15 July 2024

Supervisor: Prof. Dr. A. J. W. Scheurink
University of Groningen. *Faculty of Science and Engineering*
Thesis Life Science and Biology
Behavioural Neurosciences

Unveiling Ghrelin: Potential Pathways to a Type 2 Diabetes Cure

D. M. Z. Simon

University of Groningen, Faculty Science and Engineering

Abstract
Type 2 diabetes (T2D) remains a widespread metabolic disorder characterised by insulin resistance and pancreatic β-cell dysfunction, resulting in elevated blood glucose levels and associated complications. Ghrelin, initially identified for its role in appetite regulation, has garnered attention for its broader implications in glucose metabolism and diabetes therapy. Recent research has highlighted two primary forms of ghrelin: acylated ghrelin (AG) and unacylated ghrelin (UAG), alongside the lesser-known obestatin peptide. AG promotes appetite and contributes to insulin resistance, whereas UAG exhibits anti-diabetic properties by enhancing insulin sensitivity and protecting pancreatic β-cells. Obestatin, originating from the same gene as ghrelin, shows promise in reducing food intake and improving insulin signalling.

Efforts to harness ghrelin's therapeutic potential include the development of ghrelin receptor antagonists and immunizations targeting ghrelin, aiming to modulate appetite and glucose metabolism. While AG antagonists have shown efficacy in animal models, translating these findings to humans remains challenging. Conversely, UAG and obestatin offer novel avenues with their distinct mechanisms in diabetes management. UAG's ability to enhance insulin sensitivity and mitigate oxidative stress presents a promising therapeutic approach, while obestatin's anorexic and insulin-sensitising effects suggest potential as a T2D treatment.

This review explores the evolving status of ghrelin-based therapies, highlighting the complexities and promising paths for combatting T2D. Further research is essential to resolve the precise mechanisms and optimise therapeutic strategies involving ghrelin, UAG, and/or obestatin, potentially paving the way for innovative treatments that improve patient outcomes and quality of life.

1. Introduction

Type 2 diabetes (T2D) is a prevalent metabolic disorder characterised by insulin resistance and pancreatic β-cell dysfunction, leading to elevated blood glucose levels (hyperglycemia) (Hameed et al, 2015). This condition is associated with significant morbidity and mortality due to its complications, including cardiovascular disease and nerve damage (Inzucchi et al., 2016).

The pathogenesis of T2D involves a complex interplay of genetic predisposition and environmental factors, primarily obesity. Insulin resistance, a hallmark of T2D, results in impaired glucose uptake by tissues such as skeletal muscle and liver, while pancreatic β-cells fail to maintain adequate insulin secretion to compensate for this resistance (Togliatto et al., 2010)

Ghrelin, primarily known for its role in appetite regulation, emerges as a potential player in glucose metabolism and diabetes (Gauna et al., 2005, Dezaki et al., 2008, Vestergaard et al., 2008). Initially discovered as a growth hormone secretagogue, ghrelin is produced predominantly in the stomach and acts via the growth hormone secretagogue receptor (GHSr) in various tissues including the brain, pancreas, and adipose tissue (Kojima et al., 1999).

Recent studies have delineated ghrelin into two primary forms: acylated ghrelin (AG) and unacylated ghrelin (UAG). AG, the active form, stimulates appetite and contributes to insulin resistance and glucose intolerance. In contrast, UAG was initially considered inactive but has since shown promise in counteracting AG's effects. UAG exhibits anti-diabetic properties by improving insulin sensitivity, reducing oxidative stress, and protecting pancreatic β-cells (Granata et al., 2010).

Research indicates that AG promotes glucose intolerance and insulin resistance by affecting insulin secretion and glucose metabolism pathways (Kouno et al., 2016). Conversely, UAG has been found to improve glucose tolerance and insulin sensitivity in animal models and human studies (Tong et al., 2013). Moreover, UAG shows potential in protecting endothelial progenitor cells and reducing oxidative stress, crucial factors in diabetes-associated complications (Togliatto et al., 2010).

Efforts to harness ghrelin's potential as a therapeutic target include the development of ghrelin receptor antagonists and immunizations against ghrelin. These approaches aim to modulate appetite and glucose metabolism, potentially offering new avenues for managing T2D and obesity (Nakazato et al., 2001).

The different forms of ghrelin show great potential due to their anti-diabetic properties. The aim of this analytic overview is to find the best potential treatment for T2D, out of different forms of ghrelin.

2. Type 2 diabetes

Type 2 diabetes, T2D, is a well-known metabolic disorder. Over the years, it has been rapidly increasing, reaching epidemic proportions. It is a worldwide problem, where the prevalence and incidence represents over 90% of all diabetes patients (Laakso, M., 2019).

This disorder is characterised by insulin resistance and dysfunction of the pancreatic β-cells. These symptoms are caused by hyperglycemia(Hameed et al, 2015). Along with these symptoms come several complications. Commonly found complications are nerve damage, cardiovascular disease (CVD) and dementia. The complications, or a combination of them, can result in morbidity and mortality in T2D patients (Inzucchi et al., 2016).

T2D is the variant of diabetes mellitus that is precipitated by an interplay between genetic factors and environment. Patients with insulin resistance, IR, are of high risk for T2D. IR can be a result of being overweight. A patient is considered obese when their BMI is over 30kg/m². The body resides in an insulin resistant state (Langenberg et al., 2018). Therefore, obese people are likely to become T2D patients. This insulin resistant state is caused by hyperglycemia and is tissue specific. Blood glucose levels increase as a result of increased food intake. As a response, the body secretes insulin to take up and store the circulating glucose. This insulin-mediated glucose uptake is reduced in patients suffering from IR. The ability of the body to take up glucose via the skeletal muscle, adipose tissue and liver is reduced, whereas the glucose production in the liver is increased (Gutch et al, 2015). The glucose production by the liver is normally suppressed by insulin and decreases gluconeogenesis, formation of glucose, by inhibiting the encoding genes (Laakso, M., 2019). A lack

of response causes shifts in blood glucose levels, resulting in an overall increase. Additionally, insulin prevents accelerated lipolysis in the fat cells, keeping the free fatty acids and glycerol levels in the blood low (Laakso, M., 2019).

Insulin is secreted by the β-cells of the pancreatic islets of Langerhans. These glucose-sensing cells respond to shifting blood glucose levels (Gelback et al, 2022). Dysfunctioning of the β-cells causes a disbalance in blood glucose levels and the whole glucose metabolism. An important effector molecule for β-cell dysfunction are free fatty acids. FFAs decrease the amount of functioning β-cells by lipoapoptosis, a metabolic cause of programmed cell death (Hammeed et al., 2015). Insulin keeps the amount of blood FFAs low, however, a disbalance in the glucose metabolism and insulin secretion causes a decrease in insulin. This will result in an increase in blood FFAs, which results in increased dysfunctioning of the β-cells (Togliatto et al., 2010).
Another important symptom of diabetes is an imbalance in UAG/AG ratio together with an impairment of circulating endothelial progenitor cells (EPC). Functioning of EPCs are impaired in diabetes (Tepper et al., 2002). Endothelial injury can increase the risk for atherosclerotic vascular disease. These events rely mainly on NADPH oxidase-mediated reactive oxygen species (ROS) production (Gao et al., 2009).

ROS, including superoxide anion (O2-) and hydrogen peroxide (H2O2), play a critical role in the pathogenesis of insulin resistance in skeletal muscle, a key feature of T2D. Increased ROS levels can induce oxidative stress, which interferes with insulin signalling pathways and impairs glucose uptake in skeletal muscle cells (Kim et al., 2006).

Evidence suggests that NADPH oxidase-mediated ROS production contributes significantly to the onset of skeletal muscle insulin resistance in diabetes. NADPH oxidase is a major source of ROS in many cell types, including skeletal muscle cells, and its overactivity in diabetes leads to excessive ROS production (Gao et al., 2009). These ROS can directly impair insulin signalling by causing oxidative modifications to insulin receptor substrate (IRS) proteins and other key signalling molecules involved in glucose uptake (Houstis et al., 2006).

Moreover, ROS-induced oxidative stress in skeletal muscle cells can promote inflammation and mitochondrial dysfunction, further exacerbating insulin resistance and impairing glucose homeostasis (Anderson et al., 2009).

Much is known about the mechanisms and important pathways that contribute to T2D, however, a cure has been hard to find.

3. Present treatments for type 2 diabetes

Finding a treatment for T2D has been an ongoing field in research. Compared to type 1 diabetes, the form of diabetes mellitus that has a more genetic basis and is often presented in early life, a cure for T2D has been harder to find. Since lifestyle and environment are big contributing factors, and over 400 genetic variants have been found, there are many aspects to take into consideration (Mahajan et al., 2018). The 400 genetic variants all have a moderate or small effect on the prevalence of T2D, influencing different pathways, processes in several tissues and cells. This includes the β-cells, skeletal muscle, liver and fat cells (Laakso, M., 2019). Because of the significant amount of genetic variants, two previous studies have divided patients into two subgroups. One is characterised by β-cell dysfunction and one by insulin resistance (Dimas et al., 2014, Udler et al., 2018). Even after subdivision, it is still hard to find a cure that has a positive influence on the involved pathways, with as little side effects possible.

Several classes of drugs have been used and tried for T2D, such as glucose-lowering medications, weight-loss medications, drugs promoting both glucose-lowering and weight-loss (Majety et al., 2023). Majety et al has made a list with several forms of medication, which has been listed below.
As a glucose-lowering medication, metformin is often used. It decreases the glucose production in the liver by inhibiting the gluconeogenesis. It has positive effects on the insulin sensitivity in the skeletal muscle. This is a result of increasing insulin receptor tyrosine kinase activity and translocation of glucose transporter 4 (GLUT 4)

to the cell membranes (TDPPRG, 1999). Additionally, it corrects the glucotoxicity, improving β-cell sensitivity to glucose shifts. It does not cause hypoglycemia and has beneficial effects on blood pressure and lipid concentration (Bailey et al., 1996). Another often used medicine is thiazolidinediones. These types of insulin sensitisers are an active gamma isoform of PPARγ (Majety et al., 2023). It has a positive influence on the glucose uptake of the skeletal muscles and the adipocytes, lowering blood glucose. Additionally, it improves insulin sensitivity and pancreatic β-cell functioning. The trials show promising results, unfortunately there have been multiple side effects found which include fluid retention, an increased risk of heart failure, weight gain and an increased risk of bone fracture by decreased bone density.

Alpha-glucosidase inhibitors play a significant role in managing blood glucose levels by competitively inhibiting the alpha-glucosidase enzyme, which decreases carbohydrate absorption in the small intestine, ultimately reducing postprandial glucose levels. Clinical studies have shown that these inhibitors can effectively lower the percentage of individuals transitioning from T2D and impaired glucose tolerance (IGT) to normal glucose tolerance.

Sodium-glucose co-transporter-2 (SGLT2) inhibitors are primarily expressed in the proximal tubule of the kidney, mediating the reabsorption of almost 90% of the filtered glucose load. These inhibitors promote renal excretion of glucose, thereby lowering elevated blood glucose levels. SGLT2 inhibitors not only reduce the onset of T2D but also offer benefits in prediabetes, protecting pancreatic beta cells from glucotoxicity, inducing weight loss, and improving hepatic insulin sensitivity. However, their efficacy in patients with heart failure and prediabetes has not shown significant benefits.

Weight-loss medications also play a crucial role in managing T2D and prediabetes. Orlistat, which reversibly inhibits gastric and pancreatic lipases, has been shown to increase GLP-1 levels, stimulating insulin secretion. Despite its effectiveness, its usage is often limited due to numerous side effects. Phentermine/topiramate stimulates norepinephrine release in the hypothalamus, suppresses appetite, and increases satiety. Though its mechanism of action is not fully understood, it is believed to work on AMPA receptors to reduce cravings and GABA receptors to increase energy expenditure. Naltrexone/bupropion combines the effects of bupropion, a norepinephrine and dopamine reuptake inhibitor that stimulates POMC neurons in the hypothalamus to increase satiety, and naltrexone, which prevents the rebound inhibition of POMC neurons by β-endorphin, working synergistically with bupropion. This combination treatment has been shown to decrease fasting plasma glucose and improve glucose homeostasis.

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) mimic GLP-1, enhancing glucose-dependent insulin secretion and suppressing glucagon production from pancreatic alpha cells, thereby achieving glycemic control. In addition to their glucose-regulating properties, GLP-1 RAs reduce neuroinflammation, promote nerve growth, improve cardiac function, suppress appetite, delay gastric emptying, regulate lipid metabolism, and reduce fat deposition. Tirzepatide, a dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist, has shown remarkable efficacy, with over 95% of prediabetic patients reverting to normoglycemia compared to 60% with a placebo. However, there was no observed decrease in fasting insulin levels, which might be attributed to weight loss.

For now, there is no cure that has no to little side effects that makes the cure dangerous or unpleasant. The medications all focus on a hand-full of aspects. Most influence the glucose pathway, insulin, the pancreatic β-cells and the hunger and or satiety feeling. A potential medicine and cure must act on all these aspects. Preferably a medicine that is not foreign for the body to limit side effects. When examining the body's mechanisms that influence these aspects, one hormone stands out: ghrelin.

4. Ghrelin; one gene, three peptide hormones

The initial mention of ghrelin emerged in 1999 in a scientific publication entitled "*Ghrelin is a growth-hormone-releasing acylated peptide from stomach*" by Kojima et al. In the search for the ligand that acts on the growth-hormone secretagogues receptor (GHSr). They found that the unknown ligand must act on the regulation of growth hormone (GH) release. What they found was a 28 amino acid long peptide, in which the serine 3 residue is n-octanoylated (Kojima et al., 1999). The O-n-octanoylation at serine 3 is of great importance for the functioning of ghrelin (Lundgren, O., 2000).

The researchers created the name ghrelin, “ghre” meaning grow, given its growth hormone-releasing function. They identified its primary production site as the stomach. Subsequently, it was discovered that ghrelin is also partly synthesised in the pancreas, intestine, hypothalamus, and pituitary (Kosowicz et al., 2011). Additionally, expression in the heart and lungs has been found (Lundgren, O., 2000). Lundgren hypothesised a likely association between ghrelin and food intake, conjecturing that chemical and/or mechanical triggers may be involved. This hypothesis was based on the observation that increased food consumption would cause an increase in the secretion of GH (Kojima et al., 1999). Further research has brought additional aspects of this growth hormone-releasing peptide to light.

One of the most significant and intriguing discoveries about ghrelin is that its gene product undergoes post-translational acylation, influencing the activity of ghrelin (Delporte., 2013). The gene coding for ghrelin results in different hormones, with different functions. This post-translational acylation is regulated by ghrelin O-acyltransferase (GOAT). The end product of post-translational acylation is acyl-ghrelin (AG). When this enzyme is inactive, the product will be called unacylated-ghrelin (UAG). The acylation of ghrelin determines its type of activity (Granata et al., 2010). AG is considered “active” ghrelin and UAG is considered “inactive” ghrelin.

The activated form of ghrelin does have the ability to bind to the growth hormone secretagogue receptor 1a (GHSr1a), whereas the inactive form lacks this resource (Granata et al., 2010). This finding implies that the ligand that was discovered by Kojima in 1999 was AG. Since this was found a decade after the discovery of ghrelin, most articles do not take this difference into consideration. Most articles have studied AG while using the name ghrelin. As a consequence, not much is known about the “inactive” UAG.

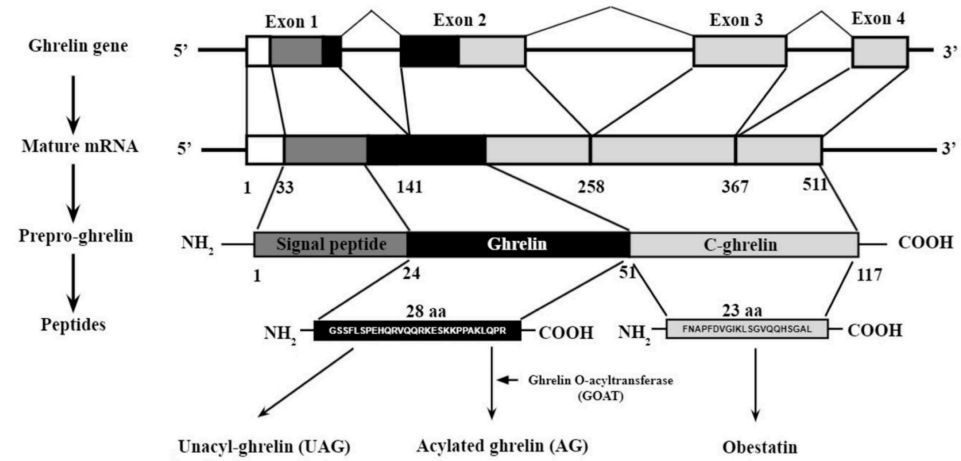
GHSr1a, the receptor to which AG can bind, is located in many tissues throughout the body, such as the brain and the thyroid (Poher et al., 2018). In the brain, GHSR1a is mostly found in the pituitary, hypothalamus and hippocampus (Airapetov et al., 2021). The hypothalamus contains the feeding centre of the brain, where the GHSR1a is localised in the neurons that express neuropeptide Y (NPY) and Agouti-related peptide (AgRP); the NPY/AGrP neuron (Poher et al., 2018). These peptides play a major role in stimulating appetite and therefore increasing food intake, making them orexigenic. Research from Qi et al have analysed the effects of NPY in the AgRP neurons. NPY and AgRP are co-localised, as well as co-expressed (Qi et al., 2022). When looking at both peptides, both are increased when the body has a negative energy balance. In the experiment of Qi et al, researchers looked at the specific functions and effects of NPY in the AgRP neurons concerning the control of feeding behaviour as well as energy homeostasis. They created a knockout for the NPY in the AgRP neurons. The results show that a deletion in NPY induced increased body weight in the mice, which may be caused by the increased food intake (Qi et al., 2022). However, obese mice show an increase in both NPY and AGrP levels (Mayer et al., 2009).
Additionally, insulin plays a role in the regulation of the orexigenic peptides, it decreases the NPY/AgrP expression in the neurons to decrease the peptide levels (Mayer et al., 2009). When food has been taken in, the body will respond to the increased glucose levels and insulin will be secreted. As a consequence, the hunger feeling mediated by NPY and AgRP will be decreased and therefore the expression of the NPY/AgRP neurons decreases. The activation of the NPY/AgrP neurons mediates an orexigenic response (Nasrallah et al, 2014). As a result, the hunger feeling is promoted, increasing appetite and food intake.
Ghrelin stimulates the NPY/AgRP neurons. When ghrelin is present, it will bind to the GHSr, leading to the activation of the NPY/AgRP neurons (Andrews et al., 2008). The NPY hormone plays a major role in maintaining energy balance, in relation to insulin (Loh et al, 2017). A lack of insulin receptor signalling or insulin deficiency increases the hypothalamic NPY expression, which also leads to an increased appetite and a decrease in energy expenditure. Additionally, the insulin resistant state, that can be a result of obesity, leads to an increased NPY levels in the hypothalamus (Loh et al., 2017). Living in a world where food is always accessible, combined with the feeling of hunger, it can result in increased body weight, and in severe cases to obesity .Conversely, insulin signalling also modulates POMC/CART neurons (Loh et al., 2017). Insulin activates these neurons, leading to enhanced satiety. This effect is antagonistic to the action of NPY/AgRP neurons.

Ghrelin has an influence on many parts of the glucose metabolism (Tong et al., 2010). AG mainly influences the regulation of glucose and lipid metabolism. Increased levels of AG have a negative effect on glucose-stimulated insulin secretion and intravenous glucose tolerance (Tong et al., 2010). A clamp study in 2008 showed that AG infusion resulted in hyperglycemia and increased FFA levels (Gauna et al., 2005, Dezaki et al., 2008, Vestergaard et al., 2008). These findings show the diabetogenic effects of AG.
When analysing the food consumption behaviour of GOAT knock-out (KO) mice, it showed that the AG has an influence on the glucose-containing carbohydrate intake (Kouno et al., 2016). Additionally, the same study fed the mice a diet rich in starch and fat for several weeks. The KO mice had an aberrant behaviour from the wild type (WT) mice, showing a decrease in food intake and weight gain. Besides that, it showed increased glucose tolerance and increased insulin sensitivity (Kouno et al., 2016). These findings show that ghrelin also plays a role in glucose metabolism and that the different types of ghrelin have different influences on insulin sensitivity. Acylated ghrelin can be considered as a diabetogenic and orexigenic gastric peptide (Togliatto et al., 2010).

These findings and characteristics are based on AG. While AG administration suppresses insulin secretion, induces peripheral insulin resistance, and impairs glucose tolerance in humans (Tong et al., 2014), UAG has opposite effects. In a research of Granata et al it was found that there is more UAG in the circulation than AG. Ratios vary between 2:1 to 9:1 (Tong et al., 2013). At first, UAG was considered “inactive”, but the research of Granata et al showed that it affects the body throughout several pathways. UAG has been found to counteract the glucogenic effect of AG, along with the hyperglycemia resulting from increased AG levels (Gortan Cappellari et al., 2016). In the same study it was found that UAG lowers the ROS production in skeletal muscle via several pathways.

Then there was the discovery of a third peptide that derived from the ghrelin gene. Obestatin is the third, and last discovered variant of the ghrelin gene, perhaps for now. It originates from the ghrelin gene, the same as AG and UAG. Prepro-ghrelin consists of ghrelin and c-ghrelin. Ghrelin forms AG and UAG, whereas c-ghrelin forms the obestatin peptide (fig. 1). Obestatin showed, just like UAG, anti-diabetic properties and counteracting the orexigenic effects of AG.

These findings suggest that the ghrelin system contributes to the regulation of β-cell function and the glucose metabolism and could be used as a cure for type 2 diabetes.



	Acylated ghrelin (AG)	Obestatin
Expression	GI tract (Stomach, Intestine), Brain, Pancreas, etc.	GI tract, Pancreas, etc.
Receptor	GHS-R1a	GPR39?, GLP-1R?, GHS-R?

Figure 1. Post-translational processing of preproghrelin to unacylated ghrelin, ghrelin, and obestatin. The figure was taken from Villarreal et al.

5. Ghrelin receptor antagonist // anti-ghrelin

Since ghrelin is a stimulator of appetite and increases food intake, contributing to increased weight and diabetogenic effects, an antagonist could be a solution to prevent or reverse the effects of diabetes. There have been attempts at ghrelin antagonists, or anti-ghrelin, vaccinations. These consisted of passive antibody transfer. The polyclonal anti-ghrelin antibodies were administered within the brain ventricles. The administration led to a reduction in fast-induced feeding as well as a suppression of food intake in rats (Nakazato et al., 2001).

Ghrelin receptor antagonists show improving glucose tolerance, suppressing appetite, and promoting weight loss in animal models. These findings correlate with the hypothesis of anti-ghrelin being a prevention and/or treatment for obesity. For example, in rodents, a single oral dose of a GHSr-1a antagonist resulted in improved glucose homeostasis, as a result of increased insulin secretion. When given daily, a GHSr-1a antagonist significantly reduced food intake and led to a weight loss of up to 15%, primarily due to a selective reduction in fat mass in obese mice. The significant weight loss was a result of the reduced food intake. The GHSr-1a showed no significant effects on gastric emptying. Given the highest dose showed little effect, whereas the lowest dose showed no significant effect. However, weight loss was still measurable. This concludes that the effects of the GHSr-1a are linked to brain exposure (Elser et al., 2007).

Another study used ghrelin receptor antagonist [D-Lys(3)] GHRP-6, a modified form of Growth Hormone Releasing Peptide-6 (GHRP-6), where the third amino acid is replaced with D-lysine. The antagonist was subcutaneously administered, so that the substance can be slowly absorbed into the bloodstream. After seven days, mice on both high-fat and standard diets, showed a decrease in food intake. Additionally, a decrease in body weight, blood glucose, insulin, and leptin levels was found. An increase of the ketone body concentrations and uncoupling-protein-1 mRNA in brown adipose tissue implies that fat tissue has been broken down. These effects of [D-Lys(3)] GHRP-6 are anorexigenic, as confirmed by pair-feeding studies (Maletínská et al., 2011). Furthermore, anti-ghrelin antibodies effectively inhibited acute ghrelin-mediated orexigenic effects in mice.

Efforts to explore ghrelin as a vaccine target involved synthesising ghrelin analogs bound to haptens, a small molecule that can bind to a larger carrier molecule to stimulate antibody production. This was done to induce an autoimmune response. For instance, vaccines based on the 28-amino acid sequence of ghrelin showed that immunised Wistar rats had a reduction in weight gain, with reduced fat mass and reduced circulating leptin levels. Leptin is considered a satiety hormone, a decrease in this hormone might not be beneficial in T2D patients. The ratio of brain/serum ghrelin levels was also lower, suggesting reduced central ghrelin passage (Zorilla et al., 2006).

In another study, pigs were actively immunised against ghrelin. They looked at the ratio of bound ghrelin in the plasma. The increase in bound ghrelin results showed a more than 15% decrease in voluntary food intake compared to controls. However, the weight loss was not significant. Immunised pigs weighed approximately 10% less than control animals. This suggested that ghrelin immunisation could induce mild anorexia, potentially serving as a strategy for controlling caloric intake and obesity (Vizcarra et al., 2007).

A novel approach using a non-infectious viral vector carrying ghrelin demonstrated that such vaccines could induce specific autoantibodies, neutralising disease-related proteins. This concept was extended to design an anti-ghrelin vaccine aimed at obesity treatment, using a chemical conjugate of active ghrelin with protein tubules of NS1 from the bluetongue virus. In a randomised study, immunised mice showed decreased food intake and enhanced energy expenditure, although body weight did not significantly change. This suggested that the anti-ghrelin vaccine positively impacted energy homeostasis and could be used alongside diet and exercise for obesity treatment (Andrade et al., 2013). However, as previously mentioned, diet and exercise normally had little to no effect in treatments for T2D patients.

Human trials for immunisation against ghrelin have shown mixed results. A randomised, double-blind, placebo-controlled trial in 2006 involving 87 obese patients found no significant additional weight loss compared to controls, despite a strong response in ghrelin autoantibodies. The median weight loss was 3.6 kg over six months for both groups, indicating the treatment was safe but not more effective than placebo (Colon-Gonzalez et al., 2013).

Conversely, another study suggested that IgG anti-ghrelin autoantibodies might protect ghrelin from degradation, enhancing its orexigenic effects. However, not all obese subjects show increased IgG affinity for ghrelin, indicating that this phenomenon is not universally linked to obesity. The production of ghrelin-reactive IgG might involve microbial factors suggesting a complex interplay between gut microbiota and immune response (Takagi et al., 2013, Fetissov et al., 2008).

6. Unacylated ghrelin

Unacylated ghrelin was considered inactive as it is unable to bind to GSHr (Granata et al., 2010). Once it was found that it has opposing effects to AG, more research was done. UAG has anti-diabetogenic and anorexic properties, making it an interesting gastric polypeptide (Togliatto et al., 2010). These properties made the peptide an interesting candidate for a potential cure for diabetes.
In a study of Togliatto et al in 2010 these potentials were measured. They investigated the potential of AG and UAG to reverse the defects of diabetes. They found that systemic administration in healthy subjects prevented diabetes induced EPC damage. This prevention had a positive effect on the vasculogenic, blood vessel formation, potential in patients with T2D. Additionally, the mobilisation of bone marrow EPC was recovered. They also found that EPCs express an UAG-binding site, to which AG cannot bind. The specific functions and reasoning for this binding is not yet found. Systemic administration of UAG for 6 hours resulted in a significant reduction of ROS production in T2D patients. Additionally, the administration also showed an increase in the amount of viable cells. UAG has the ability to prevent p53 protein accumulation. The p53 protein plays a crucial role in cell apoptosis, cell death. Prevention of the accumulation of the protein shows that UAG enhances cell survival and influences the cellular response to stress and damage (Ugwu et al., 2017). This also includes cell recovery of dCACs (early circulating angiogenic cells) and dEPCs, which have been reported to be immobilised in diabetes patients. This increase of the amount of cells was not found in healthy patients (Togliatto et al., 2010). By protection of diabetes EPCs from apoptosis and increasing mobilisation of the EPCs, Togliatto et al concluded that UAG does revert diabetes induced EPC damage.

Another study, from Tong et al, looked into the effects of acute administration of UAG on insulin secretion in healthy humans. Over-expression of UAG in the adipose tissue had positive effects on the glucose tolerance and insulin sensitivity in mice (Zhang et al., 2008). Therefore, Tong et al also tested the dose-dependence of UAG administration. Insulin secretion in rats, after UAG administration, was dose-dependent. In humans, an overnight UAG administration in healthy subjects had many positive effects. A higher dose did not change the effects that UAG administration had. They measured increased glucose tolerance and insulin secretion and a decrease in the blood FFA levels. T2D patients showed an increase in glucose tolerance and insulin secretion, however these results have not been consistent compared to other studies. These positive results of UAG were only found when it was administered in combination with AG. Their conclusion was that UAG had no effect on the insulin sensitivity, and secretion and that there was no change in the glucose tolerance when administered either alone or in combination with AG. However, it did have effects on the feeding behaviour on the healthy subjects. Healthy subjects consumed less food that was high in glucose and fructose (Tong et al., 2014). .
UAG administration for 16 hours in T2D patients did show a decrease in blood glucose levels, unfortunately insulin secretion was not affected (Özcan et al, 2014). An explanation can be that short administration was not sufficient enough to stimulate certain and needed brain centres (Tong et al., 2014).
From these findings, Tong et al concluded that acute administration of UAG has no significant effect on insulin secretion and glucose and FFA regulation.

Tong et al might not have found a significant influence of UAG on the glucose metabolism, but Granata et al concluded that it does have beneficial effects on the β-cell destruction and glucose homeostasis in rats administered with streptozotocin. Streptozotocin is administered to induce a diabetic state. They examined the effects and differences between AG, UAG and obestatin (Ob). Most significant differences and results were found when the compounds were given in combinations with one another. Administration of UAG together with Ob resulted in many anti-diabetic and anorexic effects. Granata et al concluded that UAG was most effective and that it did show a significant effect when administered alone (Granata et al., 2010)..

7. Obestatin

Obestatin in the third, and last discovered variant of the ghrelin gene, for now.. It originates from the ghrelin gene, the same as AG and UAG. Prepro-ghrelin consists of ghrelin and c-ghrelin. Ghrelin forms AG and UAG, whereas c-ghrelin forms the obestatin peptide. It affects the gastrointestinal system, pancreas, adipose tissues, and cardiovascular system (Villarreal et al., 2022). The receptors on which Ob acts and which enzymes are involved in the whole process of forming the peptides is still unknown. There are some proposals of potential receptors such as G-protein coupled receptor 39 (GPR39), glucagon-like peptide-1 receptor (GLP-1R), and GHSr. Villarreal et al proposed GPR39 based on the observation that GPR39 KO mice had no c-fos expression compared to wildtype rats. Ob has lipogenesis inhibiting abilities in adipose tissues via this receptor. Ob has satiety and anorexic properties, which might be linked by the GLP-1 receptor. It has been reported that binding in human cells promoted islet survival in human β-cells. These suggestions were refuted when overexpression of GLP-1r showed no Ob interactions. When the body is in a hyperglycemic state, Ob may have the ability to interact with GHSr in the human β-cells. Binding of Ob increases insulin secretion, lowering the blood glucose levels. Whether this interaction is by direct binding or downstream signalling via another receptor is yet unknown (Villarreal et al., 2022). When chronically administered, Ob showed many anorexic properties. It has positive effects on insulin signalling in the liver, as well as a reduction of lipid accumulation in the liver. An increase in circulating adiponectin, a hormone crucial for regulating glucose levels, lipid metabolism, and reducing inflammation. It plays a protective role in metabolic health, with lower levels associated with obesity, insulin resistance, type 2 diabetes, and cardiovascular diseases (Yamauchi et al., 2013). Ob even has inhibiting properties towards ghrelin acylation. This inhibition decreases the amount of plasma AG levels, reducing the diabetogenic effects. In the study of Granata et al where rats were administered with STZ, Ob reduced blood glucose levels, confirming earlier findings of Zang et al (Granata et al., 2010). Administration of Ob in combination with a satiety signal, CK8, produced in response to dietary fat, results in significant body weight reduction by 29%, while Ob alone only reduces body weight by 13% (Villarreal et al., 2022).

Decreased pancreatic weight, which can be linked to loss of β-cells, was also found after Ob administration. Even with the decreased pancreatic weight, pancreatic insulin levels were restored to basal levels in STZ rats. The number of pancreatic islets were also increased, compared to AG and UAG administration, Ob had the most significant difference. The perseverance of the islets can prevent diabetes at adult age in the rats.

8. Overview

To be able to compare the three analysed peptides, all information can be found in table 1.

Peptide	Positive effects		No effect	Negative effects	Side notes
Ghrelin antagonist	reduced FI; suppressed appetite	Enhanced energy expenditure	Gastric emptying	Decreased leptin	Dose dependent
	Improved glucose tolerance	Decreased bound ghrelin (15%)		In human little effects	
	Weight loss (up to 15%)	Reduced serum ghrelin			
	Increased insulin secretion				
Unacylated Ghrelin	preventing EPC damage	Decreased β-cell destruction			Not dose dependent
	Recovering of EPC movement	Increased glucose tolerance			No consistent results
	Reduction ROS production	Increased insulin sensitivity			Positive effects when administered with AG
	Prevention cell apoptosis	Decreased blood glucose			

	Better response to cellular stress and damage				
Obestatin	Lipogenesis inhibition	Satiety and anorexic properties			Decreased pancreatic weight
	Promotion islet survival	Reduced lipid			
	Increased insulin secretion	Increased glucose homeostasis			
	Decreased blood glucose	Reduced inflammation			
	Positive insulin signalling	Decreased ghrelin acylation			

Table 1. An overview of the analysed peptide hormones: ghrelin antagonist, unacylated ghrelin and obestatin. For each peptide, the positive and negative effects are shown together with aspects on which no effect was found as well as side notes.

9. Conclusion

Type 2 diabetes was and still is a metabolic disorder with detrimental effects. Unfortunately, in the world we live in, the chances of becoming overweight and obese are becoming more and more prominent. The ongoing search for the right and fitting prevention methods and a cure is never ending. Ghrelin, the hunger hormone of the body may have seemed like a good solution, however, research remains inconclusive. Looking at the properties of the three analysed peptides, no right cure can be chosen. Ghrelin is relatively recently discovered, the working mechanisms are not fully understood. Looking into a ghrelin antagonist, UAG and obestatin showed that these mechanisms are even poorer understood. All three peptides do show positive effects on important pathways related to T2D. More detailed information and research has been hard to find. They all have quite similar effects, via different pathways. The ghrelin antagonist has little effects on humans, even with a high dosage, making the peptide no longer a potential cure.

UAG showed great anti-diabetic effects, however, the results differ per study. If it would work as some study has found, it would be a great cure. Given the inconsistent results, UAG may not prove to be an effective cure.

Obestatin is not yet well analysed. There are still many questions about its mechanisms. Looking at its properties, it has greatly anti-diabetic effects. Since little research has been done on this peptide, the consistency of the effects are hard to determine.

Looking at the overall results of the analysis, obestatin seems to have the highest chance of being a potential cure for type 2 diabetes.

10. Future research and perspective

Since not much is known about obestatin, more research might be done. The peptide hormone has much potential, by looking further into the mechanisms more can be known. It clearly has a great influence on the glucose metabolism. To what degree is yet to be discovered. Dose-dependency will give a better look into the working of Ob. Acute or long administration can give an insight into the working properties of Ob on the brain and its permeation through the blood. Additionally, Ob can be further analysed by administration to T2D patients. Co-administration of AG and UAG can be used to look at the best working ratios.

Because of the inconsistent results of UAG, more research can be done on this hormone as well. Once more is known about the factors that contribute to this inconsistency, the true potential of the hormone can be determined.

11. References

Airapetov, M. I., Eresko, S. O., Lebedev, A. A., Bychkov, E. R., & Shabanov, P. D. (2021, November 1). Expression of the growth hormone secretagogue receptor 1a (GHS R1a) in the brain. Physiological Reports; Wiley-Blackwell. https://doi.org/10.14814/phy2.15113

Altabas, V., & Zjačić-Rotkvić, V. (2015). Anti-ghrelin antibodies in appetite suppression: recent advances in obesity pharmacotherapy. ImmunoTargets and therapy, 4, 123–130. https://doi.org/10.2147/ITT.S60398

Anderson, E. J., Lustig, M. E., Boyle, K. E., Woodlief, T. L., Kane, D. A., Lin, C., Price, J. W., Kang, L., Rabinovitch, P. S., Szeto, H. H., Houmard, J. A., Cortright, R. N., Wasserman, D. H., & Neufer, P. D. (2009). Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. the Journal of Clinical Investigation/the Journal of Clinical Investigation, 119(3), 573–581. https://doi.org/10.1172/jci37048

Andrade, S., Pinho, F., Ribeiro, A. M., Carreira, M., Casanueva, F. F., Roy, P., & Monteiro, M. P. (2013). Immunisation against active ghrelin using virus-like particles for obesity treatment. Current pharmaceutical design, 19(36), 6551–6558. https://doi.org/10.2174/13816128113199990506

Andrews, Z. B., Liu, W., Walllingford, N., Erion, D. M., Borok, E., Friedman, J. M., Tschöp, M. H., Shanabrough, M., Cline, G., Shulman, G. I., Coppola, A., Gao, B., Horvath, T. L., & Diano, S. (2008). UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature*, *454*(7206), 846. https://doi.org/10.1038/nature07181

Artasensi, A., Pedretti, A., Vistoli, G., & Fumagalli, L. (2020). Type 2 diabetes mellitus: A Review of Multi-Target Drugs. Molecules/Molecules Online/Molecules Annual, 25(8), 1987. https://doi.org/10.3390/molecules25081987

Asakawa, A., Inui, A., Kaga, T., Katsuura, G., Fujimiya, M., Fujino, M. A., & Kasuga, M. (2003). Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut*, *52*(7), 947–952. https://doi.org/10.1136/gut.52.7.947

Bailey, C. J., & Turner, R. C. (1996). Metformin. The New England journal of medicine, 334(9), 574–579. https://doi.org/10.1056/NEJM199602293340906

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. Lancet, 389(10085), 2239–2251. https://doi.org/10.1016/s0140-6736(17)30058-2

Colon-Gonzalez, F., Kim, G. W., Lin, J. E., Valentino, M. A., & Waldman, S. A. (2013). Obesity pharmacotherapy: what is next?. Molecular aspects of medicine, 34(1), 71–83. https://doi.org/10.1016/j.mam.2012.10.005

Delporte C. (2013). Structure and physiological actions of ghrelin. *Scientifica*, *2013*, 518909. https://doi.org/10.1155/2013/518909

Dezaki K, Sone H & Yada T 2008 Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. Pharmacology and Therapeutics 118 239–249.

Dimas, A. S., Lagou, V., Barker, A., Knowles, J. W., Mägi, R., Hivert, M., Benazzo, A., Rybin, D., Jackson, A. U., Stringham, H. M., Song, C., Fischer-Rosinsky, A., Boesgaard, T. W., Grarup, N., Abbasi, F. A., Assimes, T. L., Hao, K., Yang, X., Lecoeur, C., Prokopenko, I. (2014). Impact of Type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. Diabetes, 63(6), 2158–2171. https://doi.org/10.2337/db13-0949

Esler, W. P., Rudolph, J., Claus, T. H., Tang, W., Barucci, N., Brown, S. E., Bullock, W., Daly, M., Decarr, L., Li, Y., Milardo, L., Molstad, D., Zhu, J., Gardell, S. J., Livingston, J. N., & Sweet, L. J. (2007). Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss. Endocrinology, 148(11), 5175–5185. https://doi.org/10.1210/en.2007-0239

Fetissov, S. O., Hamze Sinno, M., Coëffier, M., Bole-Feysot, C., Ducrotté, P., Hökfelt, T., & Déchelotte, P. (2008). Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora. Nutrition (Burbank, Los Angeles County, Calif.), 24(4), 348–359. https://doi.org/10.1016/j.nut.2007.12.006

Gao, L., & Mann, G. E. (2009). Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling. Cardiovascular Research, 82(1), 9–20. https://doi.org/10.1093/cvr/cvp031

Gauna, C., Kiewiet, R. M., Janssen, J. A., van de Zande, B., Delhanty, P. J., Ghigo, E., Hofland, L. J., Themmen, A. P., & van der Lely, A. J. (2007). Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditions. American journal of physiology. Endocrinology and metabolism, 293(3), E697–E704. https://doi.org/10.1152/ajpendo.00219.2007

Gelbach, P. E., Zheng, D., Fraser, S. E., White, K. L., Graham, N. A., & Finley, S. D. (2022). Kinetic and data-driven modelling of pancreatic β-cell central carbon metabolism and insulin secretion. PLoS computational biology, 18(10), e1010555. https://doi.org/10.1371/journal.pcbi.1010555

Gortan Cappellari, G., Zanetti, M., Semolic, A., Vinci, P., Ruozi, G., Falcione, A., Filigheddu, N., Guarnieri, G., Graziani, A., Giacca, M., & Barazzoni, R. (2016). Unacylated Ghrelin Reduces Skeletal Muscle Reactive Oxygen Species Generation and Inflammation and Prevents High-Fat Diet-Induced Hyperglycemia and Whole-Body Insulin Resistance in Rodents. Diabetes, 65(4), 874–886. https://doi.org/10.2337/db15-1019

Granata, R., Volante, M., Settanni, F., Gauna, C., Ghè, C., Annunziata, M., Deidda, B., Gesmundo, I., Abribat, T., Van Der Lely, A. J., Muccioli, G., Ghigo, E., & Papotti, M. (2010). Unacylated ghrelin and obestatin increase islet cell mass and prevent diabetes in streptozotocin-treated newborn rats. Journal of Molecular Endocrinology, 45(1), 9–17. https://doi.org/10.1677/jme-09-0141

Granata, R., Settanni, F., Gallo, D., Trovato, L., Biancone, L., Cantaluppi, V., Nano, R., Annunziata, M., Campiglia, P., Arnoletti, E., Ghe, C., Volante, M., Papotti, M., Muccioli, G., & Ghigo, E. (2008). Obestatin promotes survival of pancreatic B-Cells and human islets and induces expression of genes involved in the regulation of B-Cell mass and function. Diabetes, 57(4), 967–979. https://doi.org/10.2337/db07-1104

Gutch, M., Kumar, S., Razi, S. S., Gupta, K. S., & Gupta, A. (2015). Assessment of insulin sensitivity/resistance. Indian Journal of Endocrinology and Metabolism, 19(1), 160. https://doi.org/10.4103/2230-8210.146874

Hameed, I., Masoodi, S. R., Mir, S. A., Nabi, M., Ghazanfar, K., & Ganai, B. A. (2015). Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. World journal of diabetes, 6(4), 598–612. https://doi.org/10.4239/wjd.v6.i4.598

Houstis, N., Rosen, E. D., & Lander, E. S. (2006). Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature, 440(7086), 944–948. https://doi.org/10.1038/nature04634

Inzucchi, S. E., Viscoli, C. M., Young, L. H., Furie, K. L., Gorman, M., Lovejoy, A. M., Dagogo Jack, S., Ismail-Beigi, F., Korytkowski, M. T., Pratley, R. E., Schwartz, G. G., Kernan, W. N., & IRIS Trial Investigators (2016). Pioglitazone Prevents Diabetes in Patients With Insulin Resistance and Cerebrovascular Disease. Diabetes care, 39(10), 1684–1692. https://doi.org/10.2337/dc16-0798

Kim, J., Wei, Y., & Sowers, J. R. (2008). Role of mitochondrial dysfunction in insulin resistance. Circulation Research, 102(4), 401–414. https://doi.org/10.1161/circresaha.107.165472

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature, 402(6762), 656–660. https://doi.org/10.1038/45230

Kosowicz, J, K., Baumann-Antczak, A., Ruchała, M., Gryczyñska, M., Gurgul, E., & Sowiński, J. (2011c, February 1). *Thyroid Hormones Affect Plasma Ghrelin and Obestatin Levels*. Hormone and Metabolic Research; Thieme Medical Publishers (Germany). https://doi.org/10.1055/s-0030-1269853

Laakso, M. (2019). Biomarkers for type 2 diabetes. Molecular metabolism, 27S(Suppl), S139–S146. https://doi.org/10.1016/j.molmet.2019.06.016

Langenberg, C., & Lotta, L. A. (2018). Genomic insights into the causes of type 2 diabetes. Lancet (London, England), 391(10138), 2463–2474. https://doi.org/10.1016/S0140-6736(18)31132-2

Loh, K., Zhang, L., Brandon, A., Wang, Q., Begg, D., Qi, Y., Fu, M., Kulkarni, R., Teo, J., Baldock, P., Brüning, J. C., Cooney, G., Neely, G. G., & Herzog, H. (2017). Insulin controls food

intake and energy balance via NPY neurons. Molecular metabolism, 6(6), 574–584 https://doi.org/10.1016/j.molmet.2017.03.013

Lundgren O. (2000). The stomach talks with the pituitary. Gut, 47(2), 168–169. https://doi.org/10.1136/gut.47.2.168

Mahajan, A., Taliun, D., Thurner, M., Robertson, N. R., Torres, J. M., Rayner, N. W., Payne, A. J., Steinthorsdottir, V., Scott, R. A., Grarup, N., Cook, J. P., Schmidt, E. M., Wuttke, M., Sarnowski, C., Mägi, R., Nano, J., Gieger, C., Trompet, S., Lecoeur, C., Preuss, M. H., … McCarthy, M. I. (2018). Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nature genetics, 50(11), 1505–1513. https://doi.org/10.1038/s41588-018-0241-6

Majety, P., Orquera, F. a. L., Edem, D., & Hamdy, O. (2023). Pharmacological approaches to the prevention of type 2 diabetes mellitus. Frontiers in Endocrinology, 14. https://doi.org/10.3389/fendo.2023.1118848

Maletínská, L., Matyšková, R., Maixnerová, J., Sýkora, D., Pýchová, M., Spolcová, A., Blechová, M., Drápalová, J., Lacinová, Z., Haluzík, M., & Zelezná, B. (2011). The Peptidic GHS-R antagonist [D-Lys(3)]GHRP-6 markedly improves adiposity and related metabolic abnormalities in a mouse model of postmenopausal obesity. Molecular and cellular endocrinology, 343(1-2), 55–62. https://doi.org/10.1016/j.mce.2011.06.006

Mayer, C. M., & Belsham, D. D. (2009). Insulin directly regulates NPY and AgRP gene expression via the MAPK MEK/ERK signal transduction pathway in mHypoE-46 hypothalamic neurons. *Molecular and cellular endocrinology*, *307*(1-2), 99–108. https://doi.org/10.1016/j.mce.2009.02.031

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. Nature, 409(6817), 194–198. https://doi.org/10.1038/35051587

Nasrallah, C. M., & Horvath, T. L. (2014). Mitochondrial dynamics in the central regulation of metabolism. Nature Reviews Endocrinology, 10(11), 650–658. https://doi.org/10.1038/nrendo.2014.160

Nogueira, J. P., Maraninchi, M., Béliard, S., Lorec, A. M., Berthet, B., Bégu-Le Corroller, A., Dubois, N., Grangeot, R., Mattei, C., Gaudart, J., Nicolay, A., Portugal, H., Vialettes, B., & Valéro, R. (2012). Unacylated Ghrelin is associated with the isolated low HDL-cholesterol obese phenotype independently of insulin resistance and CRP level. Nutrition & metabolism, 9(1), 17. https://doi.org/10.1186/1743-7075-9-17

Özcan, B., Neggers, S. J., Miller, A. R., Yang, H. C., Lucaites, V., Abribat, T., Allas, S., Huisman, M., Visser, J. A., Themmen, A. P., Sijbrands, E. J., Delhanty, P. J., & van der Lely, A. J. (2014). Does des-acyl ghrelin improve glycemic control in obese diabetic subjects by decreasing acylated ghrelin levels?. European journal of endocrinology, 170(6), 799–807. https://doi.org/10.1530/EJE-13-0347

Pernicova, I., & Korbonits, M. (2014). Metformin—mode of action and clinical implications for diabetes and cancer. Nature Reviews. Endocrinology, 10(3), 143–156. https://doi.org/10.1038/nrendo.2013.256

Poher, A. L., Tschöp, M. H., & Müller, T. D. (2018). Ghrelin regulation of glucose metabolism. *Peptides*, *100*, 236–242. https://doi.org/10.1016/j.peptides.2017.12.015

Qi, Y., Lee, N. J., Ip, C. K., Enriquez, R., Tasan, R., Zhang, L., & Herzog, H. (2022). NPY derived from AGRP neurons controls feeding via Y1 and energy expenditure and food foraging behaviour via Y2 signalling. Molecular metabolism, 59, 101455. https://doi.org/10.1016/j.molmet.2022.101455

Takagi, K., Legrand, R., Asakawa, A., Amitani, H., François, M., Tennoune, N., Coëffier, M., Claeyssens, S., do Rego, J. C., Déchelotte, P., Inui, A., & Fetissov, S. O. (2013). Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans. Nature communications, 4, 2685. https://doi.org/10.1038/ncomms3685

Tepper, O. M., Galiano, R. D., Capla, J. M., Kalka, C., Gagne, P. J., Jacobowitz, G. R., ... & Gurtner, G. C. (2002). Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation, 106(22), 2781-2786.

The Diabetes Prevention Program Research Group (TDPPRG) (1999). The Diabetes Prevention Program. Design and methods for a clinical trial in the prevention of type 2 diabetes. Diabetes care, 22(4), 623–634. https://doi.org/10.2337/diacare.22.4.623

Togliatto, G., Trombetta, A., Dentelli, P., Baragli, A., Rosso, A., Granata, R., Ghigo, D., Pegoraro, L., Ghigo, E., & Brizzi, M. F. (2010). Unacylated ghrelin rescues endothelial progenitor cell function in individuals with type 2 diabetes. Diabetes, 59(4), 1016–1025. https://doi.org/10.2337/db09-0858

Tong, J., Dave, N., Mugundu, G. M., Davis, H. W., Gaylinn, B. D., Thorner, M. O., Tschöp, M. H., D'Alessio, D., & Desai, P. B. (2013). The pharmacokinetics of acyl, des-acyl, and total ghrelin in healthy human subjects. European journal of endocrinology, 168(6), 821–828. https://doi.org/10.1530/EJE-13-0072

Tong, J., Davis, H. W., Summer, S., Benoit, S. C., Haque, A., Bidlingmaier, M., Tschöp, M. H., & D'Alessio, D. (2014). Acute administration of unacylated ghrelin has no effect on Basal or stimulated insulin secretion in healthy humans. Diabetes, 63(7), 2309–2319. https://doi.org/10.2337/db13-1598

Tong, J., Prigeon, R. L., Davis, H. W., Bidlingmaier, M., Kahn, S. E., Cummings, D. E., Tschöp, M. H., & D'Alessio, D. (2010). Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes*, *59*(9), 2145–2151. https://doi.org/10.2337/db10-0504

Udler, M. S., Kim, J., Von Grotthuss, M., Bonàs-Guarch, S., Cole, J. B., Chiou, J., Boehnke, M., Laakso, M., Atzmon, G., Glaser, B., Mercader, J. M., Gaulton, K., Flannick, J., Getz, G., & Florez, J. C. (2018). Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: A soft clustering analysis. PLoS Medicine, 15(9), e1002654. https://doi.org/10.1371/journal.pmed.1002654

Ugwu, F. N., Yu, A. P., Sin, T. K., Tam, B. T., Lai, C. W., Wong, S. C., & Siu, P. M. (2017). Protective Effect of Unacylated Ghrelin on Compression-Induced Skeletal Muscle Injury Mediated by SIRT1-Signalling. Frontiers in physiology, 8, 962. https://doi.org/10.3389/fphys.2017.00962

Vizcarra, J. A., Kirby, J. D., Kim, S. K., & Galyean, M. L. (2007). Active immunisation against ghrelin decreases weight gain and alters plasma concentrations of growth hormone in growing pigs. Domestic animal endocrinology, 33(2), 176–189. https://doi.org/10.1016/j.domaniend.2006.05.005

Yamauchi, T., & Kadowaki, T. (2013). Adiponectin receptor as a key player in healthy longevity and Obesity-Related diseases. Cell Metabolism, 17(2), 185–196. https://doi.org/10.1016/j.cmet.2013.01.001

Zhang, W., Chai, B., Li, J. Y., Wang, H., & Mulholland, M. W. (2008). Effect of des-acyl ghrelin on adiposity and glucose metabolism. Endocrinology, 149(9), 4710–4716. https://doi.org/10.1210/en.2008-0263

Zhang, J. V., Ren, P. G., Avsian-Kretchmer, O., Luo, C. W., Rauch, R., Klein, C., & Hsueh, A. J. (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science (New York, N.Y.), 310(5750), 996–999. https://doi.org/10.1126/science.1117255

Zorrilla, E. P., Iwasaki, S., Moss, J. A., Chang, J., Otsuji, J., Inoue, K., Meijler, M. M., & Janda, K. D. (2006). Vaccination against weight gain. Proceedings of the National Academy of Sciences of the United States of America, 103(35), 13226–13231. https://doi.org/10.1073/pnas.0605376103