Effects of acylated and unacylated ghrelin on lipid metabolism

Bachelor thesis

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Contents

Abstract

The “hunger hormone” ghrelin is one of the hormones that is known to influence energy regulation and therefore potential target to fight obesity. Ghrelin occurs in different forms. The two major forms known are acylated ghrelin (AG) and unacylated ghrelin (UAG). For a long time UAG is seen as the inactive form of ghrelin, while nowadays experiments show that it is an active protein with its own and possibly antagonizing effects on AG. The aim of the present study is to give an overview of the effects of AG and UAG on the lipid metabolism. The main focus will be on the consideration which of the ghrelin forms, AG or UAG, would be most usable in the treatment against obesity.

AG elevates food intake, inguinal and retroperitoneal white adipose tissue volumes and the activity of genes involved in lipid uptake and lipogenesis. UAG decreases food intake, epididymal and perianal white adipose tissue volumes and suppresses genes that encode for regulatory enzymes involved in adipogenesis, lipogenesis and sterol synthesis in white adipose tissue.

In conclusion, both AG and UAG have influence on the lipid metabolism of individuals. It was thought that UAG had antagonizing effects on AG. In certain perspectives this agrees with studies about AG and UAG, but there is no consensus in current literature. It is important to make a clear difference between AG and UAG in experiments and literature. There is enough averment to say that understanding of the activities of both UAG and AG can bring solutions in the treatment against obesity. However, there is still further investigation needed.

Introduction

Today one of the largest health issues worldwide is obesity. Only in the Netherlands 60% of the men and 44% of the women in the age group 30-70 years are obese.[1] Obesity is a disorder of energy imbalance, whereby a long-term excess of energy intake over energy expenditure leads to fat storage. This process is influenced by many hormones, including ghrelin.

The discovery of ghrelin was a typical case of reversed pharmacology. It started with research on the development of opioid peptide derivates in the seventies. In 1976 synthetic opioid peptide derivates were found that did not exhibit any opioid activity, but had a weak Growth Hormone (GH)-releasing activity.[2] These opioid peptide derivates were demonstrated to be Growth Hormone Secretagogues (GHS).[3] These GHS did not operate on the same pathway and receptor as the GH.[4] In 1996 the GHS Receptor (GHS-R) was identified and cloned.[5] The receptor is located at chromosome 3q26.31 and encodes for a G-protein coupled 7 trans-membrane receptor.[6] The GHSR-gene, containing two exons and one intron, encodes for the GHSR-1a and GHSR-1b.[7] The natural ligand that acts through the GHSR was unknown for a couple years. Because GH is being released from the pituitary gland, it was thought that this natural ligand would be found in the hypothalamus. However, in 1999 Kojima et al. found it in stomach extracts. This ligand 'ghrelin', named after the Proto-Indo-European root word for growing 'ghre', stands for 'Growth Hormone RELeasINg.[8] Ghrelin occurs in different forms, the two major forms known are acylated ghrelin (AG) and unacylated/desacyl ghrelin (UAG). The relative ratio circulating in humans is 3-10% AG and 90-97% UAG.[9] Based on the fact that AG has an *n*-octanoylated group at the serine 3 residue, which is essential for the binding to GHS-R, and the fact that UAG doesn't contain this group, it has been suggested that AG was the active form of ghrelin and UAG the inactive form.[8,10,11,12] Nowadays experiments show that UAG is not just the inactive ghrelin, but a protein with effects on its own and possibly antagonizing effects to AG. However, UAG does not act through the GHS-R.[13]

It is known that AG has influence on energy metabolism, glucose metabolism, lipid metabolism, etc. But the role of UAG is not always clear. Articles about UAG and its relation to energy metabolism and glucose metabolism are already published. Therefore, the aim of the present thesis is to give an overview of the effects of AG and UAG on the lipid metabolism. The main focus will be on the consideration which of the ghrelin forms, AG or UAG, would be most usable in the treatment against obesity.

Lots of research has been done with regard to ghrelin. However, in just a fraction of the experiments is mentioned which form of ghrelin is used, or in which proportion different forms of ghrelin were present. Thus the challenge of this thesis will be to separate incomplete information from complete information.

The synthesis of ghrelin

Ghrelin is a stomach-derived peptide that is mainly produced in the stomach submucosa by X/A-like cells.[8,14] It is secreted to the blood in an endocrine way, based on circulating ghrelin in plasma of rats and the location of the X/A-like cells, whom are closely associated with the vascular capillaires running through the lamina propria.[14] The synthesis of ghrelin starts at the ghrelin gene. In rats this gene is located on chromosome 4q42 and in mice on chromosome 6.[15,16]

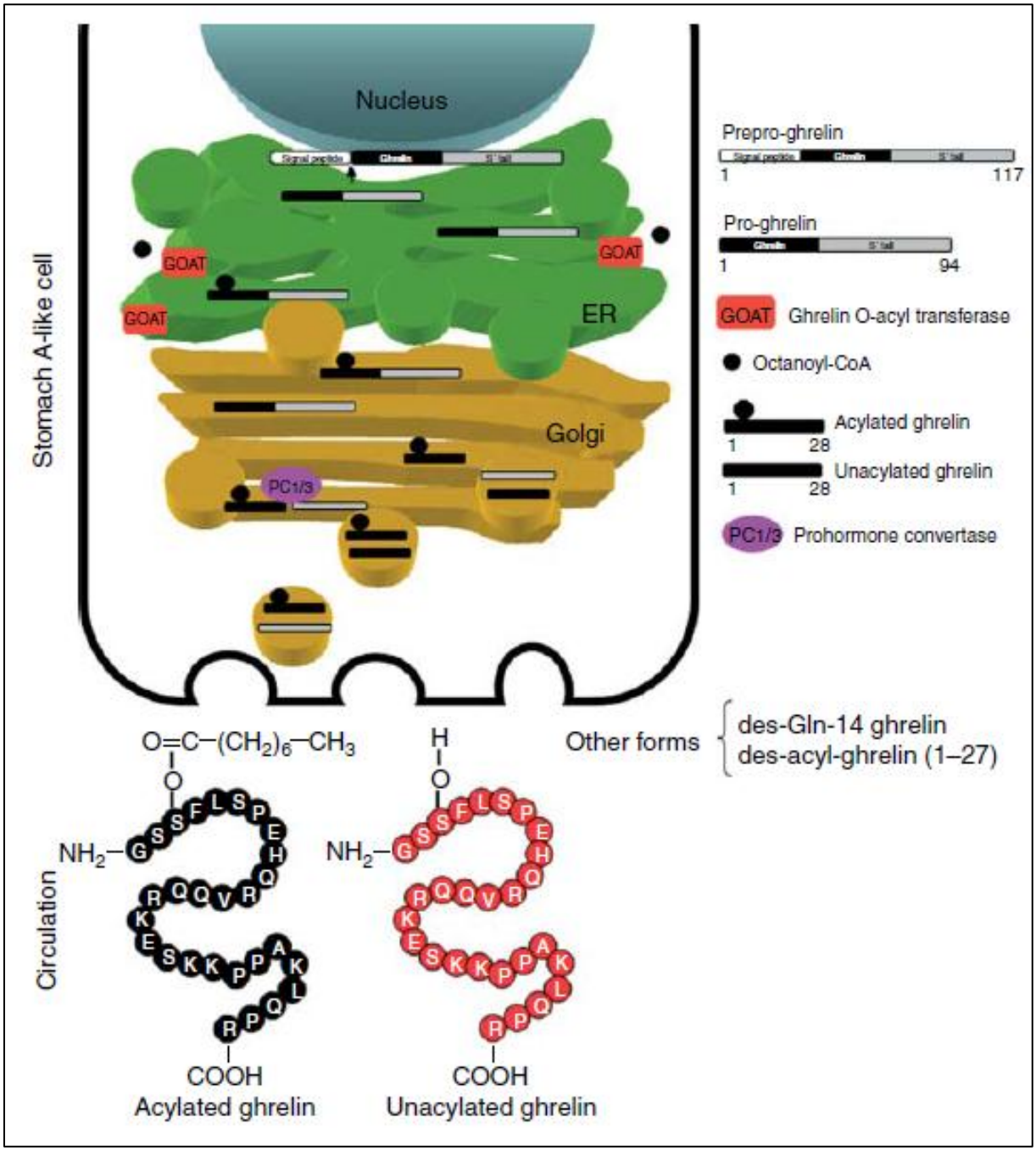

Figure 1: Posttranslational processing and acylation of the pro-ghrelin peptide.[17]

The human ghrelin gene of 7198 base pairs is located on chromosome 3p26-25.[18,19] This gene consists of four exons and three introns encoding for the ghrelin-obestatin preproprotein.[18,20,21] The basal activity of the human core promoter requires a noncoding exon 1 and a proximal sequence of intron 1, while the noncoding exon 1 is not essential for activity in rats.[22] The suspected start codon ATG of the gene is located at nucleotides 34-36, preceded by the concurrent initiations sequence, whereas, the terminal codon TAG is found at position 385-387.[23]

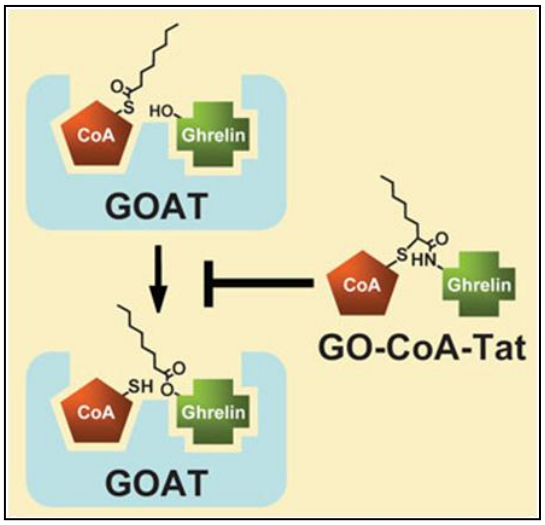

Figure 2: Go-CoA-Tat, the main inhibitor of GOAT.[24] Go-CoA-Tat prevents octanoylation of ghrelin by inhibiting GOAT.

Preproghrelin includes pro-ghrelin which consists of 94 amino acids and a signal peptide which consists of 23 amino amino acids.[4] There is evidence that prohormone convertase 1/3 (PC1/3) is responsible for the proteolytic cleavage in mice and that pro-ghrelin is being released into the lumen of the endoplasmic reticulum (ER) as shown in figure 1.[25] Pro-ghrelin includes mature ghrelin which consists of 28 amino acids and a C-terminal peptide, C-ghrelin, which consists of 66 amino acids.[8] Mature ghrelin cannot activate the GHSR-1 because first it has to octanoylate, which includes the attachment of an n-octanoylated group at the serine 3 residue. This octanoylation in the ER is catalyzed by Ghrelin O-acyltransferase (GOAT), a member of the family of membrane-bound O-acyltransferases (MBOAT).[26] GOAT is expressed in the stomach, pancreas and in smaller amounts in bones.[27,28] The bi-substrate inhibitor Go-CoA-Tat, shown in Figure 2, is one of the main known inhibitors of GOAT.[29]

Furthermore there is evidence for the presence of ghrelin de-acylase. Acyl-protein thio-esterase 1 (APT1), also known as lysophaspholipase 1, is a general intracellular dilapidation enzyme that acts both intracellular and extracellular.[30]

Thus Go-CoA-Tat and APT1 are potential tools to influence the quantity of AG and UAG in the treatment against obesity.

Acylated ghrelin

AG is a powerful stimulator of food intake and gastrointestinal motility in mice, rats and humans.[8,31,32] The hormone acts through a high affinity binding to the GHSR-1a, which causes a rapid homologous desensitization of the GHSR-1a via clathrin-mediated endocytosis.[7,33] Immunohistochemistry detects the mRNA expression of the GHSR-1a in human tissues. The highest levels of mRNA were found in the pituitary, adrenal gland and spinal cord. Additionally, by using immunohistochemistry the expression of the receptor peptide in human tissue was detected, especially in the endocrine and reproductive organs (anterior pituitary, thyroid, pancreas, testis), in parts of the CNS (cerebrum, cerebellum) and in single cells of bone marrow.[34]

Peripheral administration of AG suppresses firing of the vagal afferent pathways.[35] Intracerebroventricular administration of AG induces neuronal activities in the hypothalamus (Arcuate Nucleus (ARC) and Paraventricular Nucleus (PVN)), brain stem, hippocampus, dentate gyrus and piriform cortex.[36,37] Central treatment with AG in rats increases the mRNA expression of the neuropeptide Agouti-related protein (AgRP) and Neuropeptide Y (NPY).[38] Furthermore, in electrophysiological approaches in mice it is demonstrated that AG can activate NPY/AgRP neurons and contemporary reduce the activity of pro-opiomelanocortin (POMC) neurons as shown in Figure 3.[39] NPY and AgRP are important neurons in the communication between AG and the central melanocortin circuit in mice. AG stimulates energy intake through suppressing the hypothalamic melanocortin tone.[40] NPY inhibits POMC directly by synapses on POMC neurons and indirectly by the activation of inhibitory GABAergic interneurons.[41,42] These inhibitory interneurons innervate neurons which express POMC and the melanocortin-4 receptor (MC4-R).[42] However, NPY effects are not limited to inhibiting the melanocortin pathway because AG-induced food intake is mediated via the orexin- and anorexin pathway.[40,43] There is functional synaptic

contact of AG-containing axons with orexin-producing neurons in the hypothalamus of rats.[43] Intracerebroventricular administration of AG induces c-Fos expression in orexin-expressing neurons, but not in MCH-expressing neurons.[43,44] Thus, AG activates the orexin-producing neurons in a manner dependent of NPY. This is also indicated by the fact that AG remains incapable to induce c-fos expression in orexin-producing neurons which followed pretreatment with anti-NPY IgG.[44] AG interacts with both NPY and orexin systems to induce feeding.[45] Central and peripheral injections of acylated ghrelin induce fasted motor activity in the antrium and the duodenum of normal fed rats. These effects are mediated by the GHSR1-a on the vagal efferent nerve and NPY is involved in this regulation.[46] Thus the signaling of peripherally AG to induce the orexigenic effects is mediated by vagal efferent nerves.

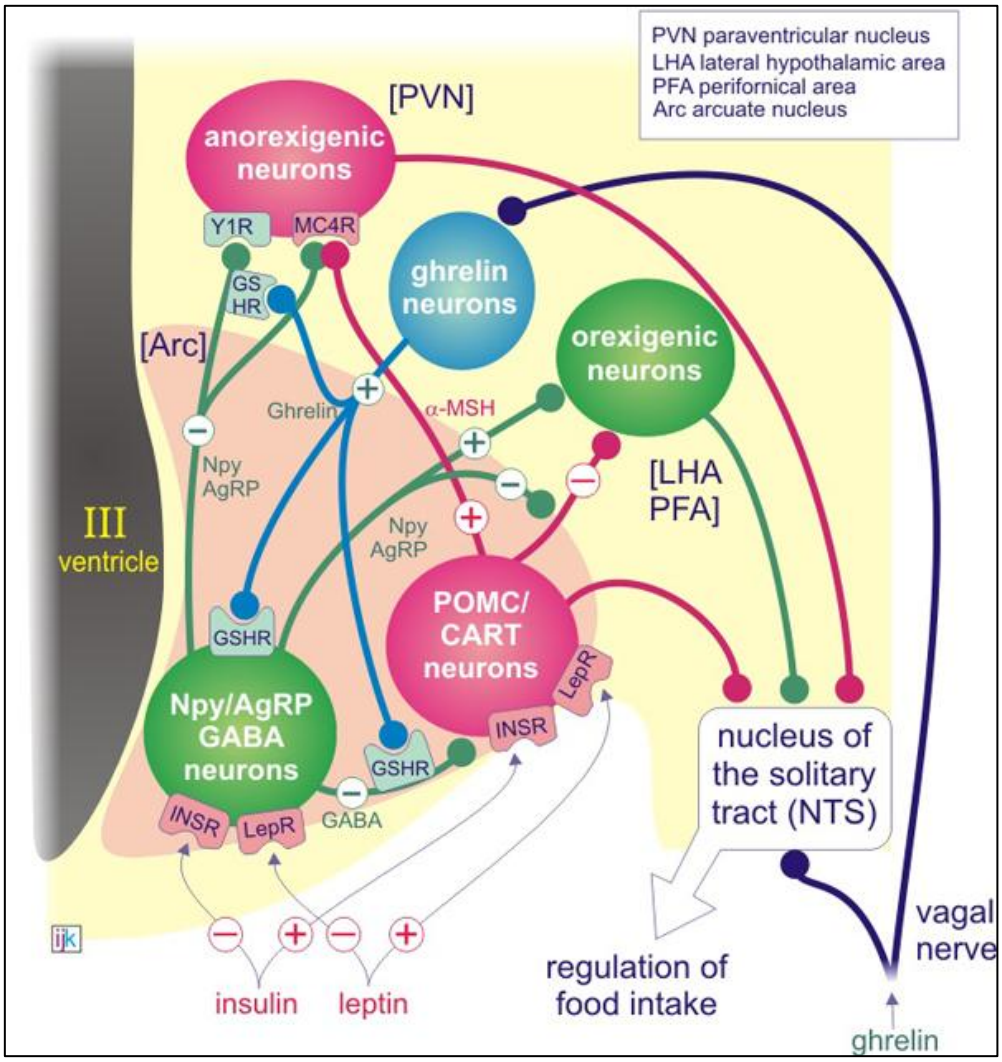

Figure 3: Regulation of food intake by ghrelin at the level of the arcuate nucleus (ARC). Ghrelin (AG) acts through the vagal nerve and stimulates via the GHSR-1 (in this picture called GSHR) NPY/AgRP neurons and inhibits POMC neurons. Furthermore NPY inhibits POMC directly by synapses on POMC neurons and indirectly by the activation of inhibitory GABA interneurons. The pink color in this picture represents "stop eating" and the green color "go eating".[47]

Infusions of AG lead to significant increases in food intake and hunger and/or appetite in human, mice and rats.[9,48,49,50] Meanwhile, Otto B et al. and Tanaka M et al. found higher fasting plasma AG levels in anorectic patients than in their healthy controls.[51,52] Another study demonstrated in anorectic patients higher levels of total fasting plasma ghrelin with an unclear composition of ghrelin.[53] This study and the study of Otto B et al. showed a decreased concentration of ghrelin plasma concentration after weight gain in anorectic patients.[51,53] Homaee HM et al. and Marzullo P et al. found lower plasma AG concentrations in obese subjects compared to lean subjects.[54,55] However, slenderizing of obese subjects increases plasma AG concentrations. In line with these findings are body weight, body fat and BMI inversely correlated with plasma AG levels.[54,56,57]

Tschöp et al. showed an increase in body weight and fat mass in rats injected with AG, while the rats got normal amounts of food.[58] This indicates that the influence of AG on body weight and fat mass can be independent of food intake.

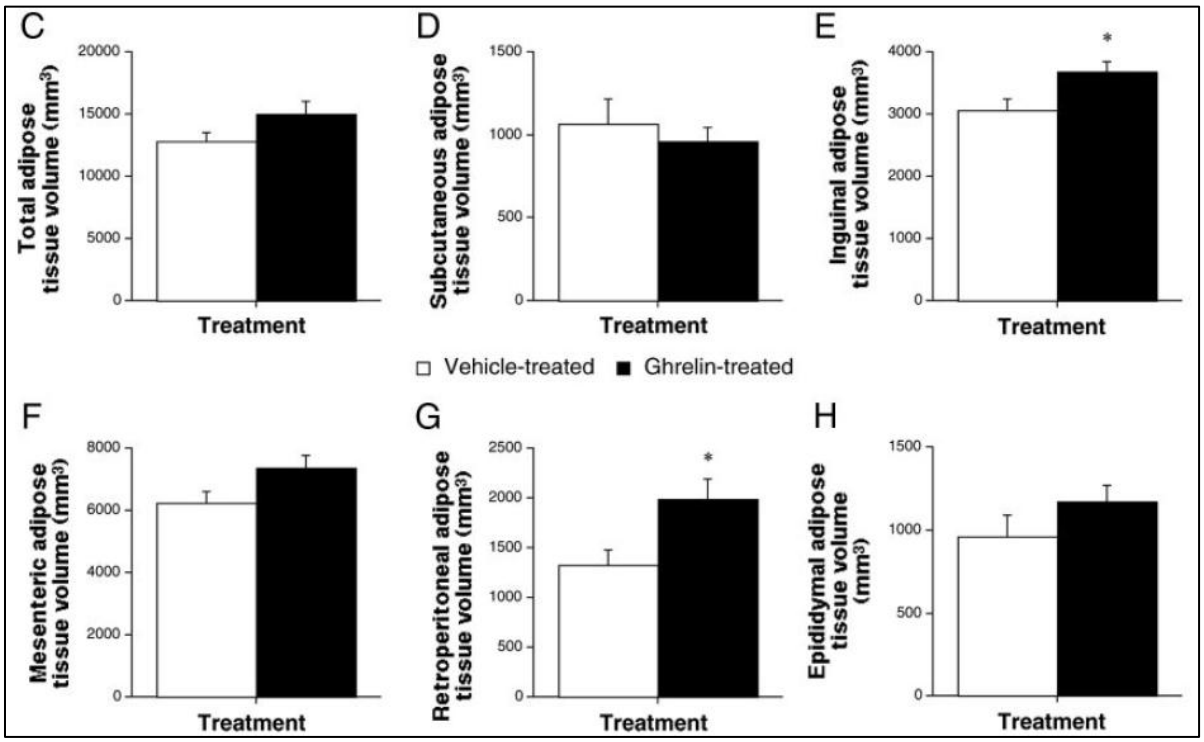

Figure 4: Adipose tissue volumes in rats. After one week continuous intravenous infusion of AG in rats are the inguinal and retroperitoneal adipose tissues significant increased.[61]

Furthermore, it is thought that AG plays a role in the regulation of lipid storage in white adipose tissue and brown adipose tissue. White adipose tissue stores energy in the form in

tryglycerides and supplies energy to the body. Visceral adiposity of white adipose tissue is a risk factor for insulin resistance and type 2 diabetes.[59] In contrast to white adipose tissue, brown adipose tissue is a key organ of thermogenesis, playing an important role in energy expenditure.[60]

Davies JS et al. showed after one week continuous intravenous infusion of AG in rats an altered abdominal white adipose tissue distribution. Although total white adipose tissue volume was not significantly elevated by the AG treatment, the inguinal and retroperitoneal white adipose tissue volumes were increased and the concentration plasma free fatty acids reduced. The weight of inguinal- and epididymal fat was elevated as well as the inguinal adipocyte size as shown in Figure 4 and 5.[61]

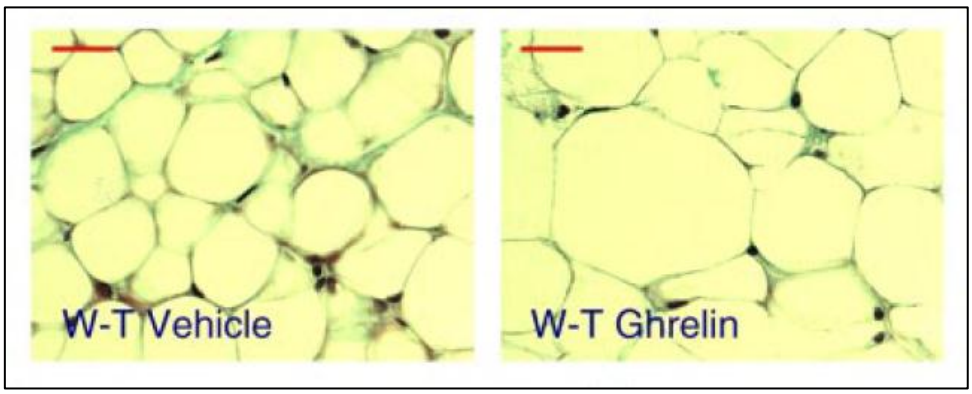

Figure 5: Adipocyte size after AG treatment. After one week continuous intravenous infusion of AG in rats is the adipocyte size elevated compared to the control.[61]

In Ghsr-/- knock-out mice, mice that cannot perceive AG, reduced levels of circulating cholesterol, triglycerides and fasting FFA levels were found. The expression levels of glucose/lipid uptake genes (GLUT4, lipoprotein lipase and CD36) in inguinal- and epididymal fat were drastically down-regulated. Similarly, expression levels of lipogenic genes (aP2, FAS, Lipin 1) were lower. This suggests that reduced glucose/lipid uptake and lipogenesis could contribute to the reduced mass of white adipose tissue in Ghsr-/- mice. Furthermore, in these mice a decrease in adiposity is shown by promoting lipid export (ABCG) and/or inhibiting lipid recycling (PEPCK).Thus, GHS-R ablation may reduce fat mass of white adipose tissue by regulating lipid uptake, lipogenesis, lipid storage, lipid recycling and/or lipid export.[62]

In contrast to white adipose tissue, the activity of glucose and lipid uptake and lipogenesis genes were elevated in the brown adipose tissue of Ghsr-/- knock-out mice, suggesting

increased lipid uptake/syntheses. Additionally GHS-R ablation improves the thermogenic function of brown adipose tissue.[62] Thus AG may decrease the lipid uptake/synthesis in brown adipose tissue which decreases the thermogenic function of brown adipose tissue.

One of the most important organs playing a role in lipid metabolism is the liver. AG treatment results in an enlarged lipid area in the liver of rats. The sums of triacylglycerol and lipid droplets were elevated, while the droplet size was equal.[61] Furthermore AG is thought to be associated with non-alcoholic steatohepatitis, but further research is necessary.[63]

Unacylated ghrelin

UAG was for a long time known as the inactive version of ghrelin. Nowadays experiments show that UAG is not just the inactive form of ghrelin, but a protein with own effects and possibly antagonizing effects to AG. However, UAG does not act trough the GHS-R, the UAG-receptor is still unknown.[13]

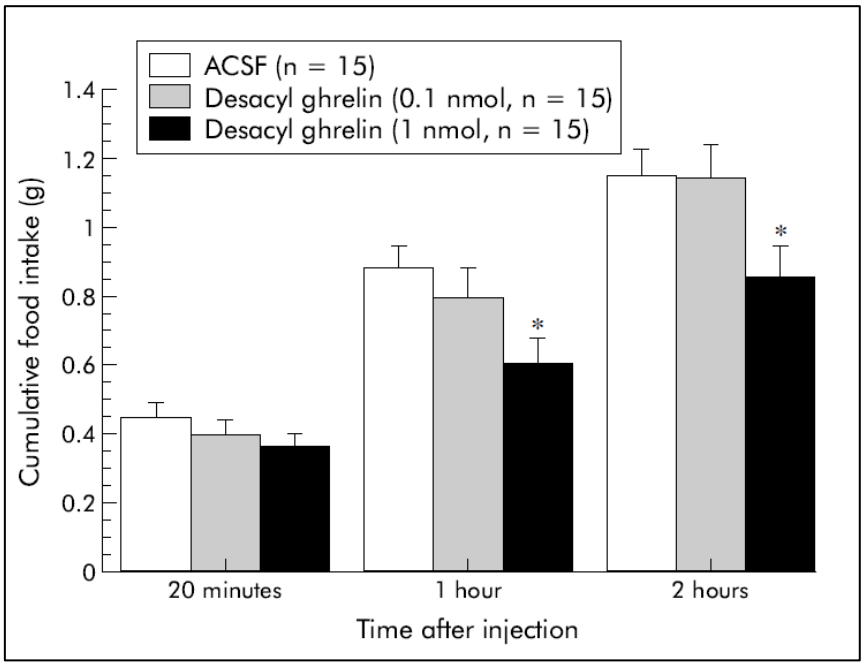

Figure 6: The effects of intracerebroventricularly administered ghrelin on cumulative food intake in sixteen hours food deprived mice. One and two hours after the injection of 1 nmol DAG results in a significant decrease in food intake (*p<0.05) compared with the artificial cerebrospinal fluid (ACSF) treated control.[70]

UAG might regulate gastric motility through a brain receptor, because UAG crosses the blood-brain-barrier via non-saturable transmembrane diffusion in mice.[64,65] In rats, UAG stimulates the expression of neuropeptides that are located in the paraventricular nucleus (PVN) and in minor amount in the arcuate nucleus (ARC). In the PVN most neurons reacting

on UAG are overlapped with CRF-neurons. CRF2 receptor antagonists block the effect of UAG on gastric motility.[57]

Urocortin is a ligand predominantly selective for the CRF2 receptor. [66,67] The gene expression of urocortin and anorexigenic Cocaine and Amphetamine Regulated Transcript (CART), endogenous satiety factors in the central nervous system, increases by administration of UAG.[31,68,69] Furthermore, UAG disrupts the fasted motor activity in the antrum in rats, but does not alter the fed motor activity. The effect of UAG on the fasted motor activity in the antrum can be blocked by truncal vagotomy, however, UAG has no effect on vagal afferent pathways.[35,57] Thus probably UAG is able to exert effects on the gastric motility via vagal efferent pathways, possibly by the CRF2-receptor.

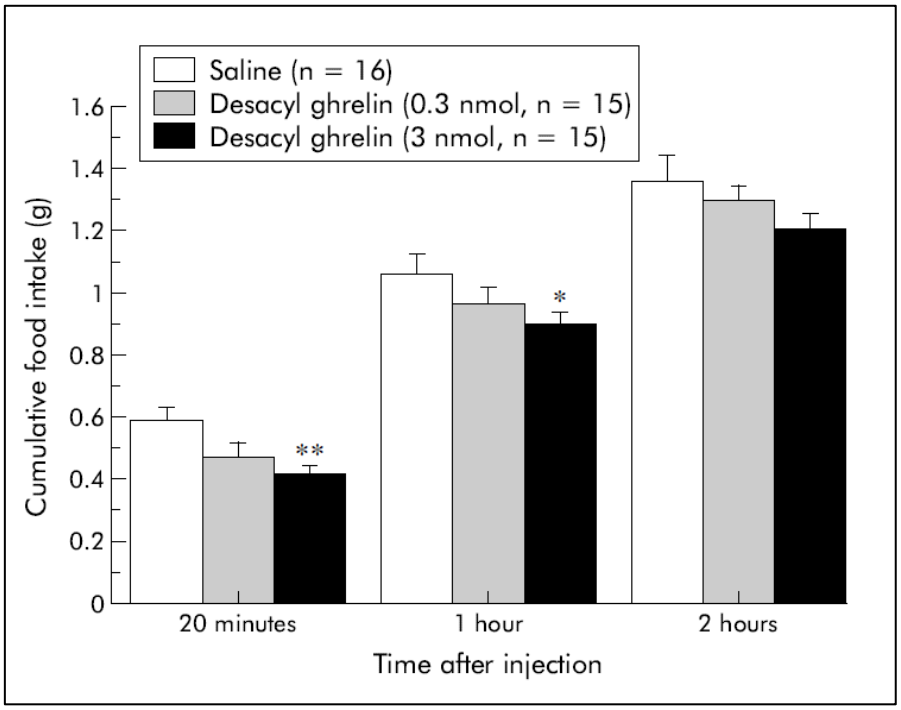

Figure 7: The effects of intracerebroventricularly administered ghrelin on cumulative food intake in sixteen hours food deprived mice. Twenty minutes and one hour after the injection of 3 nmol DAG results in a significant decrease in food intake (*p<0.05, **p<0.01)) compared with saline treated control.[70]

In mice, centrally, intraperitoneally and intracerebroventricularly administered UAG produce inhibitory effects on feeding behavior as shown in Figure 6 and 7.[70] Hotta M et al. and Koyama KI et al demonstrated higher fasting plasma UAG levels in anorectic patients than in their controls. Weight gain in the anorectic patients result in decreased fasting plasma UAG levels.[71,72] Nakahara et al. showed lower plasma UAG levels in obese subjects compared to their controls. In addition UAG has negative correlations with bodyweight and

BMI.[73] However, Zhang W et al. demonstrated no significant difference in body weight of FABP4-ghrelin transgenic mice, whom overexpresses UAG, compared with the wild-type controls. After a high-fat diet for 26 weeks, is the weight of the transgenic mice significantly lower than the wild-type mice. This is probably due to decreased adiposity in the transgenic mice, because the lean mass did not change significantly in the FABP4-ghrelin mice after the high-fat diet.[74]

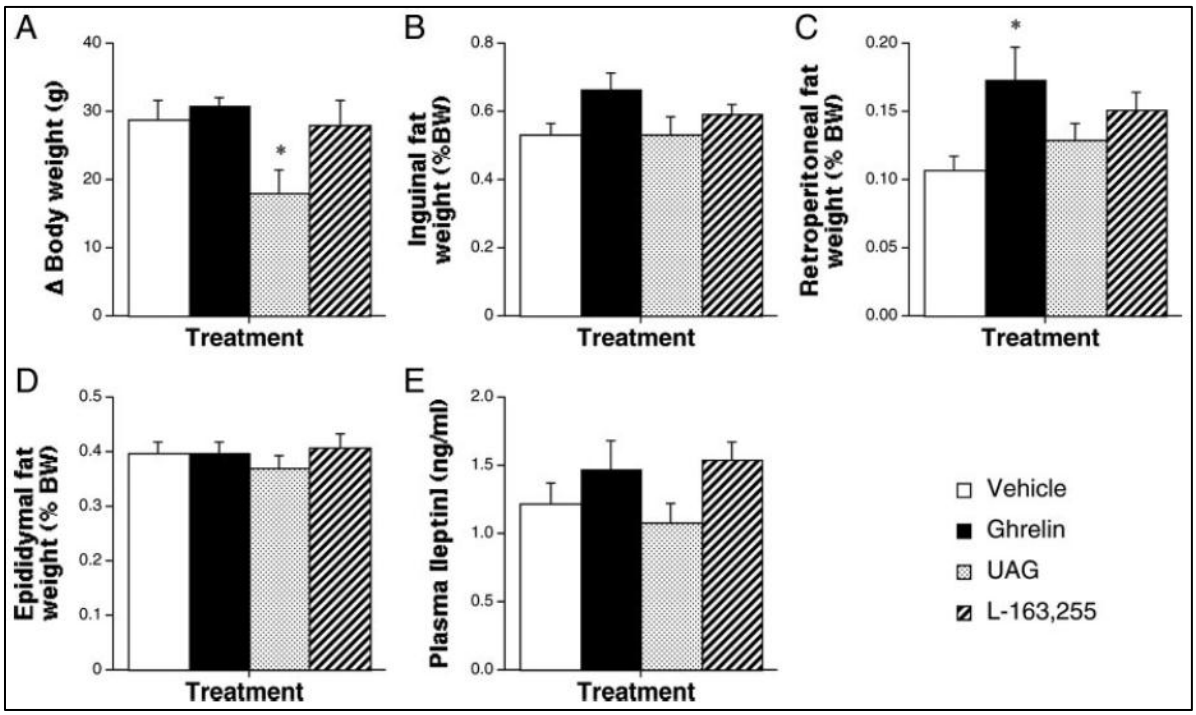

Figure 8: The effects on body weight and fat weight after one week continuous intravenous infusion of UAG in rats. The infusion of UAG does not affect any parameter of white adipose tissue adiposity, while it reduces bodyweight significantly.[61]

Zhang W et al. also demonstrated in FABP4-ghrelin transgenic mice a decrease in epididymal fat pad and perirenal fat pad compared to the wild-type controls.[74] However, Davies JS showed after a one week continuous intravenous infusion of UAG in rats no alterations in abdominal white adipose tissue distribution. Although UAG decreases plasma free fatty acids and diet-derived sterol esters in the circulation, UAG did not affect any parameter of white adipose tissue adiposity as shown in Figure 8.[61] Furthermore, no difference in brown fat tissue was found.[74]

UAG suppresses genes that encode regulatory enzymes involved in adipogenesis, lipogenesis and sterol synthesis in white adipose tissue. In the liver UAG has little effect on lipogenic pathways. It up-regulates genes than encode for components of lipid metabolic pathways. This indicates an overall improvement in metabolic profile.[75]

Discussion

The “hunger hormone” ghrelin is one of the hormones known to influence energy regulation and is therefore a potential target to fight obesity. Ghrelin occurs in different forms. The two major forms known are acylated ghrelin (AG) and unacylated ghrelin (UAG).[9] For a long time UAG was seen as the inactive form of ghrelin, while nowadays experiments show that it is an active protein with effects on its own and possibly with antagonizing effects on AG.[13] AG has influence on energy metabolism, glucose metabolism, lipid metabolism etc. But the role of UAG is not always clear. This study gives an overview of the effects of AG and UAG on the lipid metabolism.

AG and UAG are from the same origin, the ghrelin gene.[17] After some developments mature ghrelin is formed. This mature ghrelin cannot activate the GHSR, it needs to be acetylated. This acetylation in the ER is catalyzed by Ghrelin O-acyltransferase.[10] There is evidence for the presence of a ghrelin de-acylase, which acts both intracellular and extracellular.[30] The activity of ghrelin depends on the shape of the hormone, which depends on GOAT and ghrelin de-acylase. Thus, the activity of ghrelin is dependent on various factors.

Despite of the fact that UAG has antagonizing effects on AG, they do not act through the same receptor. AG acts via the GHSR-1a and UAG presumably via the CRF2-receptor.[57] Both hormones acts via vagal efferent pathways. However, AG activates NPY and AgRP and inhibits POMC and UAG activates CART.[39,68,69] In the leptin pathway activates leptin POMC/CART neurons, that decreases food intake.[76] Furthermore, both POMC and CART stimulate α-Melanocyt Stimulating Hormones (α-MSH).[77]

Infusions of AG lead to significant increases in food intake, while infusions of UAG produce inhibitory effects on feeding behavior. Anorectic patients have higher fasting plasma AG levels and higher fasting plasma UAG levels than the healthy controls.[51,52,71,72] In obese individuals are lower plasma UAG and AG levels found.[54,55,72] When anorectic individuals gain weight and obese individuals lose weight, plasma concentrations AG and UAG approach ‘normal’ values.[51,53,54,56,57,71,72] Additionally, AG is inversely correlated and UAG negatively correlated with body fat and BMI.[54,56,57] When UAG has antagonizing effects on AG it is not logical that both AG and UAG are negatively correlated with body fat and BMI. In contrast Rodriguez A et al. showed for the first time elevated plasma AG levels in obesity which could explain the high fat percentage.[9] The potential differences between the different ghrelin

forms under obese and anorectic conditions are controversial and remain largely undefined.[78,79]

AG influences on the regulation of lipid storage. It has no significant effect on the total white adipose tissue volume, but the inguinal and retroperitoneal white adipose tissue volume increase by continuous intravenous infusions of AG in rats. One week of continuous intravenous infusions of UAG in rats did not affect abdominal white adipose tissue distribution.[61] However, in the UAG-overexpressing FABP4-ghrelin transgenic mice is after a high-fat diet for 26 weeks a decrease in epididymal fat pad and perirenal fat pad demonstrated.[74] Possibly UAG shows only long-term effects during a high-fat diet because UAG, or high UAG levels, perhaps disturb the fat storage mechanism without reducing fat.

The concentration free fatty acids is reduced after continuous intravenous infusions of AG but surprisingly also by UAG.[61] Lower concentrations free fatty acids are often associated with elevated storage of fat. Infusions of UAG did not promote fat storage, but infusions of AG did. So possibly concentrations free fatty acids are results of different mechanism.

In line with the expectations of AG stimulating lipogenesis, Ghsr-/- knock-out mice show down regulation of glucose/lipid uptake genes and lipogenic genes, inhibition of lipid recycling and promoted lipid export in white adipose.[62] UAG suppresses genes that encode for regulatory enzymes involved in adipogenesis, lipogenesis and sterol synthesis in white adipose tissue.[75] Thus perhaps AG promotes and UAG suppresses lipid accumulation. This would be in line with the expectation of UAG having antagonizing effects on AG.

In contrast to white adipose tissue, the activity of glucose and lipid uptake and lipogenesis genes were elevated in the brown adipose tissue of Ghsr-/- knock-out mice, suggesting increased lipid uptake/syntheses.[72] Thus AG inhibits the formation of brown adipose tissue via the GHSR receptor. The thermogenic function of brown adipose tissue is at the expense of the energy-storing function of white adipose tissue. However, UAG has no noticed influence on brown adipose tissue.[74] So possibly AG and UAG do not keep each other in balance in brown adipose tissue.

In the liver has UAG little effect on lipogenic pathways. It up-regulates genes that encode for components of lipid metabolic pathways.[75] In the liver of rats is an enlarged lipid area shown after the AG treatment.[6]

In 2009, Kirchner H et al found an important physiological role of UAG. It seemed to be secreted in highly regulated fashion in response to caloric deprivation. In line with this they

found that GOAT is regulated by nutrient availability. Specific dietary lipids are used as acylation substrates and links ingested lipids to energy expenditure and body fat mass. Additionally Kirchner implicated the ghrelin-GOAT system as a signaling pathway that alerts the central nervous system to the presence of dietary calories.[80] This shows a whole new aspect of the ghrelin mechanism and further investigation could answer a lot more unanswered questions.

In conclusion, both AG and UAG have influence on the lipid metabolism of individuals. It was thought that UAG had antagonizing effects on AG. In certain perspectives this corresponds with studies about AG and UAG, but it cannot be applied to all results. In future experiments UAG needs to be further investigated. Furthermore, it is important to make a clear difference between AG and UAG in experiments and literature.

In this thesis the main focus was on the consideration which of the ghrelin forms, AG or UAG, would be most usable in the treatment against obesity. It seems that AG is an important factor in the development of obesity. AG is called the "hunger hormone", because after injection of it food intake elevates. Furthermore, it increases body weight and fat mass independent of food intake. Because it's easier to add a solution to a body than exiting a solution from it, an antagonist of AG could be a solution in the fight against obesity. UAG has antagonizing effects on AG, but effects of it are mediated by another mechanism than the mechanism of AG. However, the quantity of AG is dependent on the quantity of UAG, the more UAG the less AG and vice versa. By influencing the AG:UAG ratio, AG can possibly be overruled by UAG. Inhibition of activities of GOAT and/or addition of ATP1 will result in a smaller amount of AG and a larger amount of UAG. There is enough averment to say that understanding of the activities of both UAG and AG can bring solutions in the treatment against obesity. However, there is still further investigation needed.

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