastrectomy in patients with early gastric cancer. J Clin Endocrinol Metab *89,* 5392-5396.

Ji, B., Bi, Y., Simeone, D., Mortensen, R.M., and Logsdon, C.D. (2002). Human pancreatic acinar cells do not respond to cholecystokinin. Pharmacol Toxicol *91,* 327-332.

Ji, B., Bi, Y., Simeone, D., Mortensen, R.M., and Logsdon, C.D. (2001). Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. Gastroenterology *121,* 1380-1390.

Johnson, I.T., and Gee, J.M. (1981). Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. Gut *22,* 398-403.

Jordinson, M., Goodlad, R.A., Brynes, A., Bliss, P., Ghatei, M.A., Bloom, S.R., Fitzgerald, A., Grant, G., Bardocz, S., Pusztai, A. *et al.* (1999). Gastrointestinal responses to a panel of lectins in rats maintained on total parenteral nutrition. Am J Physiol *276,* G1235-1242.

Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Hogestatt, E.D., Meng, I.D., and Julius, D. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature *427,* 260-265.

Jorpes, J.E., and Mutt, V. (1961). The gastrointestinal hormones, secretin and cholecystokinin-pancreozymin. Ann Intern Med *55,* 395-405.

Juntunen, K.S., Laaksonen, D.E., Autio, K., Niskanen, L.K., Holst, J.J., Savolainen, K.E., Liukkonen, K.H., Poutanen, K.S., and Mykkanen, H.M. (2003). Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. Am J Clin Nutr *78,* 957-964.

Juntunen, K.S., Niskanen, L.K., Liukkonen, K.H., Poutanen, K.S., Holst, J.J., and Mykkanen, H.M. (2002). Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects. Am J Clin Nutr *75,* 254-262.

Karamanlis, A., Chaikomin, R., Doran, S., Bellon, M., Bartholomeusz, F.D., Wishart, J.M., Jones, K.L., Horowitz, M., and Rayner, C.K. (2007). Effects of protein on glycemic and incretin responses and gastric emptying after oral glucose in healthy subjects. Am J Clin Nutr *86,* 1364-1368.

Katsura, H., Obata, K., Mizushima, T., Yamanaka, H., Kobayashi, K., Dai, Y., Fukuoka, T., Tokunaga, A., Sakagami, M., and Noguchi, K. (2006). Antisense knock down of TRPA1, but not TRPM8, alleviates cold hyperalgesia after spinal nerve ligation in rats. Exp Neurol *200,* 112-123.

Kieffer, T.J., Huang, Z., McIntosh, C.H., Buchan, A.M., Brown, J.C., and Pederson, R.A. (1995). Gastric inhibitory polypeptide release from a tumor-derived cell line. Am J Physiol *269,* E316-322.

Kim, B.J., Carlson, O.D., Jang, H.J., Elahi, D., Berry, C., and Egan, J.M. (2005). Peptide YY is secreted after oral glucose administration in a gender-specific manner. J Clin Endocrinol Metab *90,* 6665-6671.

Kissileff, H.R., Carretta, J.C., Geliebter, A., and Pi-Sunyer, F.X. (2003). Cholecystokinin and stomach distension combine to reduce food intake in humans. Am J Physiol Regul Integr Comp Physiol *285,* R992-998.

Kissileff, H.R., Pi-Sunyer, F.X., Thornton, J., and Smith, G.P. (1981). C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr *34,* 154-160.

Koda, S., Date, Y., Murakami, N., Shimbara, T., Hanada, T., Toshinai, K., Niijima, A., Furuya, M., Inomata, N., Osuye, K. *et al.* (2005). The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. Endocrinology *146,* 2369-2375.

Koegler, F.H., Enriori, P.J., Billes, S.K., Takahashi, D.L., Martin, M.S., Clark, R.L., Evans, A.E., Grove, K.L., Cameron, J.L., and Cowley, M.A. (2005). Peptide YY(3-36) inhibits morning, but not evening, food intake and decreases body weight in rhesus macaques. Diabetes *54,* 3198-3204.

Kohnke-Godt, B., and Gabius, H.J. (1989). Heparin-binding lectin from human placenta: purification and partial molecular characterization and its relationship to basic fibroblast growth factors. Biochemistry *28,* 6531-6538.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature *402,* 656-660.

Kong, M.F., Chapman, I., Goble, E., Wishart, J., Wittert, G., Morris, H., and Horowitz, M. (1999). Effects of oral fructose and glucose on plasma GLP-1 and appetite in normal subjects. Peptides *20,* 545-551.

Korner, J., Bessler, M., Cirilo, L.J., Conwell, I.M., Daud, A., Restuccia, N.L., and Wardlaw, S.L. (2005). Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. J Clin Endocrinol Metab *90,* 359-365.

Korner, J., Inabnet, W., Conwell, I.M., Taveras, C., Daud, A., Olivero-Rivera, L., Restuccia, N.L., and Bessler, M. (2006). Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels. Obesity (Silver Spring) *14,* 1553-1561.

Krotkiewski, M. (1984). Effect of guar gum on body-weight, hunger ratings and metabolism in obese subjects. Br J Nutr *52,* 97-105.

Kwan, K.Y., Allchorne, A.J., Vollrath, M.A., Christensen, A.P., Zhang, D.S., Woolf, C.J., and Corey, D.P. (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron *50,* 277-289.

La Barre, J. (1932). Sur les possibilites d'un traitement du diabete par l'incretine. Bull Acad R Med Belg *12,* 620–634.

Lal, S., McLaughlin, J., Barlow, J., D'Amato, M., Giacovelli, G., Varro, A., Dockray, G.J., and Thompson, D.G. (2004). Cholecystokinin pathways modulate sensations induced by gastric distension in humans. Am J Physiol Gastrointest Liver Physiol *287,* G72-79.

le Roux, C.W., Batterham, R.L., Aylwin, S.J., Patterson, M., Borg, C.M., Wynne, K.J., Kent, A., Vincent, R.P., Gardiner, J., Ghatei, M.A. *et al.* (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology *147,* 3-8.

le Roux, C.W., Neary, N.M., Halsey, T.J., Small, C.J., Martinez-Isla, A.M., Ghatei, M.A., Theodorou, N.A., and Bloom, S.R. (2005). Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. J Clin Endocrinol Metab *90,* 4521-4524.

Leavitt, R.D., Felsted, R.L., and Bachur, N.R. (1977). Biological and biochemical properties of Phaseolus vulgaris isolectins. J Biol Chem *252,* 2961-2966.

Leclere, C.J., Champ, M., Boillot, J., Guille, G., Lecannu, G., Molis, C., Bornet, F., Krempf, M., Delort-Laval, J., and Galmiche, J.P. (1994). Role of viscous guar gums in lowering the glycemic response after a solid meal. Am J Clin Nutr *59,* 914-921.

Leidy, H.J., Gardner, J.K., Frye, B.R., Snook, M.L., Schuchert, M.K., Richard, E.L., and Williams, N.I. (2004). Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. J Clin Endocrinol Metab *89,* 2659-2664.

Lejeune, M.P., Westerterp, K.R., Adam, T.C., Luscombe-Marsh, N.D., and Westerterp-Plantenga, M.S. (2006). Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. Am J Clin Nutr *83,* 89-94.

Liddle, R.A. (1997). Cholecystokinin cells. Annu Rev Physiol *59,* 221-242.

Liddle, R.A., Gertz, B.J., Kanayama, S., Beccaria, L., Coker, L.D., Turnbull, T.A., and Morita, E.T. (1989). Effects of a novel cholecystokinin (CCK) receptor antagonist, MK-329, on gallbladder contraction and gastric emptying in humans. Implications for the physiology of CCK. J Clin Invest *84,* 1220-1225.

Lin, H.C., Chey, W.Y., and Zhao, X. (2000). Release of distal gut peptide YY (PYY) by fat in proximal gut depends on CCK. Peptides *21,* 1561-1563.

Linderoth, A., Biernat, M., Prykhodko, O., Kornilovska, I., Pusztai, A., Pierzynowski, S.G., and Bjorn, W.R. (2005). Induced growth and maturation of the gastrointestinal tract after Phaseolus vulgaris lectin exposure in suckling rats. J Pediatr Gastroenterol Nutr *41*, 195-203.

Linderoth, A., Prykhod'ko, O., Ahren, B., Fak, F., Pierzynowski, S.G., and Westrom, B.R. (2006a). Binding and the effect of the red kidney bean lectin, phytohaemagglutinin, in the gastrointestinal tract of suckling rats. Br J Nutr *95*, 105-115.

Linderoth, A., Prykhod'ko, O., Pierzynowski, S.G., and Westrom, B.R. (2006b). Enterally but not parenterally administered Phaseolus vulgaris lectin induces growth and precocious maturation of the gut in suckling rats. Biol Neonate *89*, 60-68.

Little, T.J., Doran, S., Meyer, J.H., Smout, A.J., O'Donovan, D.G., Wu, K.L., Jones, K.L., Wishart, J., Rayner, C.K., Horowitz, M. *et al.* (2006). The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent upon the length of small intestine exposed. Am J Physiol Endocrinol Metab *291*, E647-655.

Little, T.J., Feltrin, K.L., Horowitz, M., Meyer, J.H., Wishart, J., Chapman, I.M., and Feinle-Bisset, C. (2008). A high-fat diet raises fasting plasma CCK but does not affect upper gut motility, PYY, and ghrelin, or energy intake during CCK-8 infusion in lean men. Am J Physiol Regul Integr Comp Physiol *294*, R45-51.

Lotan, R., Skutelsky, E., Danon, D., and Sharon, N. (1975). The purification, composition, and specificity of the anti-T lectin from peanut (Arachis hypogaea). J Biol Chem *250*, 8518-8523.

Lundberg, J.M., Tatemoto, K., Terenius, L., Hellstrom, P.M., Mutt, V., Hokfelt, T., and Hamberger, B. (1982). Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. Proc Natl Acad Sci U S A *79*, 4471-4475.

Lundberg, J.M., Terenius, L., Hokfelt, T., and Tatemoto, K. (1984). Comparative immunohistochemical and biochemical analysis of pancreatic polypeptide-like peptides with special reference to presence of neuropeptide Y in central and peripheral neurons. J Neurosci *4*, 2376-2386.

MacIntosh, C.G., Andrews, J.M., Jones, K.L., Wishart, J.M., Morris, H.A., Jansen, J.B., Morley, J.E., Horowitz, M., and Chapman, I.M. (1999). Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. Am J Clin Nutr *69*, 999-1006.

Macpherson, L.J., Dubin, A.E., Evans, M.J., Marr, F., Schultz, P.G., Cravatt, B.F., and Patapoutian, A. (2007). Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature *445*, 541-545.

Macpherson, L.J., Geierstanger, B.H., Viswanath, V., Bandell, M., Eid, S.R., Hwang, S., and Patapoutian, A. (2005). The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol *15*, 929-934.

Maier, C., Schaller, G., Buranyi, B., Nowotny, P., Geyer, G., Wolzt, M., and Luger, A. (2004). The cholinergic system controls ghrelin release and ghrelin-induced growth hormone release in humans. J Clin Endocrinol Metab *89*, 4729-4733.

Malaisse-Lagae, F., Carpentier, J.L., Patel, Y.C., Malaisse, W.J., and Orci, L. (1977). Pancreatic polypeptide: a possible role in the regulation of food intake in the mouse. Hypothesis Experientia *33*, 915-917.

Manji, N., Bistrian, B.R., Mascioli, E.A., Benotti, P.A., and Blackburn, G.L. (1989). Gallstone disease in patients with severe short bowel syndrome dependent on parenteral nutrition. JPEN J Parenter Enteral Nutr *13*, 461-464.

Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., Al-Sahab, S., Bush, D., Wright, J., and Fillery-Travis, A.J. (2000). Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. J Nutr *130*, 122-127.

Marciani, L., Gowland, P.A., Spiller, R.C., Manoj, P., Moore, R.J., Young, P., and Fillery-Travis, A.J. (2001). Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol Gastrointest Liver Physiol *280*, G1227-1233.

McLaughlin, J., Grazia Luca, M., Jones, M.N., D'Amato, M., Dockray, G.J., and Thompson, D.G. (1999). Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. Gastroenterology *116*, 46-53.

McLaughlin, J.T., Lomax, R.B., Hall, L., Dockray, G.J., Thompson, D.G., and Warhurst, G. (1998). Fatty acids stimulate cholecystokinin secretion via an acyl chain length-specific, Ca2+-dependent mechanism in the enteroendocrine cell line STC-1. J Physiol *513*, 11-18.

McMahon, L.R., and Wellman, P.J. (1998). PVN infusion of GLP-1-(7-36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. Am J Physiol *274*, R23-29.

McMinn, J.E., Sindelar, D.K., Havel, P.J., and Schwartz, M.W. (2000). Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. Endocrinology *141*, 4442-4448.

Meeran, K., O'Shea, D., Edwards, C.M., Turton, M.D., Heath, M.M., Gunn, I., Abusnana, S., Rossi, M., Small, C.J., Goldstone, A.P. *et al.* (1999). Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. Endocrinology *140*, 244-250.

Meier, J.J., Gallwitz, B., Salmen, S., Goetze, O., Holst, J.J., Schmidt, W.E., and Nauck, M.A. (2003). Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab *88*, 2719-2725.

Meyer, B.M., Werth, B.A., Beglinger, C., Hildebrand, P., Jansen, J.B., Zach, D., Rovati, L.C., and Stalder, G.A. (1989). Role of cholecystokinin in regulation of gastrointestinal motor functions. Lancet *2*, 12-15.

Meyer, J.H., Gu, Y.G., Jehn, D., and Taylor, I.L. (1988). Intragastric vs intraintestinal viscous polymers and glucose tolerance after liquid meals of glucose. Am J Clin Nutr *48*, 260-266.

Mohlig, M., Koebnick, C., Weickert, M.O., Lueder, W., Otto, B., Steiniger, J., Twilfert, M., Meuser, F., Pfeiffer, A.F., and Zunft, H.J. (2005). Arabinoxylan-enriched meal increases serum ghrelin levels in healthy humans. Horm Metab Res *37*, 303-308.

Mohlig, M., Spranger, J., Otto, B., Ristow, M., Tschop, M., and Pfeiffer, A.F. (2002). Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. J Endocrinol Invest *25*, RC36-38.

Monteleone, P., Bencivenga, R., Longobardi, N., Serritella, C., and Maj, M. (2003). Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. J Clin Endocrinol Metab *88*, 5510-5514.

Moore, B., Edie, E.S., Abram, J.H. (1906). On the treatment of diabetes mellitus by acid extract of duodenal muocous membrane. Biochem J *1*, 28-38.

Moran, T.H., and Bi, S. (2006). Hyperphagia and obesity in OLETF rats lacking CCK-1 receptors. Philos Trans R Soc Lond B Biol Sci *361*, 1211-1218.

Moran, T.H., and McHugh, P.R. (1982). Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am J Physiol *242*, R491-497.

Moran, T.H., Smedh, U., Kinzig, K.P., Scott, K.A., Knipp, S., and Ladenheim, E.E. (2005). Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. Am J Physiol Regul Integr Comp Physiol *288*, R384-388.

Morinigo, R., Moize, V., Musri, M., Lacy, A.M., Navarro, S., Marin, J.L., Delgado, S., Casamitjana, R., and Vidal, J. (2006). Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. J Clin Endocrinol Metab *91*, 1735-1740.

Morley, J.E., and Flood, J.F. (1991). Amylin decreases food intake in mice. Peptides *12*, 865-869.

Morley, J.E., Levine, A.S., Grace, M., and Kneip, J. (1985). Peptide YY (PYY), a potent orexigenic agent. Brain Res *341,* 200-203.

Morton, G.J., Blevins, J.E., Williams, D.L., Niswender K.D., Gelling R.W., Rhodes C.J., Baskin D.G., and Schwartz, M.W. (2005). Leptin action in the forebrain regulates the hindbrain response to satiety signals. J Clin Invest *115,* 703–710.

Mundinger, T.O., Cummings, D.E., and Taborsky, G.J.,Jr. (2006). Direct stimulation of ghrelin secretion by sympathetic nerves. Endocrinology *147,* 2893-2901.

Murdolo, G., Lucidi, P., Di Loreto, C., Parlanti, N., De Cicco, A., Fatone, C., Fanelli, C.G., Bolli, G.B., Santeusanio, F., and De Feo, P. (2003). Insulin is required for prandial ghrelin suppression in humans. Diabetes *52,* 2923-2927.

Murray, C.D., le Roux, C.W., Gouveia, C., Bassett, P., Ghatei, M.A., Bloom, S.R., Emmanuel, A.V., and Gabe, S.M. (2006). The effect of different macronutrient infusions on appetite, ghrelin and peptide YY in parenterally fed patients. Clin Nutr *25,* 626-633.

Nagata, K., Duggan, A., Kumar, G., and Garcia-Anoveros, J. (2005). Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci *25,* 4052-4061.

Nakabayashi, H., Nishizawa, M., Nakagawa, A., Takeda, R., and Niijima, A. (1996). Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am J Physiol *271,* E808-813.

Nakagawa, A., Satake, H., Nakabayashi, H., Nishizawa, M., Furuya, K., Nakano, S., Kigoshi, T., Nakayama, K., and Uchida, K. (2004). Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. Auton Neurosci *110,* 36-43.

Naslund, E., Barkeling, B., King, N., Gutniak, M., Blundell, J.E., Holst, J.J., Rossner, S., and Hellstrom, P.M. (1999). Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. Int J Obes Relat Metab Disord *23,* 304-311.

Naslund, E., Bogefors, J., Gryback, P., Jacobsson, H., and Hellstrom, P.M. (2000). Gastric emptying: comparison of scintigraphic, polyethylene glycol dilution, and paracetamol tracer assessment techniques. Scand J Gastroenterol *35,* 375-379.

Naslund, E., King, N., Mansten, S., Adner, N., Holst, J.J., Gutniak, M., and Hellstrom, P.M. (2004). Prandial subcutaneous injections of glucagon-like peptide-1 cause weight loss in obese human subjects. Br J Nutr *91,* 439-446.

Nauck, M.A., Niedereichholz, U., Ettler, R., Holst, J.J., Orskov, C., Ritzel, R., and Schmiegel, W.H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol *273,* E981-988.

Neary, N.M., Druce, M.R., Small, C.J., and Bloom, S.R. (2006). Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect. Gut *55,* 135.

Nedvidkova, J., Krykorkova, I., Bartak, V., Papezova, H., Gold, P.W., Alesci, S., and Pacak, K. (2003). Loss of meal-induced decrease in plasma ghrelin levels in patients with anorexia nervosa. J Clin Endocrinol Metab *88,* 1678-1682.

Nelson, D.K., Glasbrenner, B., Dahmen, G., Riepl, R.L., Malfertheiner, P., and Adler, G. (1996). M1 muscarinic mechanisms regulate intestinal-phase gallbladder physiology in humans. Am J Physiol *271,* G824-830.

Niederau, C., Heintges, T., Rovati, L., and Strohmeyer, G. (1989). Effects of loxiglumide on gallbladder emptying in healthy volunteers. Gastroenterology *97,* 1331-1336.

Nitsche, R., Hinrichsen, H., Herzig, K.H., Hopfner, M., Nustede, R., and Folsch, U.R. (1998). Evaluation of a cacao drink as a simple oral stimulus to assess gallbladder contraction. Z Gastroenterol *36,* 135-141.

Nogueiras, R., Pfluger, P., Tovar, S., Arnold, M., Mitchell, S., Morris, A., Perez-Tilve, D., Vázquez, M.J., Wiedmer, P., Castañeda, T.R. *et al.* (2007). Effects of obestatin on energy balance and growth hormone secretion in rodents. Endocrinology *148,* 21-26.

Obata, K., Katsura, H., Mizushima, T., Yamanaka, H., Kobayashi, K., Dai, Y., Fukuoka, T., Tokunaga, A., Tominaga, M., and Noguchi, K. (2005). TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. J Clin Invest *115,* 2393-2401.

Oesch, S., Ruegg, C., Fischer, B., Degen, L., and Beglinger, C. (2006). Effect of gastric distension prior to eating on food intake and feelings of satiety in humans. Physiol Behav *87,* 903-910.

Ogden, C.L., Yanovski, S.Z., Carroll, M.D., and Flegal, K.M. (2007). The epidemiology of obesity. Gastroenterology *132,* 2087-2102.

Olson, B.H., Anderson, S.M., Becker, M.P., Anderson, J.W., Hunninghake, D.B., Jenkins, D.J., LaRosa, J.C., Rippe, J.M., Roberts, D.C., Stoy, D.B. *et al*. (1997). Psyllium-enriched cereals lower blood total cholesterol and LDL cholesterol, but not HDL cholesterol, in hypercholesterolemic adults: results of a meta-analysis. J Nutr *127,* 1973-1980.

Otte, J.M., Chen, C., Brunke, G., Kiehne, K., Schmitz, F., Folsch, U.R., and Herzig, K.H. (2001). Mechanisms of lectin (phytohemagglutinin)-induced growth in small intestinal epithelial cells. Digestion *64,* 169-178.

Otto, B., Heldwein, W., Otto, C., Huptas, S., and Parhofer, K.G. (2006). Effect of a high-fat meal on the postprandial ghrelin response. Am J Clin Nutr *84,* 664-5; author reply 665-6.

Panahi, S., Ezatagha, A., Temelli, F., Vasanthan, T., and Vuksan, V. (2007). Beta-glucan from two sources of oat concentrates affect postprandial glycemia in relation to the level of viscosity. J Am Coll Nutr *26,* 639-644.

Parker, B.A., Doran, S., Wishart, J., Horowitz, M., and Chapman, I.M. (2005). Effects of small intestinal and gastric glucose administration on the suppression of plasma ghrelin concentrations in healthy older men and women. Clin Endocrinol (Oxf) *62,* 539-546.

Parkin, G.J., Smith, R.B., and Johnston, D. (1973). Gallbladder volume and contractility after truncal, selective and highly selective (parietal-cell) vagotomy in man. Ann Surg *178,* 581-586.

Pasman, W.J., Saris, W.H., Wauters, M.A., and Westerterp-Plantenga, M.S. (1997). Effect of one week of fibre supplementation on hunger and satiety ratings and energy intake. Appetite *29,* 77-87.

Pedersen-Bjergaard, U., Host, U., Kelbaek, H., Schifter, S., Rehfeld, J.F., Faber, J., and Christensen, N.J. (1996). Influence of meal composition on postprandial peripheral plasma concentrations of vasoactive peptides in man. Scand J Clin Lab Invest *56,* 497-503.

Penuelas, A., Tashima, K., Tsuchiya, S., Matsumoto, K., Nakamura, T., Horie, S., and Yano, S. (2007). Contractile effect of TRPA1 receptor agonists in the isolated mouse intestine. Eur J Pharmacol *576,* 143-150.

Perez-Tilve, D., Gonzalez-Matias, L., Alvarez-Crespo, M., Leiras, R., Tovar, S., Dieguez, C., and Mallo, F. (2007). Exendin-4 potently decreases ghrelin levels in fasting rats. Diabetes *56,* 143-151.

Peters, J.H., Simasko, S.M., and Ritter, R.C. (2006). Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. Physiol Behav *89,* 477-485.

Pfluger, P.T., Kampe, J., Castaneda, T.R., Vahl, T., D'Alessio, D.A., Kruthaupt, T., Benoit, S.C., Cuntz, U., Rochlitz, H.J., Moehlig, M. *et al.* (2007). Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. J Clin Endocrinol Metab *92,* 583-588.

Pittler, M.H., and Ernst, E. (2001). Guar gum for body weight reduction: meta-analysis of randomized trials. Am J Med *110,* 724-730.

Pittner, R.A., Moore, C.X., Bhavsar, S.P., Gedulin, B.R., Smith, P.A., Jodka, C.M., Parkes, D.G., Paterniti, J.R., Srivastava, V.P., and Young, A.A. (2004). Effects of PYY[3-36] in rodent models of diabetes and obesity. Int J Obes Relat Metab Disord *28,* 963-971.

Porikos, K., and Hagamen, S. (1986). Is fiber satiating? Effects of a high fiber preload on subsequent food intake of normal-weight and obese young men. Appetite *7,* 153-162.

Poykko, S.M., Kellokoski, E., Horkko, S., Kauma, H., Kesaniemi, Y.A., and Ukkola, O. (2003). Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes *52,* 2546-2553.

Pusztai, A., Clarke, E.M., and King, T.P. (1979). The nutritional toxicity of Phaseolus vulgaris lectins. Proc Nutr Soc *38,* 115-120.

Pusztai, A., Ewen, S.W., Grant, G., Peumans, W.J., van Damme, E.J., Rubio, L., and Bardocz, S. (1990). Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion *46 Suppl 2,* 308-316.

Raben, A., Agerholm-Larsen, L., Flint, A., Holst, J.J., and Astrup, A. (2003). Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. Am J Clin Nutr *77,* 91-100.

Raben, A., Christensen, N.J., Madsen, J., Holst, J.J., and Astrup, A. (1994a). Decreased postprandial thermogenesis and fat oxidation but increased fullness after a high-fiber meal compared with a low-fiber meal. Am J Clin Nutr *59,* 1386-1394.

Raben, A., Tagliabue, A., Christensen, N.J., Madsen, J., Holst, J.J., and Astrup, A. (1994b). Resistant starch: the effect on postprandial glycemia, hormonal response, and satiety. Am J Clin Nutr *60,* 544-551.

Ranganath, L.R., Beety, J.M., Morgan, L.M., Wright, J.W., Howland, R., and Marks, V. (1996). Attenuated GLP-1 secretion in obesity: cause or consequence? Gut *38,* 916-919.

Raybould, H.E., Roberts, M.E., and Dockray, G.J. (1987). Reflex decreases in intragastric pressure in response to cholecystokinin in rats. Am J Physiol *253,* G165-170.

Raybould, H.E., and Tache, Y. (1988). Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. Am J Physiol *255,* G242-246.

Reeve, J.R.,Jr, Green, G.M., Chew, P., Eysselein, V.E., and Keire, D.A. (2003). CCK-58 is the only detectable endocrine form of cholecystokinin in rat. Am J Physiol Gastrointest Liver Physiol *285,* G255-265.

Rehfeld, J.F. (2004). Clinical endocrinology and metabolism. Cholecystokinin. Best Pract Res Clin Endocrinol Metab *18,* 569-586.

Rehfeld, J.F., Sun, G., Christensen, T., and Hillingso, J.G. (2001). The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. J Clin Endocrinol Metab *86,* 251-258.

Reidelberger, R.D., Hernandez, J., Fritzsch, B., and Hulce, M. (2004). Abdominal vagal mediation of the satiety effects of CCK in rats. Am J Physiol Regul Integr Comp Physiol *286,* R1005-1012.

Reimer, R.A., and McBurney, M.I. (1996). Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-1 and insulin in rats. Endocrinology *137,* 3948-3956.

Reinehr, T., Roth, C.L., Schernthaner, G.H., Kopp, H.P., Kriwanek, S., and Schernthaner, G. (2007). Peptide YY and glucagon-like peptide-1 in morbidly obese patients before and after surgically induced weight loss. Obes Surg *17,* 1571-1577.

Rettenbacher, M., and Reubi, J.C. (2001). Localization and characterization of neuropeptide receptors in human colon. Naunyn Schmiedebergs Arch Pharmacol *364,* 291-304.

Rindi, G., Grant, S.G., Yiangou, Y., Ghatei, M.A., Bloom, S.R., Bautch, V.L., Solcia, E., and Polak, J.M. (1990). Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. Am J Pathol *136,* 1349-1363.

Ritter, R.C. (2004). Gastrointestinal mechanisms of satiation for food. Physiol Behav *81,* 249-273.

Romon, M., Gomila, S., Hincker, P., Soudan, B., and Dallongeville, J. (2006). Influence of weight loss on plasma ghrelin responses to high-fat and high-carbohydrate test meals in obese women. J Clin Endocrinol Metab *91,* 1034-1041.

Roth, C.L., Enriori, P.J., Harz, K., Woelfle, J., Cowley, M.A., and Reinehr, T. (2005). Peptide YY is a regulator of energy homeostasis in obese children before and after weight loss. J Clin Endocrinol Metab *90,* 6386-6391.

Roth, J.D., Coffey, T., Jodka, C.M., Maier, H., Athanacio, J.R., Mack, C.M., Weyer, C., and Parkes, D.G. (2007). Combination therapy with amylin and peptide YY[3-36] in obese rodents: anorexigenic synergy and weight loss additivity. Endocrinology *148,* 6054-6061.

Rüdiger, H. (1993). Isolation of plant lectins. In Lectins and Glycobiology, H.J. Gabius, and S. Gabius, eds. (Springer, Heidelberg), pp. 31-46.

Ryttig, K.R., Tellnes, G., Haegh, L., Boe, E., and Fagerthun, H. (1989). A dietary fibre supplement and weight maintenance after weight reduction: a randomized, double-blind, placebo-controlled long-term trial. Int J Obes *13,* 165-171.

Sakata, I., Nakamura, K., Yamazaki, M., Matsubara, M., Hayashi, Y., Kangawa, K., and Sakai, T. (2002). Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. Peptides *23,* 531-536.

Schaller, G., Schmidt, A., Pleiner, J., Woloszczuk, W., Wolzt, M., and Luger, A. (2003). Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebocontrolled crossover clamp study. Diabetes *52,* 16-20.

Schirra, J., Nicolaus, M., Roggel, R., Katschinski, M., Storr, M., Woerle, H.J., and Goke, B. (2006). Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut *55,* 243-251.

Schmidt, W.E., Creutzfeldt, W., Schleser, A., Choudhury, A.R., Nustede, R., Hocker, M., Nitsche, R., Sostmann, H., Rovati, L.C., and Folsch, U.R. (1991). Role of CCK in regulation of pancreaticobiliary functions and GI motility in humans: effects of loxiglumide. Am J Physiol *260,* G197-206.

Schwartz, G.J., McHugh, P.R., and Moran, T.H. (1993). Gastric loads and cholecystokinin synergistically stimulate rat gastric vagal afferents. Am J Physiol *265,* R872-876.

Shaffer, E.A. (1982). The effect of vagotomy on gallbladder function and bile composition in man. Ann Surg *195,* 413-418.

Shebani, K.O., Stucchi, A.F., Fruin, B., McClung, J.P., Gee, D., Beer, E.R., LaMorte, W.W., and Becker, J.M. (2002). Pouchitis in a rat model of ileal J pouch-anal anastomosis. Inflamm Bowel Dis *8,* 23-34.

Sitzmann, J.V., Pitt, H.A., Steinborn, P.A., Pasha, Z.R., and Sanders, R.C. (1990). Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans. Surg Gynecol Obstet *170,* 25-31.

Sjolund, K., Ekman, R., Lindgren, S., and Rehfeld, J.F. (1996). Disturbed motilin and cholecystokinin release in the irritable bowel syndrome. Scand J Gastroenterol *31,* 1110-1114.

Sloth, B., Davidsen, L., Holst, J.J., Flint, A., and Astrup, A. (2007a). Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. Am J Physiol Endocrinol Metab *293,* E604-609.

Sloth, B., Holst, J.J., Flint, A., Gregersen, N.T., and Astrup, A. (2007b). Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. Am J Physiol Endocrinol Metab *292,* E1062-1068.

Soffer, E.E., and Adrian, T.E. (1992). Effect of meal composition and sham feeding on duodenojejunal motility in humans. Dig Dis Sci *37,* 1009-1014.

Spannagel, A.W., Green, G.M., Guan, D., Liddle, R.A., Faull, K., and Reeve, J.R.,Jr. (1996). Purification and characterization of a luminal cholecystokinin-releasing factor from rat intestinal secretion. Proc Natl Acad Sci U S A *93,* 4415-4420.

Stock, S., Leichner, P., Wong, A.C., Ghatei, M.A., Kieffer, T.J., Bloom, S.R., and Chanoine, J.P. (2005). Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. J Clin Endocrinol Metab *90,* 2161-2168.

Stokes, A., Wakano, C., Koblan-Huberson, M., Adra, C.N., Fleig, A., and Turner, H. (2006). TRPA1 is a substrate for de-ubiquitination by the tumor suppressor CYLD. Cell Signal *18,* 1584-1594.

Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earley, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W. *et al.* (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell *112,* 819-829.

Stunkard, A.J., and Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res *29,* 71-83.

Tanaka, T., Katsuma, S., Adachi, T., Koshimizu, T.A., Hirasawa, A., and Tsujimoto, G. (2007). Free fatty acids induce cholecystokinin secretion through GPR120. Naunyn Schmiedebergs Arch Pharmacol 2007 Oct 31; [Epub ahead of print]

Tannous dit El Khoury,D., Obeid, O., Azar, S.T., and Hwalla, N. (2006). Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. Ann Nutr Metab *50,* 260-269.

Tatemoto, K., and Mutt, V. (1980). Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. Nature *285,* 417-418.

Tatemoto, K., Nakano, I., Makk, G., Angwin, P., Mann, M., Schilling, J., and Go, V.L. (1988). Isolation and primary structure of human peptide YY. Biochem Biophys Res Commun *157,* 713-717.

Taylor-Clark, T.E., Undem, B.J., Macglashan Jr, D.W., Ghatta, S., Carr, M.J., and McAlexander, M.A. (2008). Prostaglandin-induced activation of nociceptive neurons via direct interaction with TRPA1. Mol Pharmacol *73,* 274-281.

Ternent, C.A., Staab, P., Thorson, A.G., Blatchford, G.J., Christensen, M.A., Thompson, J.S., Lanspa, S.J., Meade, P.G., Cali, R.A., Falk, P.M. *et al.* (1998). Ileoanal pouch function and release of peptide YY. Dis Colon Rectum *41,* 868-874.

Toft-Nielsen, M.B., Madsbad, S., and Holst, J.J. (1999). Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. Diabetes Care *22,* 1137-1143.

Toshinai, K., Yamaguchi, H., Sun, Y., Smith, R.G., Yamanaka, A., Sakurai, T., Date, Y., Mondal, M.S., Shimbara, T., Kawagoe, T. *et al.* (2006). Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. Endocrinology *147,* 2306-2314.

Trevisani, M., Siemens, J., Materazzi, S., Bautista, D.M., Nassini, R., Campi, B., Imamachi, N., Andre, E., Patacchini, R., Cottrell, G.S. *et al.* (2007). 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc Natl Acad Sci U S A *104,* 13519-13524.

Tsai, C.J., Leitzmann, M.F., Hu, F.B., Willett, W.C., and Giovannucci, E.L. (2004a). Frequent nut consumption and decreased risk of cholecystectomy in women. Am J Clin Nutr *80,* 76-81.

Tsai, C.J., Leitzmann, M.F., Hu, F.B., Willett, W.C., and Giovannucci, E.L. (2004b). A prospective cohort study of nut consumption and the risk of gallstone disease in men. Am J Epidemiol *160,* 961-968.

Tschop, M., Castaneda, T.R., Joost, H.G., Thone-Reineke, C., Ortmann, S., Klaus, S., Hagan, M.M., Chandler, P.C., Oswald, K.D., Benoit, S.C. *et al.* (2004). Physiology: does gut hormone PYY3-36 decrease food intake in rodents? Nature *430,* 1 p following 165; discussion 2 p following 165.

Tschop, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E., and Heiman, M.L. (2001). Circulating ghrelin levels are decreased in human obesity. Diabetes *50,* 707-709.

Turton, M.D., O'Shea, D., Gunn, I., Beak, S.A., Edwards, C.M., Meeran, K., Choi, S.J., Taylor, G.M., Heath, M.M., Lambert, P.D. *et al.* (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. Nature *379,* 69-72.

van Battum, P.L., Hopman, W.P., Salemans, J.M., Kuijpers, J.H., Nagengast, F.M., and Jansen, J.B. (1999). Impaired release of peptide YY in patients with proctocolectomy and ileal pouch-anal anastomosis. Scand J Gastroenterol *34,* 404-408.

Van Citters, G.W., and Lin, H.C. (2006). Ileal brake: neuropeptidergic control of intestinal transit. Curr Gastroenterol Rep *8,* 367-373.

van Dam, R.M., and Seidell, J.C. (2007). Carbohydrate intake and obesity. Eur J Clin Nutr *61 Suppl 1,* S75-99.

Van de Ven, M.L., Westerterp-Plantenga, M.S., Wouters, L., and Saris, W.H. (1994). Effects of liquid preloads with different fructose/fibre concentrations on subsequent food intake and ratings of hunger in women. Appetite *23,* 139-146.

Varga, G., Balint, A., Burghardt, B., and D'Amato, M. (2004). Involvement of endogenous CCK and CCK1 receptors in colonic motor function. Br J Pharmacol *141,* 1275-1284.

Vazquez Roque, M.I., Camilleri, M., Stephens, D.A., Jensen, M.D., Burton, D.D., Baxter, K.L., and Zinsmeister, A.R. (2006). Gastric sensorimotor functions and hormone profile in normal weight, overweight, and obese people. Gastroenterology *131,* 1717-1724.

Venkatachalam, K., and Montell, C. (2007). TRP channels. Annu Rev Biochem *76,* 387-417.

Verdich, C., Toubro, S., Buemann, B., Lysgard Madsen, J., Juul Holst, J., and Astrup, A. (2001). The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. Int J Obes Relat Metab Disord *25,* 1206-1214.

Vilsboll, T., Krarup, T., Sonne, J., Madsbad, S., Volund, A., Juul, A.G., and Holst, J.J. (2003). Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab *88,* 2706-2713.

Vukasin, A.P., Ballantyne, G.H., Nilsson, O., Bilchik, A.J., Adrian, T.E., and Modlin, I.M. (1992). Plasma and tissue alterations of peptide YY and enteroglucagon in rats after colectomy. Yale J Biol Med *65,* 1-15.

Wang, B.J., and Cui, Z.J. (2007). How does cholecystokinin stimulate exocrine pancreatic secretion? From birds, rodents, to humans. Am J Physiol Regul Integr Comp Physiol *292,* R666-678.

Wang, L., Newman, R.K., Newman, C.W., and Hofer, P.J. (1992). Barley beta-glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. J Nutr *122,* 2292-2297.

Weickert, M.O., Spranger, J., Holst, J.J., Otto, B., Koebnick, C., Mohlig, M., and Pfeiffer, A.F. (2006). Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety. Br J Nutr *96,* 795-798.

West, D.B., Fey, D., and Woods, S.C. (1984). Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. Am J Physiol *246,* R776-787.

Westerterp-Plantenga, M., Diepvens, K., Joosen, A.M., Berube-Parent, S., and Tremblay, A. (2006). Metabolic effects of spices, teas, and caffeine. Physiol Behav *89,* 85-91.

Westerterp-Plantenga, M.S., Smeets, A., and Lejeune, M.P. (2005). Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int J Obes (Lond) *29,* 682-688.

Wettergren, A., Maina, P., Boesby, S., and Holst, J.J. (1997a). Glucagon-like peptide-1 7-36 amide and peptide YY have additive inhibitory effect on gastric acid secretion in man. Scand J Gastroenterol *32,* 552-555.

Wettergren, A., Wojdemann, M., Meisner, S., Stadil, F., and Holst, J.J. (1997b). The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut *40,* 597-601.

Willems, M., Quartero, A.O., and Numans, M.E. (2001). How useful is paracetamol absorption as a marker of gastric emptying? A systematic literature study. Dig Dis Sci *46,* 2256-2262.

Williams, D.L., and Cummings, D.E. (2005). Regulation of ghrelin in physiologic and pathophysiologic states. J Nutr *135,* 1320-1325.

Williams, D.L., Cummings, D.E., Grill, H.J., and Kaplan, J.M. (2003). Meal-related ghrelin suppression requires postgastric feedback. Endocrinology *144,* 2765-2767.

Williams, D.L., Grill, H.J., Cummings, D.E., and Kaplan, J.M. (2006). Overfeeding-induced weight gain suppresses plasma ghrelin levels in rats. J Endocrinol Invest *29,* 863-868.

Williams, D.L., Grill, H.J., Cummings, D.E., and Kaplan, J.M. (2003). Vagotomy dissociates short- and long-term controls of circulating ghrelin. Endocrinology *144,* 5184-5187.

Willis, S., Kisielinski, K., Klosterhalfen, B., and Schumpelick, V. (2002). Morphological and functional adaptation of the small intestine after colectomy and ileal pouch-anal anastomosis in rats. Int J Colorectal Dis *17,* 85-91.

Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., and Collins, M.W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br J Nutr *72,* 731-743.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A., and Bloom, S.R. (2001). Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab *86,* 5992.

Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L., Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G., Ghatei, M.A. *et al.* (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology *141,* 4325-4328.

Wu, Z.S., Yu, L., Lin, Y.J., Jun, Z.J., Min, W.S., Jun, Y., and Hua, Z.B. (2000). Rapid intravenous administration of amino acids prevents biliary sludge induced by total parenteral nutrition in humans. J Hepatobiliary Pancreat Surg *7,* 504-509.

Wynne, K., Park, A.J., Small, C.J., Patterson, M., Ellis, S.M., Murphy, K.G., Wren, A.M., Frost, G.S., Meeran, K., Ghatei, M.A. *et al.* (2005). Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes *54,* 2390-2395.

Yang, J., Brown, M.S., Liang, G., Grishin, N.V., and Goldstein, J.L. (2008). Identification of the Acyltransferase that Octanoylates Ghrelin, an Appetite-Stimulating Peptide Hormone. Cell *132,* 387-396.

Zhang, J.V., Ren, P.G., Avsian-Kretchmer, O., Luo, C.W., Rauch, R., Klein, C., and Hsueh, A.J. (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science *310,* 996-999.

Zurborg, S., Yurgionas, B., Jira, J.A., Caspani, O., and Heppenstall, P.A. (2007). Direct activation of the ion channel TRPA1 by Ca(2+). Nat Neurosci *10,* 277-279.

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