# Effects of palatable diets on appetite regulation, appetite peptides and neurogenesis.

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Abstract

Obesity is increasing throughout the world at an epidemic rate, partly due to increased consumption of diets rich in fat and/or sugar. Ghrelin and leptin are hormones, originating from the periphery, involved in the regulation of food intake and of maintaining energy homeostasis. Circulating ghrelin levels increase in times of energy deficiency, such as fasting, signalling hunger whereas circulating leptin levels increase during food intake, signaling termination of feeding. In rats offered a diet rich in sucrose, the pre- and postprandial difference in circulating leptin and ghrelin was abolished. This difference in leptin was also abolished in rats offered a high fat diet. Circulating concentrations of leptin was increased whereas ghrelin concentrations were decreased in the fasted state of rats offered a diet rich in both fat and sucrose. This suggests that the animals are trying to defend themselves against, or ameliorate the effects of, the energy-dense diets. This attempt to protect themselves is, however, not efficient enough to prevent the rats from overconsumption of the diets and gaining weight. Interestingly, fructose, a form of sugar very commonly used as a sweetener in soft drinks and other sweetened beverages, was found to increase fasting levels of ghrelin, thus promoting increased caloric consumption.

The removal of the stomach (gastrectomy) in mice resulted in an 80% reduction in circulating ghrelin (hypoghrelinemia), in decreased amount of adipose tissue and decreased thermogenesis and ghrelin, when given daily over a period of eight weeks, normalised the amount of adipose tissue and thermogenesis in gastrectomised mice.

A high fat diet was offered to rats in order to study the effect of such a diet on hippocampal neurogenesis. The high fat diet was found to impair neurogenesis in male rats, as indicated by a 40% reduction in newborn neurons. This effect was not observed in female rats. The high fat diet was also found to stimulate corticosterone in male rats (but not female rats), suggesting corticosterone to be responsible for the impairment in hippocampal neurogenesis.



Key words: obesity, high-fat diet, ghrelin, leptin, hippocampus, neurogenesis, UCP1, fructose, sucrose and glucose

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# Effects of palatable diets on appetite regulation, appetite peptides and neurogenesis

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## ORIGINAL PAPERS

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**I.** **Andreas Lindqvist,** Charlotta Dornonville de la Cour, Anna Stegmark, Rolf Håkanson and Charlotte Erlanson-Albertsson. *Overeating of palatable food is associated with blunted leptin and ghrelin responses.*

Regulatory Peptides. 2005 Sep. 15; 130 (3):123-132.

**II.** Charlotta Dornonville de la Cour, **Andreas Lindqvist**, Emil Egecioglu, YC Loraine Tung, Vikas Surve, Claes Ohlsson, Charlotte Erlanson-Albertsson, Suzanne L. Dickson and Rolf Håkanson. *Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomised mice.*
Gut. 2005 Jul.; 54 (7):907-913.

**III.** **Andreas Lindqvist,** Paul Mohapel, Brenda Bouter, Helena Frielingsdorf, Donald Pizzo, Patrik Brundin and Charlotte Erlanson-Albertsson. *High-fat diet impairs hippocampal neurogenesis in male rats.*
European Journal of Neurology 2006. In press.

**IV.** **Andreas Lindqvist**, Charlotta Dornonville de la Cour, Rolf Håkanson and Charlotte Erlanson-Albertsson. *Ghrelin affects gastrectomy-induced decrease in UCP1 and $\beta_3$-AR mRNA expression in mice.*
Manuscript.

***V.*** **Andreas Lindqvist**, Annemie Baelemans and Charlotte Erlanson-Albertsson. *Effect of sucrose, glucose and fructose in liquid form on serum ghrelin and leptin levels.*
Manuscript.

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## ABBREVIATIONS

| | |
|---|---|
| 5-HT | serotonin |
| AgRP | agouti-related protein |
| ARC | arcuate nucleus |
| ATP | adenosine triphosphate |
| BAT | brown adipose tissue |
| BBB | blood-brain barrier |
| BMI | body mass index |
| BrdU | 5′-bromo-2′-deoxyuridine |
| cAMP | cyclic adenosine monophosphate |
| CART | cocaine- and amphetamine regulated transcript |
| CB | cannabinoid receptor |
| CCK | cholecystokinin |
| cDNA | complementary deoxyribonucleic acid |
| CNS | central nervous system |
| DEXA | dual energy x-ray analysis |
| DMH | dorsomedial hypothalamus |
| DNA | deoxyribonucleic acid |
| ELISA | enzyme-linked immunosorbent assay |
| GH | growth hormone |
| GHS-R | growth hormone secretagogue receptor |
| GLP-1 | glucagon-like peptide 1 |
| Gx | gastrectomy |
| HF | high fat |
| HFS | high fat sucrose |
| LF | low fat |
| LFS | low fat sucrose |
| LH | lateral hypothalamus |
| mRNA | messenger ribonucleic acid |

| | |
|---|---|
| MSH | melanocyte-stimulating hormone |
| NAc | nucleus accumbens |
| NeuN | neuronal specific nuclear protein |
| NMRI | naval marine research institute |
| NPY | neuropeptide Y |
| NTS | nucleus of the solitary tract |
| POMC | proopiomelanocortin |
| PPAR-γ | peroxisome proliferating activated receptor- γ |
| PVN | paraventricular nucleus |
| PYY | peptide YY |
| RIA | radioimmunoassay |
| RNA | ribonucleic acid |
| RYGB | Roux-en-Y gastric bypass |
| SCN | suprachiasmatic nucleus |
| SD | Sprague-Dawley |
| Socs3 | suppressor of cytokine signaling 3 |
| SON | supraoptic nucleus |
| SNS | sympathetic nervous system |
| THC | $\Delta^9$-tetrahydrocannabinol |
| UCP1 | uncoupling protein 1 |
| WAT | white adipose tissue |
| VMH | ventromedial hypothalamus |

# INTRODUCTION

## Obesity

Obesity is a multifactoral disorder influenced by genetic, behavioral, environmental and cultural factors. The prevalence of overweight and obesity is increasing rapidly in both the Western world and the developing world [1] and is now regarded as a global epidemic, affecting approximately one billion people worldwide. Since overweight and obesity are major risk factors for the development of type 2 diabetes and other pathological states, such as hypertension, dyslipidemia and atherosclerosis [2], the rapid increase in overweight and obesity is a major global health concern. Data indicate that almost 66% of the US population are overweight (BMI>25 kg/$m^2$) [3], half of which are obese (BMI>30 kg/$m^2$). In the US, more than 10% of all 2-through 5-year olds, and more than 15% of all 6-through 19-year olds are obese [4]. The increase in obesity is not exclusive to the US. Although numbers are lower in Europe, the trend is towards an earlier onset of obesity and increase in obesity in the European population. For instance, approximately 35% of the Swedish population between ages 16 and 84 were overweight or obese as of 2001. However, the increase in juvenile and adolescent obesity cannot be ascribed to genetic predisposition alone, as the increase has been far too rapid [5]. It is more likely a consequence of altered food habits (increased consumption of energy-dense diets) and lack of exercise. Numerous clinical [6, 7] and experimental studies [8, 9] have demonstrated that both *in utero* (i.e. maternal nutrition and gestational diabetes) and early postnatal (i.e. diet and energy availability) environment can influence body weight and energy homeostasis.

Using laboratory animal models it has been found that sugar and fat are powerful factors inducing reward aspects of feeding [10, 11]. Foods that are energy-dense generate a deeper sense of reward than foods that are less energy-dense [12-14]. Not surprisingly, many studies have shown a positive correlation between the amount of ingested fat and obesity [15, 16]. Diet, reduced physical activity and increased compostion of caloric-dense foods can, however, not be held responsible for all cases of obesity. Five single gene disorders resulting in obesity have been identified, suggesting the presence of a genetic component. These genes are those encoding leptin

[17, 18], the leptin receptor [19], pro-opiomelanocortin [20], prohormone convertase 1 [21] and melanocortin receptor 4 [22, 23]. However, over 600 genes, markers and chromosomal regions have been associated with or linked to obesity phenotypes [24]. Obesity is thus a multifactoral disease including factors as diverse as environment, food habits, physical exercise and genetic predisposition.

## Treatments of obesity

### *Pharmacotherapy*

All individuals should initiate weight loss treatment with diet modification, exercise and behavioral therapy. If these life style changes do not result in a 10% loss of initial weight or 0.5 kg/week over a period of six months, pharmacotherapy may be considered. Pharmacotherapy should be restricted to individuals with a BMI > 30 $kg/m^2$ or BMI > 27 $kg/m^2$ if the patient also displays hypertension, dyslipidemia, atherosclerosis (e.g. coronary artery disease), type 2 diabetes or sleep apnea. The pharmacotherapy should result in a weight loss of 2 kg in the first four weeks otherwise the dose of the drug should be adjusted or a different medication should be considered. On the Swedish market, there are currently two drugs available: Xenical® and Reductil®, and one about to be released, Acomplia®.

#### *Xenical®*

Orlistat is the active substance in Xenical®. It is an inhibitor of pancreatic lipase, an enzyme required for hydrolysis of dietary lipids in the intestine. Orlistat thus decreases the uptake of dietary fat. Weight loss in individuals undergoing orlistat treatment is greater than in individuals eating a fat-restricted diet alone. Side-effects of Xenical® treatment include loose stools and increased defecation; side-effects that may affect compliance.

*Reductil®*

Sibutramine is the active substance in Reductil®. It increases the feeling of satiation by blocking the presynaptic reuptake of noradrenaline and serotonin thus potentiating the anorectic effects of these neurotransmitters in the central nervous system (CNS). Side-effects of Reductil® treatment include increased systolic and diastolic blood pressure, increased heart rate, headache, insomnia and constipation.

*Acomplia®*

The first cannabinoid receptor antagonist was characterized in 1994. This substance was named rimonabant (in early reports referred to as SR141716) and is the active substance in Acomplia®. Rimonabant binds selectively to the endocannabionid receptor 1 (CB1). The suggested effect of rimonabant, to block signaling through the interaction with CB1, may result in decreased motivation to eat palatable food and thus has an anorectic effect. Acomplia® is set to be released to the Swedish market in the beginning of 2007 (according to information as of October 2006). Side effects to Acomplia® treatment include symptoms of depression, mood swings, anxiety and memory loss.

***Bariatric surgery***

Weight loss induced by pharmacotherapy is, however, usually short-lived and weight regain is common. Hence, bariatric surgery is an alternative for obese patients, and morbidly obese patients in particular. Gastric banding and gastric bypass are the two most common procedures, where gastric banding is the least invasive of the two. This procedure involves a silicon elastomer, which can be adjusted by injecting or removing saline through a portal under the skin, being placed around the proximal stomach of the patient, thus creating a small pouch (15-20 ml) that restricts the amount of food being ingested. The Roux-en-Y gastric bypass (RYGB) is considered the golden standard of obesity surgery. RYGB divides the stomach into two compartments by the use of surgical staples. The upper compartment is very small (28 to 56 ml), restricting food intake and is joined end-to-end with the duodenum. The lower compartment of the stomach is thus bypassed but remains functional.

**Appetite peptides**

The regulation of appetite and feeding is a very complex process, involving the participation of many agents, produced peripherally as well as centrally. I will here give a brief introduction to some of these agents, both peripheral and central ones. I acknowledge that there are many other agents and factors involved in the regulation of appetite and feeding than the ones presented below. By reviewing the literature I have chosen these agents since it is my opinion that they are among the most thoroughly studied and most established ones involved in the regulation of appetite and feeding. The order of which the chosen agents appear in this introduction is an alphabetical one and indicates no ranking between them.

***Orexigenic peptides***

*Endocannabinoids*

The natural compound $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), derived from *Cannabis sativa*, has long been known to stimulate appetite [25]. It is therefore used as an appetite enhancer for patients with AIDS and cancer [26]. $\Delta^9$-THC binds to two different cannabinoid receptors, CB1 [27, 28] and CB2 [29]. CB1 is widely expressed throughout the CNS, in sites including the hippocampus and the hypothalamus [30], and also in peripheral tissues [31-33]. CB2, on the other hand, is expressed in immune cells primarily [34]. Endogenous cannabinoids (endocannabinoids) were first discovered in 1992 when anandamide was described [28]. Three years later, in 1995, 2-arachidonoyl glycerol (2-AG) was described [35]. These two main endocannabinoids are both derived from arachidonic acid (anandamide is its amide and 2-AG is its ester). Similar to $\Delta^9$-THC, endocannabinoids were found to stimulate appetite [26, 36-38]. As the hyperphagia induced by endocannabinoids can be blocked by CB1 antagonists but not by CB2 antagonists it seems likely that the appetite-promoting effect is promoted through the CB1 receptor. Blocking the CB1 receptor results in decreased intake of palatable food, especially sweet food and drink [39, 40]. This correlates well to the observation that cannabis-induced hyperphagia in humans increases consumption of sweet foods, such as biscuits and chocolate.

Endocannabinoids are released from neurons in response to membrane depolarization.

They are subject to a very rapid enzymatic inactivation [41, 42] and are therefore only produced “on demand”. One such state of “demand” is food deprivation (fasting) which results in increased levels of endocannabinoids [43, 44]. Conversely, a single intravenous injection of the satiety peptide leptin leads to a suppressed endocannabinoid release from the nucleus accumbens (NAc) (and to a lesser extent from the hypothalamus) [44, 45]. There is an association between overweight and obesity and a missense polymorphism of fatty acid amide hydrolase [46], an enzyme involved in the degradation of endocannabinoids. Malfunctioning fatty acid amide hydrolase may result in increased endocannabinoid levels and prolonged duration of endocannabinoid secretion, leading to stimulated appetite, weight increase and subsequently obesity.

*Galanin*

Galanin is a 29-amino acid peptide originally isolated from the small intestine [47]. It is thought to be involved in biological processes, such as motility and secretion, in the gastrointestinal tract. Galanin is also widely expressed throughout the hypothalamus. To date, there are three known galanin receptor subtypes (GalR1, R2 and R3) [48-50]. While GalR1 and GalR2 are highly expressed in the hypothalamus, hippocampus, thalamus, brainstem and spinal cord [51-53], GalR3 is less abundantly expressed within the CNS [50, 54]. Intrahypothalamic injections of galanin [55, 56] stimulate feeding in satiated rats. However, galanin is without effect when administered intraperitoneally [57, 58]. The major site of galanin action on food intake is the paraventricular nucleus (PVN) [55, 56]. Studies with macronutrient choice tests suggest that injections of galanin into PVN stimulate fat intake [59]. Animals fed a high-fat diet have a stronger and more prolonged response to galanin than animals fed standard chow [59, 60]. Galanin mRNA expression and galanin production in the PVN are positively related to the amount of fat ingested [60, 61]. Rats preferring fat have higher levels of galanin mRNA and higher rate of galanin production in the PVN [57]. Hypothalamic galanin mRNA expression is increased in rats with dietary [62, 63] or genetic obesity [61, 64]. Neither food deprivation [57, 65, 66] nor lifelong caloric restriction [67] have been reported to affect galanin mRNA.

*Ghrelin*

Ghrelin is a 28 amino acid peptide cleaved from the precursor preproghrelin and has serine in position 3 acylated by *n*-octanoic acid [68]. The acylation allows ghrelin to bind to the growth hormone (GH) secretagogue receptor (GHS-R) type 1a [69]. Acylation of ghrelin increases hydrophobicity and seems to be a requirement for the ability of ghrelin to pass the blood-brain-barrier (BBB) [70, 71]. Des-acyl ghrelin and des-$Gln^{14}$ ghrelin are two other forms of ghrelin which have been identified and studied.

Although the initial report on ghrelin [68] indicated that the stomach is the major source of ghrelin, its cellular storage site was not presented. Reports later revealed that the X/A-like cells were the cellular source of ghrelin in the rat stomach [72, 73] and the $P/D_1$-cells in humans [74]. Apart from the stomach, where most ghrelin is produced (approximately 80% of circulating ghrelin is lost upon gastrectomy), it has been found in other tissues including the intestine [73] and the hypothalamus [68]. Other than the first reported function of ghrelin, i.e. stimulation of GH secretion from the pituitary gland, a number of other functions have been described. In this thesis, I will focus mainly on ghrelin´s effects related to food intake, weight gain and adiposity.

Serum ghrelin levels are known to be elevated by fasting and decreased upon ingestion of food [73, 75-77]; the postprandial decrease seems to proportionally reflect the calorie load ingested [78]. Fasting levels of ghrelin are lower in obese individuals than in lean individuals [79] and elevated in patients with anorexia nervosa [77, 80, 81] and Prader-Willi syndrome [82].

Regardless of whether ghrelin is adminstered centrally or peripherally, mice and rats respond with increased food intake and adiposity [75, 83-85]. Ghrelin has orexigenic effects also in GH-deficient dwarf rats, suggesting the effect of ghrelin on food intake and adiposity to be GH-independent [75, 86]. The role of ghrelin as a potent stimulator of food intake has been challenged by studies using knockout animals. Sun *et.al.* hypothesized that genetic deletion of ghrelin would generate anorectic dwarfs. However, these mice did not show any alterations in either food intake or growth rate [87]. Wortley *et.al.* were also unable to observe any effects of genetic deletion of ghrelin ($ghrl^{-/-}$) on food intake or weight gain [88]. However, when *$ghrl^{-/-}$* mice were

fed a high fat diet a decrease in respiratory quotient was observed compared to wild-type littermates, indicating fat to be utilized to a greater extent as fuel. Accordingly, *ghrl* $^{-/-}$ mice had less body fat [88]. In another study in *ghrl* $^{-/-}$ mice, Wortley *et.al.* found ghrelin to be involved in the protection against diet-induced obesity at an early age [89]. Also, in GHS-R null mice, ghrelin administration failed to acutely stimulate food intake or to activate arcuate nucleus (ARC) neurons [90], suggesting ghrelin to participate in the regulation of food intake through GHS-R. Recently, a second product of the preproghrelin gene, obestatin, was reported [91].

*Hypocretins/orexins*

The orexins [92], also called hypocretins [93], are neuropeptides which were described independently by two separate research groups in 1998 [92, 93]. Two orexin molecules, orexin-A (33 amino acids) and orexin-B (28 amino acids) exist and are expressed mainly in the hypothalamus, especially in the perifornical area (PFA), the lateral hypothalamus (LH) and the dorsomedial hypothalamus (DMH) [92-96], but also in the gut [97]. These peptides originate from the same precursor molecule, prepro-orexin. The mRNA expression of prepro-orexin has been reported to be up-regulated by food deprivation [92]. Orexin-A, in particular, has been reported to stimulate food intake in rats when administered centrally [98, 99]. There are two receptor subtypes; $OX_1R$ and $OX_2R$, where $OX_1R$ is more selective for orexin-A than $OX_2R$ which has equal affinity for orexin-A and orexin-B [92].

*Neuropeptide Y (NPY)*

NPY was first isolated in the early 1980s [100, 101] and was found to be a 36 amino acid peptide belonging to the pancreatic polypeptide (PP) family as it has a hairpin tertiary structure called the PP-fold [102]. NPY is one of the most widely distributed neurotransmitters in the mammalian brain [103, 104]. The most abundant NPY innervation, reaching almost the entire hypothalamus, originates from the arcuate nucleus (ARC) [105, 106]. NPY is a potent stimulator of feeding, especially carbohydrate intake. It has the ability to reduce energy expenditure and induce obesity

[107-109]. Rats given NPY for ten days show increased body weight; the increased weight being a result of increased adipose tissue [110].
Several NPY receptor subtypes have been identified: NPY Y1, Y2, Y4 and Y5 receptor [111]. Y1 and Y5 are thought to be involved in the regulation of food intake and energy homeostasis. The use of NPY antibodies [112, 113] or antisense oligonucleotides [114, 115] reduces food intake, supporting the role of NPY as a stimulator of feeding.

***Anorexigenic peptides***

*Cocaine- and amphetamine regulated transcript (CART)*

CART was first described when screening brain regions for mRNAs responsive to acute administration of cocaine or amphetamine [116]. CART was subsequently found both in brain (including the hypothalamus and pituitary) [116-118] and peripheral tissues [119]. Chronic intracerebroventricular administration of CART leads to decreased food intake and body weight [120], while treatment with CART antibodies reverses the CART-induced hypophagia [121, 122]. Also, injection of CART into the fourth ventricle results in decreased intake of sucrose solution [123]. Furthermore, central administration of CART to both normal rats and rats with NPY-induced hyperphagia results in decreased food intake [121, 122]. A receptor for CART has not yet been identified but it is thought that CART may mediate its effects via a G-protein coupled receptor [124].

*Cholecystokinin (CCK)*

CCK was discovered in 1971 when Mutt and Jorpes isolated this 33-amino acid hormone from the duodenum [125]. CCK is found in multple, biologically active forms; CCK-58, CCK-33 and CCK-8 [126]. CCK occurs in both the gastrointestinal tract [127], particularly abundant in the duodenum and jejunum, and in the CNS where sites of expression include the hypothalamus and the nucleus of the solitary tract (NTS) [128, 129]. CCK inhibits food intake in rodents and humans by reducing meal size and meal duration [130-132]. It appears to be particularly effective in this respect when given intraperitoneally (compared to subcutaneous, intravenous or central

administration) [133]. Because CCK is a powerful inhibitor of gastric emptying [132, 134] part of its anorectic effect may be caused by retention of food in the stomach. Surprisingly, long-term peripheral administration of CCK did not result in decreased overall energy intake or weight loss, granted that the rats did decrease their meal sizes but on the other hand increased their number of meals [135]. Hence indicating that CCK acts as a short-term satiety signal. CCK acts via two receptors; CCK-A and CCK-B. It is suggested that CCK exerts its anorectic effect through CCK-A receptors, as CCK-A receptor deficient rats [136] and the use of CCK-A receptor antagonists [137] abolishes the effect of CCK. It also exerts its effect via the vagus nerve; the effect of CCK on food intake is lost after vagotomy [138]. In the rat, CCK-A receptors are found in the pancreas, on vagal afferent and enteric neurons, and in the brain (including the NTS, area postrema and DMH).

*Enterostatin*

Enterostatin is a 5 amino acid peptide, which is produced through tryptic cleavage of the precursor molecule procolipase in the intestine [139, 140]. Apart from the pancreas [140, 141], immunoreactivity for enterostatin has been reported in the stomach [142], duodenal mucosa [143] and specific brain regions [144]. The proline residues at positions 2 and 4 seem to be of importance since they are conserved between species. Central [145, 146], peripheral [147] and intragastric [148] administration of enterostatin results in decreased intake of fat. Both central and peripheral responses to enterostatin have been observed. The central response is thought to be mediated through opioid [149] and serotoninergic [150, 151] pathways, whereas the peripheral response is thought to be mediated through the vagus nerve [152]. The β-subunit of $F_1F_o$-ATPase, which has been reported to be expressed in the plasma membrane of a number of cell types [153-155], has been found to be a target molecule for enterostatin [156] and has thus been suggested to be the enterostatin receptor [156, 157].

*Glucagon-like peptide 1 (GLP-1)*

GLP-1 is produced by processing of preproglucagon and is secreted from L-cells in the intestine, where it is colocalised with PYY [158]. GLP-1 is also produced in the brain

[159, 160]. GLP-1 secretion is increased in response to a meal in proportion to the caloric content [161]. The active form of GLP-1 is GLP-1(7-36). GLP-1(7-36) is a powerful incretin, with the ability to stimulate insulin biosynthesis and secretion [162, 163]. GLP-1(7-36) binds to the GLP-1 receptor which is present not only in the periphery [164, 165] but also in the NTS and the hypothalamus [166, 167]. Chronic intracerebroventricular [168, 169] or peripheral administration [170, 171] of GLP-1 to rodents results in decreased food intake and weight loss. GLP-1(7-36) given subcutaneously to obese individuals prior to every meal results in a decrease in energy intake and decreased body weight [172, 173].

*Insulin*

Insulin is produced in the pancreas by the β-cells of the islets of Langerhans. Apart from its very well established role in peripheral glucose metabolism, insulin has been found to affect food intake and body weight. When given centrally, insulin or insulin mimetics decreases food intake and body weight [174-177]. Conversely, administration of antibodies against insulin into the ventromedial hypothalamus (VMH) results in increased food intake and body weight [178, 179]. Insulin is transported across the BBB [180] and binds to its receptors, insulin receptor subtype A and subtype B, which are widely expressed throughout the brain, including the ARC [181]. By studying mice with either insulin receptor substrate-1 (IRS-1) or insulin receptor substrate-2 (IRS-2) deficiency, it was concluded that the anorectic effect of insulin is mediated through IRS-2 [182].

*Leptin*

Leptin, a product from the *ob* gene, was described in 1994 and was identified as a 16 kDa protein produced primarily in white adipose tissue (WAT) [183]. Administration of leptin results in decreased body weight; the weight loss reflecting the loss of adipose tissue [184, 185]. Mice with a mutation in the *ob* gene (the *ob/ob* mice) display hyperphagia and obesity [183], which can be reduced by leptin adminstration [184-186]. Rats infused with a recombinant adenovirus containing rat leptin cDNA,

resulting in hyperleptinemia, showed a 30-50% reduction in food intake and gained only 16-19% of the weight gained by the control rats [187].

The leptin receptor was found to be a member of the cytokine family of receptors [188]. There are five known splice variants of this receptor; Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Re [188, 189]. Ob-Rb is the active isoform of the leptin receptor and mutations in this receptor (the *db/db* mice) result in a phenotype similar to that of the *ob/ob* mouse [189]. Ob-Rb has been found in several hypothalamic nuclei involved in the regulation of body weight [190, 191].

Apart from WAT, leptin has been identified in the stomach [192], pituitary gland [193, 194], hypothalamus [195], muscle [196] and placenta [197]. The function of this extra-adipose tissue leptin remains unclear.

Plasma levels of leptin are highly correlated with fat mass and decrease in both humans and mice after weight loss [198]. In fact, most models of rodent and human obesity are associated with hyperleptinemia [199, 200]. These observations led investigators to suggest that obese individuals develop a resistence towards the effect of leptin (leptin resistence). The rate at which exogenously administered leptin crosses the BBB is reduced in obese animals [201, 202]. Hypertriglyceridemia, a state often associated with obesity, has been reported to inhibit leptin transport across the BBB [203]. This can explain why peripherally administered leptin fails to affect body weight and food intake, while leptin administered directly into the brain produces an anorectic response [204, 205]. Also, suppressor of cytokine signaling-3 (Socs3), a leptin-induced suppressor of leptin [206-208], is involved in leptin resistence. $Socs3^{+/-}$-mice [209] and Socs3-deficient mice [210] have been found to display greater sensitivity to leptin than wild-type mice and are thus protected against the development of diet-induced obesity. Recently, it was suggested that C-reactive protein (CRP), involved in inflammation, may be involved in leptin resistence by binding to leptin [211].

*Melanocortins*

The melanocortin peptides (α-, β and γ-melanocyte-stimulating hormone) are derived from the precursor molecule proopiomelanocortin (POMC) by prohormone

convertases. α-melanocyte-stimulating hormone (α-MSH) is a 13 amino acid peptide which is mainly expressed in the LH. Administration of α-MSH results in decreased food intake in both rats and mice [212, 213]. Also, administration of α-MSH to obese POMC-deficient mice [214] and diet-induced obese mice [215] decreases body weight. Whether β- or γ-MSH has any effect on feeding and appetite is still under debate [216-218]. Among the five known receptors for the MSHs, MC3R and MC4R are commonly referred to as the central receptors. MC3R is expressed in several hypothalamic nuclei including the VMH, ARC and LH [219]. MC4R, on the other hand, is more widely expressed (e.g. the hypothalamus, the hippocampus and the brain stem) [220, 221]. In the hypothalamus, MC4R is highly expressed in the DMH and the PVN [220, 221]. Intracerebroventricular injections of MC3/4R-antagonists increase food intake in both rats and mice [222-224]. The melanocortin system also includes two endogenous antagonists, agouti [225] and agouti-related protein (AgRP) [226, 227], both being stimulators of feeding.

*Peptide YY (PYY)*

PYY is a 36 amino acid gastrointestinal hormone, belonging to the PP family, first isolated from porcine small intestine in 1980 [228]. Most PYY is produced in the L-cells of the ileum, colon and rectum [229]. PYY has also been found in the central and the peripheral nervous system [230]. Two major forms of PYY exist; $PYY_{1-36}$ and $PYY_{3-36}$. The latter is generated through the action of the enzyme dipeptidyl peptidase IV [231]. $PYY_{1-36}$ activates at least three different receptors (Y1, Y2 and Y5), while $PYY_{3-36}$ activates the Y2 receptor specifically [232]. PYY levels rise in response to a meal [229, 233] in proportion to the caloric content of the meal ingested [234]. Intraperitoneal administration of $PYY_{3-36}$ to rodents and humans has been reported to decrease food intake and body weight [235-237]. These effects have been proposed to be mediated by POMC activation. However, mice lacking POMC were found to respond normally to $PYY_{3-36}$, i.e. with a reduced food intake [238]. Reports have suggested that the effect of $PYY_{3-36}$ on food intake reflects conditioned taste aversion [239]. Indeed, one of the reported side effects of $PYY_{3-36}$ in human subjects is nausea [236]. It should be noted that the anorexigenic effects of PYY is widely debated and

questioned. Numerous laboratories have have failed to observe a decrease in food intake and body weight upon PYY treatment (See Ref. [240]).

*Serotonin (5-Hydroxytryptamine, 5-HT)*

Serotonin is a monoamine neurotransmitter synthesized from tryptophan and has been found to decrease food intake and lower body weight when administered either centrally or peripherally [241]. Serotonin exerts its effect on food intake by reducing the duration and number of meals [242, 243]. Serotonin is released from the hypothalamus during food intake [244]. Several receptor subtypes have been identified of which $5HT_{1a}$, $5\text{-}HT_{1b}$, $5\text{-}HT_{2c}$ and $5\text{-}HT_3$ have been implicated in the regulation of food intake [245, 246]. Mice with a targeted disruption of the $5\text{-}HT_{2c}$ receptor are hyperphagic and consume larger meals than wild-type mice which results in obesity [247]. The anorectic behaviour of the *anx* mouse is to some extent attributed to an overactive serotonin innervation in the hypothalamus [248]. There are studies suggesting that serotonin suppresses carbohydrate intake [249, 250], while other reports suggest it to reduce fat intake [245, 251, 252].

**Regulation of food intake**

A role of the hypothalamus in the regulation of food intake was suggested over 50 years ago by the use of hypothalamic lesions and electrical stimulation of hypothalamic nuclei [253, 254]. It was suggested that the hypothalamus contains specific and discrete distinguishable centres with an impact on feeding behaviour; VMH being the satiety center and LH being the feeding center (**Figure 1**). Today it is assumed that the regulation of food intake in the hypothalamus involves neural circuits rather than specific hypothalamic nuclei. The ARC has been ascribed a particularly important role in the integration of signals regulating food intake as it is located in a part of the brain which lies outside of the BBB [255] and is in a position to act as a sensor for peripheral signals (**Figure 1**). The ARC contains two populations of neurons involved in food intake; the POMC/CART neurons (suppressors of food intake) [121, 256] and the NPY/AgRP neurons (stimulators of food intake) [257]. NPY/AgRP neurons regulate the activity of the POMC/CART neurons via two mechanisms: Firstly, AgRP blocks the release of POMC, explaining why intracerebroventricular injection of AgRP causes an increase in food intake [258]. Secondly, POMC neurons are innervated by NPY terminals [259] and express the Y1 receptor [260], through which NPY can inhibit the firing of the POMC neurons [261]. No reciprocal innervation has been found [261]. However, $PYY_{3-36}$, which is a selective agonist of the inhibitory Y2 autoreceptor [232], can exert an inhibitory control over NPY/AgRP neurons.

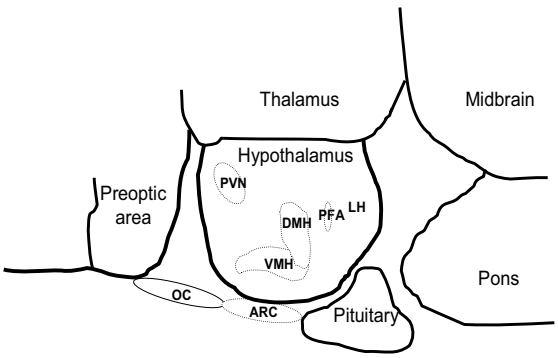


**Figure 1.** Cartoon showing the localisation of the different hypothalamic nuclei and position of the hypothalamus in relation to other brain regions. ARC (arcuate nucleus), DMH (dorsomedial hypothalamus), LH (lateral hypothalamus), OC (optic chiasm), PFA (perifornical area), PVN (paraventricular nucleus), VMH (ventromedial hypothalamus).

The lack of BBB makes the ARC an ideal site for the convergence of peripheral signals. A number of receptors for both anorexigenic and orexigenic peptides have been found in the ARC (e.g. Ob-Rb, the insulin receptor, MC3/4R, GHS-R1a and GLP-1 receptor). Ob-Rb mRNA is expressed on both NPY/AgRP neurons [262] and POMC/CART neurons [263] thereby allowing leptin to exert its effect on these neurons. Leptin acts to inhibit NPY/AgRP neurons and stimulate POMC/CART neurons, thus food intake is stimulated in the fasted state when circulating leptin is low and the orexigenic NPY/AgRP neurons can be activated [256, 264, 265]. Conversely, high levels of leptin activate the anorexigenic POMC/CART neurons [121, 266-268]. Insulin receptors are expressed in ARC, PVN and DMH where insulin acts to inhibit feeding. Also the ghrelin receptor, GHS-R1a, is expressed in ARC and PVN. Accordingly, administration of ghrelin has been found to activate neurons in the ARC and PVN [269] and to increase NPY expression in the hypothalamus [270]. Due to the

ability of GLP-1 to stimulate insulin secretion, the anorectic properties of GLP-1 may be mediated through increased levels of insulin reaching the ARC. Peripheral CCK may cross the BBB [271] and act on the DMH to suppress NPY levels [272].
The PVN and the DMH integrate signals (such as NPY and AgRP) in that these nuclei receive projections from numerous other brain sites such as the ARC and the brain stem. Lesions in the VMH and DMH produce hyperphagia and obesity. In contrast, lesions in the LH produce severe hypophagia/aphagia and weight loss which will subsequently lead to death if force feeding and hydration is not performed.

Reciprocal connections exist between the hypothalamus and the brainstem, particularly the NTS (**Figure 2**). The NTS is situated in close proximity to the area postrema, an area which has an incomplete BBB [273]. Like the ARC, the NTS is therefore ideally located for responding to peripheral circulating signals. In addition, the NTS also receives vagal afferents from the gastrointestinal tract [274, 275] (**Figure 2**). Satiety signals that reach the NTS are initiated by mechanical or chemical stimulation of the stomach and the small intestine during food ingestion. It has been demonstrated that the NTS contains NPY-binding sites [276], and there is also evidence that a melanocortin system exists, as POMC-derived peptides are synthesized in this nuclei [277-279].

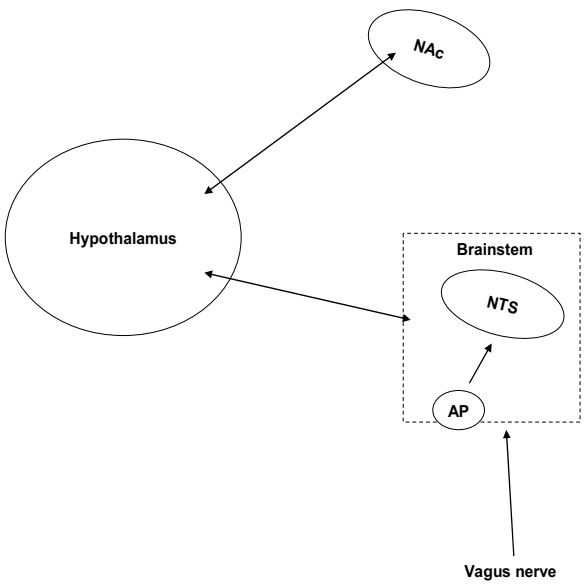


**Figure 2.** Schematic picture illustrating the communication between different brain regions involved in the regulation of food intake. AP (area postrema), NAc (nucleus accumbens), NTS (nucleus of the solitary tract).

Clearly, peripheral signals are involved in the central regulation of food intake. Such signals may also affect food intake peripherally, by for instance, affecting gastric emptying. Orexigenic peptides may increase the rate of gastric emptying [280, 281], thus signaling hunger to the CNS whereby feeding is initiated. Also, orexigenic peptides may decrease the activity of the sympathetic nervous system causing a decrease in thermogenesis [282-284]. Conversely, anorexigenic peptides may decrease the rate of gastric emptying [285, 286] and increase sympathetic nerve activity [287]. Nutrients also participate in regulating food intake. Hypoglycemia is a state associated with hunger and increased food intake [288]. Certain hypothalamic nuclei contain glucose-sensitive neurons [289, 290] which have been suggested to contain orexin [291]. Amino acids, such as tryptophan [292] and phenylalanine [293] have been reported to suppress food intake. Increased levels of free fatty acids also suppress food

intake [294]. Free fatty acids have been found to decrease circulating ghrelin levels [295] and to increase serum leptin levels [296].

It is important to bear in mind that our choice of food is not simply a balance between energy demand and supply. Feeding behavior is also linked to reward. By adding an aspect of reward to feeding, in the form of food which appeals to smell, sight and taste, the consumption of spoiled food can be avoided (or promoted). Reward is associated with addiction and activation of dopamine neurons in the NAc [297]. The rewarding aspect of food is well illustrated in animals unable to produce dopamine which become aphagic and die unless force fed by gavage [298]. Also, opioids play an important role in the rewarding aspect of feeding. Mice deficient in either β-endorphin or enkephalin lose the reinforcing property of food, regardless of the palatability of the food [299]. Microinjections of opioids into the NAc stimulate the consumption of palatable diets [300, 301], while administration of opioid antagonists reduces sucrose consumption [300].

**Neurogenesis**

Neurogenesis is the process by which neuronal stem cells generate nerve cells. Neurogenesis occurs throughout life in the subgranular zone of the dentate gyrus of the hippocampus (**Figure 3**). Outside the hippocampus, neurogenesis has been reported in the olfactory bulb [302], the hypothalamus [303] and neocortex [304].

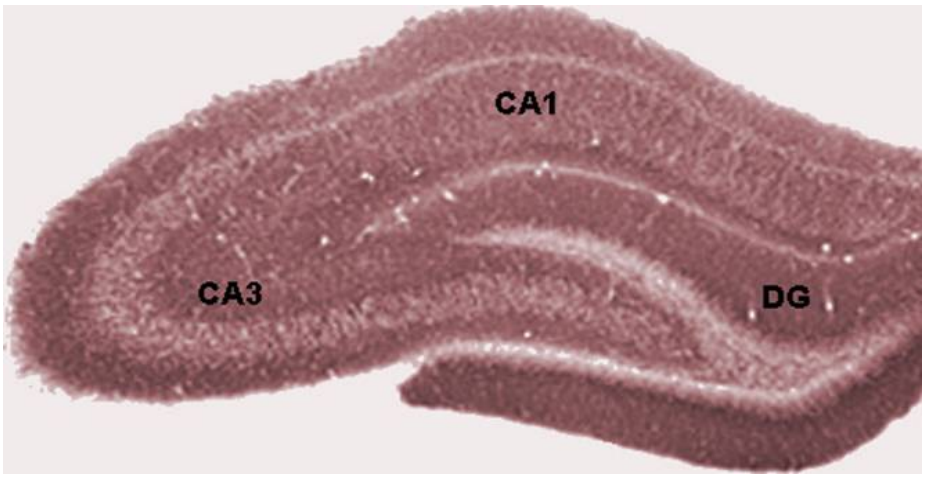


**Figure 3.** Photomicrograph showing a slice of the rat hippocampus. CA1 (Cornu Ammonis; Horn of Ammon), DG (dentate gyrus).

Neurogenesis is required for the hippocampus to maintain its plasticity and is under the control of both intrinsic and extrinsic factors (here I will focus on extrinsic factors). Extrinsic factors are factors originating peripherally and found in the circulation, for instance corticosterone. It was shown by Cameron *et.al.* in 1994 that administration of corticosterone to male rats reduced neurogenesis in the dentate gyrus of the hippocampus [305] and that hippocampal neurogenesis was accelerated following adrenalectomy (removal of the adrenal gland) [305]. Moreover, peripherally administered insulin-like growth factor I (IGF-I) [306], physical exercise [307, 308]

and an enriched environment [308, 309] stimulate hippocampal neurogenesis, whereas social isolation has been reported to delay the positive effect of running [310].

**Thermogenesis**

Energy derived from the diet must equal energy expenditure otherwise obesity will result. Generation of heat is an important aspect of energy expenditure and thermogenesis is the process by which a normal body temperature is maintained even under extreme conditions, such as cold exposure and restricted food intake. Thermogenesis can be classified as either obligatory or adaptive. Obligatory thermogenesis is achieved through the basal metabolic rate. Diet-induced thermogenesis is a form of adaptive thermogenesis and describes the marked increase in heat production which serves to reduce or prevent the development of obesity. In small rodents and also the human neonate, the main site of adaptive thermogenesis is brown adipose tissue (BAT) (in the adult human BAT is interspersed with WAT). This view is supported by the fact that mice with genetically ablated BAT become obese and hyperphagic [311]. The heat production in BAT is due to its unique ability to uncouple phosphorylation from ATP production, allowing energy to be dissipated as heat. The primary protein involved in diet-induced thermogenesis is uncoupling protein 1 (UCP1) [312], which is located exclusively to the inner mitochondrial membrane of BAT, accounting for 10% of the total membrane protein content [312]. It prevents the production of energy-rich ATP by providing an alternative pathway for proton re-entry into the mitochondria [313]. Consistently, UCP1 knockout mice have impaired cold resistence, thus supporting a role for UCP1 in thermogenesis [314]. UCP1 is under the control of the sympathetic nervous system (SNS) [315]. Factors involved in the stimulation of UCP1 activity, hence also thermogenesis, include noradrenaline and adrenaline [316], free fatty acids [313], the consumption of a high-fat diet [317], leptin [318] and triiodothyronine ($T_3$) [319] whereas fasting [320] and corticosterone [318] seem to suppress UCP1 activity. By binding to the β-adrenergic receptors located on the brown adipocytes, noradrenaline activates UCP1 activity through increased lipolysis and free fatty acids. Mice lacking the three known β-adrenergic receptors have been reported to have lower levels of UCP1 mRNA than

wild-type littermates [321] and fail to respond to cold exposure. Also, mice genetically deficient in dopamine β-hydroxylase, thus being unable to produce noradrenaline and adrenaline, have lower levels of UCP1 mRNA than their wild-type littermates [316].

## AIMS OF THE STUDY

To study the effects of diets rich in fat and/or sucrose on the secretion and mRNA expression of leptin and ghrelin.

To investigate the consequences of hypoghrelinemia (induced by gastrectomy) and hyperghrelinemia (administration of exogenous ghrelin) on food intake, body weight gain and adipose tissue.

To investigate the impact of a high-fat diet on the development of new neurons in the hippocampus.

To study the role of ghrelin in the regulation of thermogenesis in both intact and gastrectomised mice.

To study circulating levels of leptin, ghrelin, free fatty acids, triglycerides and cholesterol after ingestion of different sugar solutions.

## MATERIALS AND METHODS

*Animals*

All animal experiments were conducted in accordance with the guidelines presented by the Local Ethics Committee, Lund University, Sweden. Animals were from B&K (Sollentuna, Sweden). Upon arrival, they were provided with free access to standard chow and tap water and housed in rooms maintained at a temperature of 21±1°C with a 12 hour light-dark cycle (lights on at 0600). Female Sprague-Dawley (SD) rats were used in **Papers I** and **V**. In **Paper III**, both female and male SD-rats were used. In **Papers I** and **V**, the rats weighed 200-225 g at the start of the studies, whereas in **Paper III** they weighed approximately 140 g at the start of the study. Female Naval Marine Research Institue (NMRI) mice were used in **Papers I, II** and **IV**. In **Paper I**, the mice weighed approximately 25 g at the start of the study, whereas in **Paper II** and **IV** they weighed 30-33 g at the start of the study. The rats used in **Paper I** and **III** were housed individually in Macrolon cages, in **Paper III** and **V** the rats were housed three per cage. All mice were housed two-three per cage (**Paper I, II** and **IV**).

*Experimental diets and feeding regimes*

Four different diets were used in this thesis, standard pellet diet (LF; R36, Lactamin, Kimsta, Sweden), low-fat high sucrose (LFS), high-fat diet (HF) and high-fat high sucrose diet (HFS). All diets used, except LF, were custom-made in our laboratory. The diets were baked into cookies approximately 5 cm in diameter and 1 cm thick and dried for 48 h at 70°C. As the HF-diet has a tendency to crumble, all cages were carefully monitored for spillage on a daily basis. The composition of the diets are shown in **Table 1**. In **Paper I**, a 23% sucrose solution to drink was used in combination with either the LF- or the HF-diet and in **Paper V** the rats were offered either 23% sucrose, glucose or fructose solutions in combination with LF. All animals had free access to their respective diets and drinking solutions at all times, except in **Paper I** where both rats and mice were on a restricted feeding regime which allowed them access to the diets for an eight hour period (0800-1600).

**Table 1.** Composition and energy content of the various diets used (**Papers I and III**).

| Content | LF | LFS | HF | HFS |
|---|---|---|---|---|
| | g/100g | g/100g | g/100g | g/100g |
| **Protein** | 21 | 22.3 | 26 | 26 |
| **Total carbohydrate content** | **62** | **61.6** | **38** | **38** |
| *Starch* | 62 | 39.8 | 38 | 12.3 |
| *Sucrose* | 0 | 21.8 | 0 | 25.7 |
| **Total fat content** | **5** | **4.2** | **21** | **21** |
| *Corn oil* | * | 2.5 | 3 | 3 |
| *Coconut butter* | * | 1.7 | 18 | 18 |
| **Fibers** | 7 | 7.4 | 9.5 | 9.5 |
| **Vitamins** | 1.1 | 0.75 | 1 | 1 |
| **Salt** | 3.6 | 3.4 | 4 | 4 |
| **Minerals** | 0.3 | 0.4 | 0.5 | 0.5 |
| **Energy derived from:** | **kcal%** | **kcal%** | **kcal%** | **kcal%** |
| *Protein* | 28% | 24.4% | 23.9 | 23.9 |
| *Carbohydrate* | 57% | 65.6%** | 34 | 34*** |
| *Fat* | 15% | 10% | 42.1 | 42.1 |
| **kcal/g** | 3.2 | 3.9 | 4.7 | 4.7 |

* The fat in the standard diet consists of 70% lard (3.5g/5g) and 30% linoleic acid (1.5g/5g).

** The energy content constituted by carbohydrates in this diet is 42.4 kcal% starch and 23.2 kcal% sucrose.

*** The energy content constituted by carbohydrates in this diet is 11 kcal% starch and 23 kcal% sucrose.

*RNA extraction and Northern Blotting*

Tissues (fundus in **Paper I** and BAT in **Paper IV**) were dissected out and immediately frozen in liquid nitrogen and stored at -80°C until extraction of total RNA as described previously [322]. The quality and concentration of RNA was determined by measuring the absorbance at 260 and 280 nm, where $A_{260/280} > 1.7$ was considered sufficient purity. RNA was separated on 1.0 % agarose-formaldehyde gels and transferred to

nylon membranes (Zeta-Probe, Bio-Rad). Hybridization was carried out overnight at 65°C. The probes were labeled using [$\alpha$-$^{32}$P] deoxycytidine triphosphate (10μCi/μl; Amersham Pharmacia Biotech UK, Buckinghamshire, UK) according to a Nick translation kit (Roche Applied Science, Basel, Switzerland). After each hybridization the membranes were stripped in boiling 0.1x Standard Sodium Citrate (SSC) and 0.5% Sodium Dodecyl Sulphate (SDS) for 2x30 minutes. The levels were compared to that of an internal control, 18S, and expressed as arbitrary units (**Paper I**) or as percentage of the control group (**Paper IV**). The 18S probe was end-labelled with [$\gamma$-$^{32}$P] ATP (10μCi/μl) and hybridized overnight at 37°C. The membranes were analyzed with a phosphoimager (FLA 3000, FUJIFILM) and the software Image Reader (Fuji).

*Serum analysis*

Blood was collected as described in the papers included in this thesis. Serum was stored at -80°C until analysis. Serum ghrelin (**Papers I, II, IV and V**) was analysed by the use of a commercially available radioimmunoassay (RIA) from Phoenix Pharmaceuticals (Belmont, CA, USA). In **Paper II**, active (n-octanoylated) ghrelin and insulin-like growth factor I (IGF-I) was measured in serum using an enzyme-linked immunosorbent assay (ELISA) kit from Linco Research (St Charles, Missouri, USA) and a RIA from Mediagnost (Reutlingen, Germany), respectively. Serum leptin (**Papers I and V**) was analysed by a commercially available ELISA from Crystalchem (Downers Grove, IL, USA). Serum corticosterone (**Paper III**) was analysed using a commercially available RIA from MP Biochemicals (Orangeburg, NY, USA). A commercially available colorimetric kit for the detection of serum free fatty acids (NEFA C, Wako Chemicals, Richmond, VA, USA) was used in **Paper V**. Serum triglycerides were analysed by a GPO Trinder kit (Sigma, St Louis, MO, USA) and insulin was measured using a rat insulin ELISA from Crystalchem. Serum glucose was analysed using a kit from ThermoElectron (Melbourne, Australia).

*Analysis of body composition*

In **Paper I**, dual-energy x-ray analysis (DEXA; PIXImus from GE Healthcare, USA) was used for the assessment of the amount of total body fat in the mice. DEXA is mostly used for measurement of bone mineral density and content but can also be used to measure fat and lean tissue.

*Immunostaining for BrdU and NeuN*

In **Paper III**, we have determined the impact of dietary fat on hippocampal neurogenesis. In order to detect newborn neurons we injected the rats with 5′-bromo-2′-deoxyuridine (BrdU). BrdU is a thymidine analogue and can thus replace thymidine in newly synthesized DNA. After denaturation of DNA, BrdU is detected using an immunohistochemical approach. BrdU was injected intraperitoneally at a dose of 50 mg/kg, every two hours over a six hour period. This dose is based on optimal dose determination in embryonic mice [323]. Since BrdU is incorporated into the S-phase of the cell cycle, detecting BrdU alone would only give us a number of newborn cells (proliferation). Hence, markers for the identification of these newborn cells are needed. In **Paper III**, we double-stained for BrdU and neuronal specific nuclear protein (NeuN), a marker for mature neurons. A representative photomicrograph of such a staining is shown in **Figure 4**. In this way we could establish the identity of the BrdU-immunoreactive cells, i.e. cells found to be positive for both BrdU and NeuN were neurons.

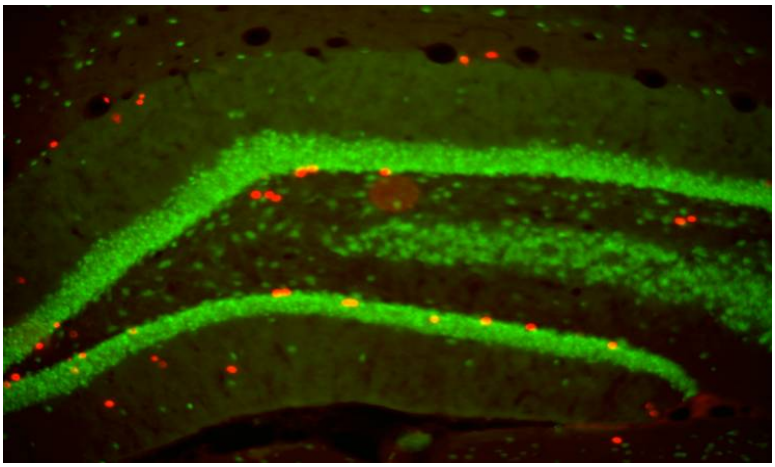

**Figure 4.** Representative photomicrograph of BrdU (red) and NeuN (green) staining of the dentate gyrus of the hippocampus.

*Statistical analysis*

Analysis of statistical significance was performed using Student´s unpaired t-test or analysis of variance (ANOVA). Results were expressed as mean±SEM and a $p<0.05$ was considered significant.

## RESULTS AND COMMENTS

*Paper I*

The aim of this paper was to investigate how a diet rich in fat and/or sucrose affects circulating levels of leptin and ghrelin. Rats were offered one of three different diets custom-made in our laboratory (see **Table 1** for composition of the diets), while controls received standard rat chow. Blood was collected from fasted (16 h) and refed rats (3 h) and serum leptin and ghrelin concentrations were measured. As was expected, control animals (LF) showed that leptin was decreased when the animals were fasted and increased postprandially (**Figure 5A**).

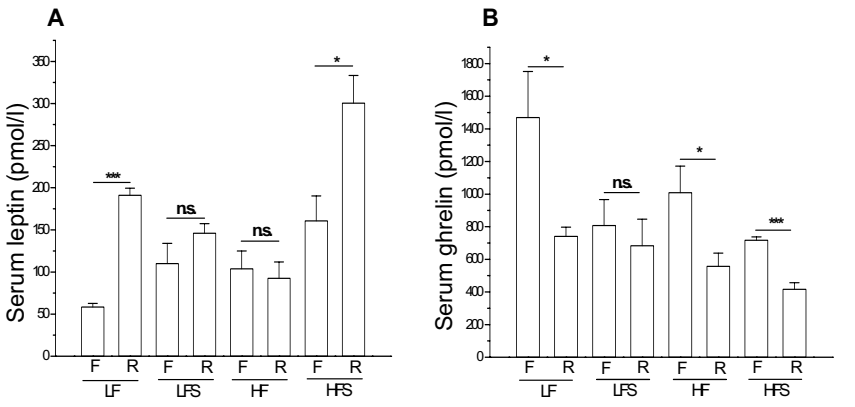


**Figure 5.** In rats fed standard rat chow (LF), leptin levels were elevated postprandially, whereas ghrelin levels were reduced postprandially. In rats fed a diet low in fat but high in sucrose (LFS) or high in fat (HF) the difference between pre- and postprandial serum leptin (A) was abolished. The LFS-diet decreased the fasting levels of ghrelin abolishing the difference between pre- and postprandial serum ghrelin. LF (standard rat chow); LFS (low fat sucrose diet); HF (high fat diet); HFS (high fat sucrose diet); F (fasting); R (refeeding).*, $p<0.05$; ***, $p<0.005$; n.s. (not significant)

Conversely, ghrelin was increased in response to fasting and decreased postprandially (**Figure 5B**). After five weeks of feeding a diet rich in sucrose (LFS) or fat (HF), the difference between fasting and postprandial serum leptin was abolished (**Figure 5A**). Leptin levels in rats receiving a diet rich in fat and sucrose (HFS) were elevated approximately two-fold, although still responding to a meal (**Figure 5A**).

The increase in circulating leptin (HFS) may reflect increased amount of fat mass or that the rats have entered a state of leptin resistance or it may be a signal to stop eating. The difference in fasting and postprandial levels of serum ghrelin was lost (LFS) or decreased (HF or HFS), mainly as a result of impaired ghrelin secretion in response to fasting (**Figure 5B**). Postprandial concentrations of circulating ghrelin in HF- and HFS-rats were reduced compared to LF-rats (**Figure 5B**). The changes in secretion pattern and circulating concentrations of leptin and ghrelin signal the rats to stop eating. Clearly, these signals are, however, not sufficient to stop the rats from consuming more calories and gaining more weight. The fact that the rats are hyperphagic indicate the presence of signals of greater power which promote feeding. Such signals may be reward signals, like dopamine or opioids.
In another experiment we investigated how sucrose in solution affected serum leptin and ghrelin. For this study we used female mice which were divided into four groups; the control group received standard chow and water, the second group standard chow and a sucrose solution, the third group high-fat diet and water and the fourth group high-fat diet and a sucrose solution. Serum leptin levels were elevated in the mice which had been offered the sucrose solution, regardless of diet (**Figure 6A**). Circulating levels of ghrelin were reduced in all groups compared to controls (LF) (**Figure 6B**). It seems that leptin secretion is affected by sucrose and that ghrelin secretion is affected by both fat and sucrose. The changes in leptin and ghrelin signal the mice to stop them from eating. The results in **Paper I** show that leptin and ghrelin concentrations respond to energy-dense diets by trying to prevent caloric overconsumption.

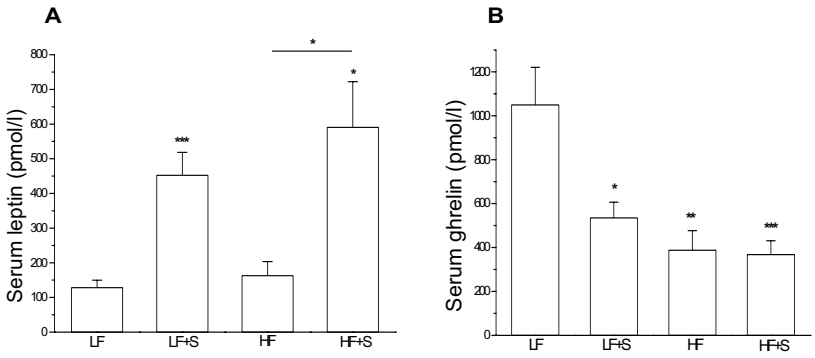


**Figure 6**. Mice offered sucrose to drink (LF+S and HF+S) had elevated fasting leptin (A). All groups (LF+S, HF and HF+S) had decreased fasting ghrelin levels (B) compared to standard chow fed mice offered water to drink (LF). LF (standard chow and water); LF+S (standard chow and sucrose); HF (high fat diet and water); HF+S (high fat diet and sucrose). *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$.

*Paper II*

Ghrelin-deficient mice are expected to be anorectic dwarfs. However, ghrelin-deficient mice exhibited normal feeding and growth [87, 88]. Although, in one study ghrelin-knockout mice were shown to be more resistent to diet-induced obesity than wild-type littermates [89]. The failure to demonstrate the anticipated phenotype may reflect the activation of compensatory systems. We therefore decided to study the effect of hypoghrelinemia using a different method. As most ghrelin is produced in the stomach, an alternative approach is to perform gastrectomy (surgical removal of the stomach). We gastrectomised mice (Gx mice) and studied the effect of hypoghrelinemia and of exogenous administration of ghrelin to Gx mice (and sham-operated mice) on daily food intake, weight gain and body fat mass. We found that Gx mice receiving saline injections had less adipose tissue than sham-operated mice receiving saline injections. Daily ghrelin administration to Gx mice normalised the amount of adipose tissue, elevating it to the level of sham-operated mice receiving

saline injections **(Figure 7)**. Previous studies have reported ghrelin to both suppress and induce adipogenesis [324-326]. The data presented in this study (**Paper II**) favour the view that ghrelin is an adipogenic agent. There was no effect of either hypoghrelinemia or hyperghrelinemia on daily food intake. The role of ghrelin as a meal initiator has been put forward previously by others [83, 84]. These studies do not report the food intake over a 24 h period, instead the intake over a period of 2 h, at the most, is reported. We did not measure the acute response by the animals to the ghrelin injections and cannot draw the conclusion that the data presented in our study (**Paper II**) are in conflict with the previously published work [83, 84]. The data presented in this paper (**Paper II**) suggest that ghrelin is adipogenic, stimulating the accumulation of adipose tissue both in intact and Gx mice via other mechanisms than increased food intake.

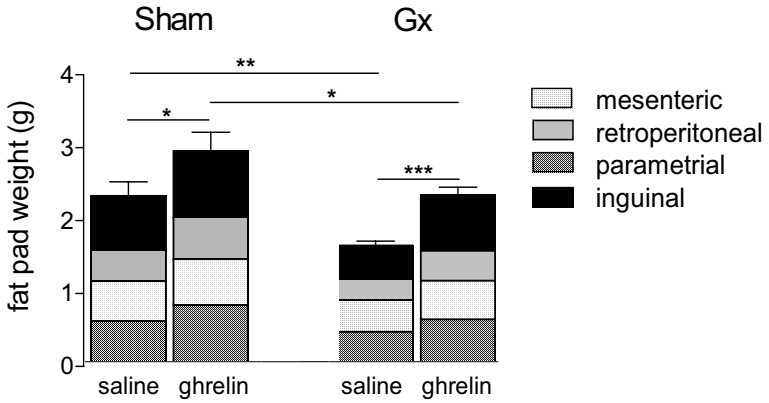


**Figure 7**. Ghrelin treatment for eight weeks to both intact sham-operated and Gx mice resulted in increased fat mass compared to mice receiving saline injections. Ghrelin-treated Gx mice had the same amount of fat mass as saline-treated sham-operated mice. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.005$.

*Paper III*

The consequences of a diet rich in fat on hippocampal neurogenesis is not well investigated. There are a few studies showing the impairment of memory formation and spacial memory in rats offered diets rich in fat and/or sucrose [327-329]. In **Paper III**, we fed rats of both gender either standard rat chow (LF) or high-fat diet (HF; see **Table 1** for diet composition) *ad libitum* for four weeks. After two weeks, the rats were injected with BrdU, a marker for dividing cells. At the end of the study, the rats were perfused transcardially with 4% paraformaldehyde and brain slices were analysed for BrdU- and NeuN-immunoreactivity, thus enabling the quantification of newborn neurons. We found no difference in body weight in male rats, while female rats fed HF weighed slightly, although significantly, more than female rats fed LF. We found male rats fed HF to have a reduced number of newborn neurons in the dentate gyrus of the hippocampus (**Figure 8**) (approximately 40% reduction), indicating an important effect of the diet on neurogenesis.

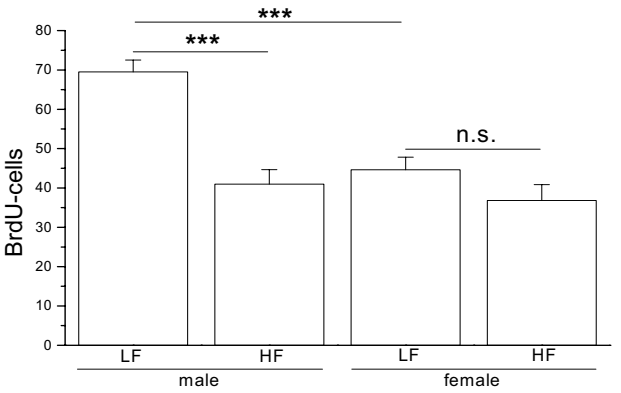


**Figure 8.** Male rats fed high-fat diet *ad libitum* for four weeks have approximately 40% less newborn neurons in the dentate gyrus of the hippocamus, indicating an impaired neurogenesis in these rats. ***, $p<0.005$; n.s. (not significant).

The reduction in number of BrdU/NeuN-immunoreactive cells in the dentate gyrus of the hippocampus was inversely related to circulating levels of corticosterone. Males fed LF had low circulating levels of corticosterone whereas those fed HF had increased levels of corticosterone. The reduction in neurogenesis was not observed in female rats. We found female rats to have elevated levels of serum corticosterone compared to male rats. Hence, the lack of effect of diet in female rats on neurogenesis may be explained by the elevated levels of corticosterone. In line with the findings in **Paper III**, diets rich in fat have been shown to increase levels of circulating corticosterone [330, 331], which in turn has been shown to decrease hippocampal neurogenesis [305, 332-334].

*Paper IV*

We found gastrectomy to lower thermogenesis considerably in mice, as indicated by decreased UCP1 mRNA expression. Daily ghrelin administration over a period of two weeks resulted in a decrease of UCP1 mRNA expression in BAT in Gx mice compared to Gx mice receiving saline (**Figure 9A**). As ghrelin has orexigenic properties it was surprising to find that eight weeks of daily ghrelin administration to Gx mice resulted in an up-regulation of UCP1 mRNA expression, reaching levels close to that of intact mice receiving ghrelin or saline (**Figure 9B**).

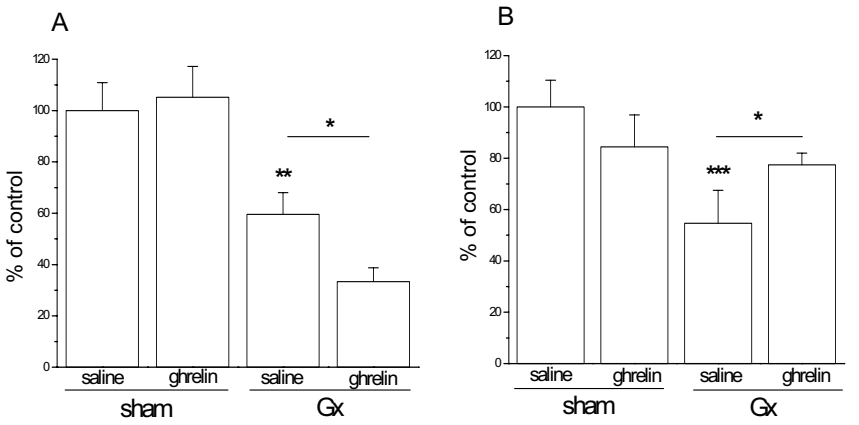


**Figure 9.** Gastrectomy results in decreased UCP1 mRNA expression after both two (A) and eight (B) weeks. Ghrelin treatment to Gx mice for two weeks resulted in a decrease of UCP1 mRNA compared to saline-treated Gx mice. Conversely, eight weeks of daily ghrelin treatment resulted in an increased expression of UCP1 mRNA, reaching the level of intact mice receiving ghrelin. In intact mice, ghrelin was without effect on UCP1 mRNA expression. *, $p<0.05$; **, $p<0.01$; ***,$p<0.005$.

These observations suggest that the effect of ghrelin may be dependent on the metabolic state of the mice. Two weeks after surgery, the mice are still adapting to their reduced capacity to store food and are adjusting their meal sizes and feeding frequencies thereafter and can therefore not "waste" more energy than absolutely necessary. In this situation ghrelin seems to function in an adipogenic manner allowing the mice to store all the energy they can. After eight weeks, the mice have adjusted to the gastrectomy and can afford to "waste" energy as heat and ghrelin seems to have thermogenic properties striving towards normalising thermogenesis. In contrast to Tsubone *et.al.* [283], ghrelin failed to affect UCP1 mRNA expression in sham-operated (intact) mice. This may be explained by differences in ghrelin dose, administration route (subcutaneuos vs. intraperitoenal) or duration of studies (two and eight weeks vs. one week).

*Paper V*

Soft drinks are basically sweet, carbonated solutions. In **Paper V**, we set out to investigate the effects of three different sugars in solution on consumption, food intake, body weight, blood lipid profile, serum ghrelin and leptin levels in rats. The sugars used were sucrose, glucose and fructose and they were provided to the animals *ad libitum* as 23% solutions. The study lasted for two weeks, which is a relatively short period of time for a feeding study. We found that all rats offered sugar solutions decreased their food intake. This may reflect an attempt to balance the excess calories provided by the solutions. However, all rats offered the palatable solutions consumed more total calories and gained more weight than rats offered water to drink. Despite the relatively short duration of the study, we found all palatable solutions to have significant impact on most parameters studied.

In **Paper V** when using rats, ghrelin levels were unchanged in response to sucrose solution whereas in **Paper I,** serum ghrelin to was found to be decreased in mice. Surprisingly, we found fructose to increase serum ghrelin levels (**Figure 10A**). This may suggest that rats and mice respond differently to sugars and/or that sucrose and fructose have different effects on ghrelin secretion. Leptin levels were increased in all groups of rats offered sugar solutions to drink (**Figure 10B**). The increased levels in leptin may reflect increased amount of body fat. Furthermore, serum levels of free fatty acids (FFA) were increased in glucose- and fructose-drinking rats (**Figure 10C**) and all sugar solutions increased serum triglycerides levels (**Figure 10D**). Fructose was the form of sugar that affected the most parameters analysed, in fact fructose affected all parameters analysed. This is interesting since fructose is very commonly used as a sweetener in soft drinks and other sweetened beverages. The results presented in **Paper V** underscore the rapid and detrimental effects of daily consumption of soft drinks.

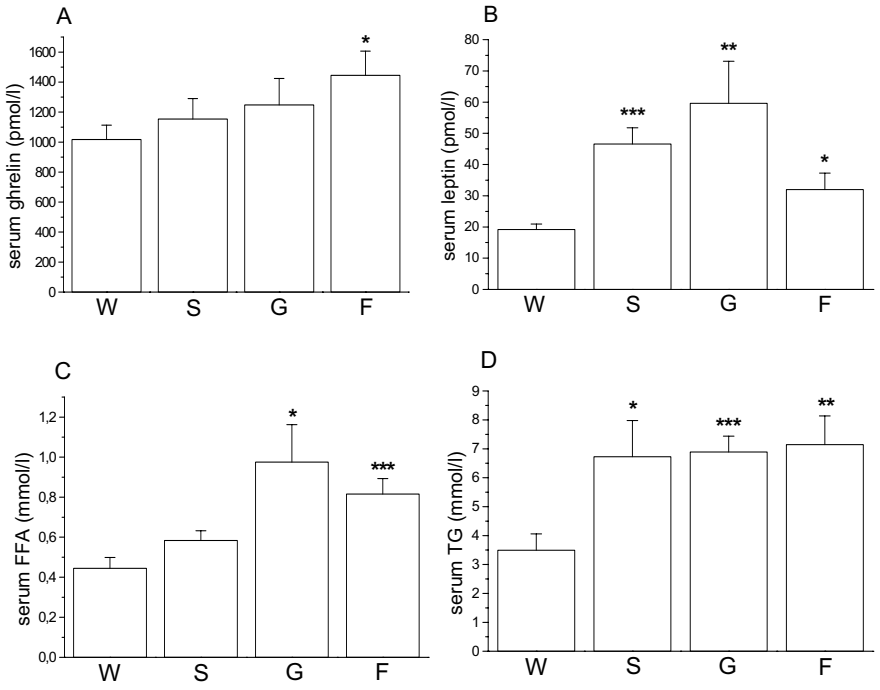


**Figure 10.** Fructose consumption over a two-week period resulted in increased fasting serum ghrelin levels (A). All sugars affected serum leptin levels by increasing them compared to water (B). Glucose and fructose increased serum free fatty acid and triglyceride levels (C and D, respectively). Serum triglycerides were also increased by sucrose (D). W (water); S (sucrose); G (glucose); F (fructose). *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$.

## GENERAL DISCUSSION

The fluctuating levels of serum ghrelin and leptin and of numerous other gastrointestinal peptides are essential for maintaining a proper regulation of food intake; high levels of ghrelin trigger meal initiation and high levels of leptin signal meal termination. A disruption in this balance results in either hypophagia or hyperphagia. Tasty diets rich in fat and/or sugar (energy-dense diets) are likely to induce overconsumption. In **Paper I**, we show that the introduction of a diet rich in sucrose leads to the abolishment of the pre- and postprandial difference in circulating leptin and ghrelin. We observed an increase in fasting leptin levels and a decrease in fasting ghrelin levels. This may reflect a defense mechanism against caloric overconsumption. Nonetheless, the animals became hyperphagic and gained more weight than control animals. The question arises if the hyperphagia and resulting weight gain would be even more pronounced if pre- and postprandial leptin and ghrelin levels had remained normal. A system promoting caloric overconsumption seems to be activated, overpowering the effects of high leptin and low ghrelin. Such a system could be the dopamine or the opioid system, systems that are both closely linked to the phenomenon of reward. Increase in dopamine release has been reported in response to intermittent consumption of sucrose solutions [335] and microinjections of opioids and opioid receptor agonists have been found to increase sucrose drinking in rats [336]. The involvement of ghrelin and leptin in the attempt to "normalise" food intake can also be seen in studies of obese individuals as well as in patients suffering from anorexia nervosa (AN). Obese people have low serum levels of ghrelin [337, 338] and high levels of leptin [339], signal the termination of feeding. Patients suffering from AN have high circulating ghrelin levels [80] and low leptin levels [340, 341], which would signal to initiate feeding. In both cases (obesity and AN) there seems to be systems operating in the opposite direction, i.e. systems promoting the obese person´s feeding and systems inhibiting the AN-patient´s feeding. Although ghrelin and leptin are not powerful enough to stop obese persons´ food intake (or induce food intake in AN-patients), they may help ameliorate the effects of the opposing systems. If ghrelin and leptin did not change in the way they did in the two

disorders (and in the rats in **Paper I**), the disorders may perhaps be even more pronounced; with time the obese person would become even more obese and the AN-patient even thinner.

The study of Gx mice and rats is an important complement to the study of genetically ghrelin-deficient mice. Genetically ghrelin-deficient mice lack all ghrelin, but Gx mice are hypoghrelinemic (approximately 15% ghrelin remains after Gx). By deleting ghrelin, a number of other systems which require ghrelin to function properly may be affected. Ghrelin has been found to be expressed in the developing pancreas of both rodents and humans [342, 343]. By genetically deleting ghrelin, it is not present during development and the ghrelin-requiring systems are affected by the absence of ghrelin. Also, compensatory systems may be activated and the real effect of ghrelin deletion may be masked. Gx may be a better way of studying the role of ghrelin without affecting other important systems which require ghrelin during development. We have shown that the removal of the stomach (Gx mice; **Paper IV**) resulted in decreased thermogenesis (as indicated by reduced UCP1 mRNA expression). Furthermore, we showed that the stomach-derived peptide hormone, ghrelin, affected thermogenesis (**Paper IV**) and adipogenesis (**Paper II**). As the stomach is a very endocrine organ, harboring many endocrine cell types, Gx means the loss of many hormones and peptides (known and unknown), resulting in an altered endocrine profile. This is illustrated by the fact that Gx has been reported to induce osteopenia in rats [344, 345] and a reduced insulin response to glucose [346]; effects that have been shown to be ghrelin-independent (**Paper II** and [347]). Bariatric surgery means anti-obesity surgery and involves mainly gastric bypass which is a common tool for inducing rapid weight loss in obese individuals. Investigating the long-term effects of bariatric surgery on appetite regulation and energy homeostasis is of interest and are being studied [348]. An interesting question is how bariatric surgery affects bone metabolism in the long-term.

Hippocampal neurogenesis is a process known to be affected by learning [349], social isolation [310] and physical exercise [307, 310, 350]. Not much is known about the role of the diet in hippocampal neurogenesis. It has previously been shown that rats fed a diet rich in fat and sugar do not perform as well as control animals in spatial memory tasks [328]. In **Paper III**, we show that the diet can affect neurogenesis in the hippocampus of male rats. The reduction in hippocampal neurogenesis in response to a high fat diet may explain why rats offered such diets do not perform as well as control rats in memory tasks [328, 329]. The impaired neurogenesis was explained by increased corticosterone levels in male rats fed high fat diet. We found no effect of diet in female rats and believe that this is caused by elevated levels of serum corticosterone in females rats offered standard rat chow, suggesting that females have higher corticosterone levels than males. So far, all studies on neurogenesis and corticosterone have been performed in male rats. Females, thus, seem to have elevated "basal" corticosterone levels which might mask the effects of the diet on neurogenesis. Further evidence for this comes from the fact that the corticosterone levels in male rats receiving standard chow in **Paper III** were only approximately 33% of that of the females receiving standard rat chow. Thus, male rats seem to be more susceptible to the effects of diet-induced increases in corticosterone. The results presented in **Paper III** adds yet another harmful effect of diets rich in fat to the list, further supporting the fact that overconsumption of these diets is unhealthy. The observations made in **Paper III**, raises the question how other types of diets would affect neurogenesis. In a time of increasing incidence of obesity, more and more people go on diets in order to lose weight. One popular diet is the Atkins diet, which advocates the consumption of diets rich in fat and protein. It would be interesting to study the effects of such a diet on neurogenesis. Offering rats sugar solutions to drink would also be of great interest. This would give an indication of the effect of soft drinks and sugary beverages on neurogenesis.

In conclusion, the papers presented in this thesis illustrate the harmful effects of overconsumption of fat and/or sugar. These diets abolish physiologically important differences in serum levels of appetite peptides. The involvement of such a peptide,

namely ghrelin, in adipogenesis and thermogenesis has been suggested by the data presented here. Furthermore, the involvement of the stomach in the regulation of thermogenesis has been demonstrated. This is a finding which may be of clinical relevance in the post-operative treatment of patients who have undergone bariatric surgery. The effect of a high fat diet on the production of new neurons in the hippocampus, a brain region involved in spatial memory and learning, has been demonstrated. This a finding which opens the door to further studies regarding the role of the diet in neurogenesis. We have shown the consumption of sugar solutions, fructose in particular, to have very rapid effects on the blood levels of appetite peptides, blood lipids, body composition and feeding pattern. The results suggest that overconsumption of soft drinks has a number of harmful effects on the body.

## MAJOR CONCLUSIONS

- Overeating of palatable diets is associated with increased fasting levels of circulating leptin and decreased fasting levels of ghrelin.

- Ghrelin is an adipogenic agent, powerful enough to normalise the loss of adipose tissue resulting from gastrectomy.

- A high fat diet reduces hippocampal neurogenesis in young male rats (but not in young females). This reduction is suggested to be a consequence of increased serum corticosterone levels induced by the high-fat diet.

- The loss of the stomach results in decreased thermogenesis, indicated by the reduction of UCP1 mRNA expression. Ghrelin seems to possess thermogenic properties under certain circumstances (e.g. after Gx).

- Rats offered sugar solutions to drink alter their feeding pattern so that approximately 50% of their daily caloric intake comes from the drink. Sugars, and fructose in particular, have very powerful effects on circulating leptin, ghrelin, free fatty acids, triglycerides and cholesterol.

## POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Övervikt och fetma har de senaste åren ökat kraftigt i hela världen och klassas nu som en global epidemi. Överviktiga och feta människor har en ökad risk för att drabbas av följdsjukdomar, såsom typ 2 diabetes och hjärt-kärlsjukdomar. En oroande trend är också att allt fler barn är överviktiga och drabbas av typ 2 diabetes, som tidigare kallades för ”åldersdiabetes”. Väldigt förenklat blir man överviktig om man tillför kroppen mer energi från mat än vad man gör av med (t ex genom motion). Regleringen av hur mycket mat man tillför kroppen är väldigt komplex och involverar många olika faktorer, såsom miljö, sinnestillstånd och genetiska anlag. Kroppen innehåller en mängd olika substanser som är inblandade i regleringen av hunger och mättnad. Substanserna som produceras både centralt (i hjärnan) och perifert (i olika organ ute i kroppen) transporteras i cirkulationen till en del av hjärnan som kallas hypothalamus, vilket resulterar i ett ökat eller minskat födointag. I denna avhandling har jag fokuserat på två hormon som produceras perifert, leptin och ghrelin. Ghrelin produceras främst i magsäcken och främjar födointag medan leptin produceras i fettväv och hämmar födointag. Eftersom ghrelin främjar födointag är det logiskt att nivåerna av ghrelin i cirkulationen är höga när man är hungrig (vid fasta) och låga efter en måltid. Leptinnivåerna är precis tvärtom, d v s låga vid fasta och höga i samband med en måltid. I avhandlingens första arbete (**Artikel I**) ville vi undersöka hur nivåerna av ghrelin och leptin påverkades av dieter med ett högt fett- och/eller sockerinnehåll. Vi tog blod från råttor efter både fasta och efter måltid. Detta gjorde vi i sambandet med att studien startade, två veckor in i studien och vid avslutandet av studien fem veckor senare. Vi fann att skillnaderna i leptin och ghrelin vid fasta och efter måltid försvann i de råttor som fått äta en diet innehållande lite fett och mycket socker. Detta tolkar vi som ett försök av dessa råttor att försvara sig mot den överkonsumption som dieterna som resulterade i. Det faktum att de trots allt åt för mycket av dieterna i och med att de gick upp i vikt visar att det finns kraftfullare system som driver djuren att fortsätta äta. Vi var även intresserade av hur söta drycker påverkade ghrelin och leptin i närvaro och frånvaro av en fettrik diet. I denna studie använde vi möss som fick dricka antingen

vatten eller en 23%-ig sockerlösning med fettsnål eller fettrik diet. Efter fem veckors utfodring analyserade vi ghrelin och leptin. Vi fann att socker oberoende av dieten höjde fasteleptinnivåerna och att alla dieter sänkte ghrelinnivåerna. Detta är i linje med vad vi fann i råttorna; nämligen att en försvarsmekanism mot överkonsumption föreligger. Dock konsumerade även mössen som fick sockerrik diet eller socker att dricka mer energi än kontrollmössen.

Avlägsnande av magen (gastrektomi) resulterar i en kraftig sänkning av mängden ghrelin i cirkulationen (ungefär 80% försvinner). Med denna vetskap undersökte vi effekterna av en lång tids ghrelinbrist på födointag, viktutveckling och fettmängd (**Artikel II**). Hälften av mössen behandlades mot sin ghrelinbrist genom att få dagliga injektioner av ghrelin. Vi undersökte även hur ghrelin påverkade intakta möss. Vare sig gastrektomi eller ghrelinbehandling påverkade det dagliga födointaget. De gastrektomerade mössen hade mindre kroppsfett jämfört med kontrollerna. Vi fann att daglig ghrelinbehandling motverkade denna minskning av kroppsfett. Vi fann även att ghrelin ökade mängden kroppsfett i kontrollmössen. Utifrån detta drog vi slutsatsen att ghrelin stimulerar fettbildning.

Kopplingen mellan fettrik diet och nyproduktionen av nervceller är ett område som är föga utforskat. Det finns några arbeten som rapporterar att råttor som blivit utfodrade en fett- eller sockerrik diet presterar sämre i minnestester än de råttor som fått äta en vanlig fettsnål standarddiet. Vi bestämde oss därför för att utfodra råttor under en fyraveckorsperiod med en fettrik diet och jämföra produktionen av nervceller med råttor som fått en standarddiet. Konsumption av den fettrika dieten ledde inte till större viktökning i hanråttor, utan bara i honråttor. Fyra veckors konsumption av den fettrika dieten ledde till en 40%-ig minskning i nybildningen av nervceller i hanråttor. Analys av hormonet kortikosteron i cirkulationen visade att de råttor som hade många nervceller, d v s kontrolldjuren som fått standarddiet, hade lägre nivåer av kortikosteron. De råttor som fått fettrik diet och hade ett lägre antal nervceller hade betydligt högre serum kortikosteronnivåer. Sedan tidigare finns det rapporter som påvisar en skadlig inverkan av kortikosteron på nervceller och att en fettrik diet

stimulerar utsöndringen av kortikosteron. Utifrån de resultat vi presenterat i **Artikel III** och tidigare publicerade observationer drog vi slutsatsen att en fettrik diet påverkar nybildningen av nervceller negativt genom att stimulera kortikosteronfrisättningen.

I **Artikel IV** ville vi undersöka magens betydelse i sig men också ghrelins betydelse vid regleringen av termogenesen. Termogenes är den process i cellen som kontrollerar kroppstemperaturen. I gnagare (men även i spädbarn) sker den huvudsakliga termogenesen i en vävnad som kallas brun fettväv. I den vävnad uttryckts ett protein som kallas uncoupling protein 1 (UCP1, svenska: urkopplande protein 1). Detta protein är ansvarigt för den värmeproduktion som krävs för att reglera termogenesen. Precis som i **Artikel II** använde vi oss av möss med bortopererad magsäck. Avlägsnande av magsäcken resulterade i en kraftig minskning av UCP1 mRNA (mRNA är den molekyl som kodar för protein) efter både två och åtta veckor. Två veckor av ghrelinbehandling till de gastrektomerade mössen ledde till ytterligare sänkning av UCP1 mRNA. Ghrelinbehandlade gastrektomerade möss hade normala UCP1 mRNA-nivåer (jämfört med kontrollmöss) efter åtta veckors behandling. Utöver de tidigare födointagsstimulerande och fettvävsstimulerande egenskaperna som rapporterats för ghrelin föreslår vi i och med de resultat presenterade i **Artikel IV** att ghrelin också har betydelse vid regleringen av termogenesen.

I **Artikel V** undersökte hur olika typer av socker i lösning påverkade nivåerna av ghrelin och leptin i cirkulationen. Vi gav råttor fri tillgång till antingen vatten (kontrolldjur), sukros-, glukos- eller fruktoslösning i två veckor. Eftersom sockerlösningarna innehåller kalorier såg vi att råttorna som drack dessa lösningar åt mindre fast föda än kontrollråttorna. Vid studiens slut fick sukros- och glukosråttorna cirka 50% av sitt totala kaloriintag från sina respektive lösningar, medan fruktosråttorna fick cirka 36%. Det faktum att råttorna som fick sockerlösning åt mindre fast föda såg vi som en kompensationsmekanism för att försöka förhindra övervikt och fetma. Råttorna som fick socker fick dock i sig mer total mängd kalorier än kontrolldjuren och gick följdaktligen upp mer i vikt. Fruktos var det socker som hade störst påverkan på de parametrar vi analyserade. Fria fettsyror i cirkulationen var

fördubblade hos fruktosråttorna jämfört med kontrollråttorna. Detta observerades även för triglycerider, kolesterol, ghrelin och leptin.

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