Medical and surgical use of the gut in the treatment of obesity

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Cover design: Rosalie Kiewiet-Kemper & Optima Grafische Communicatie, Rotterdam
Lay-Out: Optima Grafische Communicatie, Rotterdam
Printed by: Optima Grafische Communicatie, Rotterdam

Publication of this thesis was financially supported by:
Allergan, Amgen, AstraZeneca, Eli Lilly, Ferring, Ipsen Farmaceutica, Novartis Pharma, Novo Nordisk, Pfizer, ProStrakan, Sanofi-Aventis, Vifor Pharma, Zambon.

Medical and surgical use of the gut in the treatment of obesity

Medisch en chirurgisch gebruik van de darm voor de behandeling van obesitas.

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 5 januari 2011 om 15.30 uur

door
Rosalia Marije Kiewiet-Kemper

geboren te Dordrecht

PROMOTIECOMMISSIE

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Introduction

Chapter 1

Obesity

1.1 INTRODUCTION

For centuries, obesity was a sign of wealth and well-being, and therefore a condition found in the happy few only. This, however, changed drastically in the 20th century. At the end of the 20th century obesity had grown into a worldwide epidemic that threatened to overwhelm both developed and developing countries,[1] which stimulated medical profession and politics to regard obesity as a serious health concern. In 1995, the World Health Organization accepted the Body Mass Index (BMI) as the appropriate method to discern healthy weight from overweight and obesity.[2] Despite being arbitrary, a BMI of 25 kg/m^2 is generally accepted as cut-off point for overweight, while obesity is defined as a BMI of 30 kg/m^2 or higher. Using these criteria, the International Obesity Task Force estimated that at least 1.1 billion adults are overweight world-wide.[3] In the Netherlands, 46.9% of adults were overweight in 2008,[4] while at least 10% was obese.[5]

1.2 COMPLICATIONS OF OBESITY

The major burden of obesity to both patients and public health is the significantly increased morbidity and mortality.[6, 7] Overweight and obesity are associated with large decreases in life expectancy. For example, a Dutch study based on the Framingham Heart Study shows that female and male forty-year-old non-smokers loose 3.3 and 3.1 years of life expectancy because of overweight, while obese subjects loose 7.1 and 5.8 years, respectively.[8] On average, each 5 kg/m^2 increase in BMI is associated with about 30% higher all-cause mortality.[6]

Diseases associated with obesity can be classified into two pathophysiological categories: co-morbidity due to an absolute increase in fat mass and co-morbidity due to metabolic changes resulting from excess fat mass.[9] The last category, dominated by cardiovascular disease and type 2 diabetes and, to a smaller extent, malignancy, accounts for the largest part of increase in morbidity and mortality.[3, 6] Although it is likely that many factors are still unknown, several pathophysiological mechanisms that account for the development of co-morbidity in obesity have been identified.

Type 2 diabetes is the disease with the strongest correlation with obesity: both insulin secretion and insulin sensitivity are negatively influenced by obesity.[9] Indeed, the risk of type 2 diabetes already increases from a BMI of 21 kg/m^2 and correlates strongly with BMI.[10] For example, the Nurses Health Study shows that at a BMI above 35 kg/m^2, the age-adjusted relative risk for diabetes increases to 4000%.[10] Additionally, weight gain is known to increase the risk of type 2 diabetes whereas after a moderate weight loss of 5-11 kg the risk decreases by nearly 50%.[11] Insulin resistance is induced by an increase in the amount of fatty acids that infiltrate tissues (e.g. liver, skeletal muscle) and by an increase in circulating toxic adipokines (e.g. interleukin-1 (IL-1), IL-6 and tumor necrosis factor α (TNFα)) produced by an increased amount of hypertrophic

adipocytes.[3, 12, 13] These cytokines promote a chronic inflammatory state and have a negative impact on cellular insulin sensitivity in peripheral tissues with increased intracellular lipids.[13] In addition to that, infiltration of fat into the pancreatic islet cells diminishes the islets' capacity to maintain the increased insulin output demanded by insulin resistance.[3] Finally, adiponectin, which has a strong insulin sensitizing effect, is known to be decreased in obesity.[14-16]

Hypertension and heart disease account for a large part in obesity associated morbidity and mortality as well. In a large meta-analysis and a large prospective study, hypertension was present in 38% and 55% of patients, respectively, and the risk of hypertension is up to five times higher among obese people.[17-19] BMI and mortality from ischemic heart disease are strongly positively correlated, and each 5 kg/m^2 increase in BMI is associated with 40% higher ischemic heart disease mortality.[6] Multiple factors contribute to the development of hypertension in obesity: increased angiotensinogen release from adipocytes, an increase in blood volume associated with greater body mass and an increase in blood viscosity as a result of increased release of procoagulant factors.[3] Obesity associated heart disease results from both cardiac failure due to altered hemodynamics, and coronary heart disease, which is mainly caused by obesity-induced dyslipidemia.[3]

Excess body weight is increasingly recognized as an important risk factor for several types of cancer. The mechanistic background of the observed association between malignancy and overweight is not fully understood, but this link is thought to be the result of changes in the insulin and Insulin-like Growth Factor (IGF) system, in sex steroids and in adipokines.[20] BMI is positively correlated with cancer mortality: an increase of 5 kg/m^2 accounts for 10% higher neoplastic mortality.[6] A large meta-analysis by Renehan et al. demonstrated that in men increased BMI was associated with an increased relative risk ratio (RR) in oesophageal adenocarcinoma (RR 1.52), thyroid (RR 1.33), colon (RR 1.24) and renal cancer (RR 1.24). A weaker but still significant correlation was shown between increased BMI and melanoma, multiple myeloma, rectal cancer, leukemia and non-Hodgkin lymphoma. In women, increased BMI was positively associated with endometrial (RR 1.59), gallbladder (RR 1.59) and renal (RR 1.34) cancer, and esophageal adenocarcinoma (RR 1.51). Weaker correlations were demonstrated in leukemia, non-Hodgkin lymphoma and thyroid, pancreas, colon and postmenopausal breast cancer.[21]

The main diseases resulting from increased fat mass are psychosocial and psychiatric disorders, obstructive sleep apnea and bone and joint disorders.

1.3 CAUSES OF OBESITY

The discussion on the causes of the epidemic is ongoing, especially on which environmental factors can be held responsible for this major change in average body weight. It is generally acknowledged that a decrease in physical activity in combination with relative overeating leads to a chronic positive energy balance, thereby causing an increase in body weight.[5] Indeed, in

the last decades of the 20th century the availability of automobiles, computers and mechanical aids removed the physical demands from daily life.[1] Additionally, feeding habits changed rigorously: food is easily available and generally high in energy density and low in satiating fibers, leading to high energy meals.[1]

Nevertheless, many wonder whether energy dysbalance in the present 'obesogenic society' is the only explanation for the increasing prevalence of obesity, since large inter-individual variability despite similar environmental factors still remains. Common observations that relatives display the same tendency to become obese suggest that inherited factors may play an important role as well. The importance of genetics has been confirmed in twin and adoption studies. Studies in adult identical twins reared apart show heritabilities up to 70%,[22, 23] while a recent study in children demonstrates a heritability of BMI of 77%.[24] On the other hand, adoption studies or general family studies give significantly lower results of 30-60%.[25] Surprisingly, the influence of a shared childhood environment effect is relatively low (10%)[24] or even absent.[23]

At present, several forms of monogenic obesity have been identified, all based on mutations in genes involved in the leptin-melanocortin pathway: leptin (*Lep*), leptin receptor (*Lepr*), proopiomelanocortin (*Pomc*), melanocortin 4 receptor (*MC4R*), neurotrophic tyrosine kinase receptor (*TRKB*) and single-minded homolog 1 (*SIM1*).[25-28] Mutations in these genes all result in severe, often childhood onset, obesity. Most mutations are extremely rare with the exception of the MC4R mutation: this is present in about 1% of obese adults and in 5.8% of severe childhood obesity.[25, 29]

On the other hand, polygenic obesity arises when an individual's genetic pattern is susceptible to an environment that promotes energy consumption over energy expenditure. This unfavorable genetic makeup is mostly based on single nucleotide polymorphisms (SNPs), and several genome wide association studies have been performed to identify involved genes.[30, 31] At present, common variants at two loci, FTO and MC4R, have been reproducibly shown to be modestly associated with BMI,[32, 33] but it is expected that many more will follow. In this respect, the recently formulated concept of nutrigenetics, which studies the role of genetic variation on interactions between diet and health, is a challenging new area. In the future, it could possibly provide us with personalized strategies to prevent or treat obesity.[34]

Additionally, in recent years the knowledge on adipose tissue, the digestive tract and the hypothalamus, and on their role in energy balance has increased dramatically. The adipokines (e.g. leptin and adiponectin), the gut hormones (e.g. ghrelin, peptide tyrosine tyrosine (PYY), glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK)) and the hypothalamic pathways involving neuropeptide Y (NPY) and agouti-related peptide (AgRP) constitute a complex mechanism that is designed to regulate short-term meal intake and long term body weight.[35] Therefore, it is hypothesized that deregulation of this system contributes to the development of obesity. Up to now, disruption of energy homeostasis as a cause of obesity has only been shown in the above-mentioned monogenic disorders interfering with downstream pathways of leptin signaling within the brain.[27, 28] Since dysfunction of this pathway mostly interferes with adequate food

intake, it is challenging to hypothesize that factors contributing to inter-individual variation in bodyweight are more likely to change food intake than to influence the efficiency with which ingested nutrients are stored or disposed, as was previously assumed.[28]

1.4 TREATMENT OF OBESITY

Since obesity is regarded as a physical and psychological burden to most patients, establishing effective treatment modalities for this condition has the highest priority. Although patients generally regard weight reduction as their primary goal of therapy, reduction of (the risk of) co-morbidity is equally important. Therefore, effective anti-obesity treatment should be able to induce significant and persistent weight loss, resulting in improvement of present co-morbidity and reduction of the risk to develop obesity-associated diseases. At present, three different treatment modalities have been proven to be more or less effective: lifestyle modification, pharmacotherapy and bariatric surgery.

1.4.1 Lifestyle intervention

Mammals, including men, possess a powerful and complex orexigenic system to protect them in periods of food deprivation.[35] However, there appears to be no effective counter-regulatory mechanism to protect individuals from caloric overabundance, a condition that is present in large parts of the world. Therefore, a decrease in physical activity in combination with relative overeating is regarded as the central cause of obesity.[5] Based on this hypothesis, the cornerstone of anti-obesity treatment should be dietary modification (i.e. reduced-calorie diet, regardless of macronutrient composition)[36] together with increased physical exercise.[1, 37]

Lifestyle intervention is proven to be effective in establishing moderate but relevant weight reduction,[38] thereby resulting in improvement in insulin sensitivity, blood pressure and lipid profile.[39-41] Physical activity acts directly by improving metabolic parameters and indirectly by promoting weight reduction.

One of the main concerns of lifestyle changes is its poor long-term adherence.[40, 42] While treatment is effective on short-term, on long-term patients tend to revert to their former obesity promoting lifestyle, maintaining only part of the changes achieved or returning to their initial status before treatment. Active long-term follow-up seems to positively influence long-term adherence.[40, 43]

1.4.2 Pharmacotherapy

In the Netherlands, only orlistat is currently available for the treatment of obesity. Orlistat is a gastrointestinal lipase inhibitor that reduces dietary fat absorption by 30% by preventing the hydrolysis of ingested triglycerides.[44] A large meta-analysis has demonstrated that orlistat reduced weight by 2.9 kg more than placebo did.[45] Additionally, orlistat significantly reduced

waist circumference, BMI, blood pressure, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and fasting glucose.[45, 46] Incidence of type 2 diabetes was reduced in patients with impaired glucose tolerance. Unfortunately, data on morbidity and mortality are not available.[45, 46] As a result of its mechanism of action, the main side effects of orlistat are fatty stools, fecal urgency and oily spotting.[45, 46]

In the last decade two other drugs have been registered as a treatment for obesity: rimonabant and sibutramine. After a promising start, both have been withdrawn due to unacceptable side effects. Sibutramine was a centrally acting specific reuptake inhibitor for norepinephrine and serotonin, reducing food intake by enhancing satiety.[47, 48] However, it has recently been shown to increase cardiovascular death and was withdrawn in January 2010. Rimonabant was a selective blocker of the cannabinoid receptor CB1, thereby reducing appetite. Blockade of this receptor, however, appeared to be related to severe depression and the prevalence of suicide has been shown to be significantly higher in patients using rimonabant.[49] In 2008, the European Medicines Agency advised against the prescription of rimonabant.

In conclusion, the effects of pharmacological intervention on weight loss are limited. Additionally, results on morbidity and mortality are lacking, while two formerly registered drugs had unacceptable side effects. At present, it is advised to restrict the use of pharmacotherapy to patients with insufficient weight loss during participation in a lifestyle intervention program.[5]

1.4.3 Bariatric surgery

In the 1950s, surgery was introduced to treat obesity. Bariatric surgery is based on either restriction of food intake or malabsorption of ingested food.[50] The most frequently used restrictive procedure is gastric banding: a laparoscopic adjustable gastric band (LAGB) is placed around the stomach to reduce the gastric volume, thereby decreasing the amount of food possible to ingest. On the other hand, biliopancreatic diversion with duodenal switch (BPD-DS) induces malabsorption by bypassing the duodenum and jejunum by means of a newly formed anastomosis between stomach and ileum. Additionally, (Roux-en-Y) gastric bypass (GB) combines restriction and malabsorption. In this procedure, the stomach is divided into a small proximal reservoir accompanied by bypass of the remaining stomach, duodenum and proximal jejunum. The small bowel is divided as well and re-arranged into a Y-configuration, to enable outflow of food from the small upper stomach pouch, via a "Roux limb".[50] These three surgical techniques account for 90% of bariatric procedures performed worldwide.[51]

1.4.3.1 Effectivity

All bariatric procedures result in substantial and clinically relevant weight loss, with a mean of 55.9% to 61.2% of excess weight loss (EWL).[52, 53] In general, malabsorptive procedures are more effective in weight reduction than purely restrictive surgery. One year after surgery, EWL is 25% higher in favor of GB vs LAGB.[54] Indeed, pooled data of a large meta-analysis show average weight loss of 46.2% EWL after LAGB, 59.5% after laparoscopic GB and 63.6% after BPD.[52]

However, morbidity and mortality are slightly higher after laparoscopic GB than after LAGB. Biertho et al. reported major perioperative complication rates of 2.0% in laparoscopic GB versus 1.3% in LAGB, early postoperative major complication rates were 4.2% versus 1.7% respectively, and mortality rate was 0.4% versus 0%, respectively.[55] Recently, Flum et al. reported 30-day major complication (deep-vein thrombosis, venous thromboembolism, reintervention and failure to be discharged) rates of 1.0% in LAGB vs. 4.8% and 7.8% in laparoscopic and open GB, respectively.[19] The 30-day mortality rates were 0.0%, 0.2% and 2.1%, respectively.[19] These data show that bariatric surgery is highly effective in reducing weight accompanied by relatively low morbidity and mortality.

Nevertheless, the main parameter of efficacy of bariatric surgery is its effect on improvement of co-morbidity. A large meta-analysis by Buchwald et al. demonstrated that hypertension resolved in 61.7% of patients, while either resolution or improvement was present in 78.5%.[53] The same study showed that hyperlipidemia improved in at least 70% of patients.[53] These improvements are clearly of clinical relevance. Nevertheless, the beneficial effects of bariatric surgery on type 2 diabetes are most impressive. Notably, the ability to induce complete resolution of type 2 diabetes (defined as the ability to discontinue all diabetes-related medication) depends on the type of operative procedure: after BPD the resolution is 98.9%, after GB 83.7% and after LAGB 47.9%.[53]

At present bariatric surgery is by far the most effective long-term treatment of obesity. The Swedish Obese Subjects (SOS) study shows that after 2 years follow-up, weight loss in surgically treated patients (LAGB, laparoscopic GB and vertical banded gastroplasty (VBG)) was -23.4% vs. +0.1% in a contemporaneously matched conventionally treated control group, while after 10 years weight loss was -13.2% (LAGB), -16.5% (VBG) and -25.0% (laparoscopic GB) vs. +1.6%, respectively.[56] This difference in long-term weight change had significantly beneficial effect on co-morbidity. Recovery rate of type 2 diabetes after 2 and 10 years was 72% and 36% in the surgically treated group vs. 21% and 13% in the conventionally treated group.[56] Less impressive, but still significantly different was the recovery rate of hypertension: 34% and 19% in the surgically treated group vs. 21% and 11% in the conventionally treated group.[56] Finally, overall mortality in the surgically treated group was significantly lower with a hazard ratio of 0.76, as compared to the control group.[57] These favorable long-term results have been confirmed by Adams et al., who demonstrated that during a mean follow-up of 7.1 years, all-cause mortality decreased by 40% after surgery, as compared with that in a non-treated severely obese population. Cause-specific mortality in the surgery group decreased by 56% for coronary artery disease, by 92% for diabetes, and by 60% for cancer.[58]

1.4.3.2 Mechanism of action

While surgical procedures are based on food restriction, malabsorption, or both, it becomes increasingly likely that additional mechanisms are involved. Several observations, especially regarding the dramatic improvement in glycemic control after bariatric surgery, have

necessitated the search for alternative explanations. At first, type 2 diabetes often resolves within several days to weeks after GB, long before substantial weight loss has occurred. Secondly, GB and BPD have been shown to achieve greater glycemic improvement than other weight reduction interventions (either lifestyle intervention of LAGB) with equivalent weight loss. Finally, GB and BPD result in almost complete resolution of type 2 diabetes, despite the fact that patients are still overweight.[53, 59, 60] These observations have led to the hypothesis that the improvements in glycemic control, reduction in appetite and subsequent weight loss following GB and BPD result from changes in gut hormone profiles.[60, 61]

Several hypotheses regarding changes in gut hormone profiles mediating the effects of bariatric surgery have been postulated. For example, concentrations of the orexigenic gut hormone ghrelin, which is almost exclusively produced by the stomach, have been observed to remain extremely low after GB, although ghrelin concentrations are generally known to increase after weight loss. Since ghrelin is known to induce insulin resistance, a decrease in ghrelin concentrations could contribute to the improvement of insulin sensitivity after bariatric surgery.[60] Additionally, gastrointestinal bypass could lead to expedited delivery of nutrients to the lower bowel, resulting in early secretion of GLP-1 and PYY. Both peptides induce satiety, and GLP-1 additionally stimulates food-dependent insulin secretion.[60, 62]

All hypotheses regarding changes in gut hormone profiles after bariatric surgery demand confirmation. Nevertheless, it is challenging that bariatric surgery seems to extend beyond mechanically restricting food intake and/or inducing malabsorption and that in the future it should be regarded as 'metabolic surgery'.

Chapter 2

Metabolic aspects of obesity:

ghrelin, obestatin and adiponectin

2.1 GHRELIN

2.1.1 Introduction

Ghrelin, a 28-amino acid peptide produced mainly by the stomach, was originally discovered as the natural ligand of the Growth Hormone Secretagogue Receptor type 1a (GHS-R1a).[63] Its unique molecular structure is characterized by *n*-octanoylation of serine at position 3 (acylated ghrelin, AG), which is essential for binding to the GHS-R1a.[63] However, in vivo, most circulating ghrelin is unacylated (UAG), which was consequently thought to be devoid of any endocrine action.[64] Indeed, UAG does not share with AG its potent growth hormone (GH) stimulating effect,[63, 65, 66] but more recent studies have shown that UAG does have intrinsic biological effects.[67-70] However, a receptor through which UAG exerts its effects is not identified yet.

Despite being primarily identified as a potent GH stimulating factor, ghrelin has been demonstrated to have a wide spectrum of biological activities, such as stimulation of prolactin and adrenocorticotropic hormone (ACTH) secretion, promotion of gastric motility and acid secretion, and modulation of cardiovascular function.[71-75]

2.1.2 Regulation of energy homeostasis

The identification of the stomach as the principal site of production of the most important endogenous growth hormone secretagogue (GHS), having its main effect in the pituitary region, was surprising.[63] It was therefore hypothesized that ghrelin functioned as an endocrine link between the digestive tract and the hypothalamus-pituitary system. Indeed, ghrelin was demonstrated to play an important role in energy balance. Acute administration of ghrelin to rodents induced an increase in food intake and body weight.[76, 77] In agreement, human subjects experienced appetite after administration of ghrelin.[78] Eventually, ghrelin was shown to display a preprandial rise, followed by a sharp decrease after food intake, supporting the hypothesis that ghrelin plays a physiological role in meal initiation in humans.[79-81] In conclusion, ghrelin was found to be one of the most powerful orexigenic and adipogenic agents known in mammalian physiology.

Ghrelin functions as a short-term meal regulator, but on the other hand, ghrelin concentrations are affected by long-term energy homeostasis. At first, excess ghrelin concentrations were thought to cause obesity. However, studies comparing plasma ghrelin concentrations in obese and normal weight subjects showed opposite results: obesity was associated with low ghrelin concentrations.[82] Additionally, diet induced weight loss resulted in an increase of ghrelin concentrations.[83, 84] Therefore, low ghrelin concentrations in obesity rather seem compensatory than causative.

In contrast to other potent orexigenic agents, such as NPY and AgRP, which are solely active when administered intracerebroventricular, ghrelin exerts an orexigenic and adipogenic effect when administered both in the brain and peripherally.[76, 85] The exact position of ghrelin within the extremely complex network of the regulation of energy balance in which the hypothalamus

plays a central role in appetite regulation, is not completely elucidated yet. Transfer of peripheral signals to hypothalamic activation is most likely mediated in the ventromedial arcuate nucleus, where neurons co-expressing NPY-, AgRP- and GHS-R are demonstrated.[86] Indeed, the arcuate nucleus is not protected by the blood-brain barrier.[87] Finally, it remains to be demonstrated whether ghrelin solely exerts its adipogenic and orexigenic effect through the GHS-R1a or that another, not yet identified receptor is involved as well.

2.1.3 Glucose/insulin metabolism

So far, it is not known which mechanism is responsible for the increase during fasting and the postprandial decrease in ghrelin concentrations. The main focus of ghrelin production being the stomach suggests food to inhibit ghrelin secretion after a meal. Indeed, ingestion of carbohydrates strongly suppresses ghrelin secretion, in a larger extent than protein and fat do.[88] This inhibitory effect of glucose on ghrelin is at least partly mediated by insulin, since insulin as well was demonstrated to have a direct negative effect on ghrelin concentrations during hyperinsulinemic euglycemic clamps in humans.[89]

Vice versa, ghrelin is reported to have an impact on insulin secretion and glucose homeostasis as well. In humans, peripheral injection of AG was followed by an acute and significant increase in glycemia.[90, 91] Since the effects of AG on glucose and insulin concentrations lasted significantly longer than the short transient GH peak, it was suggested that this effect was GH-independent.[90] Indeed, *in vitro* AG was shown to hamper the inhibitory effect of insulin on gluconeogenesis in a hepatoma cell line. Additionally, AG was shown to induce a rapid increase in glucose and insulin concentrations in GH deficient subjects.[68, 91]

The effect of UAG on glucose and insulin metabolism is less clear. Since UAG is not able to bind to the GHS-R1a, it was assumed not to have any endogenous effect on glucose and insulin, which was initially confirmed in a human study.[65] However, UAG appeared to be able to counteract the decrease in insulin sensitivity induced by AG in GH deficient subjects. Acute co-administration of AG and UAG in a 1:1 ratio was even demonstrated to significantly improve insulin sensitivity.[91] Additionally, continuous intravenous administration of UAG was shown to decrease glucose concentrations without affecting insulin concentrations, which suggests an increase in insulin sensitivity.[92]

In conclusion, available results suggest that AG and UAG, although being derived from the same molecule, are able to modify each other's actions on glucose homeostasis. The receptor to which UAG is able to bind, and that might mediate AG's effect on glucose and insulin metabolism as well, needs to be identified.

2.1.4 Ghrelin, aim of the thesis

2.1.4.1 Chapter 4

Obesity is a condition characterized by insulin resistance eventually leading to type 2 diabetes.[93] Subjects suffering from obesity usually display very low GH concentrations.[94] Since the study by Gauna et al. reported a significant improvement in insulin sensitivity after co-administration of AG and UAG to GH deficient subjects,[91] we evaluated whether this effect could be reproduced in obese subjects as well. Being able to improve insulin sensitivity in obese subjects may implicate a first step towards a new treatment modality for type 2 diabetes. Additionally, we intended to clarify the role of UAG in glucose and insulin homeostasis.

2.1.4.2 Chapter 5

Both AG and UAG are predominantly produced in the stomach but the pancreas produces both peptides as well.[95-97] This means that they are primarily secreted into the portal circulation and that they pass the liver before entering the systemic circulation. Since both AG and UAG are reported to have hepatic effects as well, we hypothesized that measuring portal insulin and glucose concentrations may be more informative than measurements in the systemic circulation. Therefore, we used a rat model in which both the jugular and the portal vein were cannulated, allowing us to simultaneously measure glucose and insulin concentrations in the systemic and portal circulation. In the present model we assessed whether blockade of endogenous AG action (by blocking the GHS-R1a), or administration of exogenous AG, UAG, or their combinations differentially affect glucose and insulin concentrations in the portal and systemic circulation after an intravenous glucose tolerance test (IVGTT).

2.2 OBESTATIN

2.2.1 Introduction

In 2005 Zhang et al. discovered a second peptide derived from the preproghrelin polypeptide.[98] Using a bioinformatic approach, they were able to identify a second conserved region in the ghrelin gene, encoding a 23 amino acid peptide, which they called obestatin.[98] Plasma ghrelin and obestatin appeared not to be strictly correlated and were even differentially regulated in fasted and fed conditions, which supported the hypothesis that obestatin had endogenous physiological effects.[98] This hypothesis seemed to be confirmed when obestatin was demonstrated to be the natural ligand of the G protein-coupled receptor 39 (GPR39).[98]

2.2.2 Anorexigenic effect

One of the most intriguing functions of obestatin was its anorexigenic effect in rodents. Acute intracerebroventricular and intraperitoneal administration of obestatin suppressed food intake,

while daily administration of obestatin suppressed body weight gain and induced delayed gastric emptying.[98] This implicated that obestatin and ghrelin, despite being derived from the same prohormone, were functional antagonists. However, the majority of subsequent studies were not able to replicate this anorexigenic effect.[99-104] Additionally, obestatin proved not to be the ligand for GPR39,[105-107] which was later indeed confirmed by the original authors.[108] Since positive studies on the inhibitory effect of obestatin on food intake are still reported as well,[109, 110] the discussion on this topic is not closed yet.

2.2.3 Glucose/insulin metabolism

Since AG is known to induce insulin resistance,[68, 90, 111] it could be hypothesized that obestatin does influence glucose and insulin homeostasis as well. Up to now, data on this subject are limited. Two previous studies have extensively evaluated the effects of obestatin administration on glucose and insulin levels in rodents.[109, 112] The effects they observed were small, if any. However, a problem that they may have encountered in evaluating the effect of obestatin on glucose and insulin metabolism is its short half-life.[87] Obestatin is mainly produced in the stomach and might accordingly exert its effect primarily in the portal system.[98] Therefore, assessment of systemic insulin and glucose concentrations may fail to demonstrate its effect.

2.2.4 Obestatin, aim of the thesis, chapter 6

To evaluate the acute effects of intravenously administered obestatin, we used the previously described rat model, which allowed us to simultaneously measure glucose and insulin concentrations in the systemic and portal circulation.[113, 114] The aim of this study was to evaluate whether obestatin plays a role in glucose and insulin metabolism, and if so, whether it acts as a functional antagonist of (acylated) ghrelin.

2.3 ADIPONECTIN

2.3.1 Introduction

Adiponectin (previously also known as Acrp30, AdipoQ or GBP28) is the most abundant adipokine, representing approximately 0.05% of total serum protein.[15, 115-117] It is exclusively produced by white adipose tissue (WAT).[115] In contrast to other adipokines like resistin and leptin that parallel fat cell mass, adiponectin concentration is decreased in obesity.[14, 15] Hypertrophic adipocytes in obesity have been shown to display decreased adiponectin action.[118]

Adiponectin's molecular structure shows striking homology with complement 1q (C1q).[15, 115] Corresponding to the complement 1q family, adiponectin forms trimers connected by disulfide bonds.[115] In circulation, adiponectin exists in three isoforms: a trimer (low molecular weight, LMW), a hexamer (trimer-dimer, medium molecular weight, MMW) and an oligomer (high molecular weight, HMW).[119] It has been suggested that HMW adiponectin is the active isoform.[119, 120]

Two receptors through which adiponectin exerts its effects have been identified: AdipoR1, which is ubiquitously expressed and mediates 5' adenosine monophosphate-activated protein kinase (AMPK) activation, and AdipoR2, which is mostly expressed in liver and mediates peroxisome proliferator-activated receptor α (PPARα) activation.[16]

2.3.2 Insulin sensitivity

Both functional and genetic studies on adiponectin strongly suggest that reduced adiponectin levels play a causal role in the development of insulin resistance, metabolic syndrome and type 2 diabetes.[118] Low circulating adiponectin levels correlate strongly with markers of insulin resistance and metabolic syndrome (e.g. systolic blood pressure, plasma glucose, HDL-cholesterol, triglyceride (TG) and Homeostasis Model Assessment for Insulin Resistance (HOMA-IR)) and low levels have been shown to be a strong risk marker for metabolic syndrome and type 2 diabetes, independent of obesity.[121-124] Additionally, mutations in human adiponectin resulting in low plasma concentrations or impaired multimerisation are related to type 2 diabetes.[125]

Adiponectin improves insulin sensitivity by reducing tissue TG content, thereby improving insulin signal transduction, by activating PPARα, which leads to fatty-acid combustion, and finally by activating AMPK, which induces β-oxidation and glucose uptake.[16] While adiponectin strongly improves insulin sensitivity, insulin on the other hand has been demonstrated to be a strong suppressor of adiponectin concentration.[126, 127]

In conclusion, it has been hypothesized that low adiponectin levels and high insulin levels display a vicious cycle in the early stages of obesity: obesity leads to low circulating adiponectin concentrations which results in increased insulin resistance. To overcome relative insulin insufficiency insulin levels will increase, which in turn decreases adiponectin levels even further.[126] Therefore, adiponectin might play a crucial causal role in the development of insulin resistance and type 2 diabetes in obesity.[16]

2.3.3 Adiponectin, aim of the thesis, chapter 7

Energy homeostasis and body weight are regulated by a highly complex network involving brain, digestive tract and WAT.[35] Circulating gut hormones (e.g. ghrelin, GLP-1, CCK) and adipokines (e.g. leptin and adiponectin) connect digestive tract and WAT with hypothalamic centers, thereby modulating food intake and energy expenditure.[35, 128, 129]

Signaling pathways connecting digestive tract and WAT are less known. Both ghrelin and adiponectin concentrations are decreased in human obesity, a condition characterized by insulin resistance.[93] Therefore, we used human obesity as a model to study the effects of acute intravenous administration of UAG and the combination of AG and UAG on adiponectin concentration, either directly or indirectly through changes in plasma insulin concentrations. Since HMW adiponectin has been suggested to be the most active isoform we measured both total and HMW adiponectin plasma concentrations.

Chapter 3

Outcome of surgical treatment of obesity: gallstones and quality of life

3.1 GALLSTONES

3.1.1 Introduction

Cholelithiasis is a common condition among the overweight and obese, and it is well known that obesity is a major risk factor for the development of gallstones.[130, 131] The Nurses' Health Study cohort demonstrated an age-adjusted RR for development of gallstones of 6.0 for women with a BMI > 32 kg/m^2, compared with women whose BMI was < 20 kg/m^2. The incidence rate of gallstones is linearly associated with BMI.[132, 133] Although the incidence of gallstones is high in obesity, most of the patients are asymptomatic and do not require treatment.[131, 134] In the general population, the mean likelihood of symptoms occurring by 5 years is 17%.[135] However, it is unknown whether these results can be extrapolated to the obese subpopulation.

The majority of gallstones (87%) in obesity appear to be cholesterol stones.[136] At least three physical conditions are necessary for the formation of cholesterol gallstones: unphysiologic cholesterol supersaturation of hepatic bile, presence of nucleating factors promoting cholesterol crystal precipitation, and gallbladder hypomobility causing stasis of bile.[131, 137] The mechanism of increased cholesterol stone formation in obesity is a combination of excessive hepatic cholesterol secretion accompanied by increased gallbladder volumes, and possibly decreased gallbladder contractility, facilitating precipitation of cholesterol into stones.[131, 138-140]

3.1.2 Gallstones after weight loss

While obesity is a major risk factor for the development of gallstones, rapid weight loss, induced by either dieting or bariatric surgery, further increases the risk. Additional to the above mentioned mechanism of increased cholesterol gallstone formation in obesity, weight loss induces a further increase in cholesterol clearance into the gallbladder due to cholesterol mobilization from adipose tissue.[130, 139-141] Furthermore, it has been suggested that reduced food intake, especially after bariatric surgery, causes less frequent and less effective stimulation of gallbladder contraction, resulting in bile stasis which facilitates gallstone formation.[140, 142] However, unchanged gallbladder kinetics have been observed by others.[139] Nevertheless, it has been established that the rate and amount of weight loss (> 1.5 kg/week, or > 24% of initial body weight) plays a crucial role in the development of gallstones.[130, 131, 140, 142-144]

Many studies have evaluated the incidence of gallstones after weight loss, especially when induced by bariatric surgery. Surprisingly, reported postoperative prevalence of asymptomatic gallstones or incidence of symptomatic gallstones after surgery varies widely. Screening for gallstones by ultrasound results in postoperative prevalences of 27% to 71%.[130, 140, 142, 145, 146] Symptomatic gallstones (i.e. patients requiring cholecystectomy) are reported in 3% to 40.5%.[130, 142, 145-153]

Since the rate of weight loss has been shown to be an important risk factor for the development of gallstones after bariatric surgery, it is likely that most gallstones develop in the first period after surgery. Stone formation has been reported as early as 6 weeks after surgery,[140]

with a mean time to detection of 8 to 14 months.[142, 151] Almost no gallstone formation has been reported beyond two years after surgery, which exactly matches the period of most rapid weight loss.[136, 151] However, this should be interpreted with care, since most studies describe a follow-up shorter than two years. When weight stabilizes at a significantly lower level, cholesterol saturation of bile returns to normal, allowing spontaneous stone dissolution in some cases.[130, 131, 154]

3.1.3 Gallstones, aim of the thesis, chapter 8

Several different management strategies concerning the risk of gallstone formation after bariatric surgery have been advocated: concomitant cholecystectomy in all patients, wait-and-see policy, or prophylactic treatment with ursodeoxycholic acid to prevent gallstone formation. Realistic choices in management can only be made when exact figures concerning incidence and prevalence of gallstones after surgery are available, especially concerning incidence of symptomatic cholelithiasis.

Therefore, we evaluated a population of previously morbidly obese patients, who had been treated by LAGB 1.3 to 8.5 years earlier, for the prevalence of symptomatic and asymptomatic gallstones. None of the patients underwent prophylactic cholecystectomy, and ursodeoxycholic acid was not prescribed, which enabled us to study long-term natural history of gallstone disease after surgically induced weight loss. Additionally, we compared the prevalence of gallstones in this population with a morbidly obese population on a waiting list for bariatric surgery. Finally, the presence of other risk factors for development of gallstones besides rapid weight loss was assessed as well to evaluate whether individuals at high risk could be identified.

3.2 QUALITY OF LIFE

3.2.1 Introduction

Severity of disabling conditions is generally described in objective criteria. However, these criteria bear limited relation to how patients are feeling and how much impact the disease has on their daily life. Therefore, it might be useful to evaluate severity of disease in terms of quality of life (QoL). QoL refers to the overall effects of medical conditions on physical, mental, and social functioning and well-being as subjectively evaluated and reported by the patient.[155, 156] The most reliable and reproducible manner to quantify highly subjective QoL is by the use of standardized and validated questionnaires, which are either generic (applicable to the general population) or disease-specific.[157-159]

In individuals suffering from obesity, QoL is typically severely impaired compared to the general population.[156, 160-162] As discussed previously, individuals suffering from obesity are prone to develop a wide variety of serious health consequences, leading to increased disability, morbidity, and mortality. Additionally, the prevalence of psychiatric disorders, mainly

depression and anxiety disorders, is very high among obese subjects, with reported rates between 20 to 50%.[162-165] The high prevalence of both serious physical and psychological impairment seems to be an acceptable explanation for the observed deterioration of QoL. However, in this respect, obesity does not necessarily differ from other serious chronic conditions. Nevertheless, patients suffering from obesity are likely to rate their condition as more disabling than other major handicaps. Rand et al. studied a group of morbidly obese subjects who successfully lost weight after bariatric surgery and described that all patients would prefer to be normal weight with a major handicap (e.g. deafness, heart disease, one leg amputated) than to be morbidly obese again.[166] All patients said they would rather be normal weight than a morbidly obese multi-millionaire.[166]

The most generally accepted explanation for the aggravated psychosocial dysfunction in obesity compared to other chronic conditions is the social stigmatization and discrimination obese individuals experience in society.[164, 167-170] As a result of this discrimination, overweight individuals are less educated, are less likely to be married, and have lower household incomes, while indeed other chronic conditions did not affect these outcomes.[169]

Not every individual suffering from morbid obesity experiences the same negative impact on QoL. In general, women, young individuals, and those with greater rates of comorbidity experience the greatest burden.[162-164] Additionally, as BMI increases, greater impairment in QoL is observed.[162, 171] Finally, treatment-seeking individuals appear to be more impaired than nontreatment-seeking individuals.[171]

3.2.2 Effect of bariatric surgery on QoL

Traditionally, results of bariatric surgery have been quantified in the amount of weight lost. However, as discussed above, changes in QoL might be a more important factor to the individual patient. During the last two decades, increasing attention has been paid to improvement in QoL after bariatric surgery. Virtually all studies report significant improvement after bariatric surgery, regardless of the surgical procedure.[156, 162, 165, 167, 172-180]

Significant improvement in QoL has been observed as early as 2 to 4 weeks postoperatively, while weight loss in this period is almost negligible.[174] The most important improvement in QoL is generally reported in the first year after surgery. Some studies even report normalization of QoL, although patients are still severely overweight.[156, 174, 178] The few available long-term follow-up studies, however, suggest that improvement in QoL levels off or even reverts toward preoperative levels starting from 2 years after surgery.[175, 179, 180] It remains to be established whether this is the result of waning optimism in a period of weight stabilization or disappointment about only limited improvement in everyday life.[167, 180] Additionally, it has been suggested that the decrease in frequency and intensity of clinical visits might play a role as well.[179] Finally, weight regain, which is observed especially in restrictive types of bariatric surgery, might be a causal factor as well.[181, 182]

3.2.3 Quality of life, aim of the thesis, chapter 9

To evaluate whether LAGB has beneficial effects on QoL in morbid obesity after long-term follow-up, we compared a previously morbidly obese population who had undergone LAGB at least five years earlier, with morbidly obese subjects on a waiting list for bariatric surgery. Additionally, the use of a generic questionnaire enabled us to compare the patient groups with Dutch community norm values, to evaluate whether QoL normalizes after surgical treatment for morbid obesity. Finally, determinants influencing QoL in morbidly obese patients having undergone LAGB were identified.

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Part I

Metabolic aspects of obesity: ghrelin, obestatin and adiponectin

Chapter 4

Effects of acute administration of acylated and unacylated ghrelin on glucose and insulin concentrations in morbidly obese subjects without overt diabetes

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European Journal of Endocrinology 2009; 161: 567-573

ABSTRACT

Objective

To investigate the effects of unacylated ghrelin (UAG) and co-administration of acylated ghrelin (AG) and UAG in morbid obesity, a condition characterized by insulin resistance and low growth hormone (GH) levels.

Design and Methods

Eight morbidly obese non-diabetic subjects were treated with either UAG 200µg, UAG 100µg in combination with AG 100µg (Comb), or placebo in 3 episodes of 4 consecutive days in a double-blind randomized crossover design. Study medication was administered as daily single i.v. bolus injections at 0900h after an overnight fast. At 1000h a standardized meal was served. Glucose, insulin, GH, free fatty acids (FFA) and ghrelin were measured up to 4 h after administration.

Results

Insulin concentrations significantly decreased after acute administration of Comb only, reaching a minimum at 20 min: 58.2 ± 3.9% of baseline, vs. 88.7 ± 7.2% and 92.7 ± 2.6% after administration of placebo and UAG, respectively ($P < 0.01$). After 1 h, insulin concentration had returned to baseline. Glucose concentrations did not change after Comb. However, UAG administration alone, did not change glucose, insulin, FFA or GH levels.

Conclusion

Co-administration of AG and UAG as a single i.v. bolus injection causes a significant decrease in insulin concentration in non-diabetic subjects suffering from morbid obesity. Since glucose concentration did not change in the first hour after Comb administration, our data suggest a strong improvement in insulin sensitivity. These findings warrant studies in which UAG with or without AG is administered for a longer period of time. Administration of a single bolus injection of UAG did not influence glucose and insulin metabolism.

INTRODUCTION

Ghrelin, a 28-amino acid peptide produced mainly by the stomach, was originally discovered as the natural ligand of the Growth Hormone Secretagogue Receptor type 1a (GHS-R1a).[1] Its unique molecular structure is characterized by *n*-octanoylation of serine at position 3 (acylated ghrelin, AG), which is essential for binding to the GHS-R1a.[1] However, *in vivo*, most circulating ghrelin is unacylated (UAG), which was consequently thought to be devoid of any endocrine action.[2] Indeed, UAG does not share with AG its potent GH-stimulating effect,[1, 3, 4] but more recent studies have shown that UAG does have biological effects.[5-8]

Despite being primarily identified as a potent GH-stimulating factor, ghrelin has been demonstrated to have a wide spectrum of biological activities, such as stimulation of prolactin and ACTH secretion, promotion of gastric motility and acid secretion, and modulation of cardiovascular function.[9-13] One of its most intriguing functions is the long-term and short-term regulation of energy balance. Continuous administration of ghrelin to rodents induces increased food intake resulting in weight gain, whereas in humans 24-h plasma profiles show marked preprandial increases and postprandial decreases in circulating ghrelin concentrations, which suggests an orexigenic effect.[8, 14-17] Since insulin displays an exactly opposite meal-related pattern, the interaction between insulin and ghrelin has been extensively studied. In general, it is assumed that insulin has a negative effect on ghrelin concentrations,[18, 19] whereas administration of AG results in insulin resistance.[6, 20-22] On the other hand, the effect of UAG on insulin metabolism is still a matter of debate.

Since the main biological difference between AG and UAG is its ability to bind to the GHS-R1a, the question arises whether this receptor and consequently GH release is involved in ghrelin effects on glucose and insulin metabolism. To answer this question, our group has previously studied the effects of administration of AG, UAG and a combination of AG and UAG in adult-onset GH-deficient subjects.[23] Surprisingly, the combination of AG and UAG strongly improved insulin sensitivity in these individuals, whereas AG as well as UAG alone was shown to increase glucose concentration at constant insulin levels.[23]

Since decreased insulin sensitivity plays a key role in the pathophysiology of type 2 diabetes, ways to improve insulin sensitivity could be beneficial to individuals prone to develop this disease. Obesity is typically associated with insulin resistance and, in a later phase, with type 2 diabetes.[24] Additionally, obesity is characterized by low GH levels, comparable with GH-deficient subjects.[25]

In the present study, we therefore evaluated the effects of UAG and co-administration of AG and UAG on glucose and insulin metabolism in individuals suffering from morbid obesity, a condition characterized by insulin resistance and low GH levels. As we were only interested in potential ways to improve insulin sensitivity, we did not study the effects of AG administration only, as this substance is known to worsen insulin sensitivity in all animal and human models studied so far.

MATERIALS AND METHODS

Study population

Eight morbidly obese female Caucasian subjects (age 45.4 ± 10.3 (mean ± SD), range 28-62 years, mean body mass index (BMI) 42.4 ± 4.8 kg/m^2) were recruited from an affiliated clinic for bariatric surgery. All were on a waiting list to undergo gastric banding or gastric bypass (criteria: BMI > 40 kg/m^2 or BMI > 35 kg/m^2 in combination with relevant comorbidity).[26] Exclusion criteria for the present study were: overt diabetes mellitus, liver enzyme test abnormalities, pregnancy and previous bariatric surgery. All subjects gave their written informed consent to participate in the study, which had been approved by the ethical committee of our hospital. Two participants were suffering from hypertension, for which they were treated with antihypertensive drugs. Six were healthy, not suffering from any relevant comorbidities.

Study design

The present double-blind randomized crossover study design consisted of three study episodes in which three treatment regimens were administered: i) UAG 200 µg (UAG), ii) UAG 100 µg in combination with AG 100 µg (Comb), and iii) placebo (placebo). Every patient underwent all treatment regimens, which were separated by a wash out period of at least 2 weeks. Every study episode consisted of 4 consecutive days. Study medication was administered as a single daily intravenous bolus injection.

After an overnight fast, an indwelling catheter was placed in the forearm and kept patent by a slowly running saline infusion. At 0900 h study medication was administered as an acute bolus injection. Blood samples were taken before administration of study medication and at regular intervals up to 240 min: at 10, 20, 30, 45, 60, 75, 90, 120, 180 and 240 min. Subjects were kept fasted during the first hour after administration of study medication. At 1000 h they received a standard breakfast containing 595 kcal (23 g protein, 27 g fat and 65 g carbohydrate), and at 1300 h, they received a standard lunch, comparable with breakfast. After lunch up to midnight, patients were free to choose their food intake.

Study medication

Both AG and UAG were obtained from Bachem AG, Bubendorf, Switzerland. To prevent degradation of ghrelin vials were stored at -80°C up to 90 min before administration. To prevent interaction of AG and UAG *in vitro*, two separate samples were administered to the patients, followed by 5 ml of saline after each infusion. Samples were blinded and randomized.

Assessments

Blood samples for total ghrelin and AG measurements were collected in EDTA tubes. Samples were stored on ice until centrifuging. After centrifuging, serum samples were stored at -20°C until processing. Acylated and total ghrelin levels were determined using a commercially

available RIA (Linco Research, St. Charles, MO, USA). Intra- and interassay variation of the AG assay are 7 and 13% respectively, and of the total ghrelin assay 6% and 16% respectively.

Both insulin and GH were measured using a chemiluminescent immunometric assay (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Intra- and interassay variation of the insulin assay are 4% and 5% respectively, while intra- and interassay variation of the GH assay are 4% and 6% respectively. Glucose was measured on a Hitachi 917 (Roche Diagnostics) by a glucose oxidase method. Free fatty acids (FFA) concentrations in the presence of tetrahydrolipstatin (final concentration 1 mg/L, prepared from Xenical capsules) were measured in EDTA plasma on a Hitachi 912 using the Wako Chemicals kit (Wako Chemicals GmbH, Neuss, Germany).[27]

Statistical analysis

Results are presented as mean ± SEM unless otherwise specified. $P < 0.05$ was considered significant. Differences between the three study periods were calculated using the Friedman test, the non-parametric equivalent of a one-sample repeated-measures design. To determine correlations between various parameters, a two-tailed Spearman's rank test was used. Areas under the curve (AUC) were calculated using the trapezoid rule.

Statistic calculations were performed using Statistical Package for the Social Sciences (SPSS release 14.0; SPSS Inc, Chicago, IL, USA).

UAG concentrations were determined calculating the difference between total ghrelin and AG. Glucose-to-insulin ratio was used as an estimate of insulin sensitivity.

RESULTS

Concentrations of AG and UAG

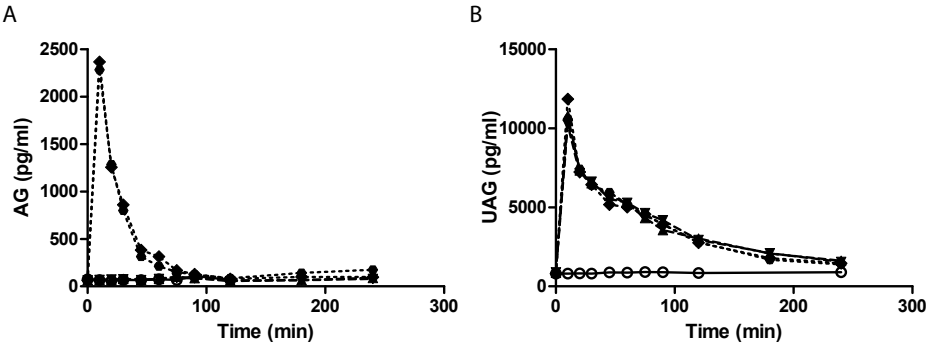
After acute administration of AG 100 µg i.v. (in combination with UAG 100 µg), baseline AG concentration of 64 pg/ml increased to a peak of 2325 pg/ml after 10 min. The half-life was short: AG concentrations returned to baseline 100 min after administration (Fig. 1A). Baseline concentrations of UAG were 844 pg/ml, increasing to 10499 pg/ml and to 11205 pg/ml 10 min after administration of UAG 200 µg i.v. alone and 100 µg i.v. in combination with AG 100 µg respectively. At termination of the measurements, 4 h after administration, UAG concentrations had not completely returned to baseline (Fig. 1B).

Effects of administration of UAG

During fasting, first hour after administration

Acute administration of UAG 200 µg did not induce any change in GH concentration.

Figure 1 Changes in plasma concentrations of acylated and unacylated ghrelin after administration of study medication.



(A) AG plasma concentration. (B) UAG plasma concentration

T_0, administration of treatment: placebo (—○—); UAG 200 µg day 1 (- -▲- -); UAG 200 µg day 4 (- -▼- -); UAG 100 µg + AG 100 µg day 1 (···♦···); UAG 100 µg + AG 100 µg day 4 (···●···).

Fasting baseline insulin concentrations were 166.8 ± 32.6 and 145.8 ± 30.4 pmol/l on day 1 and day 4 respectively. No changes in insulin concentrations were observed in the first hour after administration of UAG. Additionally, insulin concentrations after UAG administration were not different from placebo (Fig. 2A).

Figure 3A demonstrates corresponding results in glucose. Fasting baseline glucose concentrations were 4.4 ± 0.47 and 4.8 ± 0.4 mmol/l on day 1 and 4 respectively. Glucose concentrations did not change during the first hour after UAG administration and were not different from placebo.

UAG did not have any acute effects on FFA levels (data not shown).

After breakfast, 1 – 4 h after administration

As shown in figure 2C and 3B, UAG did not have any effects on glucose and insulin concentrations in fed conditions, starting 1 h after administration. Additionally, no effects on FFA metabolism were observed (data not shown).

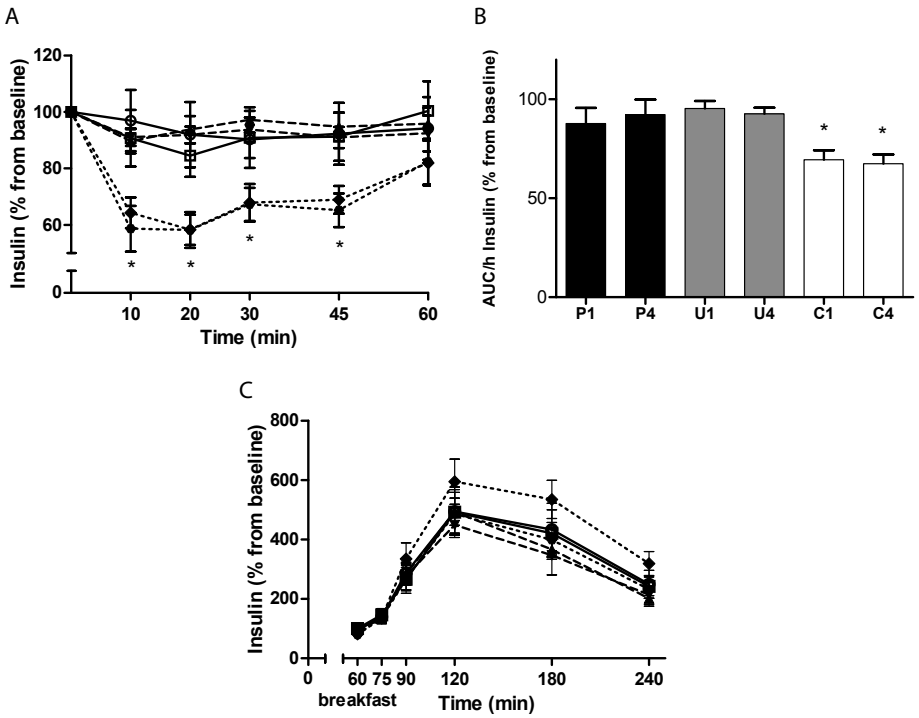
Effects of administration of UAG in combination with AG (Comb)

During fasting, first hour after administration

Administration of Comb induced a rapid and significant peak in GH levels. Maximum concentration of GH was reached at 20 min after administration: 20.9 ± 3.37 and 13.1 ± 2.70 µg/l on day 1 and 4 respectively, versus placebo 0.6 ± 0.12 and 0.6 ± 0.21 µg/l respectively, and UAG 0.6 ± 0.21 and 0.3 ± 0.08 µg/l respectively ($P < 0.001$, data not shown).

Insulin concentrations decreased strongly after acute administration of Comb, reaching a minimum at 20 min (Fig. 2A). Insulin concentrations at T_{20} were 58.3 ± 5.4 and 58.2 ± 6.3%

Figure 2 Serum insulin concentration



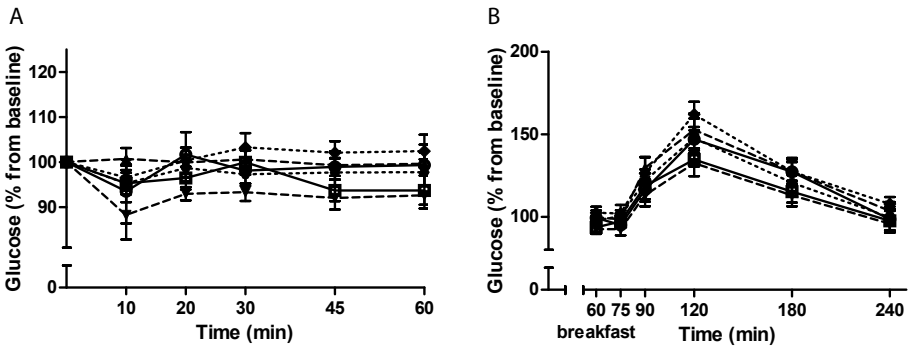
(A) First hour after administration of study medication. Concentration presented as % from baseline, before administration. T_0, administration of treatment: placebo day 1 (—○—); placebo day 4 (—□—); UAG 200 µg day 1 (- -▲- -); UAG 200 µg day 4 (- -▼- -); UAG 100 µg + AG 100 µg day 1 (···♦···); UAG 100 µg + AG 100 µg day 4 (···●···). * $P < 0.05$ Comb day 1 and 4 versus placebo day 1 and 4, UAG day 1 and 4.
(B) Area under the curve/hour of insulin concentration, presented as % from baseline, in the first hour after administration of study medication. Treatment: placebo day 1 (P1), placebo day 4 (P4), UAG 200 µg day 1 (U1), UAG 200 µg day 4 (U4), UAG 100 µg + AG 100 µg day 1 (C1), UAG 100 µg + AG 100 µg day 4 (C4). * $P < 0.05$ C1 and C4 versus P1, P4, U1 and U4.
(C) After breakfast. Concentration presented as % from baseline, before administration. T_0, administration of treatment. T_{60}, breakfast.

of baseline on day 1 and 4 respectively, whereas after administration of placebo and UAG on day 1 and day 4, insulin concentrations were 92.0 ± 11.6, 84.5 ± 7.4, 93.7 ± 4.8 and 91.8 ± 3.0% respectively ($P < 0.01$) Fig. 2B shows AUC/h, which demonstrates that insulin concentration is significantly lower throughout the first hour after administration of Comb, compared with both placebo and UAG ($P < 0.05$).

Comb administration did not have any effect on glucose concentration (Fig. 3A). Therefore, calculating glucose over insulin ratio resulted in a strong improvement in insulin sensitivity after Comb administration: at T_{20} insulin sensitivity is 184.3 ± 19.7 and 169.3 ± 16.7% of baseline on day 1 and 4, respectively.

Comb administration did not have any effect on FFA levels (data not shown).

Figure 3 Serum glucose concentration



(A) First hour after administration of study medication. Concentration presented as % from baseline, before administration. T_0, administration of treatment: placebo day 1 (—○—); placebo day 4 (—□—); UAG 200 µg day 1 (- -▲- -); UAG 200 µg day 4 (- -▼- -); UAG 100 µg + AG 100 µg day 1 (···♦···); UAG 100 µg + AG 100 µg day 4 (···●···).
(B) After breakfast. Concentration presented as % from baseline, before administration. T_0, administration of treatment. T_{60}, breakfast.

After breakfast, 1 – 4 h after administration

After breakfast, the suppressing effect of Comb on insulin concentration could not be observed anymore. Insulin concentration after Comb administration was not significantly different from either placebo or UAG (Fig. 2C). However, no rebound effect was observed as well. Again, no effects on glucose (Fig. 3B) and FFA metabolism were observed (data not shown).

Tachyphylaxis

We did not observe any change in effects after repeated administration of study medication, especially no reduction of improvement in insulin sensitivity after Comb administration. Results on day 1 were not different from day 4 in the UAG period as well as in the Comb period.

Correlations with change in insulin sensitivity

None of the subjects studied was suffering from diabetes mellitus, but nevertheless both baseline insulin concentration as well as 2-h postprandial insulin concentration had a high interindividual variability. Baseline insulin concentration in the placebo period varied from 72.9 to 365.8 pmol/l, whereas 2-h postprandial insulin concentration varied from 222.5 to 1513.8 pmol/l. Additionally, GH responses to Comb administration varied strongly as well, with a GH peak range 20 min after administration of 9.3 – 31.2 µg/l. To evaluate which individuals would benefit the most of the positive effect of Comb on insulin sensitivity, a correlation study was performed. Neither baseline and postprandial insulin concentrations nor GH response showed any correlation with change in insulin sensitivity after Comb administration.

Side effects

Three patients experienced a short episode of flushing and dizziness shortly after administration of Comb. They all developed this mild and self-limiting side effect on one day, randomly in 4 days during the Comb study period.

DISCUSSION

This study demonstrates that co-administration of AG and UAG induces a strong decrease in insulin concentration in morbidly obese subjects without overt diabetes. A single injection of AG + UAG resulted in almost 50% reduction of insulin concentration with unaffected glucose levels, suggesting a strong improvement in insulin sensitivity. During repeated administration, no tachyphylaxis was observed. Broglio et al. previously demonstrated that in healthy young men UAG was able to counteract the insulin resistance induced by AG alone.[3] Additionally, co-administration of AG and UAG was shown to improve insulin sensitivity in GH-deficient patients.[23] Nevertheless, the present study population is the first that could actually benefit from a treatment able to improve insulin sensitivity. Since obesity induces insulin resistance and consequently causes diabetes mellitus, the present findings could lead towards a new approach in treating diabetes.

The observed decrease in insulin concentration after acute injection of AG + UAG with unaffected glucose levels suggests an improvement in insulin sensitivity, as stated above. However, glucose/insulin ratio is only partially correlated with the variation in insulin action and insulin sensitivity, since insulin levels also depend on secretion, distribution and degradation of insulin.[28] Nevertheless, in the present study, we at least replicated the effect of AG + UAG on insulin concentration as previously observed in our study in GH-deficient subjects.[23] Therefore, future studies evaluating the effect of AG + UAG on insulin sensitivity using an euglycaemic insulin clamp are warranted and indicated.

Co-administration of AG and UAG affected insulin concentration in the first hour after administration only. The most likely explanation of this short-lived effect is the observed short half-life of AG and, to a smaller extent, UAG. Additionally, plasma concentrations of UAG were comparable 10 min after administration of UAG 200 µg and UAG 100 µg + AG 100 µg respectively. Therefore, the AG plasma peak concentration must have been significantly earlier than 10 min, followed by a rapid degradation of AG to UAG. Since subjects were fasted during the first hour of the study protocol and insulin concentrations had returned to baseline at breakfast, no conclusions can be drawn about the acute effect of AG + UAG on insulin sensitivity in fed conditions. Nevertheless, at least no rebound effect was observed after breakfast.

In considering co-administration of AG and UAG as a treatment of insulin resistance, it is important to be aware of the risk of tachyphylaxis. To date, no data are available on the long-term effects of AG and UAG administration. In the present study, AG + UAG was administered on

4 consecutive days, while no decrease in effect was observed. We found the sustained effects of the combination of AG and UAG after 4 days of once-daily administration suggesting the absence of acute tachyphylaxis reassuring.

Shortly after the discovery of the orexigenic effect of ghrelin, it was hypothesized that obese subjects would have elevated ghrelin concentrations that could contribute to the pathogenesis of obesity.[29] On the contrary, total ghrelin concentrations were found to be decreased in obesity.[29] More recent studies, however, have assessed both AG and UAG levels and AG/UAG ratios. UAG, but not AG, is decreased in obesity, while insulin-resistant obese subjects display a higher AG/UAG ratio than equally obese insulin-sensitive subjects.[30, 31] These data suggest that relatively high AG levels combined with lower UAG levels might contribute to insulin resistance in obesity. In the present study, however, we administered UAG and AG in a 1:1 ratio, which is much higher than *in vivo* where UAG/AG is about 9:1.[32] Since this 1:1 ratio was previously observed to improve insulin sensitivity,[23] we decided to continue using these concentrations. Nevertheless, future studies are needed to evaluate the effect on insulin resistance of co-administration of AG and UAG in different proportions.

Since the present study did not evaluate the effects of AG in morbidly obese subjects, it could be discussed that the observed decrease in insulin concentration is the result of AG alone more than of the co-administration of AG and UAG. Three studies have evaluated the effect of ghrelin administration in obesity. One study did not show any change in glucose and insulin concentrations,[33] while two studies reported an increase in glucose concentration with a slight decrease in insulin levels.[9, 21] These results are not in accordance with the present findings that show a highly significant decrease in insulin concentrations without a reciprocal increase in glucose concentrations. This difference suggests that the present findings do result from the co-administration of AG and UAG more than of AG alone, which is supported by the study in GH deficient subjects as well.[23]

In the present study, UAG administration had no effect on glucose and insulin levels despite the presence of pharmacological concentrations. It is still unclear whether acute changes in UAG levels do have intrinsic effects on glucose and insulin concentrations. Some reports on acute effects of UAG described an increase in glucose levels,[23] while other studies, like the present, did not observe any effect.[3] However, continuous administration of UAG, on the contrary, seems to improve insulin sensitivity.[34] Therefore, possible explanations for the observed effects of co-administration of AG + UAG remain speculative. Since UAG is not able to bind to the GHS-R1a, it is not likely that antagonism on this receptor plays a role. Additionally, GHS-R1a does not mediate ghrelin's effects on hepatic glucose output by primary porcine hepatocytes.[6] Whether a yet unidentified receptor to which both AG and UAG are able to bind mediates these effects needs to be studied.

Our study clearly opens new perspectives in the approach of insulin resistance in obesity. As mentioned before, euglycaemic insulin clamp studies are needed to evaluate whether the present changes in glucose and insulin concentrations are mainly the result of improvement

in insulin sensitivity, as currently expected. Further research is needed to evaluate whether the present findings can be extrapolated to fed conditions. However, attention must be paid to the possible adverse effects of continuous administration of AG, such as its impact on adipogenesis and food intake.[17] Finally, the effects of co-administration of AG and UAG in subjects suffering from diabetes should be studied.

In conclusion, the present study demonstrates that co-administration of AG and UAG in a 1:1 molar ratio in fasted morbidly obese subjects without overt diabetes, strongly decreases insulin concentrations at unchanged glucose levels, suggesting an improvement in insulin sensitivity. Further studies are needed to provide information on the effects in fed conditions and in diabetic subjects.

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Chapter 5

Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditions

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American Journal of Physiology – Endocrinology and Metabolism 2007; 293: E697-E704

ABSTRACT

Acylated and unacylated ghrelin (AG and UAG) are gut hormones that exert pleiotropic actions, including regulation of insulin secretion and glucose metabolism. In this study, we investigated whether AG and UAG differentially regulate portal and systemic insulin levels after a glucose load. We studied the effects of the administration of AG (30 nmol/kg), UAG (3 and 30 nmol/kg), the ghrelin receptor antagonist [D-Lys^3]GHRP-6 (1 µmol/kg), or various combinations of these compounds on portal and systemic levels of glucose and insulin after an intravenous glucose tolerance test (IVGTT, D-glucose 1 g/kg) in anesthetized fasted Wistar rats. UAG administration potently and dose-dependently enhanced the rise of insulin concentration induced by IVGTT in the portal and, to a lesser extent, the systemic circulation. This UAG-induced effect was completely blocked by the coadministration of exogenous AG at equimolar concentrations. Similarly to UAG, [D-Lys^3]GHRP-6, alone or in combination with AG and UAG, strongly enhanced the portal insulin response to IVGTT, whereas exogenous AG alone did not exert any further effect. Our data demonstrate that, in glucose-stimulated conditions, exogenous UAG acts as a potent insulin secretagogue, whereas endogenous AG exerts a maximal tonic inhibition on glucose-induced insulin release.

INTRODUCTION

Ghrelin is a guthormone predominantly produced in the stomach and, to a lesser extent, in other regions of the gastrointestinal tract.[1-3] Ghrelin circulates in the bloodstream in two different forms: acylated (or *n*-octanoylated) and unacylated (or des-octanoylated or des-acylated).[2] Acylated ghrelin (AG) has a unique feature: a posttranslational esterification of a fatty (*n*-octanoic or, to a lesser extent, *n*-decanoic) acid on serine residue at position 3.[2] This acylation is considered necessary for AG's actions via the growth hormone secretagogue receptor type 1a (GHS-R1a), also called ghrelin receptor (GRLN-R).[2, 4] However, normally AG accounts for less than 10% of the total ghrelin in the circulation. The majority of circulating ghrelin is unacylated (UAG), which binds with high affinity to a receptor, different from GHS-R1a and yet unknown.[2, 5]

Both AG and UAG have pleiotropic activities, including regulation of insulin secretion and glucose metabolism. It has been shown that endogenous AG and UAG are also produced in the endocrine pancreas, which also expresses the GHS-R1a.[6-10] It has been found that endogenous AG in the pancreas inhibits the glucose-induced insulin release via the GHS-R1a,[7] as demonstrated by the marked increase of insulin response to glucose after blockade of endogenous AG (i.e., via receptor antagonism, anti-AG antiserum, deletion of the ghrelin gene).[1, 7, 11] Moreover, ablation of the ghrelin gene improved glucose tolerance, insulin secretion, and insulin sensitivity in genetically leptin-deficient (*ob/ob*) obese mice.[11] Administration of exogenous AG suppressed further insulin secretion both in fasting and in glucose-stimulated conditions, and it worsened insulin sensitivity and glucose tolerance after a meal or a glucose load.[1, 11-13] UAG administration neither had effects on glucose-induced insulin release in a perfused pancreas model,[1] nor did it induce significant changes in systemic fasting levels of insulin and glucose in vivo.[1, 7, 13, 14] However, UAG increased insulin release in vitro by insulinoma cell lines exposed to high glucose concentrations,[15, 16] and overexpression of (endogenous) UAG in pancreatic islets improved the insulin sensitivity to an intraperitoneal glucose load in mice.[17] Moreover, when coadministered with AG, UAG completely prevented the AG-induced increase in circulating glucose levels and worsening of insulin sensitivity.[13, 18, 19]

Together, these data elucidate the role of AG in the negative regulation of insulin secretion, insulin sensitivity, and glucose metabolism. On the other hand, they show that an excess of endogenous UAG improves insulin sensitivity and suggest that UAG, or more likely the ratio AG/UAG, might be implicated in the modulation of insulin release. However, at present, the metabolic role of UAG remains to be defined. The reported effects of AG and UAG on glucose and insulin levels in vivo are based on measurements of systemic blood samples, whereas both AG and UAG are secreted into the portal circulation before they reach the systemic circulation. Moreover, these peptides also have hepatic effects. Therefore, we hypothesized that, concerning insulin secretion, assessment of insulin concentration in the portal vein might be more informative than that in the systemic circulation.

The aim of this study was to investigate whether the blockade of endogenous AG action (i.e., blockade of the GHS-R1a) or the administration of exogenous AG and UAG differentially regulates the portal and systemic insulin response to glucose and/or modulates hepatic insulin clearance. We therefore studied in rats the effects of the administration of AG, UAG, the ghrelin receptor antagonist [D-Lys^3]GHRP-6, or their combinations on portal and peripheral glucose and insulin levels during an intravenous glucose tolerance test (IVGTT).

MATERIALS AND METHODS

Materials

Plasma glucose levels were measured using a glucose oxidase method (Instruchemie, Delfzijl, The Netherlands). Rat insulin was measured using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). Total and acylated ghrelin were measured using radioimmunoassays (RIAs) from Linco Research (St. Charles, MO). Rat acylated and unacylated (des-octanoyl) ghrelin, as well as [D-Lys^3]GHRP-6, were obtained from NeoMPS (Strasbourg, France). Pentobarbital sodium (250 mg/5 ml) was prepared and provided by the hospital pharmacy (Erasmus MC, Rotterdam, The Netherlands). EDTA-containing tubes were obtained by Greiner Bio-One (Alphen aan den Rijn, The Netherlands). Silicone catheters (3-French size) were provided by UNO Roestvaststaal (Zevenaar, The Netherlands); suture needles (Dafilon 8/0) were by B. Braun Melsungen (Melsungen, Germany).

Animals

Male Wistar rats (age 10–12 wk, weight 350–400 g; Harlan Netherlands, Horst, The Netherlands) were housed in groups in a temperature-controlled room under a 12:12-h light-dark cycle and maintained on pelleted chow with free access to water. The animals were housed for at least 1 wk before the start of the experiments to allow for acclimatization. Animal protocols were in compliance with the principles of laboratory animal care and Dutch regulations on animal welfare and were approved by the institutional Animal Welfare Committee.

Surgery and Experimental Design

All studies were performed after a fasting period of 18 h (overnight). Studies were performed under anesthesia, and the rats were euthanized at the end of the experiment.

Animals were anesthetized using an intraperitoneal (ip) injection of pentobarbital sodium (60 mg/kg induction, 20 mg/kg maintenance administered at the end of the surgical procedure, before the start of the experimental session). Deep anesthesia was confirmed by the absence of reflexes. Animals were kept on a warming mat to maintain core body temperature and were connected to a breathing apparatus (O2, 1 l/min), to improve oxygenation, for the entire duration of the experiment (including surgical procedure).

The surgical procedure was performed under aseptic conditions, as follows.

Cannulation of the jugular vein. An incision was made just above the right clavicle, the connective and adipose tissues were pushed aside, and the jugular vein was exposed. After the jugular vein was mobilized, a catheter previously connected to a syringe and filled with saline solution was pushed inside the vessel until it reached the right atrium. Patency of the catheter was checked by aspirating blood and flushing the catheter with saline solution. The free end of the catheter was used for saline injection, treatment administration, and sampling.

Cannulation of the portal vein. A midline incision was made from the level of the symphysis pubis to the xiphoid cartilage. The intestines were lifted out and laid next to the animal on gauze moistened with warm saline solution to minimize dehydration. A purse-string (diameter 1 mm) was made in the wall of the portal vein, opposite the gastroduodenal vein. The center of the purse-string was cut, and the catheter was inserted into the portal vein and pushed in for a few millimeters with the tip secured 1 mm caudal to the liver. The patency of the catheter was checked by aspirating blood and injecting saline. The free end of the cannula was used for sampling procedure during the experiment.

Treatment Administration and Sampling

Rats (fasted overnight) were assigned to one of the following treatment groups:

1. Saline (1 ml), $n = 12$.
2. IVGTT, $n = 12$. IVGTT was performed by injecting D-glucose at a dose of 1 g/kg (50%, 1 ml maximal volume) through the jugular catheter. The dose of 1 g/kg was chosen taking into account the reduction of insulin sensitivity caused by abdominal surgery[20] and the possible interference due to anesthesia.[21, 22] Pentobarbital sodium was used, since compared with other anesthetics it has been shown to interfere less with insulin secretion and glucose metabolism in both the fed and the fasted conditions,[21, 22] in accord with our previous observations (unpublished data).
3. IVGTT + rat AG (30 nmol/kg), $n = 7$.
4. IVGTT + rat UAG (3 nmol/kg), $n = 6$.
5. IVGTT + UAG (30 nmol/kg), $n = 10$.
6. IVGTT + [D-Lys^3]GHRP-6 (1 µmol/kg), $n = 6$.
7. IVGTT + [D-Lys^3]GHRP-6 (1 µmol/kg) + AG (30 nmol/kg), $n = 6$.
8. IVGTT + [D-Lys^3]GHRP-6 (1 µmol/kg) + UAG (30 nmol/kg), $n = 7$.
9. IVGTT + AG (30 nmol/kg) + UAG (30 nmol/kg), $n = 7$.

After baseline samples had been taken from both catheters, treatments were administered through the jugular cannula at time 0, and samples were taken from both catheters at 1, 5, 10, 20, 30, and 50 min after treatment administration to measure glucose and insulin levels. At baseline, total and acylated ghrelin levels were also measured in 24 rats (before they were

assigned to different treatment groups). At every time point, the blood volume withdrawn from each catheter (350 µl) was replaced by an equal volume of saline solution.

Blood samples were collected using ice-cold EDTA-containing tubes, to which aprotinin (Trasylol, 500,000 KIE, 40 µl/ml) was added. Samples were immediately centrifuged, and plasma aliquots for AG measurements were acidified with 1 N HCl (1:10 vol/vol). All aliquots were kept at 4°C until the end of the experiment and then stored at –20°C. Multiple freeze-thaw cycles were avoided, and aliquots were thawed only for the ghrelin assay. This procedure has been indicated by Hosoda et al.[23] and by Groschl et al.[24] as a standard procedure for collection of blood samples to determine ghrelin concentrations.

At the end of each experiment the animals were killed by exsanguination under deep anesthesia.

Serum total ghrelin and AG levels (pg/ml) were measured using RIA kits that utilize 125I-labeled ghrelin as a tracer. The specificity for rat ghrelin (total and AG, respectively) is 100%. Total ghrelin is recognized by polyclonal rabbit antibodies raised against full-length ghrelin. This antibody recognizes intact and des-octanoyl ghrelin and ghrelin residues 14–28. The sensitivity of the assay is 93 pg/ml; the intra-assay coefficient of variation (CV) averages 6.4%, the interassay CV 16.3%. AG is recognized by a guinea pig anti-ghrelin specific for the ghrelin molecule octanoylated at its Ser3 residue. This antibody recognizes octanoyl ghrelin, intact and residues 1–10. Cross-reactivity with UAG is <0.1% and with ghrelin fragments (residues 14–28) is zero. The sensitivity of the assay is 7.8 pg/ml; the intra-assay CV is 7.4% and the interassay CV is 13.5%.

Insulin was measured using a rat insulin ELISA kit according to the manufacturer's instructions. The sensitivity of the assay is 0.07 µg/l.

Calculations

UAG. UAG levels were calculated by subtracting AG from total ghrelin concentrations at every time point either in the portal or in the peripheral (i.e., right atrium) vein samples.

Hepatic clearance. To estimate whether the liver might play a role in the clearance of ghrelin produced by the gut, we calculated the percentage of hepatic clearance by using a method originally proposed by Kaden et al.[25] The percentage of hepatic extraction of any given hormone is calculated as (hormone presented to the liver – hormone leaving the liver) x 100/(hormone presented to the liver). The ratio of the relative contribution of a "hormone presented to the liver" by the portal vein vs. the hepatic artery (concentration x flow) is assumed to be 3:1.[26] The percentage of portal hormone extraction is calculated as (hormone concentration in the portal vein – hormone concentration in hepatic vein) x 100/(hormone concentration in the portal vein). Since the contribution to posthepatic insulin levels due to tissues that do not drain in the portal vein is negligible, we assumed that the insulin gradient between portal vein and right atrium is a valid proxy of hepatic clearance, although in the right atrium insulin

concentration may be affected by a greater dilution (due to the ancillary venous return) than in the hepatic vein.

Results are expressed as absolute changes vs. baseline (means ± SE) and as areas under the curve (AUCs) (means ± SE).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows 10.0 (Chicago, IL). The one-way analysis of variance (ANOVA) was used to compare the several treatment groups for baseline levels and AUC of each parameter. The one-way repeated-measures ANOVA was used to verify whether, for each group and each parameter, there was an overall difference over the 50-min time course. An independent t-test was performed to compare two groups, whereas a paired t-test was also run to compare changes vs. baseline and jugular vs. portal values within each group. A difference was considered significant when $P < 0.05$.

RESULTS

AG and UAG Baseline Levels

The AG concentration in the portal vein was 1.7-fold higher than in the systemic circulation (108 ± 13 vs. 63 ± 5 pg/ml, respectively, $P < 0.001$), whereas the portal-peripheral gradient of UAG was 1.1 (1,449 ± 92 vs. 1,286 ± 71 pg/ml). The AG/UAG ratio was already very low in the portal vein, and it decreased further in the systemic circulation (0.075 ± 0.006 vs. 0.049 ± 0.003, respectively, $P < 0.01$).

Effects of IVGTT, Alone or Combined With Different Treatments, on Glucose and Insulin Levels

Baseline glucose and insulin levels were not significantly different among all groups both in the portal and in the systemic circulation (Table 1).

After saline injection (1 ml), insulin levels showed a small and transient decrease in both the portal and the peripheral circulation ($\Delta_{5\text{-}0}$, $P < 0.01$ and $P < 0.05$ vs. baseline, respectively; Fig. 1, A and C), whereas glucose levels did not show significant variations at any time point (Fig. 2, A and C, represent Δvariations during the time course; ΔAUCs are reported in Table 2).

As expected, IVGTT induced a prompt increase in insulin levels in both the portal and in the jugular samples. The insulin peak occurred at 1 min of our time course and was larger in the portal vein than in the systemic circulation (Fig. 1, A and C). Insulin levels were higher in the IVGTT than in the saline group during the whole time course (ΔAUC, $P < 0.0005$; Fig. 1, A and C). Of course, IVGTT promptly increased glucose levels, which were higher in the systemic than in the portal circulation and were reduced by the elevated circulating insulin, although they had not normalized yet after 50 min ($P < 0.0005$ vs. baseline and vs. saline; Fig. 2 and Table. 2).

Table 1. Baseline absolute levels of glucose and insulin in portal and in systemic circulation

	Glucose, mmol/l		Insulin, µg/l	
Groups	*Portal*	*Systemic*	*Portal*	*Systemic*
Saline ($n = 12$)	7.6±1.1	9.9±1.2	4.2±0.9	1.7±0.4
IVGTT controls ($n = 12$)	7.9±0.8	10.1±0.7	5.3±0.8	1.8±0.3
IVGTT + AG, 30 nmol/kg ($n = 7$)	10.2±1.0	10.1±0.8	4.6±0.1	1.6±0.3
IVGTT + UAG, 3 nmol/kg ($n = 6$)	9.1±1.2	9.5±1.4	3.6±0.4	1.7±0.4
IVGTT + UAG, 30 nmol/kg ($n = 10$)	6.2±0.8	7.7±1.1	4.3±0.8	1.4±0.2
IVGTT + AG + UAG ($n = 7$)	6.9±0.9	7.5±1.2	3.5±0.9	1.0±0.1
IVGTT + [D-Lys^3]GHRP-6 ($n = 6$)	9.8±1.0	9.8±1.0	3.1±0.6	1.3±0.4
IVGTT + [D-Lys^3]GHRP-6 + AG ($n = 6$)	9.5±1.9	10.5±1.2	2.9±0.7	1.1±0.2
IVGTT + [D-Lys^3]GHRP-6 + UAG ($n = 7$)	8.2±0.9	10.1±2.2	3.5±0.5	1.8±0.4

Values are means ± SE; n = no. of animals. IVGTT, iv glucose tolerance test; AG, acylated ghrelin; UAG, unacylated ghrelin. Baseline absolute levels of glucose and insulin were not significantly different among the treatment groups in either the portal or the systemic circulation.

Fig. 1. Unacylated ghrelin (UAG) dose-dependently stimulated the second-phase insulin response to an iv glucose load (IVGTT, 1 g/kg), whereas exogenous acylated ghrelin (AG) did not modify insulin levels. This insulin-secretagogue effect of UAG was much larger in the portal vein (*A* and *B*) than in the systemic circulation (*C* and *D*). *Left*: values during the time course relative to the baseline value, which was set as 0 (△). *Right*: △AUCs of all parameters after treatment administration. Vertical dotted line, treatment administration at $t = 0$. ◇, saline ($n = 12$); □, IVGTT ($n = 12$); ○, IVGTT + AG (30 nmol/kg; $n = 7$); ♦: IVGTT + UAG (3 nmol/kg; $n = 6$); ●, IVGTT + UAG (30 nmol/kg; $n = 10$). *$P < 0.01$ vs. IVGTT. Other P values are reported in the figure; differences were considered significant for $P < 0.05$.

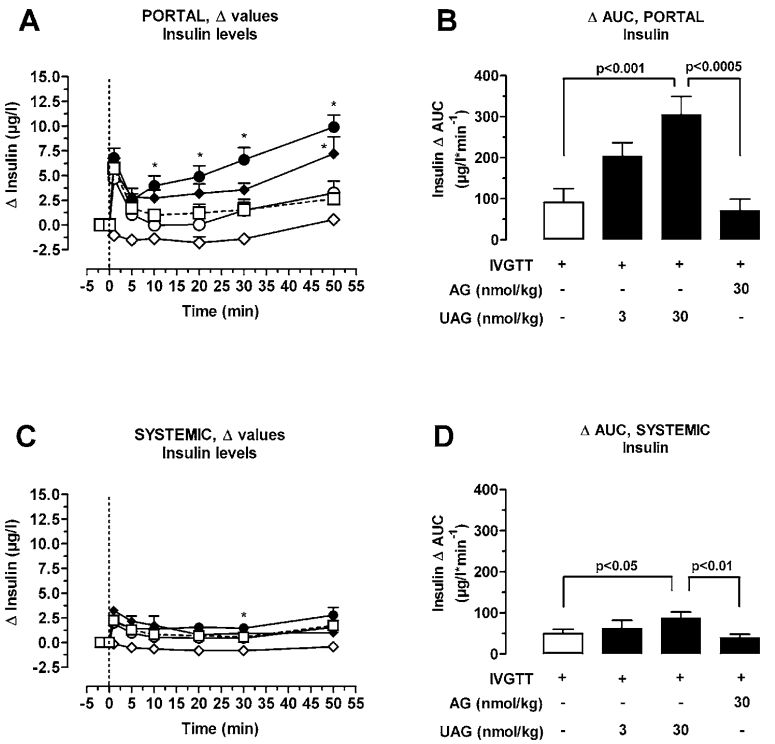
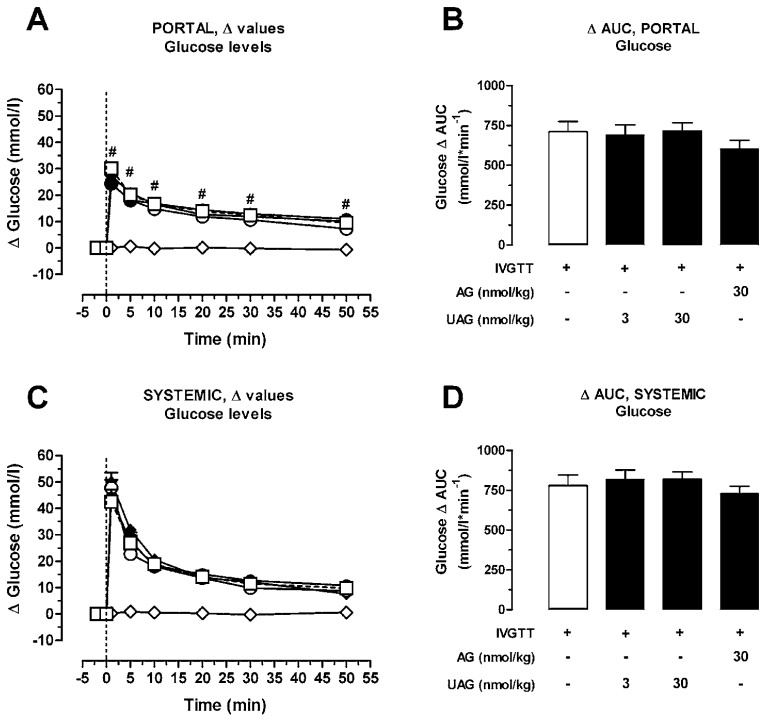


Fig. 2. Administration of exogenous AG (30 nmol/kg) or UAG (3 and 30 nmol/kg) did not modify glucose levels either in the portal vein (*A* and *B*) or in the peripheral circulation (*C* and *D*). *Left*: values during the time course relative to the baseline value which was set as 0 (△). *Right*: △AUCs of all parameters after treatment administration. Vertical dotted line, treatment administration at $t = 0$. ◇, saline ($n = 12$); □, IVGTT ($n = 12$); ○, IVGTT + AG (30 nmol/kg; $n = 7$); ♦, IVGTT + UAG (3 nmol/kg; $n = 6$), ●, IVGTT + UAG (30 nmol/kg; $n = 10$). #$P < 0.001$ vs. IVGTT. Differences were considered significant for $P < 0.05$.



The administration of exogenous AG (30 nmol/kg) did not change the insulin response to IVGTT significantly, although a small and transient decrease was recorded in portal, but not in systemic, insulin levels (Fig. 1, A–D). Moreover, the administration of AG did not modify glucose levels (excursion curves and ΔAUCs) after IVGTT either in the portal or in the systemic samples (Fig. 2 and Table 2).

Administration of UAG dose-dependently increased the second-phase insulin response to IVGTT in the portal vein. In fact, after peaking at 1 min, insulin decreased and started gradually to rise again at 10 min and reached the highest level at 50 min ($\Delta_{50\text{-}0}$, IVGTT + UAG 3 nmol/kg vs. IVGTT, $P < 0.004$; IVGTT + UAG 30 nmol/kg vs. IVGTT, $P < 0.0005$; Fig. 1A). The insulin response to IVGTT during the whole time course (ΔAUC) was clearly and dose-dependently increased by UAG, although statistical significance was reached only at 30 nmol/kg ($P < 0.001$ vs. IVGTT; Fig. 1B). In the systemic circulation, the stimulatory effect of UAG at 30 nmol/kg was still detectable, although much less than in the portal vein (ΔAUC, $P < 0.05$; Fig. 1, C and D). However, portal and systemic glucose levels after IVGTT were not modified significantly by UAG (Fig. 2 and Table 2).

Table 2. Glucose and insulin (levels △AUC) in portal and systemic circulation

Groups	Glucose △AUC, mmol·l^{-1}·min		Insulin △AUC, μg·l^{-1}·min	
	Portal	*Systemic*	*Portal*	*Systemic*
Saline ($n = 12$)	−4±27	14±16	−53±20	−31±11
IVGTT controls ($n = 12$)	711±65	778±68	91±33	50±11
IVGTT + AG, 30 nmol/kg ($n = 7$)	604±55	730±44	72±27	40±9
IVGTT + UAG, 3 nmol/kg ($n = 6$)	693±61	818±60	204±33	63±19
IVGTT + UAG, 30 nmol/kg ($n = 10$)	716±51	819±46	305±44 $^{P<0.001}$	88±15 $^{P<0.05}$
IVGTT + AG + UAG ($n = 7$)	666±50	855±59	73±35	39±12
IVGTT + [D-Lys^3]GHRP-6 ($n = 6$)	815±65	997±107	280±68 $^{P<0.01}$	68±18
IVGTT + [D-Lys^3]GHRP-6 + AG ($n = 6$)	785±66	734±74	234±54 $^{P<0.03}$	69±13
IVGTT + [D-Lys^3]GHRP-6 + UAG ($n = 7$)	652±35	703±67	257±81 $^{P=0.05}$	60±26

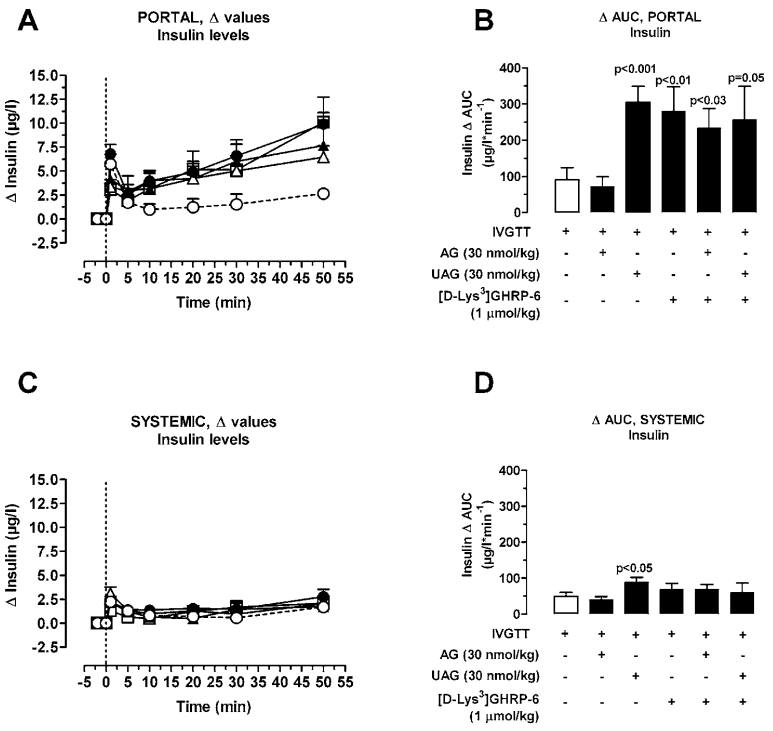
The GHS-R1a antagonist [D-Lys^3]GHRP-6 (1 μmol/kg), like UAG, enhanced the second-phase insulin response to glucose in the portal vein. Portal insulin levels gradually increased from 20 min ($P < 0.05$) to 50 min ($\Delta_{50\text{-}0}$, [D-Lys^3]GHRP-6+IVGTT vs, IVGTT, $P < 0.03$; Fig. 3A). Portal insulin ΔAUC was significantly higher ($P < 0.01$) in rats treated with [D-Lys^3]GHRP-6 + IVGTT than in those that received IVGTT alone (Fig. 3B). In the systemic circulation, the stimulatory effect on insulin release induced by the GHS-R1a antagonist was lost, and the ΔAUC of the whole time course was similar to that in the IVGTT group. (Fig. 3, C and D). Moreover, the effect exerted by [D-Lys^3]GHRP-6 + IVGTT on glucose-induced insulin secretion was not modified by the simultaneous administration of AG or UAG. Figure 3, A and B, clearly shows that [D-Lys^3] GHRP-6, alone or coadministered with AG or UAG, stimulated the second-phase portal insulin response to IVGTT and that this effect was again similar in extent, pattern, and timing to that observed after UAG (30 nmol/kg) alone. ΔAUC of portal insulin concentrations in the group treated with [D-Lys^3]GHRP-6, alone or combined with AG and UAG, was similar and higher than in the control (IVGTT) animals ($P < 0.01$, $P = 0.05$, and $P < 0.03$, respectively). Furthermore, glucose-stimulated portal insulin levels (ΔAUC) in all the groups treated with [D-Lys^3]GHRP-6, alone or in combination with AG and UAG, were higher ($P < 0.005$, $P < 0.01$, and $P < 0.04$, respectively) than in animals that received exogenous AG alone (Fig. 3, B and D).

No effects were observed on peripheral insulin levels in rats treated with the GHS-R1a antagonist, alone or in combination with AG or UAG, compared with the IVGTT or the IVGTT+AG group (Fig. 3, C and D).

Despite the observed increase of insulin levels, after administration of the GHS-R1a antagonist [D-Lys^3]GHRP-6, alone or in combination with AG or UAG, this was not accompanied by any significant changes in portal or peripheral glucose levels in terms of AUC (Table 2) and curve profile (data not shown).

Interestingly, the coadministration of AG (30 nmol/kg) with UAG (30 nmol/kg) completely abolished the UAG-mediated increase in the second-phase insulin release both in portal

Fig. 3. Insulin-secretagogue effect of UAG in glucose-stimulated conditions was similar to that of [D-Lys^3]GHRP-6, alone or in combination with AG or UAG (*A* and *B*), whereas in peripheral circulation only a slight stimulatory effect of UAG was still detectable (*C* and *D*). *Left*: values during the time course relative to the baseline value which was set as 0 (△). *Right*: △AUCs of all parameters after treatment administration. Vertical dotted line, treatment administration at $t = 0$. ○, IVGTT; ●, IVGTT + UAG (30 nmol/kg; $n = 10$); □, IVGTT+[D-Lys^3]GHRP-6 (1 μmol/kg; $n = 6$); △, IVGTT+[D-Lys^3]GHRP-6 + AG (30 nmol/kg; $n = 6$); ▲, IVGTT+[D-Lys^3]GHRP-6 + UAG (30 nmol/kg; $n = 7$). *P* values are reported in the figure; differences were considered significant for $P < 0.05$.



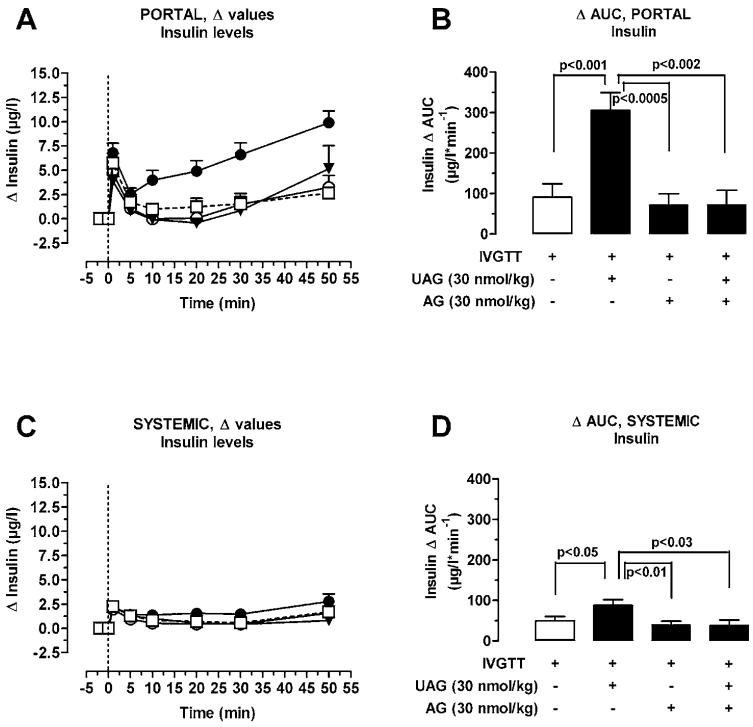
(ΔAUC,: $P < 0.002$) and in peripheral ($P < 0.03$) circulation (Fig. 4, A–D), but this did not modify portal and peripheral glucose levels after IVGTT (Table 2).

Hepatic Insulin Clearance

Since modulation of insulin levels observed in the portal vein by various treatments were (severely) blunted in the systemic circulation, we hypothesized that the administered compounds might not only affect insulin secretion in the portal vein but also modify insulin cleared by the liver and thereby increase the portal-peripheral gradient of insulin.

Insulin clearance after saline injection (%AUC) was 63 ± 3%, and it did not change significantly after glucose load. UAG at 30 nmol/kg, but not at 3 nmol/kg, slightly increased hepatic insulin clearance, which was higher ($P < 0.05$) than in the IVGTT or IVGTT + AG groups (IVGTT + UAG: 69 ± 2% vs. IVGTT: 59 ± 4% and vs. IVGTT + AG: 57 ± 5%). Like UAG, [D-Lys^3]GHRP-6, alone

Fig. 4. Coadministration of AG (30 nmol/kg) and UAG (30 nmol/kg) abolished completely the UAG-induced enhancement of insulin response to glucose both in the portal vein (*A* and *B*) and in the peripheral circulation (*C* and *D*). *A* and *C*: values during the time course relative to the baseline value, which was set as 0 (△). *B* and *D*: △AUCs after treatment administration. Vertical dotted line, treatment administration at $t = 0$. □, IVGTT; ○, IVGTT + AG (30 nmol/kg; $n = 7$); ●, IVGTT + UAG (30 nmol/kg; $n = 10$); ▼, IVGTT + AG (30 nmol/kg) + UAG (30 nmol/kg; $n = 7$). *$P < 0.01$ vs. IVGTT. *P* values for △AUCs are reported in the figure; differences were considered significant for $P < 0.05$.



or combined with AG and UAG, slightly increased hepatic insulin clearance compared with rats treated with IVGTT alone or with AG. However, statistical significance was reached only by the group that received IVGTT + AG + [D-Lys^3]GHRP-6 (70 ± 3%, $P < 0.05$ vs. IVGTT, $P < 0.02$ vs. IVGTT + AG; data not shown).

DISCUSSION

The results of the present study show that UAG acts as a secretagogue of insulin in the portal vein in anesthetized rats. This UAG-induced increase in insulin levels was abolished by the coadministration of AG and was similar to that exerted by blockade of the GHS-R1a using the specific antagonist [D-Lys^3]GHRP-6. Moreover, UAG as well as [D-Lys^3]GHRP-6 slightly increased

hepatic insulin clearance. This may partly explain why we observed a marked increase in insulin levels in the portal circulation but not in the peripheral blood.

Our data demonstrate for the first time that UAG potently and dose-dependently enhances the insulin response to an intravenous glucose load in vivo. This insulin secretagogue effect of UAG was marked in the portal vein, whereas it was barely detectable in the systemic circulation, supporting the hypothesis that UAG plays an important role in glucose metabolism in the liver. In line with this, previous observations using primary hepatocyte cultures showed that UAG dose-dependently decreased glucose output and completely prevented the AG-induced and partially blocked the glucagon-dependent glucose release.[27] However, it was also found that UAG alone does not improve hepatic insulin sensitivity in a euglycemic hyperinsulinemic clamp model in mice.[19] In the present study, we estimated that UAG also slightly increased the fraction of insulin cleared by the liver, thus contributing to the augmentation of the portal-peripheral gradient of insulin. Although we did not perform real insulin clearance studies, we speculate that UAG might also influence hepatic insulin metabolism. Therefore, we suggest that UAG stimulates insulin secretion by pancreatic islets and perhaps also improves insulin action on target tissues (e.g., the liver). Interestingly, the UAG-enhanced insulin response to glucose was similar in extent, timing, and pattern to that exerted by [D-Lys^3]GHRP-6, a GHS-R1a antagonist. The effect of [D-Lys^3]GHRP-6 likely reflects the blockade of the inhibitory action of endogenous AG on β-cells. This is in accord with the evidence that endogenous AG tonically restricts glucose-induced insulin release and that pharmacological, immunological, and genetic blockade of AG action in pancreatic islets enhanced glucose-induced insulin release.[1, 7, 11] Nevertheless, by using this model, we could not detect significant effects on glucose levels in any of the treatment groups, making difficult any interpretation of these data as variations in insulin sensitivity. This may be explained by the high glucose load that we administered during the experiments, the presence of an increased counterregulatory hormonal response in the studied rats due to abdominal surgery,[20] and/or possible effects of the anesthesia.[21, 22]

We show that the administration of (exogenous) AG did not suppress insulin release any further, suggesting that after a glucose load endogenous AG at low concentrations, which we reconfirmed in our model, already exerts a maximal inhibitory effect on insulin secretion, at least under these experimental conditions. Another possible reason is that this maximal suppressive activity is due to autocrine and paracrine effects of AG produced in the pancreas. This would also explain why the coadministration of the GHS-R1a antagonist together with exogenous AG elicited the insulin response to glucose load to the same extent as [D-Lys^3]GHRP-6 alone, i.e., removing the inhibitory tone of endogenous AG on insulin secretion. Our findings differ from previous reports by Dezaki et al.,[1] who observed a suppressive effect of exogenous AG on glucose-induced insulin release, which was not modified by UAG in a perfused pancreas model. However, this discrepancy may be due to the fact that, differently from Dezaki et al., we used an *in vivo* model.

Intriguingly, when exogenous AG was coadministered with UAG, it completely blocked the insulin secretagogue effect of UAG. This finding once again reinforces the hypothesis that AG and UAG, at least at equimolar concentrations, interact with each other and have effects on glucose homeostasis. This is in agreement with previous reports in humans and in rodents, showing that the coadministration of UAG with AG was able to prevent the AG-induced decrease in circulating insulin and worsening of insulin sensitivity.[12, 13, 18, 19]

Although our data do not provide evidence regarding the possible mechanism of action of UAG, we found a striking similarity between the insulin secretagogue effect of UAG and [D-Lys^3]GHRP-6. This observation, coupled with the finding that exogenous AG could block the UAG-induced stimulation on insulin, led us to speculate that UAG may act as an antagonist of endogenous AG (i.e., removing the suppressive tone of AG on insulin release). However, since UAG, differently from [D-Lys^3]GHRP-6, does not block the GHS-R1a,[2] we suggest the existence of a putative UAG receptor (different from GHS-R1a) that mediates the stimulating effect of UAG on insulin. The fact that the actions of UAG and [D-Lys^3]GHRP-6 on glucose-stimulated insulin secretion were neither additive nor synergistic might be explained by two mechanisms: 1) either UAG or [D-Lys^3]GHRP-6 exerts a maximal antagonistic activity on endogenous AG; 2) [D-Lys^3]GHRP-6 is not only an (ant)agonist of the GHS-R1a but also an agonist of the putative UAG receptor. Indeed, the mechanisms of (inter)action of UAG, [D-Lys^3]GHRP-6, and AG on insulin release and glucose metabolism, as well as their physiological relevance, need to be further elucidated and may disclose a ghrelin system far more complex than it is currently known.

In conclusion, our data demonstrate that UAG at pharmacological concentrations is a potent insulin secretagogue. This, together with our previous observation that UAG blunts glucose output by primary hepatocytes,[27] suggests that UAG action is targeted mainly at the liver. These effects of UAG in the regulation of glucose metabolism might be of therapeutic interest for those pathological conditions characterized by insulin resistance and impaired insulin release.

Acknowledgements

We thank Piet Uitterlinden and Dr. Yolanda de Rijke for skillful technical assistance and Drs. Maarten O. van Aken and Kees Schoenderwoerd for helpful scientific discussion.

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Chapter 6

Bolus administration of obestatin does not change glucose and insulin levels neither in the systemic nor in the portal circulation of the rat

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Peptides 2008; 29: 2144-2149

ABSTRACT

Obestatin is a second peptide derived from the preproghrelin polypeptide. It was originally thought to have anorexigenic effects, thereby functioning as an antagonist of ghrelin. However, this has been a subject of debate ever since. Since acylated ghrelin strongly induces insulin resistance, it could be hypothesized that obestatin plays a role in glucose homeostasis as well. In the present study we evaluated the effect of obestatin on glucose and insulin metabolism in the systemic and portal circulation. Obestatin 200 nmol/kg was administered systemically as a single intravenous bolus injection to fasted pentobarbital anesthetized adult male Wistar rats. Up to 50 minutes after administration, blood samples were taken to measure glucose and insulin concentrations, both in the portal and in the systemic circulation. The effect of obestatin was evaluated in fasted and in glucose-stimulated conditions (IVGTT) and compared to control groups treated with saline or IVGTT, respectively. Intravenous administration of obestatin did not have any effect on glucose and insulin concentrations, neither systemic nor portal, when compared to the control groups. Only the glucose peak 1 min after administration of IVGTT was slightly higher in the obestatin treated rats: 605.8 ± 106.3% vs. 522.2 ± 47.1% in the portal circulation, respectively (*NS*), and 800.7 ± 78.7% vs. 549.6 ± 37.0% in the systemic circulation, respectively ($P < 0.02$), but it can be debated whether this has any clinical relevance. In the present study, we demonstrated that intravenously administered obestatin does not influence glucose and insulin concentrations, neither in the portal nor in the systemic circulation.

INTRODUCTION

Ghrelin, a 28-amino acid peptide produced mainly by the stomach, was originally discovered as a natural ligand of the Growth Hormone Secretagogue Receptor type 1a (GHS-R1a).[1] Despite being primarily identified as a potent GH stimulating factor, ghrelin has been demonstrated to have a wide spectrum of biological activities, such as stimulation of prolactin and ACTH secretion, promotion of gastric motility and acid secretion, and modulation of cardiovascular function.[2, 3] One of its most intriguing functions is the long-term and short-term regulation of energy balance. Continuous administration of ghrelin to rodents induces increased food intake resulting in weight gain, whereas in humans 24-h plasma profiles show marked preprandial increases and postprandial decreases in circulating ghrelin concentrations, which suggests an orexigenic effect.[4-6]

Ghrelin is derived from a 117 amino acid peptide called preproghrelin, which is predominantly produced in X/A like cells in the stomach.[1] In 2005, Zhang et al. identified a second peptide encoded by the GHRL gene, using comparative genomic analysis, and called it obestatin.[7] This amidated 23 amino acid peptide and ghrelin appeared to be differentially secreted since fasting and subsequent refeeding in rats induced a rise and subsequent fall in ghrelin concentrations, whereas no changes in obestatin concentrations were observed.[7] Additionally, acute intraperitoneal and intracerebroventricular administration of obestatin suppressed food intake, while daily administration of obestatin suppressed body weight gain and induced delayed gastric emptying.[7] These results suggested that obestatin and ghrelin had opposing effects on food intake and body weight regulation.

Following these initial results, obestatin has been the topic of an ongoing discussion. Many studies failed to reproduce the inhibiting effect on food intake and body weight gain or questioned its role in energy homeostasis.[8-13] Additionally, the hypothesis that obestatin exerted its effect by stimulating the orphan receptor GPR39, was rejected by several groups including the original authors.[14-17] On the other hand, several studies in rodents confirmed an anorexigenic effect of obestatin, either endogenous or by counteracting the orexigenic effect of ghrelin.[18, 19]

Acylated ghrelin is known to induce insulin resistance.[20-22] Therefore, it could be hypothesized that obestatin does affect insulin and glucose secretion as well. Recently, two studies have evaluated glucose and insulin responses to obestatin administration, both measuring concentrations in the systemic circulation.[19, 23] However, a problem that may be encountered in evaluating the effect of obestatin on glucose and insulin metabolism is its short half-life.[24] Obestatin is mainly produced in the stomach and might accordingly exert its effect primarily in the portal system.[7] Therefore, measurements of systemic insulin and glucose concentrations may fail to demonstrate this effect. Additionally, hepatic effects of obestatin may be overlooked when measuring systemic concentrations of glucose and insulin only.

In the present study, we used a previously validated rat model which allowed us to simultaneously measure systemic and portal insulin and glucose concentrations.[25, 26] The aim of this

study was to evaluate acute effects of intravenous administration of obestatin on glucose and insulin metabolism in fasted and glucose-stimulated conditions.

MATERIALS AND METHODS

Animals

Male Wistar rats (age: 10-12 weeks; weight: 350-400 g, Harlan Netherlands BV, Horst, The Netherlands) were housed in groups in a temperature-controlled room under a 12-h light/12-h dark cycle, and maintained on pelleted chow with free access to water. The animals were housed for at least one week before starting the experiments, in order to allow acclimatization. Animal protocols were in compliance with the Dutch regulations on animal welfare and approved by the institutional Animal Welfare Committee.

Surgery and experimental design

All studies were performed after a fasting period of 18 h (overnight). Studies were performed under anaesthesia and the rats were euthanized at the end of the experiment.

Animals were anaesthetized using an intraperitoneal (ip) injection of sodium pentobarbital (60 mg/kg induction, 20 mg/kg maintenance administered at the end of the surgical procedure, before starting the experimental session). Sodium pentobarbital was used, since, compared to other anaesthetics, it has been shown to interfere less with insulin secretion and glucose metabolism both in the fed and the fasted conditions.[27, 28]

Deep anaesthesia was confirmed by the absence of reflexes. Animals were kept on a warming mat to maintain core body temperature and were connected to a breathing apparatus (O_2, 1 l/min) to improve oxygenation, for the entire duration of the experiment (including surgical procedure).

The surgical procedure was performed under aseptic conditions, as follows:

Cannulation of the jugular vein: an incision was made just above the right clavicle, the connective and adipose tissues were pushed aside and the jugular vein was exposed. After the jugular vein was mobilized, a catheter previously connected to a syringe and filled with saline solution was pushed inside the vessel until it reached the right atrium. Patency of the catheter was checked by aspirating blood and flushing the catheter with saline solution. The free end of the catheter was used for saline injection, treatment administration and sampling.

Cannulation of the portal vein: a midline incision was made from the level of the symphysis pubis to the xiphoid cartilage. The intestines were lifted out and laid next to the animal on gauze moistened with warm saline solution to minimize dehydration. A purse-string (diameter approximately 1 mm) was made in the wall of the portal vein, opposite to the gastroduodenal vein. Then the center of the purse-string was cut and the cannula inserted into the portal vein and pushed in for a few millimetres, with the tip secured about 1 mm caudal to the liver. The

patency of the cannula was checked by aspirating blood and injecting saline. The free end of the cannula was used for sampling during the experiment.

Treatment administration and sampling

Rats were assigned to one of the following treatment groups:

1. Intravenous saline (1 ml), *n* = 12.
2. Intravenous obestatin 200 nmol/kg (in 1 ml), *n = 7*.
3. Intravenous Glucose Tolerance Test (IVGTT), *n* = 12. IVGTT was performed by injecting D-glucose at a dose of 1 g/kg (50%, 1 ml maximal volume) through the jugular catheter. The dose of 1 g/kg was chosen taking in account the reduction of insulin sensitivity caused by abdominal surgery [29] and the possible interference due to anesthesia.[27, 28]
4. Intravenous obestatin 200 nmol/kg + IVGTT 1 g/kg (1 ml maximal volume), n = 6.

After baseline samples were taken from both catheters, treatment was administered through the jugular cannula at time 0 and samples were taken from both catheters at 1, 5, 10, 20, 30 and 50 min after treatment administration to measure glucose and insulin levels. At every time point, the blood volume withdrawn from each catheter (350 µl) was replaced by an equal volume of saline solution.

Plasma samples were stored at -20ºC until the assay.

At the end of each experiment the animals were killed by exsanguination under deep anaesthesia.

Materials

Plasma glucose levels were measured using a glucose oxidase method (Instruchemie, Delfzijl, The Netherlands). Rat insulin was measured using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). The sensitivity of the assay is 0.07 µg/l, according to manufacturer's instructions.

Rat obestatin (Phe-Asn-Ala-Pro-Phe-Asp-Val-Gly-Ile-Lys-Leu-Ser-Gly-Ala-Gln-Tyr-Gln-Gln-His-Gly-Arg-Ala-Leu-NH2) was obtained from NeoMPS (Strasbourg, France).

Sodium pentobarbital (250 mg/5 ml) was provided by the hospital pharmacy. EDTA containing tubes were obtained by Greiner Bio-One BV (Alphen aan den Rijn, The Netherlands). Silicone catheters (3-french size) were provided by UNO Roestvaststaal BV (Zevenaar, The Netherlands); suture needles (Dafilon 8/0) by B. Braun Melsungen AG (Melsungen, Germany).

Statistical analysis

Results are presented as mean ± S.E.M. unless otherwise specified. $P < 0.05$ was considered significant. Group 1 (saline) was used as a control for group 2 (obestatin), and group 3 (IVGTT) was used as a control for group 4 (IVGTT + obestatin). Differences between study groups were calculated using the Mann-Whitney test. Differences over time within one group were calculated using Friedman's test. Glucose/insulin ratio was calculated as a measure of insulin sensitivity, since HOMA-IR was considered not to be appropriate in non-homeostatic conditions. Statistic

calculations were performed using Statistical Package for the Social Sciences (SPSS release 14.0; SPSS Inc, Chicago).

RESULTS

Baseline glucose and insulin levels

Baseline insulin concentrations in the portal circulation were approximately 2.5 times higher than in the systemic circulation (portal 4.47 ± 0.46 µg/l vs. systemic 1.7 ± 0.22 µg/l), while the difference in baseline portal and systemic glucose levels was small (portal 6.78 ± 0.51 mmol/l vs. systemic 8.25 ± 0.59 mmol/l). Both baseline glucose (portal 7.79 ± 0.65 mmol/l, systemic 9.96 ± 0.65 mmol/l) and insulin (portal 4.75 ± 0.60 µg/l, systemic 1.77 ± 0.24 µg/l) levels were higher in the control groups (IVGTT and saline) than in the obestatin treatment groups (glucose portal 5.02 ± 0.62 mmol/l, systemic 5.18 ± 0.60 mmol/l, insulin portal 3.92 ± 0.72 µg/l, systemic 1.61 ± 0.46 µg/l). Therefore, results are standardized and presented as percentage of baseline rather than absolute values.

Fasted conditions

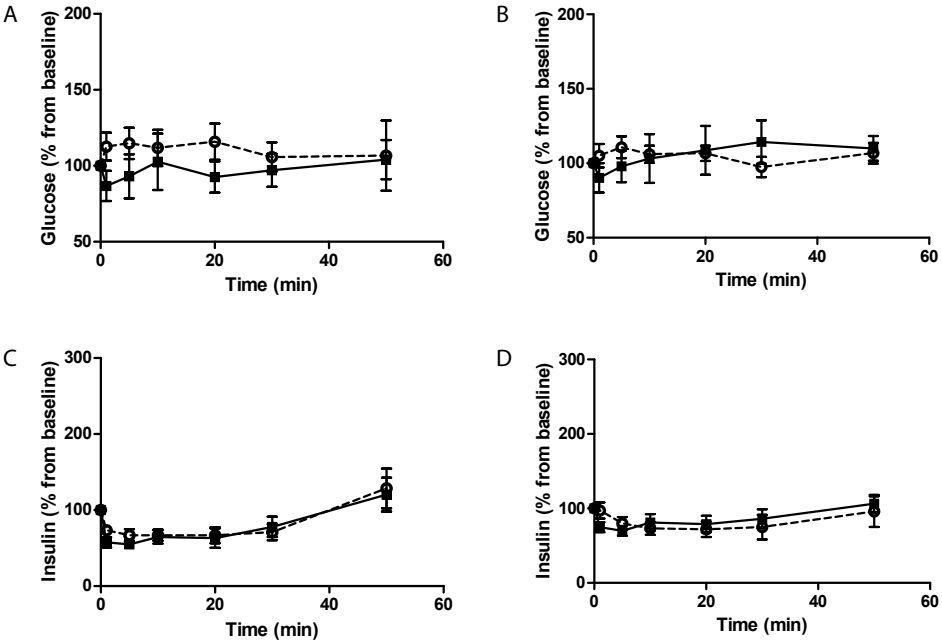
After administration of saline, no change in glucose concentration was observed. Administration of obestatin did not induce any change in glucose concentrations as well, neither in the portal nor in the systemic circulation. Indeed, glucose concentrations after obestatin treatment were not significantly different from glucose concentrations after saline administration during the 50 min time course (Fig. 1A and B). Area under the curve (AUC) of 0-50 min was not significantly different as well.

Insulin concentrations decreased slightly after administration of saline, returning to baseline after 50 min. The same effect was observed after administration of obestatin. Therefore, no significant differences in insulin concentrations were observed in comparing obestatin with saline administration, neither in the portal nor in the systemic circulation (Fig. 1C and D). AUC of 0-50 min was not significantly different as well.

Glucose stimulated conditions

Administration of glucose 1 g/kg resulted in a prompt increase in glucose concentrations. The glucose peak occurred after 1 min both in the systemic and portal circulation. Obestatin administration appeared to induce a slightly higher glucose peak compared with IVGTT alone: 605.8 ± 106.3% vs. 522.2 ± 47.1% in the portal circulation, respectively (*NS*), and 800.7 ± 78.7% vs. 549.6 ± 37.0% in the systemic circulation, respectively ($P < 0.02$). After 1 min, glucose concentrations decreased rapidly, though not returning to baseline within 50 min. During this period, no significant differences in glucose concentration were observed between IVGTT in combination with obestatin vs. IVGTT alone (Fig 2A and B). AUC of 0-50 min was not significantly different as well.

Fig 1. Glucose and insulin concentrations after administration of saline vs. obestatin 200 nmol/kg i.v.



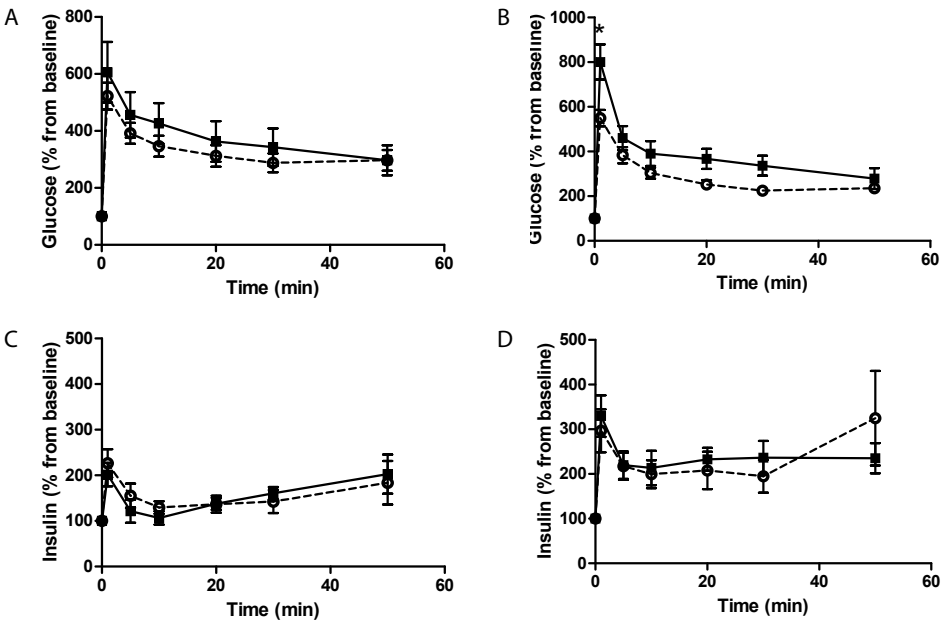
(A) Glucose concentration in the portal system. (B) Glucose concentration in the systemic circulation. (C) Insulin concentration in the portal system. (D) Insulin concentration in the systemic circulation. Data are presented as % from baseline = T_0 = before administration. Study medication: saline (---○---), obestatin 200 nmol/kg (—■—). * $P < 0.05$

Insulin displayed an equally rapid response to IVGTT as glucose did, with a peak occurring at 1 min both in the systemic and portal circulation. The insulin peak was not significantly different in the groups with or without obestatin. Insulin concentration at 1 min in the portal circulation was 201.6 ± 25.5% after obestatin treatment vs. 235.7 ± 29.6% after IVGTT alone (*NS*), whereas concentrations in the systemic circulation were 329.7 ± 46.4% vs. 296.7 ± 48.0% (*NS*), respectively. No significant differences in insulin concentration were observed up to 50 min after administration of study medication (Fig. 2C and D). AUC of 0-50 min was not significantly different as well.

Glucose/insulin ratio

The glucose/insulin ratio, as a measure of insulin sensitivity, was calculated for each time point. No significant differences between the obestatin group vs. the saline group were observed. Additionally, there were no differences between the obestatin with IVGTT vs. the IVGTT group alone (data not shown).

Fig 2. Glucose and insulin concentrations after administration of IVGTT (glucose 1 g/kg) or IVGTT + obestatin 200 nmol/kg i.v.



(A) Glucose concentration in the portal system. (B) Glucose concentration in the systemic circulation. (C) Insulin concentration in the portal system. (D) Insulin concentration in the systemic circulation. Data are presented as % from baseline = T_0 = before administration. Study medication: IVGTT (---○---), IVGTT + obestatin 200 nmol/kg (—■—). * $P < 0.05$

DISCUSSION

In the present study we demonstrated that acute intravenous administration of obestatin does not change glucose and insulin concentrations in the systemic circulation. This lack of effect was observed in fasted as well as in glucose-stimulated conditions. There was only a slight difference in peak glucose concentrations after IVGTT, but it can be debated whether this has any clinical relevance. Additionally, we measured glucose and insulin directly in the portal vein. These results, however, were not different from the systemic observations: obestatin does not change glucose and insulin concentrations in the portal circulation when administered systemically.

Obestatin was originally identified as a second conserved peptide derived from preproghrelin.[7] Plasma ghrelin and obestatin were demonstrated not to be strictly correlated and were even differentially regulated in fasted and fed conditions,[7, 12, 18] which supported the hypothesis that obestatin was a not a non-functional connective peptide, but had endogenous physiological effects. Zhang et al. demonstrated that, contrary to the orexigenic and adipogenic effects of acylated ghrelin, intraperitoneal and intracerebroventricular administration of obestatin suppressed food intake and decreased body weight gain in rodents.[7] This observation could indicate that obestatin and ghrelin function as full antagonists in vivo.

Since acylated ghrelin induces insulin resistance,[20-22] it could be hypothesized that obestatin does influence glucose and insulin homeostasis as well. Two previous studies have extensively evaluated the effects of obestatin administration on glucose and insulin levels in rodents. Green et al. demonstrated that both glucose and insulin levels were lower in obestatin treated rats after a standard meal.[19] However, since food intake in the obestatin treated group was significantly lower than in the control group, the observed effect might at least be partially attributed to this difference. Indeed, in basal and IPGTT stimulated conditions, no effect was observed.[19] Ren et al. did not observe any effect on systemic concentrations of glucose and insulin after intravenous administration of obestatin as well.[23] However, obestatin was shown to reduce insulin response after IVGTT.[23] Additionally, two studies did not observe any effect on glucose concentrations after administration of obestatin.[12, 13] In summary, previously observed effects of obestatin on glucose and insulin homeostasis were small, if any, and certainly not strong enough to regard obestatin as an antagonist of ghrelin in this system. The present results are generally consistent with these observations: we did not observe any effect of obestatin on glucose and insulin concentrations measured in the systemic circulation. However, we did not only evaluate systemic glucose and insulin concentrations, but measured portal concentrations as well. Obestatin is reported to have a very short half-life in the circulation, which suggests that most of its actions occur locally.[24] Since obestatin is mainly produced in the stomach and has been demonstrated in the pancreas as well,[30, 31] it might be discussed that its main site of action is the portal system. Therefore, measuring systemic glucose and insulin concentrations might fail to establish the local effects of obestatin. Nevertheless, in the present study we were not able to demonstrate any effects of obestatin in the portal system as well.

There are some limitations to the present study. At first, the observation that obestatin does not play a role in glucose and insulin metabolism applies for intravenous administration of obestatin in a dose of 200 nmol/kg only. These results cannot be extrapolated to different doses or administration regimens. The original study used a protocol of intraperitoneal and intracerebroventricular administration of obestatin.[7] The protocol of our rat model however, imposed intravenous administration. We selected the same high dose which was previously described to be effective when administrated intravenously as well as in the intraperitoneal dose-finding study by Lagaud et al.[18, 23] However, it still could be that the lack of observed effect is due to the selected dose of obestatin. Secondly, baseline glucose and insulin concentrations were lower in the study groups compared to the control group. This is most likely due to technical issues, such as lower perioperative stress in the study group rats than in the control group as a result of increasing experience in the surgical team, and is assumed not to have caused a bias after standardization.

In conclusion, intravenous administration of obestatin does not have any effect on glucose and insulin concentrations, neither systemically nor in the portal system. However, additional (dose-finding) studies are necessary to convincingly reject the role of obestatin in glucose and insulin homeostasis.

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Chapter 7

Acute effects of acylated and unacylated ghrelin on total and high molecular weight adiponectin in morbidly obese subjects

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J Endocrinol Invest, 2010 Oct 15 (Epub ahead of print)

ABSTRACT

Background

Energy homeostasis and body weight are regulated by a highly complex network involving the brain, the digestive tract and white adipose tissue (WAT). Knowledge about signalling pathways connecting digestive tract and WAT is limited. Gut hormone ghrelin and adipokine adiponectin are both decreased in obesity and they share a potent effect on insulin sensitivity: both adiponectin and the combination of acylated (AG) and unacylated ghrelin (UAG) improve insulin sensitivity.

Aim

In the present study, we evaluated whether acute administration of UAG alone or combined with AG affects adiponectin concentrations.

Subjects and Methods

Eight morbidly obese non-diabetic subjects were treated with either UAG 200µg, UAG 100µg + AG 100µg (Comb), or placebo in 3 episodes in a double blind randomized cross-over design. Study medication was administered as single i.v. bolus injections at 09.00h after an overnight fast. High molecular weight (HMW) and total adiponectin, glucose, insulin and total and acylated ghrelin were measured up to one hour after administration.

Results

HMW and total adiponectin concentrations did not change after administration of either UAG or Comb, nor were they different from placebo. Insulin concentrations decreased significantly after acute administration of Comb, reaching a minimum at 20 min: 58.2 ± 3.9% of baseline.

Conclusions

Acute intravenous administration of UAG and the combination of UAG and AG in morbidly obese non-diabetic subjects without overt diabetes does not affect total or HMW adiponectin concentrations, neither directly nor indirectly by changing insulin concentrations.

INTRODUCTION

Energy homeostasis and body weight are regulated by a highly complex network involving the brain, the digestive tract and white adipose tissue (WAT).[1] Hypothalamic neurons respond to hormones, produced by either the gut or WAT, by modifying the synthesis of neuropeptides that modulate food intake and energy balance. Multiple pathways connecting the gut and WAT with the brain have been characterized during the last two decades. Most gut hormones (e.g. peptide tyrosine-tyrosine (PYY), pancreatic polypeptide (PP), amylin, glucagon-like peptide-1 (GLP-1), and oxyntomodulin) display an anorexigenic effect by centrally inhibiting food intake, reducing adiposity and altering energy expenditure.[2-7] On the other hand, ghrelin is at present the only known orexigenic gut hormone, inducing food intake and adiposity by stimulating the release of the orexigenic neuropeptides neuropeptide Y (NPY) and Agouti-related peptide (AgRP).[8-11] Central effects of leptin are most extensively studied regarding signalling pathways of WAT to the brain. Leptin acts centrally as a full antagonist of ghrelin, thereby reducing food intake and body weight gain, and modulating glucose metabolism.[12-14] Additionally, central effects of the adipokines adiponectin and resistin have been suggested as well.[15-18]

While pathways connecting respectively WAT and the gut with the brain have been studied extensively, direct connections between WAT and the gut are largely unknown. Studies reporting correlations between gut hormone concentrations and adipokine concentrations add little information to our understanding of their interaction, since concentrations could well be independently influenced by another factor. Additionally, those studies reporting results of adipokine administration on gut hormone concentrations and vice versa (mostly leptin *vs* ghrelin) do not answer the question whether the observed effects are direct or indirect.[19-21] As stated above, both gut hormones and adipokines have centrally mediated effects on food intake, body composition and glucose metabolism. On the other hand, gut hormone concentrations and adipokine concentrations are largely regulated by energy intake and body composition, possibly mediated by insulin and glucose levels.[8, 16, 22-27] Therefore, it could be hypothesized that connections between the gut and WAT are either direct, i.e. effectuated locally in the gut or WAT, or indirect, i.e. mediated by central pathways or changes in insulin and glucose concentrations.

The gut hormone ghrelin and the adipokine adiponectin have some striking homologies. At first, both hormones play an important role in glucose metabolism. Acylated ghrelin (AG), which is able to bind to the receptor for which ghrelin is the natural ligand (GHS-R1a), has been shown to induce insulin resistance.[28, 29] On the other hand, unacylated ghrelin (UAG), which lacks a *n*-octanoyl group necessary for binding to the GHS-R1a, has been suggested to have an insulin-sensitizing role. At least, it is likely to counterbalance the influence of AG on insulin secretion and glucose levels.[30] Finally, the combination of AG and UAG strongly improves insulin sensitivity.[31, 32] Adiponectin has been demonstrated to strongly improve insulin sensitivity as well.[27, 33, 34] It has been suggested that high molecular weight (HMW) adiponectin is the active isoform, since low levels of HMW adiponectin, have been demonstrated to strongly correlate

with insulin resistance and development of type 2 diabetes.[35, 36] Secondly, both hormones are typically decreased in obesity.[23, 25, 26]

In the present study, we used human obesity as a model to study the effects of acute intravenous administration of UAG and the combination of AG and UAG on adiponectin concentration. It was hypothesized that ghrelin could have either a direct effect on adiponectin concentration, or an indirect effect mediated by a decrease in insulin concentration after coadministration of AG and UAG, as reported previously.[32] On the other hand, since the mechanism responsible for the significant decrease of insulin concentration at unchanged glucose levels after coadministration of AG and UAG is still unknown, adiponectin could hypothetically be the mediator of this improvement in insulin sensitivity.

MATERIALS AND METHODS

Study population

Eight morbidly obese female Caucasian subjects (age 45.4 ± 10.3 (mean ± SD), range 28-62 years, mean Body Mass Index 42.4 ± 4.8 kg/m^2) were recruited from an affiliated clinic for bariatric surgery. All were on a waiting list to undergo gastric banding or gastric bypass (criteria: Body Mass Index (BMI) > 40 kg/m^2 or BMI > 35 kg/m^2 in combination with relevant comorbidity).[37] Exclusion criteria for the study were: overt type 2 diabetes, liver enzyme test abnormalities, pregnancy and previous bariatric surgery. All subjects gave their written informed consent to participate in the study, which had been approved by the ethical committee of our hospital.

Study design

The double blind randomized study design consisted of 3 study episodes in which 3 treatment regimens were administered: 1) UAG 200 µg (UAG), 2) UAG 100 µg in combination with AG 100 µg (Comb), 3) placebo (placebo). Every patient underwent all treatment regimens, which were separated by a wash out period of 2 weeks at least. Study medication was administered as a single daily intravenous bolus injection.

After an overnight fast, an indwelling catheter was placed in the forearm and kept patent by a slowly running saline infusion. At 9.00h study medication was administered as an acute bolus injection. Blood samples were taken before administration of study medication (T_0) and at 20, 45 and 60 min. Subjects were kept fasted during the study period.

Study medication

Both AG and UAG were obtained from Bachem AG, Bubendorf, Switzerland. To prevent degradation of ghrelin vials were stored at -80°C up to 90 min before administration. To prevent interaction of AG and UAG in vitro two separate samples were administered to the patients, followed by 5 ml of saline after each infusion.

Assessments

Blood samples for total ghrelin and acylated ghrelin measurements were collected in EDTA tubes. Samples were stored on ice until centrifugation. After centrifugation serum samples were stored at -20°C until processed. Acylated and total ghrelin levels were determined using a commercially available RadioImmunoAssay (Linco Research, St. Charles, Missouri, USA). Intra- and interassay variation of the AG assay are 7 and 13% respectively, and of the total ghrelin assay 6% and 16% respectively.

Adiponectin (total and HMW) was measured by an in house ELISA that has been shown to correlate highly with other commercially available assays for adiponectin (B-Bridge total adiponectin r = 0.97 and Alpco Diagnostics total adiponectin r = 0.98 and HMW adiponectin r = 0.98) (Oxford Brookes University, Oxford, England).[38] Intra- and interassay variation was 8% and 10% respectively.

Insulin was measured using a chemiluminescent immunometric assay (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Intra- and interassay variation was 4% and 5% respectively. Glucose was measured on a Hitachi 917 (Roche Diagnostics, Mannheim, Germany) by a glucose-oxidase method.

Statistical analysis

Results are presented as mean ± SEM unless otherwise specified. $P < 0.05$ was considered significant. Differences between the three study periods were calculated using the Friedman test, the nonparametric equivalent of a one-sample repeated measures design. Areas under the curve (AUC) were calculated using the trapezoid rule. UAG concentrations were determined calculating the difference between total ghrelin and AG.

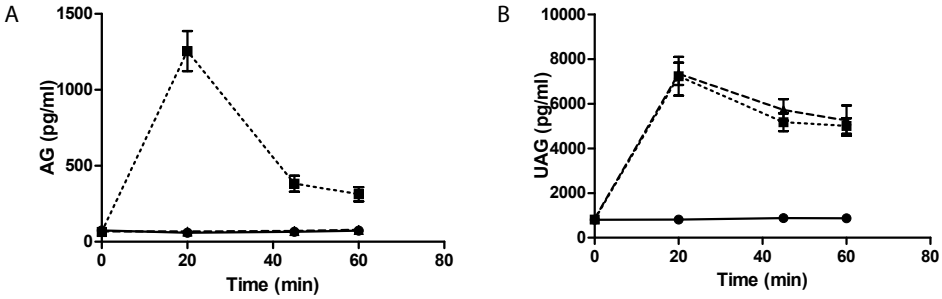
Statistic calculations were performed using Statistical Package for the Social Sciences (SPSS release 14.0; SPSS Inc, Chicago).

RESULTS

Concentrations of AG and UAG

After acute administration of AG 100 µg i.v. (in combination with UAG 100 µg) baseline AG concentration of 64 pg/ml increased to a peak of 1254 pg/ml after 20 min. The half-life was short: AG concentrations approached baseline at 60 min after administration (Fig. 1A). Baseline concentrations of UAG were 844 pg/ml, increasing to 7337 pg/ml and to 7231 pg/ml 20 min after administration of UAG 200 µg i.v. alone and 100 µg i.v. in combination with AG 100 µg respectively. At termination of the measurements, 60 min after administration, UAG concentrations had not returned to baseline (Fig. 1B).

Fig. 1 Acylated and unacylated ghrelin



A: Acylated ghrelin concentration

B: Unacylated ghrelin concentration

T_0 = administration of study medication: placebo (—●—), UAG (---▲---), Comb (···■···).

Adiponectin

Baseline concentration of total adiponectin was 20.4 ± 1.01 µg/ml. Baseline concentration of HMW adiponectin was 10.0 ± 0.65 µg/ml, which was 48.1 ± 3.34% of total adiponectin.

Both total and HMW adiponectin concentrations did not change after administration of either UAG or Comb. Additionally, concentrations of total and HMW adiponectin were never significantly different from placebo during the study periods (Fig. 2A and 2B). Figure 2 shows total (2A) and HMW (2B) adiponectin concentrations, as well as HMW/total adiponectin ratio (2C), throughout the study period displayed as percentage of baseline concentrations.

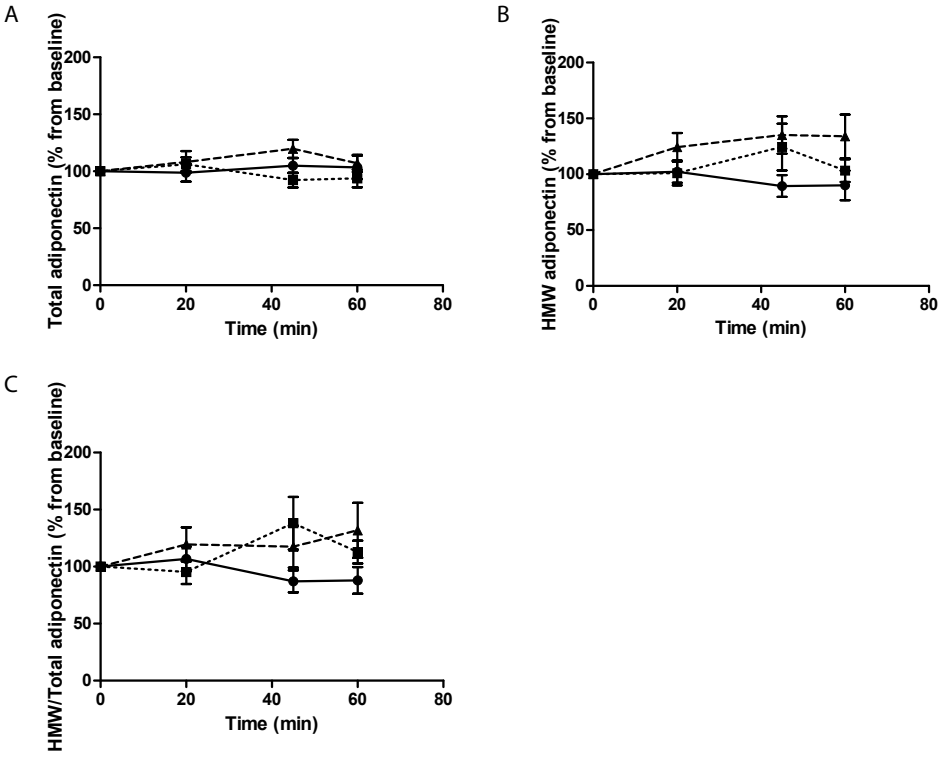
AUC of total adiponectin (percentage of baseline concentrations, i.e. $T_0 = 100$) from 0 to 60 min was 6097 ± 362.1*min, 6629 ± 420.5*min and 5944 ± 286.3*min after placebo, UAG and Comb respectively (*NS*). AUC of HMW adiponectin (percentage of baseline concentrations, i.e. $T_0 = 100$) from 0 to 60 min was 5766 ± 457.3*min, 7500 ± 736.7*min and 6591 ± 337.6*min after placebo, UAG and Comb respectively (*NS*) (data not shown).

Glucose and insulin

Baseline concentration of glucose was 4.4 ± 0.47 mmol/l. Neither UAG nor Comb induced any significant change in glucose concentrations. Concentrations of glucose were never significantly different from placebo throughout the study period. Figure 3A shows glucose concentrations displayed as percentage of baseline concentrations.

Baseline insulin concentration was 184.1 ± 24.0 pmol/l. Administration of UAG did not have any effect on insulin concentration. However, administration of Comb induced a significant decrease in insulin levels, reaching a minimum after 20 min. Fig. 3B shows that at 20 min, insulin concentration after Comb is 58.2 ± 3.9% of baseline, while after placebo and UAG administration insulin concentration is 88.7 ± 7.1% and 92.7 ± 2.6%, respectively ($P < 0.05$).

Fig. 2 Adiponectin

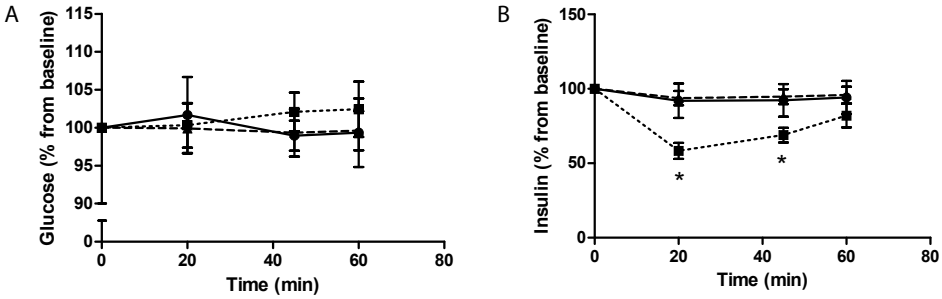


A: Total adiponectin concentration, presented as % from baseline.
B: HMW adiponectin concentration, presented as % from baseline.
C: HMW/total adiponectin concentration, presented as % from baseline.
T_0 = administration of study medication: placebo (—●—), UAG (---▲---), Comb (···■···).

DISCUSSION

Energy homeostasis and body weight are regulated by a highly complex network involving the brain, the digestive tract and WAT.[1] Circulating gut hormones and adipokines connect the digestive tract and WAT with several parts of the brain such as the hypothalamus and brain stem, thereby modulating food intake and energy expenditure.[1, 10, 17] However, signalling pathways connecting digestive tract and WAT are less well characterized. Adiponectin and ghrelin concentrations are both decreased in obesity.[23, 25, 26] Additionally, they both have important effects on insulin sensitivity. Adiponectin strongly improves insulin sensitivity, while AG decreases insulin sensitivity.[28, 34] However, the combination of AG and UAG has been demonstrated to improve insulin sensitivity as well.[31, 32] We evaluated effects of an acute intravenous administration of either UAG alone or in combination with AG on levels of total and HMW adiponectin in human

Fig. 3 Glucose and insulin



A: Glucose concentration, presented as % from baseline.

B: Insulin concentration, presented as % from baseline.

T_0 = administration of study medication: placebo (—●—), UAG (---▲---), Comb (···■···).

* $P < 0.05$

obesity. Neither UAG nor UAG + AG affected concentrations of total and HMW adiponectin in the first hour after administration.

Both ghrelin and GHS-R mRNA are expressed in adipose tissue, which suggests that ghrelin has a function in adipocyte metabolism.[39, 40] Indeed, ghrelin has been shown to promote adipogenesis by a direct peripheral action: both AG and UAG stimulate lipid accumulation in human visceral adipocytes and rat bone marrow adipocytes.[40, 41] However, apart from its function in lipid storage, adipose tissue has an additional role as an endocrine organ secreting adipokines, a function which could hypothetically be modulated by ghrelin as well. To our knowledge, *in vivo* studies evaluating this relation between WAT and the digestive tract are lacking. Only Ott et al. demonstrated that *in vitro* administration of ghrelin to a brown adipocyte model strongly decreased basal adiponectin mRNA expression.[42] In the present *in vivo* study, this effect of ghrelin on adiponectin levels could not be replicated.

Both ghrelin and adiponectin serum levels are decreased in obesity,[23, 25, 26] a condition characterized by insulin resistance. It could be hypothesized that increased insulin levels in obesity are responsible, since insulin has been shown to negatively influence both ghrelin and adiponectin concentrations.[43-45] Therefore, we hypothesized that apart from a possible direct effect on adipocyte level, ghrelin could indirectly influence adiponectin concentrations by affecting insulin levels. In the present study, insulin concentrations decreased by almost 50% at 20 min after administration of AG + UAG. However, this did not affect adiponectin concentrations, which invalidates an indirect effect of ghrelin on adiponectin concentrations. On the other hand, since we have not yet been able to elucidate the mechanism through which coadministration of UAG + AG improves insulin sensitivity, we hypothesized that this effect could be mediated by a direct increase in adiponectin concentration. The present study however did not show an increase in adiponectin concentration preceding the observed decrease in insulin

concentration. Therefore, it is less likely that adiponectin mediates the improvement in insulin sensitivity effectuated by administration of UAG + AG.

There are several limitations to the present study. First of all, the design of our study did not include an arm of AG only, which implicates that no conclusions can be drawn regarding the effect of AG on adiponectin. Secondly, we only studied acute effects of UAG and UAG + AG on adiponectin concentrations. It could be hypothesized that continuous administration of ghrelin does affect adiponectin levels or that the effect occurs more than one hour after acute administration. However, previous *in vitro* results show that ghrelin-induced decrease in adiponectin mRNA already occurred 30 minutes after administration.[42]

In conclusion, the present study shows that acute intravenous administration of unacylated ghrelin and the combination of unacylated and acylated ghrelin in morbidly obese subjects without overt diabetes does not acutely affect total or HMW adiponectin concentrations, neither directly nor indirectly by changing insulin concentrations. Studies evaluating effects of acylated ghrelin and long-term effects of (continuous) ghrelin administration on adiponectin concentrations are indicated.

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Part II

Outcome of surgical treatment of obesity: gallstones and quality of life

Chapter 8

Gallstone formation after weight loss following gastric banding in morbidly obese dutch patients

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Obesity Surgery 2006; 16: 592-596

ABSTRACT

Background

Obesity is a risk factor for the development of gallstones. Rapid weight loss may be an even stronger risk factor. We retrospectively assessed the prevalence and risk factors of gallstone formation after adjustable gastric banding (AGB) in a Dutch population.

Methods

All patients who underwent AGB between Jan 1992 and Dec 2000 for morbid obesity were invited to take part in this study. Transabdominal ultrasonography of the gallbladder was performed in those patients without a prior history of cholecystectomy (Group A). Additionally, 45 morbidly obese patients underwent ultrasonography of the gallbladder before weight reduction surgery (Group B).

Results

120 patients were enrolled in the study (group A). Prior history of cholecystectomy was present in 21 patients: 16 before and 5 after AGB. Ultrasonography was performed in 98 patients: gallstones were present in 26 (26.5%). On multivariate analysis, neither preoperative weight, nor maximum weight loss, nor the interval between operation and the postoperative ultrasonography were determinants of the risk for developing gallstone disease. Prevalence of gallstones was significantly lower in the morbidly obese patients who had not yet undergone weight reduction surgery (Group B).

Conclusions

Rapid weight loss induced by AGB is an important risk factor for the development of gallstones. No additional determinants were found. Every morbidly obese patient undergoing bariatric surgery must be considered at risk for developing gallstone disease.

INTRODUCTION

Obesity is a known risk factor for the development of gallstones.[1, 2] However, rapid loss of excess weight may be an even stronger risk factor.[3-6] The development of gallstones following weight loss is likely related to a change in cholesterol metabolism, because the percentage of cholesterol stones in this population is considerably higher than in the general population.[6]

The formation of cholesterol stones is the result of three physical conditions: supersaturation of bile with cholesterol, decreased gallbladder contractions, and acceleration of cholesterol crystal nucleation.[5, 7, 8] Each of these processes may result from weight loss. Consequently, the amount and rate of weight loss play an important role in gallstone formation as well.[8, 9]

Morbidly obese individuals are at very high risk for the development of gallstones after weight reduction surgery. In 22% to 71% of morbidly obese individuals, gallstone disease has developed after bariatric surgery.[3, 10, 11]

The prevalence of gallstones after weight reduction surgery was assessed in a Dutch morbidly obese population. We compared the presence of gallstones in two groups of morbidly obese patients: those who already had undergone weight reduction surgery and those who had not. The presence of other risk factors for development of gallstones besides rapid weight loss, was also assessed.

METHODS

Bariatric surgery has been performed in our hospital since Jan 1992. The method used has been placement of an adjustable gastric band (AGB) to mechanically restrict food intake. Patients accepted for surgery all met the criteria for morbid obesity: body mass index (BMI) >40 kg/m^2, or BMI >35 kg/m^2 in combination with relevant co-morbidity.[12]

All patients who underwent gastric banding from Jan 1992 to Dec 2000 were invited to take part in a retrospective study, evaluating weight loss, quality of life, general health and gallstone formation. In this study, we describe the results considering gallstone formation. At least 1 year had passed between surgery and participation in the study. Participants were assessed for preoperative weight, maximum weight loss, present weight, and history of cholecystectomy, including timing of gallbladder surgery either before or after AGB. Patients without a prior history of cholecystectomy underwent transabdominal ultrasonography of the gallbladder to detect gallstones or sludge (Group A).

Because in our study group no data were available regarding the presence of gallstones before AGB, we created a control group of morbidly obese subjects who had not yet undergone bariatric surgery (Group B). Consecutive non-selected morbidly obese patients entering the weight reduction surgery program were evaluated for a history of cholecystectomy, and under-

went transabdominal ultrasonography. None of these patients had a prior history of weight reduction surgery.

Statistical analysis was carried out using either analysis of variance or the χ^2-test to compare patient groups. All data are expressed as mean ± standard deviation (SD).

RESULTS

A total of 225 patients underwent AGB in our hospital between 1992 and 2000; 120 of the patients (53%; 11 male, 109 female) agreed to participate in this study (Group A). Their weight before AGB was 130.4 ± 17.4 kg, with BMI 44.5 ± 5.6 kg/m^2. The average time between AGB and participation in the study was 56.1 months (range 16-102 months).

All patients reported the same pattern in weight reduction: rapid weight loss in the first year after surgery with eventual stabilization at a slightly higher level afterwards. Maximum weight loss was 31.5 ± 11.3% of initial body weight, and BMI at the time of participating in the study was 34.2 ± 6.1 kg/m^2. None of the patients had used ursodeoxycholic acid after AGB.

A history of cholecystectomy was present at the time of evaluation in 21 (17.5%) of 120 patients: 16 (13.3%) before AGB and 5 (4.2%) after AGB. Mean time between AGB and cholecystectomy in the latter patients was 37 months (range 15 to 73 months).

The remaining 98 patients underwent transabdominal ultrasonography of the gallbladder (one man refused). Gallstones were detected in 26 (26.5%) of them (2 men, 24 women), including sludge in 2 (2.0%) who had evidence of gallstones as well. Thus, in Group A the total prevalence of gallstones after weight reduction surgery was 31 (30.1%) in 103 patients at risk: 5 cases of symptomatic gallstones after AGB who had undergone cholecystectomy, and 26 cases of gallstones detected on postoperative ultrasonography performed in 98 patients.

Although symptomatic gallstone disease appeared to be present in only 4.9% (5 in 103 who underwent cholecystectomy after AGB), another 2 patients in group A with apparently "silent" gallstones reported complaints attributable to gallbladder disease. They underwent cholecystectomy subsequently. Thus, 7 patients out of 31 (22.5%) who had gallstones after AGB developed symptoms consistent with gallbladder disease.

Table 1 shows that there was no significant difference between subjects with or without evidence of gallstones on ultrasonography regarding age (43.5 ± 11.3 years vs. 41.4 ± 8.0 years), sex (7.7% male vs 11.1% male), initial body weight (131.8 ± 17.0 kg vs 129.4 ± 17.7 kg) or BMI (45.0 ± 4.6 vs 44.0 ± 6.1). Similarly, neither the total amount of weight loss (30.6 ± 12.9% of initial body weight vs 31.4 ± 10.5%), nor the time interval between weight reduction surgery and detection of gallstones (55.4 ± 21.6 months vs 55.5 ± 20.7 months), were determinants of the risk to develop gallstones.

Table 1. Characteristics of the morbidly obese patients after weight reduction surgery (mean ± SD)

	Cholecystectomy (n=21)		Ultrasonography (n=98)	
	Before AGB	After AGB	Gallstones	No gallstones
	n = 16	n = 5	n = 26	n = 72
M : F	0 : 16	0 : 5	2 : 24	8 : 64
Age (years)	43.6 (± 9.9)	49.0 (± 10.3)	43.5 (± 11.3)	41.4 (± 8.0)
Preoperative weight (kg)	132.4 (± 14.1)	122.8 (± 16.8)	131.8 (± 17.0)	129.4 (± 17.7)
Preoperative BMI (kg/m^2)	45.6 (± 5.0)	43.3 (± 3.3)	45.0 (± 4.6)	44.0 (± 6.1)
Max weight loss (kg)	45.4 (± 19.3)	41.0 (± 23.3)	40.8 (± 19.3)	40.7 (± 15.6)
Max weight loss (% of initial body weight)	33.6 (± 12.3)	32.4 (± 13.7)	30.6 (± 12.9)	31.4 (± 10.5)
Time after surgery (months)	59.2 (± 25.4)	59.6 (± 24.6)	55.4 (± 21.6)	55.5 (± 20.7)

AGB = adjustable gastric banding

Those patients who had undergone cholecystectomy for symptomatic gallstone disease after AGB tended to be older than those patients who had gallstones identified by ultrasonography (49.0 ± 10.3 years vs 43.5 ± 11.3 years), although this difference was not statistically significant.

In the control group B of prospectively nonselected morbidly obese patients entering the weight reduction surgery program for treatment of their obesity, 45 participants underwent preoperative transabdominal ultrasonography of the gallbladder. Gallstones were found in 6 patients (13.3%). Table 2 presents the characteristics of this group (group B) compared to the previously discussed group who already had weight reduction surgery (group A). Group A patients who had undergone cholecystectomy were excluded, because gallstone formation after weight reduction surgery could not be studied in this group. The proportion of male patients in group A was lower than in group B: 9.7% vs 26.7%. There was no significant difference in body weight (129.7 ± 17.4 kg in group A vs 129.0 ± 21.8 kg in group B) or BMI (44.2 ± 5.6 kg/m^2 in group A vs 43.9 ± 7.4 kg/m^2 in group B) before AGB.

Table 2. Characteristics of the morbidly obese patients before and after weight reduction surgery (mean ± SD)

	After AGB (group A)	Before AGB (group B)
	n=103	n=45
M : F	10 : 93	12 : 33
Age (years)	42.3 (± 9.1)	38.1 (± 11.5)
Weight before AGB (kg)	129.7 (± 17.4)	129.0 (± 21.8)
BMI before AGB (kg/m^2)	44.2 (± 5.6)	43.9 (± 7.4)
Present weight (kg)	97.7 (± 19.2)	
Present BMI (kg/m^2)	34.3 (± 6.2)	
Gallstones present (%)	31 (30.1%)	6 (13.3%)

AGB = adjustable gastric banding

*Present weight and BMI represents the weight and BMI at the time of transabdominal ultrasonography.

At the time of performing ultrasonography, age of the patients was significantly higher in group A than in group B (42.3 ± 9.1 years vs 38.1 ± 11.5 years, P<0.05). On the other hand,

weight (97.7 ± 19.2 kg in group A vs 129.0 ± 21.8 kg in group B, $P<0.001$) and BMI (34.3 ± 6.2 vs 43.9 ± 7.4, $P<0.001$) were significantly lower in group A compared to group B.

The most important finding was the difference in prevalence of gallstones: 13.3% of morbidly obese patients had gallstones before AGB vs 30.1% in the group after AGB (Table 2). Using the χ^2-test, this difference is statistically significant ($P<0.05$).

DISCUSSION

The prevalence of gallstones after AGB is 30% in our Dutch population of morbidly obese patients. These results correspond with the reports of others. For example, a study by Miller et al[10] reported an incidence of 22% at 1 year after vertical banded gastroplasty (VBG) or AGB, increasing to 30% at 2 years after the surgery. Shiffman et al[13] described an incidence of gallstone formation of 38% within 6 months after Roux-en-Y gastric bypass (RYGBP).

The incidence of gallstone formation after weight loss varies widely: 10% of patients suffering from morbid obesity develop gallstones after a low calorie diet,[4, 9, 14] while up to 71% of morbidly obese patients develop gallstones after RYGBP.[11] RYGBP and especially the duodenal switch operation have the feature of bypass of the duodenum by food, decreased cholecystokinin secretion with gallbladder stasis, and more rapid and greater weight loss.[6, 13, 15, 16] Those bariatric operations are followed by a significantly higher incidence of gallstone formation than the purely gastric restrictive operations. However, AGB is also followed by supersaturation of bile with cholesterol due to mobilization of cholesterol from mobilized fat, depressed gallbladder emptying due to decreased food intake, and accelerated cholesterol crystal nucleation from bile stasis.[5, 7, 8]

It is generally considered that the risk of gallstone formation after weight loss increases sharply if the rate of weight loss exceeds 1.5 kg/week or if the total amount of weight loss is >24% of initial body weight.[3, 7, 8] Our population of morbidly obese patients treated with AGB had rapid and considerable weight loss (average 31% of initial body weight), but with the cholecystokinin mechanism intact. Nevertheless, changes in cholecystokinin secretion due to decreased food loading still result in decreased gallbladder contraction.

The prevalence of 30.1% gallstone formation after AGB in our study might be an overestimation of the true number of patients developing gallstones after AGB, because we do not know the number of gallstones before AGB in Group A. It is possible that some of group A patients already had silent gallstones before AGB. Therefore, we assessed the prevalence of gallstones in a group B, morbidly obese patients who had not yet undergone bariatric surgery. The prevalence of silent gallstones based on ultrasonography in this group was only 13.3%. Comparing these figures, we can draw two conclusions. First, the majority of the gallstones likely developed after the AGB. Second, the significant difference in prevalence of gallstones in

these two groups supports the tenet that weight loss is a major risk factor for the formation of gallstones.

There are some limitations regarding these results. The two patient groups were recruited in a different way: group A was retrospectively studied AGB patients who were invited to participate, versus group B was consecutive non-operated patients who were invited to participate prospectively. The percentage of eligible patients who agreed to participate was higher in the latter group. Also, the patients in group A were older and more often female than in group B, which are known risk factors for the development gallstones. Nevertheless, the difference in body weight at the time of ultrasonography was significantly lower in group A who had undergone AGB.

We compared the 30% prevalence of gallstones after AGB weight loss with the prevalence in the general Dutch population (not suffering from morbid obesity) assessed by Thijs et al.[17] In the general population, 4% and 10% of men and 16% and 11% of women at age 30 to 39 years and 40 to 49 years respectively have gallstone disease, which is lower than in our population after AGB. The difference is far less striking when comparing the general Dutch population with the morbidly obese patients before AGB (group B) who had a 13% prevalence of gallstones.

On multivariate analysis comparing the patients with and without gallstones after AGB, we could not identify any determinants of risk to develop gallstones. Age, sex, initial body weight and amount of weight lost were not significantly different in those who did and those who did not develop gallstones.[9, 13, 18, 19] However, Yang et al,[9] Wudel et al[11] and Shiffman et al[13] describe a correlation between gallstone formation and amount of weight lost. Papavramidis[3] and Al-Jiffry[7] state that this risk increases sharply if the rate of weight loss exceeds 1.5 to 1.7 kg/week, while Erlinger found this relationship for weight loss exceeding 24% of initial body weight.[8] One explication for the lack of relationship between amount of weight loss and stone formation in our study might be the rather long period between the AGB operation and the time of transabdominal ultrasonography, because gallstones are reported to develop in the first weeks to months after weight reduction surgery[3, 6, 7, 10, 11] and the stones may even disappear when body weight stabilizes.[5-7] Figures regarding weight loss in the first postoperative year (in which weight loss is fastest) were not available.

The percentage of newly formed gallstones after weight reduction surgery which became symptomatic, leading to cholecystectomy, is more important. Gallstones formed in 30.1% of our population, of whom 22.5% developed symptomatic gallstone disease, which is consistent with other findings reporting 12% to 40%.[3-5, 10, 11] Nevertheless, the prevalence of symptomatic gallstone disease in our total population of patients who had undergone AGB was only 6.8% (7 in 103).

Because symptomatic gallstone disease may be accompanied by significant morbidity, prevention of gallstone formation after weight reduction surgery may be a consideration. This involves simultaneous cholecystectomy during weight reduction surgery[3, 15, 18, 20, 21] or postoperative prophylactic treatment with ursodeoxycholic acid.[10, 11, 22]

None of our patients had any prophylaxis, because this was not the practice in the Netherlands in the period that they underwent surgery. Nowadays, both methods have earned their place. We prefer postoperative treatment with ursodeoxycholic acid, because it is an easy, cost-effective preventative. It would be helpful to compare these methods in a prospectively randomized controlled trial regarding development of gallstones, morbidity, and cost-effectiveness.

In conclusion, we found gallstones in 30% of morbidly obese patients after gastric banding. This was significantly higher than the prevalence of gallstones in a population of morbidly obese patients before weight reduction surgery. This finding is consistent with the concept of weight loss being a risk factor for gallstone formation. Additional risk factors for gallstone formation could not be demonstrated. In 22.5% of the patients who developed gallstones, symptomatic gallbladder disease was diagnosed, which is 6.8% of the total population who underwent AGB.

Acknowledgements

The authors wish to thank Prof.dr.Ton J.M. Cleophas for his assistance in statistical analysis.

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Chapter 9

Quality of life after gastric banding in morbidly obese dutch patients: long-term follow-up

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Obesity Research & Clinical Practice 2008; 2: 151-158

ABSTRACT

Objective

The long-term effects of gastric banding (AGB) on quality of life (QoL) in a morbidly obese population were investigated in a cross-sectional study. Additionally, determinants of QoL after AGB were assessed.

Methods

All patients treated by AGB for morbid obesity in a Dutch hospital were invited to complete the RAND 36-Item Health Survey. Of 121 participating patients 59 met the criteria for long-term follow-up (>5 years): 4 male and 55 female, mean age 42.4 ± 9.7 years, mean Body Mass Index (BMI) before surgery 44.9 ± 5.9 kg/m^2. Time since surgery was 74.7 months (range 60-107.6). The control group consisted of 28 presurgical patients. General and obesity-related parameters were assessed for correlation with QoL.

Results

Significant differences between the preoperative group and Dutch community norm (CN) values were found on five out of eight QoL subscales, in favor of CN. AGB induced significant weight loss in the postoperative group: 56.1% excess weight loss (%EWL). This group scored significantly better than the preoperative group on one out of eight subscales: physical functioning (P = 0.019). Additionally, scores on four out of eight subscales were still significantly impaired compared to CN. Postoperative BMI and %EWL influenced QoL after long-term follow-up, whereas weight regain had no negative impact.

Conclusions

This study shows that after long-term follow-up subjects treated by gastric banding to induce weight loss have a slightly better QoL than those who did not undergo surgery yet. QoL remains impaired in comparison to the general population. After long-term follow-up BMI and weight loss do influence QoL whereas weight regain does not have any negative impact.

INTRODUCTION

Obesity is an increasing worldwide health problem. The prevalence has always been the highest in the United States, with 32.2% of adults being obese (defined as a body mass index (BMI) > 30 kg/m^2) in the period 2003-2004,[1] but Europe is following with rates up to 24% among men, and 36% among women.[2] In the Netherlands, 46.5% of the general population (age > 20 years) is overweight, and 11.3% is obese.[3]

Especially individuals suffering from morbid obesity (defined as a BMI > 40 kg/m^2) are observed to have a significantly higher morbidity and mortality than the general population. A wide range of diseases are commonly associated with (morbid) obesity, such as hypertension, diabetes, hyperlipidemia, sleep apnea, asthma and degenerative joint disease.[4-6] Additionally, morbid obesity has important negative psychosocial consequences. Functional impairment, increased morbidity and especially social stigmatization typically cause depression, low self-esteem and anxiety disorders.[4, 7-10] As a result of this high prevalence of both physical and psychological complications, severe obesity clearly is associated with significantly reduced health-related quality of life (QoL).[9, 11-14]

Up to the present, bariatric surgery is the only treatment for morbid obesity which results in substantial and, more important, sustained weight loss. The effectiveness of bariatric surgery traditionally has been measured in percentage of excess body weight lost. However, since major improvements in general health can be achieved with even modest weight loss,[15, 16] absolute weight change after bariatric surgery seems not the best way to evaluate its effects. Additionally, weight loss is not a good measure for postoperative improvement in psychopathology as well.[17, 18] As a result, the effectiveness of bariatric surgery is often defined as improvement in health-related QoL. In the first years after surgery, quality of life is strongly improved compared to preoperative scores.[8-11, 19] Indeed, some studies show that quality of life scores just after surgery are comparable with community norm values, even though BMI is still significantly higher.[7, 20, 21] Nevertheless, after a period of 2 years postoperatively, quality of life has a tendency to worsen.[9, 13, 18, 19] It is unclear whether this is the result of waning optimism in a period of weight stabilization, disappointment about only limited improvement in everyday life or persistence of pre-surgical problems not related to body weight.[18, 22] In addition, weight regain, which is observed especially in restrictive types of bariatric surgery, might play a negative role.[9, 23] Unfortunately, only few studies report follow-up results longer than 5 years postoperatively describing quality of life.

The objective of this study was to evaluate the long-term effects of gastric banding on the quality of life in morbidly obese patients. Does QoL improve, compared to the preoperative situation? And if so, is QoL comparable to the general population postoperatively? Additionally, the aim was to identify determinants influencing QoL in morbidly obese patients having undergone gastric banding.

METHODS

Patient groups

Weight reduction surgery has been performed in our hospital since 1992. The surgical method used is the placement of an adjustable gastric band (AGB, LAP-BAND® System, Allergan Inc., Irvine, USA) to mechanically restrict food intake. Patients accepted for surgery all met the criteria for morbid obesity: body mass index (BMI) > 40 kg/m^2, or BMI > 35 kg/m^2 in combination with relevant comorbidity.

All patients who underwent gastric banding from 1992 to December 2000 were invited to take part in a retrospective study, evaluating different aspects of gastric banding like weight loss, quality of life, general health and gallstone formation.[24] In this study, we describe the results considering quality of life after long-term follow-up, defined as at least 5 years after surgery.

A letter of invitation was send to all patients. After confirmation of participation in the study (all participants gave their written informed consent), questionnaires were mailed to their homes, and completed in a self-administered way. A total of 121 of 225 (54%) patients agreed to participate, of whom 59 subjects met the inclusion criteria of at least 5 years interval between surgery and participation in the study. This group (group A) consisted of 4 male and 55 female patients, mean age 42.4 years (range 25-62 years). All patients returned the questionnaires on visiting the outpatient clinic for a structured interview and assessment of anthropometric measures. Preoperative information (presurgical BMI, excess weight and comorbid conditions) was based on medical records. Postoperative BMI and excess weight loss (%EWL) describe the situation at the moment of participation in the study. Lowest weight between surgery and study moment was based on patient self-report. Weight regain (%WR) was defined as the difference between reported lowest body weight after surgery and weight at the moment of participation in the study, expressed as percentage of maximum weight loss. Comorbidity score is defined as the total number of relevant comorbid conditions (diabetes, hypertension, joint pain).

Since preoperative data on quality of life was not available in our study group (group A), we chose a cross-sectional design to study differences in QoL pre- and postoperatively. Therefore, 50 additional patients who had entered the weight reduction surgery program but had not yet undergone surgery were invited to complete the questionnaire which was sent to their homes. Eventually, 28 out of 50 patients returned the questionnaire, resulting in a response rate of 56% (group B). Group B consisted of 4 male and 24 female patients, mean age was 39.8 years (range 28-62 years). Anthropometric measures and medical history were based on patient self-report, and checked in the medical records.

Questionnaire

The RAND 36-Item Health Survey is a widely used generic questionnaire assessing eight domains of subjective health status: (1) physical functioning, (2) bodily pain, (3) role limitations due to

physical health problems, (4) role limitations due to personal or emotional problems, (5) mental health, (6) social functioning, (7) vitality, and (8) general health. One additional item gives an indication of perceived change of health over the past year (health change). The RAND 36-Item Health Survey includes the same items as the MOS 36-item Short-Form Health Survey (SF-36), but differs slightly in its scoring system.[25] Transformed scores range from 0 (poor health) to 100 (good health) and were calculated for the 9 domains. The 36-item Health Survey has been proven to be a useful instrument in assessing quality of life in morbidly obese subjects, before and after bariatric surgery.[12, 26-30]

In this study, a validated Dutch version of the RAND 36-Item Health Survey was used.[31] Van der Zee reported a Cronbach's alpha for internal consistency between 0.71 and 0.92 for different domains, while test-retest reliability varied between 0.58 and 0.82 after 2 months. The guidelines also provided Dutch community norm (CN) values ($N = 1063$, 65% female, mean age 44.1 years).

Statistical analysis

Results are reported as mean ± standard deviation (SD). A P-value of <0.05 is considered significant. To compare RAND scores in group A vs. CN, and group B vs. CN, a one-sample t-test was used. Since on exploring the RAND scores in group A and B, they proved not to be normally distributed, the nonparametric Mann-Whitney test was used to identify differences in RAND scores between the postoperative group (group A) and the preoperative control group (group B). In the postoperative group (group A) multiple regression analysis was carried out to identify variables contributing to RAND domain scores. The following variables were tested: age, gender, time since surgery, preoperative BMI, postoperative BMI, %EWL, %WR and comorbidity score. Statistic calculations were performed using Statistical Package for the Social Sciences (SPSS release 14.0; SPSS Inc, Chicago).

RESULTS

Patient characteristics

Patient characteristics of group A and B are described in Table 1. In group A, mean BMI decreased significantly from 44.9 ± 5.9 kg/m^2 preoperatively to 33.3 ± 6.0 kg/m^2 at follow-up ($P < 0.001$), representing 56.1 ± 27.0% excess weight loss. Mean time since surgery was 74.7 months (range 60 – 107.6). BMI at the time of participation in the study was significantly lower in group A, compared to group B ($P < 0.001$). Additionally, the prevalence of diabetes was significantly lower in group A, compared to group B, whereas the prevalence of hypertension and joint pain was not significantly different. Both groups did not differ significantly in age, nor in male to female ratio. Comparing group A and B with CN, the most important difference is the male to female ratio which was significantly higher in CN.

Table 1. Descriptive characteristics of patients after gastric banding and presurgical patients

	Group A (*N*=59)	Group B (*N*=28)	CN (*N*=1063)	*P*
Age (years)	42.4 ± 9.7	39.8 ± 8.5	44.1	A vs. CN *NS* B vs. CN *P*<0.05 A vs. B *NS*
Sex (M:F)	4:55	4:24	372:691	A vs. CN *P*<0.05 A vs. CN *P*<0.05 A vs. B *NS*
Time since surgery (months)	74.7 ± 15.3			
Preoperative BMI (kg/m^2)	44.9 ± 5.9	41.8 ± 3.4		*P*<0.05
Postoperative BMI (kg/m^2)	33.3 ± 6.0	41.8 ± 3.4		*P*<0.001
Excess weight loss (%)	56.1 ± 27.0			
Weight regain (%)	26.1 ± 23.7			
Diabetes (%)	2	12		*P*<0.05
Hypertension (%)	26	40		*NS*
Joint pain (%)	65	50		*NS*
Comorbidity score	0.66 ± 0.63	0.89 ± 0.79		*NS*

Group A: Patients in follow-up after gastric banding for morbid obesity
Group B: Presurgical patients suffering from morbid obesity
CN: Dutch community norm population
Mean ± S.D.

Quality of life

Figs. 1 and 2 demonstrate RAND domain scores in group A, group B and CN. Group B had significantly lower scores on 6 out of 9 domains compared to CN: physical functioning, general health, social functioning, vitality, bodily pain and health change ($0.001 < P < 0.04$).

Group A scored significantly better on one domain compared to the presurgical group B, i.e. physical functioning ($P = 0.019$). Scores on the remaining eight domains (social functioning, mental health, vitality, bodily pain, role limitations due to physical or emotional problems, general health and health change) seemed higher after surgery, but were not statistically significantly different in group A.

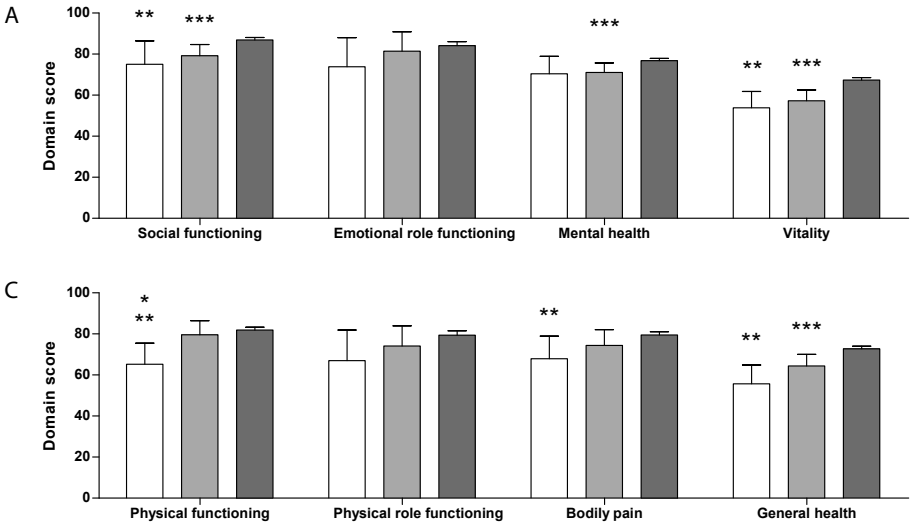
On the other hand, compared to CN values group A still scored significantly lower on four domains of the RAND questionnaire: social functioning, mental health, vitality and general health ($0.001 < P < 0.016$). Additionally, scores on the remaining domains (physical functioning, bodily pain, role limitations due to physical or emotional problems) showed a trend to be more impaired in group A as well, though not reaching significance.

Determinants of quality of life after gastric banding

The variables studied can be subdivided in three categories: general (age, gender), obesity specific (BMI preoperative and postoperative, comorbidity score) and surgery specific (%EWL, %WR, time since surgery).

On multiple regression analysis, age was the most frequent determinant of RAND-scores, negatively influencing physical functioning, social functioning, role limitations due to physical

Fig. 1 RAND-36 Domain scores



RAND-36 domain scores (95% confidence interval)
Group B: Presurgical patients suffering from morbid obesity
Group A: Patients in follow-up after gastric banding for morbid obesity
CN: Dutch community norm values
(*) significant difference in group B vs. group A; (**) significant difference in group B vs. CN; (***) significant difference in group A vs. CN

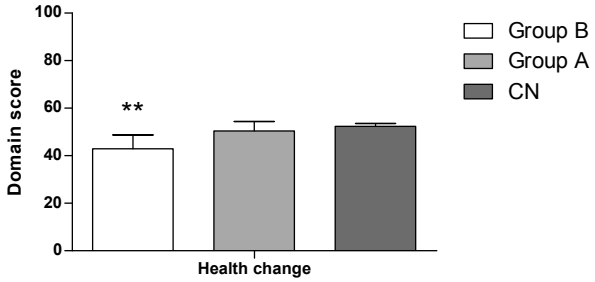
or emotional problems, mental health, general health and bodily pain. Sex had no influence on any of the RAND scores.

Regarding obesity specific variables, it showed that after long-term follow-up preoperative BMI did not influence QoL anymore. On the other hand, postoperative BMI had a significant negative correlation with domain scores on role limitations due to emotional problems and general health ($P = 0.011$, $P = 0.02$, respectively): a lower postoperative BMI resulted in better QoL on these domains. Additionally, the comorbidity score, which quantifies obesity related comorbidity, was significantly negatively associated with physical functioning. Joint pain accounted for the major part of variance attributable to comorbidity score.

The main parameter describing efficacy of bariatric surgery, i.e. %EWL, accounted for a significant proportion of variance in the domains role limitations due to emotional problems, vitality and general health ($P = 0.003$, $P = 0.021$, $P = 0.035$ respectively). More weight loss resulted in higher scores on these domains. Neither %WR nor the time since surgery showed any correlation with quality of life.

The one additional domain in the RAND questionnaire, representing perceived change of health over the last year was not influenced by any of the investigated parameters. Likewise, domain score on health change was not significantly different from the CN score, indicating a stable perceived health situation in patients after 5 years follow-up.

Fig. 2 RAND Domain score Health change



RAND-36 domain score Health change (95% confidence interval)
Group B: Presurgical patients suffering from morbid obesity
Group A: Patients in follow-up after gastric banding for morbid obesity
CN: Dutch community norm values
(*) significant difference in group B vs. group A; (**) significant difference in group B vs. CN; (***) significant difference in group A vs. CN

DISCUSSION

Health-related quality of life is one of the main measures to quantify the effect and success of bariatric surgery in treating the morbidly obese. In the present study, we compared long-term effects of gastric banding on QoL in a group of treated, previously morbidly obese patients to a group of presurgical morbidly obese patients and to Dutch community norm values. Quality of life, as measured by the RAND-36, proved to be significantly impaired in morbidly obese subjects. Long-term follow-up after gastric banding showed a slightly better QoL compared to presurgical data: only domain scores on physical functioning were significantly higher, eight additional domains were not significantly different. Additionally, postoperative QoL was persistently impaired compared to Dutch community norm values.

Many previous studies have consistently shown impaired QoL in morbidly obese patients, compared to community norm data.[12, 14, 20, 28] Only one study, by Horchner et al., was not able to demonstrate significant differences between a preoperative morbidly obese population and standardized Dutch norm data for the MOS SF-36 questionnaire.[32] The results of the present study support the hypothesis of impaired QoL in obesity. Both the presurgical morbidly obese population as well as the postsurgical obese population showed impaired QoL compared to Duch community norm data. Nevertheless, despite a significant difference in BMI, postsurgical patients had a higher score on only one QoL domain compared to presurgical patients. Scores on eight other domains displayed a trend, but were not significantly better.

In general, physical subscales appear to be more impaired in obesity than mental subscales.[11, 28] The RAND questionnaire lacks physical and mental composite scores, but all eight domains could be subdivided in a category representing either physical QoL (physical functioning,

bodily pain, role limitations due to physical health problems, general health) or mental QoL (role limitations due to personal or emotional problems, mental health, social functioning, vitality). Regarding this differentiation, we could identify a trend towards more impairment in mental domain scores in the postoperative group, whereas no differentiation could be made in the preoperative group. Compared to norm data our preoperative and postoperative groups scored lower on physical functioning, general health, bodily pain, social functioning, vitality, and general health, social functioning, vitality, mental health respectively. This might indicate that morbid obesity has a negative impact on both physical and mental QoL. However, a better QoL after gastric banding compared to presurgical data was seen in one domain representing physical QoL: physical functioning. Seven domains, i.e. bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, mental health, social functioning and vitality, were not significantly different between the pre and post surgery groups. This suggests that gastric banding has more impact on physical QoL than mental QoL.

The central issue of the present study was to evaluate the long-term effect on QoL of successful surgical treatment of morbid obesity. In our population, the results of gastric banding in terms of weight loss are consistent with other studies concerning purely restrictive types of bariatric surgery.[4, 20, 28, 33, 34] Mean BMI declined significantly from 44.9 ± 5.9 kg/m^2 preoperatively to 33.3 ± 6.0 kg/m^2 at follow-up, representing 56.1 ± 27.0% excess weight loss. Since obesity is to blame for impaired QoL, improvement could be expected after bariatric surgery. However, our study shows that despite a significant difference in body weight, QoL at least 5 years after gastric banding is only slightly better than in patients not treated for morbid obesity yet. This means, that after gastric banding, patients still experience impaired QoL comparable to presurgical patients, though being successful in losing weight. One of the most important explanations might be that despite significant weight loss, our study population still fulfils the criteria for obesity. Nevertheless, some studies report significant improvement (scores even exceeding norm values) shortly after surgery, in a period when substantial weight loss could not be observed yet.[7, 33] After longer follow-up, improvement in QoL seems to level off.[7, 28, 33, 35] Additionally, both Waters et al. and Van Gemert et al. even report a decrease after 24 and 86 months respectively.[13, 22] Due to the cross-sectional design of our study, we are not able to comment on changes over time. Nevertheless, we conclude that after long-term follow-up the results of gastric banding on QoL, despite persisting significant weight reduction, are disappointing.

Age was the most important determinant of quality of life in the population after gastric banding, negatively influencing both physical and mental domains: physical functioning, social functioning, role limitations due to physical or emotional problems, mental health, general health and bodily pain. Sex did not influence QoL in our population. Additionally, presence of comorbidity negatively influenced physical functioning, which could mostly be attributed to joint pain rather than diabetes or hypertension.

Weight loss induced by gastric banding occurs in the first few years after surgery, [13, 22, 33] and could therefore be expected to have stabilised after five years. Nevertheless, in our population %EWL still significantly influenced QoL (domain scores on role limitations due to emotional problems, vitality and general health). This means, that the more weight patients lose after gastric banding, the stronger their QoL improves, even after long-term follow-up. This statement is supported by the finding that postoperative BMI accounts for some variance in QoL as well. However, results from previous studies are inconsistent on this topic. Some authors demonstrate a significant effect of both BMI and %EWL, whereas others only show positive influence of %EWL on physical domain scores.[11, 13, 14, 19] Moreover, Dixon denied %EWL as being a predictor for QoL after bariatric surgery.[28] Based on our results, we conclude that more weight loss and a lower postoperative BMI do positively influence long-term quality of life. Additionally, after more than five years of follow-up preoperative BMI does not influence QoL anymore.

Since both %EWL and BMI influence QoL, it could be expected that %WR has, on the contrary, a negative influence on QoL. It is generally known that especially purely restrictive types of bariatric surgery are associated with slight weight regain after the first years of gradual weight loss.[9, 23] This phenomenon could theoretically be held responsible for at least some part of decreasing QoL after long-term follow-up. In our study weight regain was substantially present (23.9%), but it did not account for any negative influence on quality of life.

There are some limitations to the present study. In the first place, the cross-sectional design of the study does not allow us to draw conclusions on causality. Although the preoperative and postoperative population are not significantly different except for characteristics resulting from gastric banding (BMI and prevalence of diabetes), longitudinal studies are needed to evaluate change in QoL after surgery. In the second place, response rates were 54% and 56% in the follow-up and presurgical population respectively. Especially in studies concerning subjective topics like quality of life, lower response rates might cause significant bias. However, it is not easy to predict in what way the final results are influenced by this response bias. Then, a bias could have been introduced by using self-reported weight to calculate weight regain. Errors in reported weight, either deliberately or due to recall problems, might have led to misinterpretation of the relevance of weight regain in determining QoL. Additionally, group A and B were significantly different in one domain. However, some additional domains display differences between group A and B, which were not statistically significantly different. This might be due to the relatively small sample size of the presurgical group (Fig. 1). Additional studies with larger sample sizes are needed to establish possible differences where the present study might have failed to. Finally, the Dutch community norm population differed from our postoperative population in two respects. In the first place, the female to male ratio was higher in our study population, and since female sex is described to have a negative influence on quality of life, this might have underestimated domain scores in our population.[31] Nevertheless, we did not demonstrate any significant effect of sex on quality of life. In the second place, our study

population was slightly younger than the Dutch community norm population, which might on the contrary have caused overestimation of domain scores in our population.

In conclusion, effects of gastric banding on quality of life after long-term follow-up are disappointing: differences with preoperative quality of life are small - we only observed improvement in physical functioning - and impairment in comparison to community norm values persist. This corresponds with weight loss after bariatric surgery: weight decreases significantly, but patients will still be obese afterwards. Additionally, even after long-term follow-up weight loss and postoperative BMI do influence quality of life, whereas weight regain does not have a negative impact on quality of life. The present study confirms the relevance of describing results of bariatric surgery in terms of quality of life rather than weight loss. To evaluate improvement of quality of life immediately after surgery and possible deterioration after long-term follow-up longitudinal studies in larger populations are needed.

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General discussion, perspectives and summary

Chapter 10

General Discussion

THE EFFECTS OF ACYLATED AND UNACYLATED GHRELIN ON GLUCOSE AND INSULIN METABOLISM

Chapter 4 and 5

Ghrelin's molecular structure is characterized by *n*-octanoylation of serine at position 3 (acylated ghrelin, AG). This post-translational modification, catalyzed by Ghrelin O-Acyltransferase (GOAT), is essential for binding to the Growth Hormone Secretagogue Receptor type 1a (GHS-R1a).[1, 2] *In vivo*, most circulating ghrelin is unacylated (unacylated ghrelin, UAG), which was consequently thought to be devoid of any endocrine action.[3] However, many studies have shown that UAG does have intrinsic biological effects.[4-25] For example, it has been suggested that, analogous to AG, UAG might play a role in glucose homeostasis. While ghrelin has consistently been demonstrated to induce insulin resistance, the previously observed effects of UAG on glucose and insulin concentrations need confirmation. Additionally, identification of a receptor for UAG would add important information to the understanding of its functionality.

In the study described in chapter 4 we evaluated the effects of UAG and the combination of AG and UAG on glucose and insulin metabolism in morbidly obese subjects. Eight morbidly obese non-diabetic subjects were treated with either UAG 200µg, UAG 100µg in combination with AG 100µg (Comb), or placebo in 3 episodes of 4 consecutive days in a double-blind randomized crossover design. Study medication was administered as daily single i.v. bolus injections at 0900h after an overnight fast. At 1000h a standardized meal was served.

Insulin concentrations significantly decreased after acute administration of Comb, reaching a minimum at 20 min: 58.2 ± 3.9% of baseline, vs. 88.7 ± 7.2% and 92.7 ± 2.6% after administration of placebo and UAG, respectively ($P < 0.01$). After 1 h, insulin concentration had returned to baseline. Glucose concentrations did not change after Comb, which suggests that Comb strongly improves insulin sensitivity. On the other hand, UAG administration alone did not change glucose or insulin concentrations. In fed conditions, 1 h after administration of study medication, neither UAG nor Comb affected glucose and insulin metabolism.

In the study described in chapter 5 we investigated whether the blockade of endogenous AG action (i.e. blockade of the GHS-R1a) or the administration of exogenous AG and UAG differentially regulates the portal and systemic insulin response to glucose and/or modulates hepatic insulin clearance. We therefore studied in rats the effects of the administration of AG, UAG, the ghrelin receptor antagonist [D-Lys^3]GHRP-6, or their combination on portal and peripheral glucose and insulin levels during an intravenous glucose tolerance test (IVGTT).

UAG administration potently and dose-dependently enhanced the rise of insulin concentrations induced by IVGTT in the portal and, to a lesser extent, the systemic circulation. This UAG-induced effect was completely blocked by the coadministration of exogenous AG at equimolar concentrations. Similarly to UAG, [D-Lys^3]GHRP-6 alone or in combination with AG and UAG strongly enhanced the portal insulin response to IVGTT, whereas exogenous AG alone did not exert any further effect.

Studies on the effect of AG on glucose and insulin metabolism quite consistently report a decrease in insulin concentration accompanied by an increase in glucose concentration after acute administration of AG, which suggests that AG induces insulin resistance.[17, 26-28] The effect of UAG on glucose and insulin metabolism has been studied less extensively and results are not consistent. Gauna et al. evaluated effects of acute UAG administration and reported an increase in glucose concentration at unchanged insulin concentration in GH-deficient humans,[18] but a decrease in glucose concentration in primary hepatocytes.[17] On the other hand, several studies were unable to demonstrate any effect of UAG administration on levels of insulin and glucose.[8, 14, 15] Additional data however suggest that UAG has an insulin sensitizing effect.[20, 29] Surprisingly, UAG has been repeatedly shown to abolish AG's effects on insulin sensitivity.[8, 17, 20] Moreover, the coadministration of AG and UAG has been suggested to improve insulin sensitivity in GH-deficient subjects.[18]

We reported the effects of acute UAG administration in humans and rodents. In morbidly obese females UAG did not affect glucose and insulin concentrations in fasted conditions. However, in rodents UAG was shown to increase the second-phase insulin response to IVGTT dose-dependently. The most important insight provided by our study in rats was that the increase of insulin concentration following UAG administration measured in the portal circulation was almost not perceptible in the systemic circulation. Therefore, UAG likely establishes most of its actions in the portal system which might be an explanation why many *in vivo* studies failed to demonstrate effects of UAG on glucose and insulin metabolism.

The results of our studies described in chapter 4 and 5 confirm the previously observed results of acute coadministration of AG and UAG. Indeed, coadministration of AG and UAG in rodents completely abolished the increase in insulin concentration after UAG treatment alone, resulting in a net effect comparable to placebo. Moreover, in morbidly obese females insulin concentration was observed to decrease by almost 50% within the first hour after acute UAG and AG administration, while glucose concentration remained unchanged, resulting in an increase in glucose/insulin ratio. This change in glucose/insulin ratio again suggests an improvement in insulin sensitivity after coadministration of AG and UAG.

One factor complicating the interpretation of the biological effects and interactions of AG and UAG is that the receptor through which UAG exerts its metabolic effects has not been identified yet. Two hypotheses regarding the UAG receptor can be postulated: either UAG acts through a yet unidentified growth hormone secretagogue (GHS) receptor that, unlike the GSH-R1a, recognizes ghrelin independently of its acylation (i.e. a common receptor for UAG and AG), and/or UAG binds to another yet unidentified receptor distinct from the GHS receptor while AG mediates its effects in the same metabolic system through its known receptor, GHS-R1a.

Since the molecular structure of AG and UAG only differs in *n*-octanoylation of serine at position 3, it is not unlikely that they share a common receptor that binds structures other than the acyl group. However, when ghrelin binds to a receptor independent of its acylation, effects of receptor activation by AG and UAG can be expected to be identical. Indeed, several studies have

demonstrated identical effects and signaling pathways of AG and UAG in cardiomyocytes,[4] rat adipose tissue,[22] C2C12 skeletal myoblasts,[16] HIT-T15 beta-cells,[19] bone marrow adipocytes[23] and osteoblasts.[13] Most of these cells did not express GHS-R1a,[4, 13, 16, 19, 22] and the effect of AG and UAG was not shared with known synthetic GHS-R1a agonists,[17, 20, 23] which suggests that the observed actions are mediated by a receptor that is distinct from GHS-R1a.

However, UAG and AG do not always have corresponding effects. For example, as discussed previously, they can have antagonistic effects on glucose metabolism.[8, 17] This suggests that in some metabolic processes UAG and AG have either antagonistic effects on one common receptor or stimulate two different receptors that generate antagonistic effects. Gauna et al. have shown that AG's effects on glucose and insulin concentrations are mediated by GHS-R1a, since its action is blocked by GHS-R1a antagonists.[30] On the other hand, UAG's effects were not blocked by GHS-R1a antagonists, suggesting that UAG mediates its effect through a different receptor.[30] Additionally, Toshinai et al. demonstrated that intracerebroventricular administration of AG did not induce food intake in GHS-R1a deficient mice, while UAG did stimulate feeding in the same population, suggesting that the AG effect is mediated by GHS-R1a and the UAG effect is mediated by a different receptor.[24]

In our study described in chapter 5, we demonstrated that the GHS-R1a receptor antagonist [D-Lys^3]GHRP-6 strongly enhanced the portal insulin response to IVGTT. This effect is likely the result of blockade of the inhibitory action of endogenous AG on beta-cells mediated by GHS-R1a. Administration of UAG alone resulted in an enhanced portal insulin response which was similar to that exerted by the GHS-R1a receptor blocker and was not affected by coadministration with [D-Lys^3]GHRP-6. These results again suggest that at least in some metabolic systems AG's effects are mediated by the GHS-R1a receptor, whereas UAG's effects are mediated through a different presently unknown receptor.

Future directions

The pathophysiological basis of type 2 diabetes is an increase in insulin resistance. Therefore, if either UAG or UAG+AG could indeed improve insulin sensitivity, this might be a promising perspective in the treatment of type 2 diabetes. Presently, data on effects of continuous UAG administration with or without AG are lacking, as are long-term results of this treatment. Additionally, since most studies have evaluated the effects of UAG (with or without AG) in fasted conditions, studies in fed conditions should be performed. Finally, and most importantly, studies on the effects in patients suffering from type 2 diabetes are indicated. While a decrease in insulin concentration unaccompanied by a change in glucose concentration suggests an improvement in insulin sensitivity, clamp studies are needed to confirm whether these changes are indeed the result of an improvement in insulin sensitivity.

At present, interactions of AG and UAG are difficult to interpret: they seem to be functional antagonists in some metabolic systems while in other systems their effect is similar. Identification

of a UAG receptor (which might correspond to a second type of ghrelin receptor, as stated above) could provide important insight into the regulation and interaction within the ghrelin system.

THE EFFECTS OF OBESTATIN ON GLUCOSE AND INSULIN METABOLISM.

Chapter 6

In 2005 Zhang et al. discovered a second peptide derived from the preproghrelin polypeptide. Using a bioinformatic approach, they were able to identify a second conserved region in the ghrelin gene, encoding a 23 amino acid peptide, which they called obestatin. Surprisingly, acute intracerebroventricular and intraperitoneal administration of obestatin suppressed food intake, while daily administration of obestatin suppressed body weight gain and induced delayed gastric emptying. This implicated that obestatin was a functional peptide, and had endogenous physiological effects acting as a full antagonist of ghrelin.[31] Since ghrelin is known to play an important role in glucose and insulin metabolism,[17, 26, 28] it could be hypothesized that obestatin does affect insulin and glucose secretion as well.

In the present study we evaluated the effects of obestatin on glucose and insulin metabolism in the systemic and portal circulation. Obestatin 200 nmol/kg was administered systemically as a single intravenous bolus injection to fasted pentobarbital anesthetized adult male Wistar rats. Up to 50 min after administration, blood samples were taken to measure glucose and insulin concentrations, both in the portal and in the systemic circulation. The effect of obestatin was evaluated in fasted and in glucose-stimulated conditions (IVGTT) and compared to control groups treated with vehicle or IVGTT, respectively.

The results can be described easily: intravenous administration of obestatin did not have any effect on glucose and insulin concentrations, neither in the systemic nor in the portal circulation, when compared to the control groups.

At first, the discovery of obestatin seemed to open completely new perspectives on ghrelin metabolism. For example, if indeed ghrelin and obestatin acted as full antagonists, this could be a valid explanation why ghrelin (gene) deficient (*ghrl-/-*) mice display such a mild phenotype.[32] However, several serious issues questioning the physiological relevance of obestatin soon arose. Since ghrelin and obestatin are coexpressed in the same cell types,[31, 33, 34] which mechanisms (post-translational or alternative splicing) account for production of either obestatin or ghrelin and how is cosecretion avoided?[35-38] Secondly, concentrations of obestatin, both in the gastric fundus and in plasma, were very low compared to ghrelin concentrations, and half-life of obestatin in circulation was very short.[39, 40] Finally, obestatin was demonstrated not to influence GH secretion, which is one of the most important functions of ghrelin.[31]

Nevertheless, several studies have been able to confirm the original results of obestatin on food intake,[41, 42] while other studies showed that obestatin inhibited thirst, improved memory, regulated sleep, affected cell proliferation, increased the secretion of pancreatic juice enzymes,

promoted survival of pancreatic β-cells, and affected glucose-induced insulin secretion.[34, 43-53] On the other hand, at least as many studies failed to demonstrate any metabolic effect of obestatin administration in different areas.[37, 39, 49, 54-66]

The present study adds more negative data on the physiological and pharmacological role of obestatin: intravenous administration of obestatin does not influence glucose and insulin metabolism, neither systemic, nor in the portal circulation. So, we did not provide any convincing evidence that obestatin is a functional product of the preproghrelin gene. It becomes increasingly likely that the physiological relevance of obestatin is limited. The before mentioned issues remain counter-intuitive regarding the characteristics of a physiologically relevant peptide. Additionally, more negative than positive studies are presently available. Moreover, some of the studies demonstrated positive results only under highly specific conditions, i.e. anorexic effects were observed only with a specific diet,[54] or with exact timing after obestatin administration,[67] and effects on glucose and insulin metabolism displayed a dual or U-shaped dose-response curve.[42, 53] Besides, the study describing a U-shaped dose-response curve was recently retracted by the original authors due to lack of reproducibility of the data.[68] Finally, the original authors claimed that obestatin was the ligand for the orphan receptor GPR39, which was convincingly proved to be invalid.[57, 67, 69-71] However, one remark should be made: commercially available obestatin peptides, as currently used in biomedical investigations, were proven to be highly instable and the quality was claimed to be insufficient for *in vivo* and *in vitro* experiments, which could be an explanation for the negative results.[72, 73]

Future directions

At present, the question whether obestatin is a functional hormone or a non-functional connective peptide remains to be answered. This, however, seems easier said than done. Where one positive study with results that can be replicated is generally enough to prove an effect, the invalidation of a presumed biological effect demands a more thorough approach. At present, the main points of criticism regarding the negative studies on obestatin are the low quality of available obestatin samples (was it really obestatin that was used?) and its instability and short half-life (did obestatin reach the intended site of action?). Besides, information on effective doses is limited as well.

Therefore, additional dose-finding studies with obestatin of proven quality should be performed, although a gold-standard that defines this quality is still lacking. Recovery studies measuring obestatin concentration after administration could be of use. However, instability of obestatin might underestimate recovered concentrations of obestatin. At first, data should be obtained on effects of obestatin in areas where functionality of ghrelin is widely known, i.e. GH release, food intake, and glucose and insulin metabolism. If obestatin does not have any physiological or pharmacological effect in these areas, clinical relevance of the peptide will become increasingly unlikely, despite possible positive effects on memory and sleep.

THE EFFECTS OF ADMINISTRATION OF ACYLATED AND UNACYLATED GHRELIN ON TOTAL AND HIGH MOLECULAR WEIGHT ADIPONECTIN.

Chapter 7

Energy homeostasis and body weight are regulated by a highly complex network involving the brain, the digestive tract and white adipose tissue (WAT). Hypothalamic neurons respond to hormones, produced by either the gut (gut hormones) or WAT (adipokines), by modifying the synthesis of neuropeptides that modulate food intake and energy balance.[74] While pathways connecting respectively WAT and the gut with the brain have been studied extensively, connections between WAT and the gut are largely unknown.

We evaluated the effects of acute intravenous administration of UAG and the combination of AG and UAG on adiponectin concentration. Eight morbidly obese non-diabetic subjects were treated with either UAG 200µg, UAG 100µg + AG 100µg (Comb), or placebo in 3 episodes in a double blind randomized cross-over design. Study medication was administered as single i.v. bolus injections at 09.00h after an overnight fast. High molecular weight (HMW) and total adiponectin, glucose, insulin, and total and acylated ghrelin were measured up to one hour after administration.

HMW and total adiponectin concentrations did not change after administration of either UAG or Comb, nor were they different from placebo. Insulin concentrations decreased significantly after acute administration of Comb, reaching a minimum at 20 min: 58.2 ± 3.9% of baseline.

As indicated above, direct connections between WAT and the gut are largely unknown. Studies reporting correlations between gut hormone concentrations and adipokine concentrations add little information to our understanding of their interaction, since concentrations could well be independently influenced by another common factor.[75, 76] Few studies are available reporting effects of gut hormone administration on adipokine concentrations and vice versa (mostly evaluating connections between leptin and ghrelin) and results are not always consistent. For example, ghrelin mRNA expression in the stomach has been reported to be upregulated upon leptin administration,[77, 78] while other studies report a decrease of ghrelin concentrations at high leptin levels.[79-83] Additionally, intracerebroventricular administration of leptin and ghrelin has been reported not to influence adiponectin levels,[77] whereas leptin transgene expression in the hypothalamus was demonstrated to reduce adiponectin concentrations (indicating internal adipokine regulation).[83]

Another factor complicating the evaluation of interaction of the gut and WAT is the complexity of the signalling network regulating energy balance. It could be hypothesized that adipokines and gut hormones have local effects in resp. the gut and WAT, which is supported by for example the identification of the ghrelin receptor (GHS-R1a) in WAT and the ubiquitous expression of the leptin receptor and adiponectin receptor.[84-86] However, gut hormones and adipokines could as well indirectly regulate each others concentrations. Namely, both gut hormones and adipokines have centrally mediated effects on food intake, body composition

and glucose metabolism,[6, 87-105] while on the other hand gut hormone concentrations and adipokine concentrations are largely regulated by energy intake and body composition, which is possibly mediated by insulin and glucose levels.[78, 80, 85, 92, 106-114] Therefore, if any future study identifies effects of gut hormone administration on adipokine concentration vice versa, it remains to be established whether it is a direct effect, i.e. effectuated locally in the gut or WAT, or an indirect effect, i.e. mediated by central pathways or changes in glucose and insulin concentrations.

We did not demonstrate any acute effect of either UAG or Comb on total and HMW adiponectin concentrations, which makes our hypothesis of a direct effect of UAG or the combination of AG and UAG on adiponectin less likely. Additionally, although adiponectin concentrations have been shown to drop under hyperinsulinaemic conditions,[85, 115] the presently observed prompt and significant decrease of insulin concentration did not acutely affect adiponectin concentrations as well. Finally, since ghrelin is known to induce adiposity by stimulating hypothalamic orexigenic pathways[92, 94, 116] and adiponectin has been shown to be decreased in obesity,[107, 111] ghrelin might have an indirect negative effect on adiponectin concentrations. However, it is likely that this effect, if present, will not be observed within one hour after administration of ghrelin. Therefore, we cannot comment on this relationship between ghrelin and adiponectin based on the results of the present study.

Future directions

At present, there is no effective medical treatment for obesity available, despite all studies on agonists acting on the anorexigenic adipokine pathways, on modifying actions of gut hormones, and on antagonists of the orexigenic ghrelin pathways. One of the problems encountered in the development of anti-obesity treatment based upon interference with the homeostatic systems of the gastrointestinal tract, WAT and the brain, is the redundancy of this network. Intervention in one pathway results in up or down regulation of other pathways which eventually leads to stabilisation of body weight.[74]

Increasing knowledge of the pathways within this highly complex network might enable the development of effective anti-obesity treatment by intervening in multiple pathways, such as combining synergistically acting adipokines and gut hormones, which has been shown to be highly effective in animal studies.[117, 118] Therefore, it is important to identify connections between the gut and WAT, since these are much less known than centrally acting pathways. However, one should be aware of the possibility that gut hormones and adipokines might communicate either directly on cellular level, or indirectly, by changes in insulin and glucose concentrations or by influencing body composition via the brain. *In vitro* studies evaluating cellular effects and local receptors could identify direct actions, while *in vivo* clamp studies could evaluate effects independently of changes in insulin and glucose concentrations. Finally, since changes in body composition are relatively slow processes, long-term studies are necessary to evaluate effects mediated by changes in energy homeostasis.

CHOLELITHIASIS AFTER BARIATRIC SURGERY

Chapter 8

Rapid weight loss is an important risk factor for the development of gallstones. Therefore, it is a major concern to everyone treating morbidly obese patients by bariatric surgery. However, to be able to define an effective management strategy, it is important to have insight into the incidence of symptomatic and asymptomatic gallstones after bariatric surgery.

We evaluated a population of previously morbidly obese patients, who had been treated by LAGB 1.3 to 8.5 years earlier, for the prevalence of symptomatic and asymptomatic gallstones. None of the patients underwent prophylactic cholecystectomy, and ursodeoxycholic acid was not prescribed, which enabled us to study long-term natural history of gallstone disease after surgically induced weight loss. Additionally, we compared prevalence of gallstones in this population with a morbidly obese population on a waiting list for bariatric surgery.

Our population of 120 patients had a maximum weight loss of 31.5 ± 11.3% of initial body weight induced by LAGB. Sixteen patients had had cholecystectomy before LAGB, 5 afterwards. Ninety-eight patients underwent transabdominal ultrasonography to evaluate the presence of gallstones. Gallstones were detected in 26 (26.5%) of the subjects. Thus, the prevalence of gallstones after LAGB was 31 (30.1%) in 103 patients at risk: 5 cases of symptomatic gallstones who had already undergone cholecystectomy before participating in the study, and 26 cases of gallstones detected on ultrasonography. Two patients in this group with apparently "silent" gallstones reported complaints attributable to gallbladder disease and subsequently underwent cholecystectomy. In contrast, the prevalence of gallstones in the morbidly obese population on a waiting list for bariatric surgery was 13.3%. In conclusion, the prevalence of gallstones after LAGB was 30.1%, of whom 22.5% became symptomatic (i.e. 6.8% of all patients at risk). The prevalence of gallstones after LAGB was significantly higher than before LAGB: 30.1% vs. 13.3%, which supports the hypothesis that significant weight loss as a major risk factor for the development of gallstones.

At present, there is no consensus about the management of gallstone formation after bariatric surgery. Three different policies have been advocated: i) to perform cholecystectomy in all patients as a routine part of bariatric surgery,[119-123] ii) to investigate for gallstones as a part of the preoperative assessment and proceed to cholecystectomy if stones are present,[124, 125] iii) not to investigate routinely for gallstones, and then treating only symptomatic patients.[126-130] Additionally, prophylactic treatment with ursodeoxycholic acid to prevent gallstone formation after surgery has been proven to be effective.[126, 131-133]

Those who perform routine cholecystectomy in all patients state that their procedure adds a mean operative time of 15 to 50 min but that hospital stay and perioperative morbidity and mortality is not significantly higher.[119, 121-124] However, following this policy, the majority of patients undergo surgery for a disease they will never develop. The management strategy of performing preoperative ultrasonography and performing cholecystectomy during bariatric

surgery only in patients with gallstones seems to some extent irrational, regarding the results of the present study, demonstrating that the majority of gallstones develops after surgery. There is no data available that patients with gallstones before surgery are at a higher risk to become symptomatic. No factors, other than previous complications of gallstones, seem to predict complications of gallstones.[134] Finally, those who propagate a wait-and-see policy claim that there is no evidence to treat asymptomatic gallstones in morbidly obese patients or patients after bariatric surgery differently from those in the general population, in which cholecystectomy is only performed when symptoms are present.[130] However, treatment of symptomatic gallstones might be more difficult after bariatric surgery since the anatomical changes resulting from surgery hinder the endoscopic treatment of gallstones, and laparoscopy might be more difficult after previous surgery.[120] Additionally, they risk severe morbidity due to symptoms of cholelithiasis.

In contrast to most studies evaluating cholelithiasis shortly after bariatric surgery, the present study evaluated patients with a mean follow-up of 4.6 years after surgery. In this period, 7 of 103 patients developed symptomatic gallstone disease, i.e. one case in 67.7 patient-years. Cumulative risk to develop symptoms when having gallstones was 24.4% by 5 years. These results are not significantly different from the general population.[130, 135] Therefore, based on the present results one could incline towards the wait-and-see policy. Conclusions about the benefit of treating patients with ursodeoxycholic acid could not be drawn.

Future directions

The present study does give insight into the prevalence of symptomatic and asymptomatic gallstone disease after long-term follow-up after bariatric surgery. However, to better define the optimal management strategy concerning development of gallstone disease after bariatric surgery, a clinical study comparing concomitant cholecystectomy with a wait-and-see policy with or without ursodeoxycholic acid should be performed. Based on outcome regarding morbidity (either as a result of concomitant cholecystectomy or of symptomatic gallstone disease), mortality, and costs, an optimal policy can be established.

QUALITY OF LIFE AFTER BARIATRIC SURGERY

Chapter 9

In individuals suffering from obesity, quality of life (QoL) is severely impaired compared to the general population.[136-139] It has been shown that bariatric surgery results in a significant improvement in QoL.[137, 139-150] The most important improvement in QoL (up to normalisation of QoL) is generally reported in the first year after surgery.[137, 142, 147] The few available long-term follow-up studies, however, suggest that this improvement in QoL levels off or even reverts toward preoperative levels starting from 2 years after surgery.[144, 148, 150]

We evaluated QoL in a population of previously morbidly obese patients, who had been treated by LAGB. Of 120 participants, 59 patients met the criteria for long-term follow-up (i.e. > 5 years). Time since surgery in this subgroup was 74.7 months (range 60-107.6). We compared QoL in this population with a morbidly obese population on a waiting list for bariatric surgery. Additionally, we compared both study populations with Dutch community norm values (CN). General and obesity-related parameters were assessed for correlation with QoL. QoL was measured using a generic questionnaire (RAND 36-Item Health Survey), which quantifies QoL in scores on 9 different domains of physical and psychosocial functioning.[151]

As expected, QoL in the presurgical group was significantly impaired compared to the Dutch community: scores were lower on 6 out of 9 domains. However, in the postsurgical group, QoL was only slightly better. Compared to CN, scores were still significantly impaired on 4 out of 9 domains. Additionally, the postsurgical group scored better on only one domain compared to the presurgical group.

Several determinants of QoL after long-term follow-up have been identified in the present study. Age, postoperative BMI and comorbidity have been demonstrated to negatively influence QoL, while excess weight loss positively influences several domains of QoL. Sex, preoperative BMI, weight regain and the time since surgery did not correlate with any scores of QoL.

The present study confirms the limited effect of LAGB on QoL at long-term follow-up, which is in accordance with the few available studies on this subject.[144, 148, 150] In contrast with the reported significant improvement shortly after surgery,[137, 142, 144, 145, 147] QoL appeared to decrease with time, reverting towards preoperative levels to the extent that changes were no longer significant. These results are disappointing, since at present bariatric surgery is the most effective treatment for obesity in terms of persistent weight loss and improvement in comorbidity.[152-154] Therefore, it is important to establish why long-term effect on QoL is limited, in contrast to weight reduction and improvement in comorbidity.

Several explanations for long-term decrease of QoL have been suggested. At first, since decrease in QoL is generally reported from two years after surgery (the moment that the curve of weight loss levels off and eventually inverts), weight regain was hypothesized to be an important factor.[148-150] Secondly, it could be the result of waning optimism in a period of weight stabilization, disappointment about only limited improvement in everyday life or persistence of pre-surgical problems not related to body weight.[149, 150] Finally, it is possible that patients partly depend on frequent medical and emotional support from their clinic visits to improve psychologically.[150] Unfortunately, definite conclusions cannot be drawn yet.

On the other hand, QoL can improve early after bariatric surgery: significant improvement has been observed as early as 2 to 4 weeks postoperatively, while weight loss in this period is almost negligible.[142] Interestingly, good explanations for this unexpected finding have not been provided yet. It could be hypothesized that patients regard the moment of surgery as a final resolution of their life-long problem or that the waiting list for surgery is simply too long.

The present study confirms that at long-term follow-up after LAGB, QoL is significantly worse than Dutch norm data. Indeed, QoL is only slightly better compared to morbidly obese persons who have not yet undergone surgery. The study identified several parameters influencing QoL (age, comorbidity, BMI and percentage excess weight loss) but these parameters do not establish a good explanation for a decrease in QoL at long-term follow-up.

Future directions

At present, it is unclear why QoL increases rapidly after bariatric surgery, while a decrease (back to preoperative levels) is observed at long-term follow-up. Valid explanations for both phenomena are necessary, since our present management of patients undergoing bariatric surgery appears not to provide them with a gradual and persistent improvement in QoL. QoL is highly subjective, which makes effective and valid evaluations difficult. However, answers to questions like 'do we need to provide patients with more realistic expectations preoperatively?' or 'do we need to intensify long-term follow-up treatment?' are urgently needed.

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Summary

Obesity has become a worldwide epidemic that threatens to overwhelm both developed and developing countries, as described in **Chapter 1**. The major burden of obesity to both patients and public health as a whole is the significantly increased morbidity and mortality (due to, for example, type 2 diabetes, hypertension, cancer and psychopathology). It is generally acknowledged that a decrease in physical activity in combination with relative overeating leads to a chronic positive energy balance, thereby causing an increase in body weight. However, other factors that regulate individual susceptibility to obesity in an 'obesogenic society' must be involved as well but only a small part has been identified. For example, genetics have been shown to play an important role. Genetic mutations and single nucleotide polymorphisms have been identified that disrupt a highly complex endocrine and neuroendocrine network that regulates energy homeostasis and body weight. The main sites of (inter-)action in this network are white adipose tissue (WAT), the digestive tract and the hypothalamus.

In **Chapter 2** the physiology of the gut hormone ghrelin, the peptide obestatin, and the adipokine adiponectin are discussed. Ghrelin is a hormone principally produced in the stomach and primarily identified as a strong growth hormone secretagogue (GHS). Ghrelin circulates in two main isoforms: acylated (AG) and unacylated (UAG) ghrelin. Acylation is crucial for its binding to the known growth hormone secretagogue receptor type 1a (GHS-R1a). Apart from being a GHS, ghrelin has an important role in energy homeostasis, and in glucose and insulin metabolism. Ghrelin is derived from the polypeptide preproghrelin. The function of a second peptide derived from this prohormone, obestatin, is currently hotly debated. Initially, obestatin was described as a functional antagonist of ghrelin, but subsequent studies were not able to replicate these results. Like ghrelin, adiponectin plays an important role in glucose and insulin homeostasis. Therefore, its connection with ghrelin must be identified.

Unfortunately, treatment of obesity is difficult. Currently, bariatric surgery is the most effective treatment when quantified in terms of weight loss. However, it is at least equally important to assess its effectiveness in improving comorbidity. Additionally, any side effects of surgery should be acceptable. In **Chapter 3** the effect of bariatric surgery on quality of life (QoL), and the risk of patients developing gallstones after bariatric surgery are discussed.

AG has been shown to increase insulin resistance. On the other hand, the effect of UAG on insulin sensitivity is still not elucidated. Intriguingly, coadministration of AG and UAG to growth hormone (GH) deficient individuals improves their insulin sensitivity. **Chapter 4** describes a study in which the effects of administration of UAG, and the combination of AG and UAG in morbid obesity, a condition characterized by insulin resistance and low GH levels, is evaluated. Eight morbidly obese non-diabetic subjects were treated with an intravenous bolus injection of either UAG (200µg), UAG (100µg) in combination with AG (100µg), or placebo in 3 episodes of 4 consecutive days in a double-blind randomized crossover design. Administration of a bolus injection of UAG did not influence glucose and insulin concentrations in fasting conditions. However, coadministration of AG and UAG caused a significant decrease in insulin concentrations,

to 58.2 ± 3.9% of baseline at 20 min. Since glucose concentrations did not change in the first hour after coadministration of AG and UAG, our data suggest a marked improvement in insulin sensitivity.

AG and UAG are released principally into the hepatic portal system. Therefore, it is important to know whether AG and UAG differentially regulate portal and systemic insulin levels. In the study described in **Chapter 5** we evaluated the effects of the administration of AG (30 nmol/kg), UAG (3 and 30 nmol/kg), the GHS-R1a antagonist [D-Lys^3]GHRP-6 (1 µmol/kg), or various combinations of these compounds on portal and systemic levels of glucose and insulin after an intravenous glucose tolerance test (IVGTT, D-glucose 1 g/kg) in anesthetized fasted Wistar rats. UAG administration potently and dose-dependently enhanced the rise of insulin concentration induced by IVGTT in the portal and, to a lesser extent, the systemic circulation. This UAG-induced effect was completely blocked by the coadministration of exogenous AG at equimolar concentrations. Like UAG, [D-Lys^3]GHRP-6, alone or in combination with AG and UAG, strongly enhanced the portal insulin response to IVGTT, whereas exogenous AG alone did not. These data demonstrate that, in glucose-stimulated conditions, exogenous UAG acts as a potent insulin secretagogue, whereas endogenous AG inhibits glucose-induced insulin release.

Like ghrelin, obestatin is produced principally in the portal system and has a very short half-life. It is still unclear if obestatin is a *bona fide* hormone (and a functional antagonist of ghrelin), or simply a non-functional proteolytic derivative of the ghrelin prohormone. Since AG induces insulin resistance, it could be hypothesized that obestatin plays a role in glucose homeostasis as well. In the study described in **Chapter 6** we evaluated the effect of obestatin on glucose and insulin metabolism in the systemic and portal circulations. Fasted male Wistar rats were anesthetized with pentobarbital. Obestatin (200 nmol/kg) was administered systemically as an intravenous bolus injection either in fasted conditions or glucose-stimulated conditions (IVGTT). Sequential blood samples were then obtained from the portal and jugular veins for 50 min following administration. It was found that obestatin had no effect on glucose and insulin concentrations in the systemic and portal circulations of either fasted or glucose-stimulated animals.

The brain, the gut and WAT play important roles in regulating energy homeostasis and body weight. While connections of respectively WAT and the gut with the brain have been studied extensively, knowledge about signalling pathways connecting the digestive tract and WAT is relatively limited. Ghrelin and adiponectin share some striking homologies: both are decreased in obesity and both share a potent effect on insulin sensitivity. However, it is not known if ghrelin and adiponectin regulate each other. The study described in **Chapter 7** examines whether acute administration of UAG alone or combined with AG affects total and high molecular weight (HMW) adiponectin concentrations, either directly or indirectly by changes in insulin concentration. Eight morbidly obese non-diabetic subjects were treated with either UAG (200µg), coadministered UAG (100µg) and AG (100µg), or placebo in 3 episodes in a double blind randomized cross-over design. HMW and total adiponectin concentrations did

not change after administration of either UAG or combined UAG + AG, nor were they different from placebo. In addition, since a significant decrease in insulin concentration was observed, it can be concluded that there was no acute indirect effect of UAG and UAG + AG on adiponectin concentrations.

Bariatric surgery is currently the most effective long-term treatment for obesity. However, it has very specific complications. For example, because bariatric surgery induces rapid and substantial weight loss patients are at risk of developing gallstones. A retrospective study is described in **Chapter 8** in which 120 previously morbidly obese subjects who had undergone laparoscopic adjustable gastric banding (LAGB) and 45 morbidly obese subjects on a waiting list for bariatric surgery were evaluated for gallstones. Prior history of cholecystectomy was present in 21 post-LAGB patients; 16 before and 5 after LAGB. Of 98 patients in which ultrasonography was performed 26 (26.5%) presented with gallstones. Overall, the prevalence of gallstones after weight reduction surgery was 31 (30.1%) in 103 patients at risk. In contrast, the prevalence of gallstones in the morbidly obese population on a waiting list for bariatric surgery was 13.3% (6 out of 45 patients), which was significantly lower than in the post-surgical group. Therefore, rapid weight loss induced by LAGB should be regarded as an important risk factor for the development of gallstones. Multivariate analysis indicated that neither preoperative weight, nor maximum weight loss, nor the interval between operation and the postoperative ultrasonography were determinants of the risk for developing gallstone disease.

Effectiveness of bariatric surgery can be easily quantified as excess weight loss (EWL). However, it is important that along with the weight loss comorbidity improves as well. In relation to this we studied the effect of LAGB on quality of life (QoL), specifically after long-term follow-up, as described in **Chapter 9**. In a cross-sectional design, 59 previously morbidly obese subjects who had undergone LAGB at least 60 months earlier and 28 morbidly obese subjects on a waiting list for bariatric surgery completed a generic QoL questionnaire, the RAND 36-Item Health Survey, quantifying QoL. Scores of both groups were compared to Dutch community norm data (CN). The preoperative group scored significantly lower on five out of eight QoL subscales compared to CN, while the postoperative group scored significantly lower on four out of eight QoL subscales compared to CN. The postoperative group scored significantly higher on one out of eight subscales compared to the preoperative group. Postoperative BMI and %EWL influenced QoL after long-term follow-up, whereas weight regain had no negative impact. This study indicates that after long-term follow-up subjects treated by LAGB to induce weight loss have a slightly better QoL than those who had not yet undergone surgery. QoL remains impaired in comparison to the general population.

In **Chapter 10** the results of the studies are placed in a broader perspective and directions for future research are discussed.

Nederlandse samenvatting

OBESITAS

Introductie

In de afgelopen decennia is de prevalentie van overgewicht (gedefinieerd als een BMI > 25 kg/m^2) sterk toegenomen, aanvankelijk in de westerse wereld, maar inmiddels ook in de rest van de wereld. Dit heeft grote gevolgen voor de maatschappij, daar met name obesitas (gedefinieerd als een BMI > 30 kg/m^2) gepaard gaat met een aanzienlijke stijging van morbiditeit en mortaliteit. Diabetes mellitus type 2, hypertensie, hyperlipidemie, maligniteiten en psychopathologie, ziektebeelden die sterk geassocieerd zijn met overgewicht, zijn hiervan de belangrijkste veroorzakers.

Algemeen wordt aangenomen dat een daling van de hoeveelheid lichaamsbeweging in combinatie met de eenvoudige beschikbaarheid van energierijk voedsel de belangrijkste oorzaak is van de sterke stijging van de prevalentie van overgewicht en obesitas. Dit lijkt echter onvoldoende om de sterke interindividuele variatie in lichaamsgewicht in een zg. 'obesogene samenleving' te verklaren. Er komen langzamerhand steeds meer aanwijzingen dat er een genetische basis is die de gevoeligheid voor overgewicht bepaalt. Inmiddels zijn meerdere mutaties en SNP's gedocumenteerd die overgewicht tot gevolg hebben. Deze mutaties leiden zonder uitzondering tot een verstoring van de complexe endocriene en neuro-endocriene regulatie van de energiehomeostase. De drie systemen die hierin een belangrijke rol spelen zijn het centraal zenuwstelsel (met name de hypothalamus), de darm (via de productie van darmhormonen zoals ghreline, GLP-1, CCK etc) en het vetweefsel (via de productie van adipokines zoals leptine en adiponectine).

Wanneer wordt aangenomen dat de oorzaak van overgewicht en obesitas een gebrek aan lichaamsbeweging in combinatie met relatief te veel eten is, lijkt behandeling eenvoudig. Aanpassing van de leefstijl is helaas slechts beperkt effectief en de resultaten zijn met name op de lange termijn teleurstellend. Op dit moment is de meest effectieve behandeling de bariatrische chirurgie. Hierbij wordt door middel van chirurgisch ingrijpen in de anatomie van de darm mechanische restrictie van de voedselinname of een malabsorptie geïnduceerd. Dit leidt tot aanzienlijk gewichtsverlies, dat ook op de langere termijn persisteert.

DE EFFECTEN VAN GEACYLEERD EN ONGEACYLEERD GHRELINE OP HET GLUCOSE- EN INSULINEMETABOLISME.

Hoofdstuk 4 en 5

Ghreline is een eiwit, bestaande uit 28 aminozuren, dat in de maag geproduceerd wordt. In 1999 werd dit hormoon geïdentificeerd als groeihormoon secretagoog, een effect dat gemedieerd wordt door de Groeihormoon Secretagoog receptor type 1a (GHS-R1a). Karakteristiek voor de structuur van ghreline is een posttranslationele acylering met een *n*-octanoylgroep van serine

op positie 3, welke noodzakelijk is voor binding aan de GHS-R1a. Naast deze geacyleerde isovorm (geacyleerd ghreline, AG) kent ghreline ook een ongeacyleerde isovorm (ongeacyleerd ghreline, UAG). Daar UAG niet kan binden aan de GHS-R1a, werd aanvankelijk gedacht dat UAG biologisch inactief was. Echter, onderzoek heeft aangetoond dat ook UAG een rol van betekenis speelt in meerdere metabole processen.

Van AG is inmiddels bevestigd dat het insulineresistentie induceert. Het effect van UAG op het glucose- en insulinemetabolisme is daarentegen nog niet onomstotelijk vastgesteld. Opvallend genoeg is wel aangetoond dat behandeling met de combinatie van AG en UAG het effect van AG op insuline resistentie teniet doet en juist de insulinegevoeligheid bevordert bij patiënten lijdend aan groeihormoondeficiëntie. Daar UAG redelijkerwijs niet bindt aan de GHS-R1a, staat tevens ter discussie via welke receptor het effect van ghreline op het glucose- en insulinemetabolisme gemedieerd wordt.

In hoofdstuk 4 wordt de studie beschreven waarin onderzocht werd wat het effect is van behandeling met UAG en de combinatie van AG en UAG versus placebo op de glucose- en insulinespiegels bij proefpersonen lijdend aan morbide obesitas (Body Mass Index (BMI) > 40 kg/m^2), een conditie gekarakteriseerd door insulineresistentie en lage groeihormoonspiegels. Geen van de proefpersonen leed aan diabetes mellitus. De medicatie werd toegediend volgens een gerandomiseerd, dubbelblind, crossover protocol.

Intraveneuze toediening van 200 µg UAG aan de onderzoekspersonen gaf geen verandering in glucose- en insulineconcentraties ten opzichte van placebo, noch in nuchtere toestand noch na een maaltijd genuttigd 1 uur na toediening van de medicatie. Intraveneuze toediening van 100 µg UAG + 100 µg AG daarentegen leidde tot een significante daling van de insulineconcentratie tot een minimum van 58.2 ± 3.9% van de uitgangswaarde vóór toediening van de medicatie. Bij deze daling van de insulinespiegel werd geen verandering van glucoseconcentratie geobserveerd. Eén uur na toediening was de insulineconcentratie weer gelijk aan de uitgangswaarde. UAG + AG werd gedurende vier opeenvolgende dagen toegediend. Ook op dag 4 was het effect onverminderd waarneembaar, hetgeen bevestigt dat in deze periode geen tachyphylaxie is opgetreden.

Concluderend bevestigt deze studie het reeds eerder geobserveerde effect dat intraveneuze toediening van de combinatie van AG en UAG acuut en kortdurend een sterke afname van de insulinespiegel tot gevolg heeft, ditmaal in een groep morbide obese proefpersonen. Het gelijk blijven van de glucoseconcentratie in dezelfde periode suggereert een toename van insulinegevoeligheid, hetgeen een belangrijke winst zou kunnen betekenen in deze populatie lijdend aan morbide obesitas.

De in hoofdstuk 5 beschreven studie heeft gebruik gemaakt van een rattenmodel waarin het mogelijk is zowel portale als perifere glucose- en insulineconcentraties te meten. Daar zowel ghreline als insuline primair in het portale systeem worden gesecerneerd, is het niet

ondenkbaar dat beïnvloeding van glucose en insuline door AG en UAG met name locaal detecteerbaar is en dat perifere meting van glucose- en insulinespiegels een onderschatting van het effect tot gevolg heeft. Ter bestudering van de portale effecten van AG en UAG op glucose- en insulinespiegels tijdens een intraveneuze glucose tolerantie test (IVGTT) werd deze medicatie afzonderlijk en in combinatie intraveneus toegediend in bovengenoemd rattenmodel. Bovendien werd de rol van de GHS-R1a geëvalueerd door middel van bestudering van de effecten van toediening van de GHS-R1a blokker [D-Lys^3]GHRP-6 op het glucose-en insulinemetabolisme, alleen of in combinatie met UAG of AG.

Intraveneuze toediening van UAG induceerde een significante stimulatie van de insulinerespons op een IVGTT. Dit effect werd met name geobserveerd in de portale circulatie, maar in mindere mate ook in de systemische circulatie. Combinatie van UAG met [D-Lys^3]GHRP-6 leidde niet tot een mutatie van het effect van UAG. Het effect van toediening van [D-Lys^3] GHRP-6 alleen kwam overeen met de toediening van UAG: stimulatie van de insuline respons op een IVGTT. Toediening van AG daarentegen had geen verandering van glucose- noch van insulinespiegels tot gevolg. Wel bleek dat wanneer AG gelijktijdig met UAG toegediend werd, de toename van de insulinerespons zoals geobserveerd na UAG alleen zich niet voordeed.

Bovenstaande resultaten laten zien, dat AG onder fysiologische omstandigheden een maximaal inhiberend effect op insulinesecretie heeft, gemedieerd door de GHS-R1a. Toediening van AG leidde immers niet tot een verandering van glucose- en insulinespiegels, terwijl blokkade van de GHS-R1a een toename van de insulinerespons op een IVGTT bewerkstelligde. UAG daarentegen lijkt juist een stimulerend effect op de insulinesecretie te hebben via een nog nader te determineren systeem onafhankelijk van de GHS-R1a. Het effect van UAG blijkt zich met name in het portale systeem af te spelen. Deze bevindingen suggereren dat in het portale systeem AG en UAG functionele antagonisten zijn waarbij hun effecten gemedieerd worden via verschillende receptoren.

DE EFFECTEN VAN OBESTATINE OP HET GLUCOSE- EN INSULINEMETABOLISME.

Hoofdstuk 6

In 2005 werd een tweede peptide afkomstig van het preproghreline polypeptide geïdentificeerd. Dit 23 aminozuren lange eiwit leek aanvankelijk een belangrijke rol te vervullen als functionele antagonist van ghreline met betrekking tot het hongergevoel: obestatine bleek een sterk anorexigeen effect te hebben na intraperitoneale en intracerebroventriculaire toediening. Meerdere vervolgonderzoeken waren echter niet in staat deze oorspronkelijke bevindingen te reproduceren, zodat er een discussie ontstond of obestatine wel een functioneel hormoon was of slechts een bijproduct bij de productie van ghreline. Ook op andere gebieden, zoals

bij het glucose- en insulinemetabolisme, was er controverse aangaande de functionaliteit van obestatine.

In de studie beschreven in hoofdstuk 6 werd opnieuw het eerder beschreven rattenmodel gebruikt ter evaluatie van eventuele biologische effecten van obestatine op het glucose- en insulinemetabolisme. Daar de halfwaardetijd van obestatine erg kort is en het eiwit primair in het portale systeem gesecerneerd wordt, zouden ook hierbij effecten gemist kunnen worden wanneer uitsluitend glucose- en insulineconcentraties in de perifere circulatie gemeten zouden worden, analoog aan de in hoofdstuk 5 beschreven situatie met betrekking tot UAG.

Intraveneuze toediening van 200 nmol/kg obestatine als bolusinjectie leidde tot glucose- en insulineconcentraties niet verschillend van placebo, noch in de perifere circulatie, noch in de systemische circulatie. Ook toediening van 200 nmol/kg obestatine tijdens een IVGTT leidde tot veranderingen in glucose- en insulineconcentraties conform de veranderingen zoals die werden waargenomen na toediening van placebo tijdens een IVGTT.

De bovenbeschreven resultaten ontkrachtigen de hypothese dat obestatine, analoog aan ghreline, een belangrijk effect heeft op het glucose- en insulinemetabolisme. Dit geldt echter alleen voor de huidige dosering en omstandigheden. De resultaten kunnen niet zonder meer geëxtrapoleerd worden en eventuele functionaliteit van obestatine in het glucose- en insulinemetabolisme kan op dit moment (nog) niet definitief verworpen worden.

DE EFFECTEN VAN GHRELIN OP ADIPONECTINECONCENTRATIES.

Hoofdstuk 7

Het energiemetabolisme van de mens wordt binnen zeer stricte grenzen gereguleerd. Het systeem dat hiervoor zorgt draagt, bestaat uit drie componenten: de darm, het vetweefsel en de hersenen (met name de hypothalamus). De darm produceert darmhormonen, zoals ghreline, glucagon-like peptide 1 (GLP-1) en peptide tyrosine-tyrosine (PYY), die afhankelijk van de aanwezigheid van voedsel in de darm gesecerneerd worden. Het vetweefsel produceert adipokines, zoals leptine en adiponectine. De signalen afkomstig uit de darm en het vetweefsel worden geïntegreerd op het niveau van de hypothalamus, alwaar hongergevoelens gereguleerd worden.

Connecties tussen de darm en de hersenen en tussen het vetweefsel en de hersenen zijn uitvoerig bestudeerd en beschreven. Over de relatie tussen darmhormonen en adipokines daarentegen is relatief weinig bekend. Het darmhormoon ghreline en de adipokine adiponectine hebben een aantal opvallende overeenkomsten. Beide zijn verlaagd in geval van obesitas en beide hebben een belangrijke rol binnen het glucosemetabolisme: adiponectine (met name de hoog-moleculair gewicht (HMW) isovorm) heeft een gunstig effect op de insulinegevoeligheid en voor de rol van ghreline wordt verwezen naar hoofdstuk 4 en 5. Informatie over wederzijdse beïnvloeding ontbreekt echter vrijwel geheel, hoewel interessante hypotheses

over deze onderlinge relaties geponeerd zouden kunnen worden. De beide hormonen zouden elkaar direct, op locaal niveau, kunnen beïnvloeden, maar de interactie zou ook indirect kunnen verlopen, via modificatie van insulineconcentratie of lichaamsgewicht. Insuline en lichaamsgewicht worden immers beide beïnvloed door ghreline en adiponectine, terwijl deze twee factoren anderzijds juist de ghreline-en adiponectineconcentraties beïnvloeden.

Hoofdstuk 7 beschrijft een studie waarin de korte-termijn relatie tussen ghreline en adiponectine geëvalueerd wordt, meer specifiek de effecten van geacyleerd ghreline (UAG) en de combinatie van ongeacyleerd en geacyleerd ghreline (AG) op de concentraties van totaal en HMW adiponectine. Daar eerder reeds werd vastgesteld dat de combinatie van UAG en AG een acute sterke daling van insulineconcentraties induceert, kan tevens een eventueel indirect effect van ghreline op adiponectineconcentraties (via modificatie van de insulineconcentratie) geëvalueerd worden. Anderzijds is er nog geen sluitende verklaring voor de daling van insulinespiegels (mogelijk duidend op een toename van de insulinegevoeligheid) na toediening van UAG + AG (zie hoofdstuk 4). Gezien het feit dat adiponectine een belangrijke rol speelt bij de regulatie van insulinegevoeligheid, zou dit effect theoretisch gemedieerd kunnen worden via modificatie van adiponectinespiegels na toediening van UAG + AG.

Concentraties van HMW en totaal adiponectine werden gemeten gedurende 1 uur na de intraveneuze toediening van 200 µg UAG, 100 µg UAG + 100 µg AG of placebo aan nuchtere proefpersonen lijdend aan morbide obesitas. Noch UAG alleen, noch de combinatie van UAG + AG leidde tot verandering van de concentraties van HMW en totaal adiponectine. Er werd geen verschil geobserveerd met de resultaten na toediening van placebo. De eerder beschreven daling van de insulinespiegel werd wel geobserveerd, maar ook dit leidde niet tot verandering van de adiponectinespiegels.

De huidige studie liet geen korte-termijn effect zien van intraveneuze toediening van UAG met of zonder AG op de concentratie van HMW en totaal adiponectine. Ondanks het feit dat eerder werd vastgesteld dat insuline een belangrijke regulator van de adiponectineconcentratie is, resulteerde een UAG + AG gemedieerde daling van insuline niet in een acute verandering van adiponectinespiegels. Anderzijds zal ook de hypothese dat de geobserveerde daling van insulinespiegels na de toediening van UAG + AG gemedieerd wordt door een verandering in adiponectineconcentraties verworpen moeten worden. Desalteniettemin kan op basis van deze resultaten een connectie tussen ghreline en adiponectine niet definitief verworpen worden, met name daar het een observatie gericht op acute effecten (gedurende 1 uur na toediening) betreft en vooral indirecte effecten een langere looptijd nodig hebben.

HET ONTWIKKELEN VAN GALSTENEN NA EEN MAAGBANDOPERATIE.

Hoofdstuk 8

Overgewicht is een bekende risicofactor voor het ontwikkelen van galstenen. Gewichtsverlies echter is mogelijk zelfs een sterkere risicofactor. Dit is een belangrijke zorg in het kader van behandeling van obesitas middels bariatrische chirurgie, omdat hierbij een situatie gecreeerd wordt, waarin mensen met ernstig overgewicht in korte tijd veel gewicht verliezen en dientengevolge theoretisch een grote kans hebben op het ontwikkelen van galstenen. Er zijn effectieve profylactische behandelingen voor handen, zoals profylactische cholecystectomie tijdens bariatrische chirurgie of behandeling met ursodeoxycholzuur. Om een juiste afweging aangaande nut en noodzaak van profylactische behandeling van galstenen te kunnen maken, is het van belang geïnformeerd te zijn over de incidentie van cholelithiasis na bariatrische chirurgie, waarbij tevens onderscheid gemaakt moet worden tussen symptomatisch en asymptomatisch galsteenlijden.

In hoofdstuk 8 wordt een studie beschreven waarin 120 patiënten na maagbandoperatie (LAGB) worden geëvalueerd voor het optreden van symptomatisch en asymptomatisch galsteenlijden. Alle patiënten voldeden preoperatief aan de criteria voor morbide obesitas (BMI > 40 kg/m^2 of > 35 kg/m^2 in combinatie met relevante comorbiditeit). Deelname aan het onderzoek vond plaats gemiddeld 4,6 jaar na operatie (varierend van 1,3 tot 8,5 jaar) en het maximale gewichtsverlies bedroeg 31,5 ± 11,3% van het preoperatieve gewicht. Als controlegroep functioneerde een groep van patiënten van de wachtlijst voor LAGB.

Een groep van 16 patiënten had reeds voor LAGB een cholecystectomie ondergaan en viel derhalve af voor evaluatie. Tevens hadden 5 patiënten na LAGB (maar voor deelname aan het onderzoek) een cholecystectomie ondergaan vanwege symptomatisch galsteenlijden. 98 patiënten ondergingen een echo van de bovenbuik ter evaluatie van de aanwezigheid van galstenen. Bij 26 (26,5%) van hen werden galstenen vastgesteld, die anamnestisch in 2 gevallen symptomatisch bleken te zijn. Dit resulteerde in een prevalentie van galstenen na LAGB van 31 (30,1%) uit 103 patiënten. Van hen waren in totaal 7 (6,8%) patiënten op enig moment symptomatisch geweest. De prevalentie van galstenen in de patiëntengroep die nog geen LAGB hadden ondergaan was significant lager: 13,3%.

De prevalentie van galstenen bleek na LAGB significant hoger te zijn dan in een populatie patiënten lijdend aan morbide obesitas die nog geen bariatrische chirurgie ondergingen. Slechts 7 van de 31 patiënten ontwikkelden echter klachten in een periode van 4,6 jaar, hetgeen resulteert in een cumulatief risico van 24,4% na 5 jaar, een percentage dat overeen komt met het percentage symptomatisch worden van bekende cholelithiasis in de algemene bevolking. Op dit moment worden asymptomatische galstenen niet behandeld en dit beleid lijkt dus ook gerechtvaardigd na LAGB. Wel moet hierbij opgemerkt worden, dat de prevalentie na LAGB significant hoger was dan voor LAGB, hetgeen tevens gewichtsverlies als risicofactor voor het ontwikkelen van galstenen bevestigt. Om een juiste afweging te kunnen maken

met betrekking tot de indicatie van profylactische behandeling van galstenen na bariatrische chirurgie is uiteindelijk gerandomiseerd onderzoek nodig, waarbij met name morbiditeit en kosten van de verschillende benaderingen tegen elkaar afgezet moeten worden.

LANGE-TERMIJNEFFECTEN VAN BARIATRISCHE CHIRURGIE OP DE KWALITEIT VAN LEVEN.

Hoofdstuk 9

Het is algemeen bekend dat obesitas gepaard gaat met een verminderde kwaliteit van leven. Sociale stigmatisatie, een negatief zelfbeeld en een minder goede lichamelijke gezondheid zijn hier debet aan. Daar bariatrische chirurgie een effectieve behandeling is van obesitas, mag worden aangenomen dat deze behandeling een gunstig effect heeft op de kwaliteit van leven. Opvallend genoeg is inderdaad aangetoond dat reeds kort na de operatie, op een moment dat er van significant gewichtsverlies nog geen sprake is, de kwaliteit van leven reeds sterk verbetert. Helaas laten de schaarse lange-termijnstudies in de loop van de tijd na bariatrische chirurgie echter weer een afname van de kwaliteit van leven zien, ondanks een min of meer stabiel blijvend gewicht.

In de studie in hoofdstuk 9 werd van 59 patiënten die minimaal 5 jaar tevoren (gemiddeld 74,7 maanden, variërend van 60 tot 107,6 maanden) een maagbandplaatsing hadden ondergaan de kwaliteit van leven geëvalueerd. Als objectieve maat voor dit subjectieve gegeven werd de gevalideerde Nederlandse vertaling van een gestandaardiseerde algemene kwaliteit-van-levenvragenlijst, de RAND-36, gebruikt. Deze vragenlijst beslaat 9 categorieën aangaand fysiek en psychosociaal functioneren. Alle patiënten voldeden preoperatief aan de criteria voor morbide obesitas. Postoperatief daalde hun BMI van 44,9 ± 5,9 kg/m^2 naar 33.3 ± 6,0 kg/m^2. Als controlegroepen werden gebruikt een populatie van patiënten lijdend aan morbide obesitas die op de wachtlijst voor LAGB stonden en de Nederlandse bevolking (aan de hand van eerder gerapporteerde standaardscores). Tevens werd gezocht naar factoren die de kwaliteit van leven positief of negatief beïnvloedden.

Morbide obesitas leidde inderdaad tot een sterke afname van de kwaliteit van leven, zoals weerspiegeld werd in de bevinding dat de groep van de wachtlijst significant slechter scoorde op 6 van de 9 items vergeleken met de Nederlandse bevolking. Helaas was het resultaat na LAGB slechts beperkt beter: geopereerde patiënten scoorden op slechts 1 item significant beter dan de wachtlijstgroep en significant slechter op 4 van de 9 items vergeleken met de Nederlandse bevolking. Kwaliteit van leven werd in de geopereerde groep negatief beïnvloed door leeftijd, postoperatieve BMI en comorbiditeit en positief door de mate van gewichtsverlies na LAGB. Preoperatieve BMI, mate van stijging van het gewicht na initiële daling en de hoeveelheid tijd verstreken na de operatie hadden geen invloed op de kwaliteit van leven.

Concluderend bevestigt deze studie enerzijds dat obesitas een negatief effect heeft op de kwaliteit van leven, en anderzijds dat langere tijd na bariatrische chirurgie de kwaliteit van leven nog steeds onder de maat is. Daar het een cross-sectionele studie betreft, kan niet gedifferentieerd worden tussen een verslechtering na initiële verbetering of slechts een beperkt gunstig effect van LAGB. De beperkte gegevens uit de literatuur pleiten voor het eerste beloop. In eerdere studies werd gesuggereerd dat de afname van kwaliteit van leven in de loop der tijd het gevolg zou zijn van vermindering van intensiteit van medische controle, teleurstelling over slechts beperkt resultaat of gewichtstoename na initiële daling. De bepalende factoren zoals in onze studie werden vastgesteld hebben daarentegen opvallend weinig relatie met het tijdsbeloop na LAGB en lijken, ook op lange termijn, hoofdzakelijk gerelateerd aan de mate en gevolgen van het overgewicht zelf en het succes van de operatie gekwantificeerd als gewichtsverlies. In vervolgstudies, liefst van longitudinale opzet, zal het vooral belangrijk zijn vast te stellen welke andere beïnvloedbare factoren de kwaliteit van leven na bariatrische chirurgie bepalen, zodat hier in de follow up van geopereerde patiënten zo adequaat mogelijk op ingesprongen kan worden.

List of abbreviations

1.	ACTH	Adrenocorticotropic hormone
2.	AG	Acylated ghrelin
3.	AgRP	Agouti-related peptide
4.	AMPK	5′ adenosine monophosphate-activated protein kinase
5.	BMI	Body mass index
6.	BPD-DS	Biliopancreatic diversion – duodenal switch
7.	CB1	Cannabinoid receptor type 1
8.	CCK	Cholecystokinin
9.	EWL	Excess weight loss
10.	FFA	Free fatty acid
11.	GB	Gastric bypass
12.	GH	Growth hormone
13.	GHS	Growth hormone secretagogue
14.	GHS-R1a	Growth hormone secretagogue receptor type 1a
15.	GIP	Gastric inhibitory polypeptide
16.	GLP-1	Glucagon-like peptide-1
17.	GOAT	Ghrelin O-acyltransferase
18.	GPR39	G-protein coupled receptor 39
19.	HDL	High-density lipoprotein
20.	HMW	High molecular weight
21.	HOMA-IR	Homeostasis model assessment for insulin resistance
22.	IGF-1	Insulin-like growth factor 1
23.	IL-1	Interleukin-1
24.	IL-6	Interleukin-6
25.	IVGTT	Intravenous glucose tolerance test
26.	LAGB	Laparoscopic adjustable gastric banding
27.	LDL	Low-density lipoprotein
28.	LMW	Low molecular weight
29.	MC4R	Melanocortin 4 receptor
30.	MMW	Medium molecular weight
31.	NPY	Neuropeptide Y
32.	POMC	Pro-opiomelanocortin
33.	PPARα	Peroxisome proliferator-activated receptor α
34.	PYY	Peptide tyrosine tyrosine
35.	QoL	Quality of life
36.	RR	Relative risk ratio
37.	SIM1	Single-minded homolog 1
38.	SNP	Single nucleotide polymorphism
39.	TG	Triglyceride

TNFα	Tumor necrosis factor α
TRKB	Neurotrophic tyrosine kinase receptor
UAG	Unacylated ghrelin
VBG	Vertical banded gastroplasty
WAT	White adipose tissue

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ORAL PRESENTATIONS

2001 Regionale Klinische Avond Inwendige Geneeskunde, Rotterdam, The Netherlands
Een schokkend begin van het nieuwe jaar.

2003 Voorjaarsvergadering Nederlandse Vereniging voor Gastroenterologie, Veldhoven, The Netherlands
Een vrouw met een cholecystitis en obstructie-icterus.

Internistendagen, Maastricht, The Netherlands
Mediterranean Spotted Fever: a family outbreak.

2005 Wetenschapsdag Albert Schweitzer ziekenhuis, Dordrecht, The Netherlands
Maagbandplaatsing bij patiënten met morbide obesitas: Het risico op ontwikkeling van galstenen.

2006 Regionale Klinische Avond Inwendige Geneeskunde, Rotterdam, The Netherlands
Een halszaak.

2007 Klinische Endocrinologiedagen, Doorwerth, The Netherlands
Riedel's thyroiditis: successful treatment with prednisone and tamoxifen.

Endo-Neuro-Psycho Meeting, Doorwerth, The Netherlands
Effects of acylated and unacylated ghrelin on glucose and insulin metabolism in morbidly obese subjects without overt diabetes

2010 Rotterdamse Internistendag, Rotterdam, The Netherlands
Chirurgische behandeling van obesitas.

POSTER PRESENTATIONS

2001 United European Gastroenterology Week, Amsterdam, The Netherlands
Morbid obesity and endoscopic signs of hiatal hernia and reflux oesophagitis.

United European Gastroenterology Week, Amsterdam, The Netherlands
Morbid obesity and the prevalence of elevated liver enzymes.

2005 United European Gastroenterology Week, Copenhagen, Denmark
Gallstone formation after weight reduction surgery in morbidly obese patients.

2008 Annual Meeting of the Endocrine Society (ENDO), San Francisco, USA
Effects of acylated and unacylated ghrelin on glucose and insulin metabolism in morbidly obese subjects without overt diabetes

Dankwoord

Dit is een memorabel moment. De laatste pagina van het proefschrift nadert. Er is geen beter moment om terug te kijken op een lange en atypische onderzoeksperiode. Wat ooit begon als een 'klein onderzoekje' in het Albert Schweitzer ziekenhuis groeide uit tot het promotieonderzoek dat nu in boekvorm voor u ligt. Het lijkt ideaal om pas halverwege een onderzoekstraject te besluiten dat het eigenlijk wel een promotie waard is, want dat betekent dat het grootste deel al achter de rug is en het eind dus in zicht is. Niettemin kan het eind lang in zicht blijven en lijkt het soms nauwelijks te naderen. Maar goed, hier is het dan!

In de levensloop van dit proefschrift waren twee momenten van cruciaal belang. Het eerste moment vond plaats tijdens mijn sollicitatiegesprek naar de functie van AGNIO in het Albert Schweitzer ziekenhuis te Dordrecht. Dr. A.C.M. van Vliet, beste Adrie, jouw vraag of ik misschien belangstelling had om onderzoek te doen naar de maagbandpatiënten van de heelkundepoli en mijn positieve antwoord daarop betekende het begin van wat uiteindelijk deel 2 van dit proefschrift werd. Behalve een (kritische) onderzoeksbegeleider was je in dezelfde periode ook een (gedegen) opleider in de Interne Geneeskunde. Je bent het klassieke voorbeeld van een algemeen internist: je bent thuis in elk deelspecialisme van het vak. De eerste keer dat je mijn advies vroeg over een endocrinologisch probleem was dan ook een bijzonder moment.

Prof. dr. A.J. van der Lelij, beste Aart Jan, het tweede moment was toen we besloten dat we 'iets leuks' zouden gaan doen met 'jouw' ghreline en 'mijn' dikke mensen. Dit voornemen resulteerde in eerste instantie in het project beschreven in hoofdstuk 4. Toen ik had bedacht dat het mogelijk zou moeten zijn om te promoveren, was je direct enthousiast en kon ik aanschuiven bij het rattenproject. Is er een masterplan of komt alles toevallig goed uit? Ik heb moeten wennen aan je zeer efficiënte timemanagement systeem: je bent er als je nodig bent, maar wanneer je inschat niet nodig te zijn, ben je er niet. Ik wist aanvankelijk bijvoorbeeld niet, dat een antwoord op versie 1 van een artikel kon zijn dat het 'gewoon goed' was. Nu, als perifeer specialist, ben ik soms jaloers op dit talent. Last but not least, dank uiteraard dat je me in opleiding hebt genomen tot endocrinoloog, iets dat later heel schaars bleek te zijn.

Dr. ir. J.A. Visser, beste Jenny, dank dat je als zijinstromende copromotor direct zo enthousiast en betrokken was.

De leescommissie, bestaande uit prof. dr. J.F. Lange, prof. dr. J.A. Romijn en Prof.dr.ir. A.P.N. Themmen, wil ik hartelijk danken voor hun (zeer) snelle beoordeling van het manuscript. Beste Axel, dankzij jouw Engelse contacten kwam hoofdstuk 7 tot stand, waarvoor dank.

Gedurende alle onderzoeksjaren zijn er veel mensen op mijn pad gekomen die me ondersteund of gestimuleerd hebben. Ik waag een poging hen te noemen, met het risico mensen te vergeten. Deze laatste categorie dank ik op deze plaats alvast heel hartelijk…

Geen patiëntgebonden onderzoek zonder patiënten, dus alle mensen die van heinde en verre kwamen om deel te nemen aan het follow up onderzoek na maagbandplaatsing krijgen daarvoor alle waardering. Van hen heb ik geleerd wat het betekent om te lijden aan obesitas. Ik heb veel bewondering voor de acht patiënten die bereid waren om gedurende drie weken vier dagen per week naar het ziekenhuis te komen voor een onderzoek waar zij zelf geen voordeel van zouden ondervinden. Daarom heel hartelijk dank!

Het Albert Schweitzer ziekenhuis te Dordrecht was een van de eerste ziekenhuizen in Nederland waar bariatrische chirurgie verricht werd, wat mij vervolgens een prachtige studiegroep opleverde waarin lange-termijneffecten van maagbandplaatsingen geëvalueerd konden worden. Dr. W.L.E.M. Hesp startte destijds de bariatrische chirurgie. Beste Fried, je was altijd enthousiast over mijn project en ik vind het bijzonder dat we nu weer nauw samenwerken in de obesitaswerkgroep.

Drs. L.P.L.H. Cuijpers, beste Luc, jouw suggestie om de RAND-36 te gebruiken als kwaliteit-van-levenvragenlijst leverde onderzoeksresultaten die internationaal vergelijkbaar waren, hetgeen daarom voorspoedig resulteerde in een publicatie.

Drs. M.F. Durian, beste Marc, destijds collega arts-assistent, dank dat je (voor een deel) de interviews met de maagbandpatiënten overnam toen ik met de opleiding startte en verhuisde van locatie Amstelwijck naar locatie Dordwijk.

In 2005 arriveerde ik in het Erasmus MC, kort nadat dr. M.O. van Aken er gestart was als staflid endocrinologie. Beste Maarten, samen werkten we het protocol voor de 'ghrelintrial' uit tot het lijvige onderzoek dat het geworden is. Dank voor je laagdrempeligheid en positieve instelling. Ik miste je opmerking 'heel goed, heel goed' toen je was vertrokken naar het HagaZiekenhuis, vlak voordat ik vertrok naar Dordrecht.

Ongeveer 1000 bloedafnames en vele kilometers op en neer naar het lab, dat was jouw investering in het onderzoek, Kim. De kwaliteit van een onderzoek staat of valt met de nauwkeurigheid van de uitvoerders, dus daarom was ik heel blij toen jij als afstudeerstudent kwam om me bij te staan. Gelukkig leverde het jou uiteindelijk ook veel op: het onderzoek waar je inmiddels al een poos intensief mee bezig bent.

Duizend bloedafnames betekent een veelvoud aan laboratoriumbepalingen. Hans van Toor, maar vooral Piet Uitterlinden, hebben 'onder supervisie' van Yolanda de Rijke deze verricht op het endolab op de 5e verdieping. Beste Piet, wat bleef je bewonderenswaardig vriendelijk lachen als ik wéér kwam vragen of het al klaar was. Het boek over de Baltische staten mag je houden…

Dr. C. Gauna, cara Carlotta, abbiamo passato tante ore insieme nel centro per gli sperimenti sugli animali in mezzo ai ratti. E' stato sempre bello e ci siamo divertite (tranne quando per

sfortuna i ratti morivano alla fine dei prelievi) e nel corso delle settimane siamo diventate una squadra ben funzionante. I ratti ormai non li sogno più... e tu?

Beste Bedette van de Zande, dank voor je hulp bij de bepaling van de glucose- en insuline-waarden voor het obestatine-onderzoek. Ik heb zelf immers twee linker 'labhanden'.

Ezio Ghigo, Thierry Abribat, Patric Delhanty and Leo Hofland, the co authors who have not been mentioned yet: thank you very much for your scientific input. Dear Patric, thanks for your critical review of the English summary.

Mijn onderzoeksperiode liep parallel aan mijn opleiding tot internist-endocrinoloog. Ik kijk met veel plezier terug op mijn opleidingsperiode in het Albert Schweitzer ziekenhuis. Daar werd een goede basis in de Interne Geneeskunde gelegd. De specialisten die mij destijds opleidden in het vak, zijn nu mijn maten. Ik vind het heel bijzonder dat ik sinds mijn toetreding tot de maatschap nooit enig gevoel van ongelijkwaardigheid heb gekregen, terwijl we tevoren zo lang als meester en gezel hadden samengewerkt. Dank ook voor jullie sportieve reactie toen ik na mijn toetreden als jonge vrouw in de maatschap direct het grootste vooroordeel bevestigde.

Het academische deel van de opleiding Interne Geneeskunde duurde maar een jaar. De opleider, prof. dr. J.L.C.M. van Saase, leerde ik pas echt goed kennen tijdens de organisatie van de Rotterdamse Internistendag. Beste Jan, volgende keer kom ik eens gewoon in de zaal zitten.

De periode op de afdeling endocrinologie werd gekenmerkt door hoogstaande patiënten-zorg, wetenschap, 'sterke' verhalen, frequente congressen en veel gezelligheid. Ik heb me er de endocrinologie zeer grondig eigen kunnen maken. Aart Jan, Wouter, Richard, Joop en Carola, dank daarvoor.

Terwijl het einde van het dankwoord nadert, wordt het tijd voor de mensen die letterlijk en figuurlijk naast me staan.

Zij die letterlijk naast me staan zijn mijn paranimfen, Marieke Segboer-Joosten en Sebastian Neggers. Sebastian, we begonnen vrijwel tegelijkertijd met ons aandachtsgebied endocrinologie en belandden in hetzelfde schuitje toen jij je stortte op het acromegalie-onderzoek naast je opleiding. Toen we klaar waren met onze opleiding vertrok ik naar het Albert Schweitzer ziekenhuis en jij bleef in het Erasmus MC, waar je volgens mij prima op je plaats bent. We zullen elkaar regelmatig tegen blijven komen: op nascholingen (als collega's) en bij de bakker (als bijna-buren). Succes met het afronden van je eigen promotie. Marieke, vriendin sinds de eerste dag van de Eurekaweek in 1992, hier staan we dan, ruim 18 jaar later. We zijn inmiddels twee artsexamens, twee specialisaties, twee promoties, twee bruiloften en drie kinderen (waaronder onze bijna-tweeling) verder en ik ben benieuwd wat er allemaal nog gaat komen.

Zij die figuurlijk naast me staan zijn mijn ouders. Pappa en mamma, jullie hebben me altijd gestimuleerd om optimaal gebruik te maken van mijn capaciteiten en wanneer ik er eens toe neigde de makkelijkere weg te kiezen, wisten jullie me heel subtiel weer op het 'juiste' pad te

brengen. Bedankt ook voor alle praktische hulp door de jaren heen. Vooral alle keren dat jullie de laatste tijd op Max hebben gepast was een typisch voorbeeld van een win-win-win situatie.

En dan tot slot mijn twee mannen. Lieve Rob, we kennen elkaar al zo lang, wat zal ik nog eens zeggen. Moet ik je bedanken voor je nuchterheid en relativeringsvermogen? Of voor het feit dat je altijd mijn computerhelpdesk bent? Ik kan denk ik volstaan met de opmerking dat we nou eenmaal een heel goed team zijn. Wie kan er nou echt samen gezellig behangen? Wij dus! En voor mijn kleine man Max: "Zoooo, klaaaaarrrr. Pakke, soene, buite!!!"

Curriculum Vitae

Rosalie Kiewiet-Kemper werd geboren te Dordrecht op 25 juni 1974. Haar VWO-diploma behaalde zij (cum laude) aan het Gymnasium Camphusianum te Gorinchem in 1992. Aansluitend startte zij met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Na het (cum laude) behalen van het artsexamen in december 1998 was zij van januari 1999 tot juni 2000 werkzaam als assistent-geneeskundige niet in opleiding op de afdeling Neurologie van het Erasmus MC te Rotterdam. In juni 2000 werd zij vervolgens assistent-geneeskundige niet in opleiding op de afdeling Interne Geneeskunde van het Albert Schweitzer ziekenhuis te Dordrecht. In deze periode werd een begin gemaakt met het onderzoek naar late gevolgen van maagbandoperaties als behandeling van morbide obesitas onder supervisie van dr. A.C.M. van Vliet. Op 1 januari 2002 startte zij met de opleiding Interne Geneeskunde in het Albert Schweitzer ziekenhuis te Dordrecht (opleider dr. A.C.M. van Vliet). Na 3 jaar werd de opleiding vervolgd in het Erasmus MC te Rotterdam (opleider prof.dr. J.L.C.M. van Saase). In 2006 trad zij toe tot het aandachtsgebied Endocrinologie (opleider prof.dr. A.J. van der Lelij, later dr. W.W. de Herder). In deze periode werd een belangrijk stuk van het onderzoek naar de metabole aspecten van obesitas verricht. Per 1 januari 2008 werd de opleiding tot internist-endocrinoloog voltooid. Hierna was zij gedurende 6 maanden werkzaam als internist-endocrinoloog in het Erasmus MC. Op 1 augustus 2008 trad zij toe tot de maatschap Internisten en Maag-Darm-Leverartsen van het Albert Schweitzer ziekenhuis te Dordrecht.

Rosalie Kiewiet-Kemper is getrouwd met Rob Kemper. Zij hebben een zoon, Max.