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► To cite this version:

Miski Scerif, Anthony P Goldstone, Márta Korbonits. Ghrelin in Obesity and Endocrine Diseases. Molecular and Cellular Endocrinology, 2011, 340 (1), pp.15. 10.1016/j.mce.2011.02.011 . hal-00706320

HAL Id: hal-00706320

<https://hal.science/hal-00706320>

Submitted on 10 Jun 2012

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Accepted Manuscript

Title: Ghrelin in Obesity and Endocrine Diseases

Authors: Miski Scerif, Anthony P Goldstone, Márta Korbonits

PII: S0303-7207(11)00119-5
DOI: doi:10.1016/j.mce.2011.02.011
Reference: MCE 7774

To appear in: *Molecular and Cellular Endocrinology*

Received date: 13-2-2011
Accepted date: 14-2-2011

Please cite this article as: Scerif, M., Goldstone, A.P., Korbonits, M., Ghrelin in Obesity and Endocrine Diseases, *Molecular and Cellular Endocrinology* (2010), doi:10.1016/j.mce.2011.02.011

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Ghrelin in Obesity and Endocrine Diseases

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1. Ghrelin in Obesity

1.1.1. Appetite regulation

The hypothalamus is crucial for appetite regulation and energy homeostasis. Afferent signals from peripheral sites such as the gastrointestinal tract and adipose tissues are integrated by complex neuronal networks to produce efferent responses responsible for food intake and energy metabolism (Druce & Bloom, 2006). There are numerous hypothalamic appetite regulators. Orexigenic (appetite-stimulating) compounds include: neuropeptide Y (NPY), agouti-related peptide (AgRP), ghrelin, orexin and cannabinoids, and anorexigenic (appetite-suppressing) peptides include: pro-opiomelanocortin (POMC) and cocaine and amphetamine regulatory transcript (CART), thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH).

1.1.2 Ghrelin and appetite regulation

The appetite-stimulating function of ghrelin was identified secondary to its effect on growth hormone (GH) release from somatotroph cells of the anterior pituitary (Korbonits & Grossman, 2004), however, ghrelin is the first known peripheral hormone to display orexigenic effects through its action on the hypothalamic appetite-regulating pathways (Nakazato *et al.*, 2001). In addition, ghrelin is amongst the most powerful of the orexigenic peptides (Kojima & Kangawa, 2005). While most orexigenic peptides originate from the brain and are only active when injected into the brain, ghrelin is active even with peripheral administration leading to an increase in appetite in rodents and humans (Wren *et al.*, 2001a; Wren *et al.*, 2001b). Ghrelin activates NPY/AgRP neurons (Seoane *et al.*, 2003) of the hypothalamic arcuate nucleus (ARC) through its receptor,

GHS-R1a, promoting production and secretion of their orexigenic neuropeptides to suppress pro-POMC neuronal activity while promoting food intake (Cowley *et al.*, 2003; Egecioglu *et al.*, 2008). Interplay between ghrelin and orexin (Toshinai *et al.*, 2003) suggests that arousal and wakefulness may be required for ghrelin's orexigenic effect (Olszewski *et al.*, 2008). Mice with double knockout (KO) of NPY/AgRP genes completely lack the orexigenic action of ghrelin, confirming that these neuropeptides mediate and are essential for the orexigenic effect of this peptide hormone (Chen *et al.*, 2004).

1.2. AMPK and the orexigenic signaling pathway of ghrelin

Adenosine monophosphate-activated protein kinase (AMPK) is an evolutionally well conserved heterotrimeric serine/threonine kinase composed of a catalytic α (alpha) subunit and regulatory β (beta) and γ (gamma) subunits. AMPK is a regulator of cellular and systemic energy homeostasis and acts as a sensor of energy status (Kola *et al.*, 2006; Xiao *et al.*, 2007). Once activated, AMPK leads to a switch from anabolic to catabolic pathways; with the aim of restoring energy balance, ATP production is increased and ATP-utilising pathways are inhibited. Hypothalamic AMPK plays a vital role in the regulation of food intake (Kola *et al.*, 2006; Lim *et al.*, 2010). Constitutively active AMPK increases both food intake and body weight; whereas dominant negative AMPK expression in the hypothalamus is sufficient to reduce food intake and body weight (Minokoshi *et al.*, 2004).

The peripheral hormones ghrelin (Andersson *et al.*, 2004; Kola *et al.*, 2005) and adiponectin (Kadowaki *et al.*, 2008; Kubota *et al.*, 2007; Wen *et al.*, 2010) both have

hypothalamic AMPK implicated in their orexigenic effects. Hypothalamic AMPK has also been suggested as the mediator of the orexigenic effects of several other metabolic hormones and neuropeptides/modulators including cannabinoids (Kola *et al.*, 2005) and glucocorticoids (Christ-Crain *et al.*, 2008). Evidence that AMPK activity in the hypothalamus is also regulated by anorexigenic hormones: leptin (Andersson *et al.*, 2004; Minokoshi *et al.*, 2004) and insulin (Minokoshi *et al.*, 2004), further demonstrates that hypothalamic AMPK is directly involved in appetite regulation.

We have shown that rat and mouse hypothalamic AMPK activity is stimulated by both central (intracerebroventricular) and peripheral (intraperitoneal) ghrelin administration (Kola *et al.*, 2005; Kola *et al.*, 2008). The central orexigenic effect of ghrelin is AMPK-dependent as compound C, an inhibitor of AMPK, completely prevented ghrelin's orexigenic effect, and adenoviruses harbouring $\alpha 1/\alpha 2$ dominant negative AMPK were able to impair the stimulatory effect of ghrelin on food intake (Lopez *et al.*, 2008). The signalling pathway of ghrelin's orexigenic effect in the hypothalamus has been further elucidated with the identification of the endocannabinoid system (Kola *et al.*, 2005; Kola *et al.*, 2008) and calmodulin kinase kinase 2 (CaMKK2) (Anderson *et al.*, 2008) as upstream regulators to AMPK activation. The fatty acid pathways (Lopez *et al.*, 2008) and mitochondrial uncoupling protein 2 (UCP2) (Andrews *et al.*, 2008), have been identified as novel downstream effectors. Combining the recently described data, a pathway by which ghrelin could stimulate appetite has been suggested (Kola & Korbonits, 2009) (**Figure 1**).

1.3. Ghrelin in short- and long-term regulation of energy homeostasis

There is evidence to show that ghrelin is important in short-term regulation of appetite and energy balance. The clear pre-prandial rise and post-prandial fall in plasma ghrelin levels support the hypothesis that ghrelin acts as an initiator signal for meal consumption in humans (Cummings *et al.*, 2001). The pre-prandial increase of ghrelin levels was found to initiate meal consumption voluntarily, without time- or food-related cues (Cummings *et al.*, 2004), whilst the post-prandial ghrelin suppression is proportional to the ingested calorie load (Callahan *et al.*, 2004). Ghrelin also appears to be involved in the regulation of long-term energy homeostasis (Cummings, 2006). This peptide hormone has been described as the peripheral counterpart of insulin and leptin, and AMPK also appears to be involved in its peripheral metabolic effects (Kola *et al.*, 2005) (**Figure 2**).

Ghrelin is able to reverse the down-regulating effect of insulin on mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK), the rate limiting enzyme of gluconeogenesis (Murata *et al.*, 2002). Ghrelin reduces glucose-stimulated insulin secretion and glucose disappearance in healthy humans (Tong *et al.*, 2010) thus ghrelin leads to increase in blood glucose levels and impairs glucose tolerance (Korbonits & Grossman, 2004). Ghrelin has been shown to significantly increase sterol-regulatory element binding protein-1 (SREBP1) mRNA levels, as well as several fat storage-related proteins, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and lipoprotein lipase (LPL) leading to stimulation of intracytoplasmic lipid accumulation (Rodriguez *et al.*, 2009). Ghrelin reduces the use of fat as a metabolic fuel and promotes an increase in adipose tissue and body weight (Tschop *et al.*, 2000). Circulating ghrelin induces abdominal obesity, independently of its central orexigenic activity, via GHS-R-dependent lipid retention (Davies *et al.*, 2009; Salome *et al.*, 2009).

1.4.1. Obesity

Obesity is a condition that results from chronic disruption of energy balance; energy intake continuously exceeds energy expenditure and accumulation of body fat follows (Hill, 2006). The prevalence of obesity is on the rise and the obesity pandemic is arguably amongst the most serious public health challenges in the world today. Obesity is strongly associated with type 2 diabetes mellitus, hyperlipidemia and cardiovascular disease, and provides significant contributions to ill health in both western and developing countries (Kopelman, 2000). Extensive research into the mechanisms of appetite regulation and energy balance has unveiled complex physiological systems behind energy homeostasis, and yielded potential targets for therapeutic intervention in the fight against obesity.

1.4.2 Ghrelin and obesity

Plasma ghrelin levels inversely correlate with body mass index (BMI). Thus, ghrelin levels are reduced in those who are obese compared to normal body weight controls (Tschop *et al.*, 2001; Williams *et al.*, 2006). Recent evidence suggests that diet-induced obesity causes ghrelin resistance by reducing NPY/AgRP responsiveness to plasma ghrelin and suppressing the neuroendocrine ghrelin axis, in an attempt to limit further food intake (Briggs *et al.*, 2010). Ghrelin levels have been shown to negatively correlate with factors which are raised in obesity namely, percentage body fat, insulin and leptin levels (Tschop *et al.*, 2001). In one study ghrelin levels were not related to fat mass or intra-abdominal fat content, but showed strong negative correlation with insulin levels

and insulin resistance (Purnell *et al.*, 2003). However in an MRI study of non-obese and obese adults, ghrelin was negatively correlated with visceral adiposity, fasting insulin, and homeostasis model insulin resistance index, with visceral adiposity having a stronger inverse correlation with ghrelin than subcutaneous fat depots possibly through hyperinsulinemia, as the negative correlations with insulin resistance were even stronger (Goldstone *et al.*, 2004). Abnormal glucose homeostasis inversely correlated with, and was found to be an independent determinant of, plasma ghrelin levels in obese children and adolescents (Zhu *et al.*, 2010).

It has been shown that selective ghrelin blockade, in diet-induced obese mice, results in reduction of food intake, bodyweight and body fat mass (Shearman *et al.*, 2006). Adult ghrelin-deficient mice, compared to their wild-type littermates, display significantly lower glucose levels and increased insulin sensitivity under conditions of caloric restriction (Sun *et al.*, 2008) and are protected from the rapid weight gain induced by early exposure to a high-fat diet (Wortley *et al.*, 2005). In addition, mice lacking ghrelin receptors resist the development of diet-induced obesity (Zigman *et al.*, 2005), and antagonism of the ghrelin receptor results in reduction of food intake and weight gain (Asakawa *et al.*, 2003). Therefore, reduced levels of ghrelin activity in obese individuals, as compared with non-obese controls, should provide greater protection from further weight gain. However, obese individuals display smaller post-prandial reductions in serum ghrelin levels, perhaps leading to a reduced feeling of satiety (Goldstone *et al.*, 2005; le Roux *et al.*, 2005). In an earlier study, food intake failed to suppress ghrelin levels in obese humans (English *et al.*, 2002). In addition, weight loss is associated with a

simultaneous increase in plasma ghrelin levels (Cummings *et al.*, 2002a; Hansen *et al.*, 2002), which may make the maintenance of weight loss more difficult.

Obesity endangers the lives of millions of people worldwide, thus novel approaches to control body weight remain a high priority. Both ghrelin and GHS-R are being investigated as therapeutic targets (Schellekens *et al.*, 2010). *In vitro* generated biostable RNA-based compounds that specifically bind ghrelin have been used in animal models to inhibit ghrelin-mediated GHS-R activation (Helmling *et al.*, 2004). Zorilla *et al.* have developed an anti-ghrelin vaccine, providing a novel method to block ghrelin action (Zorrilla *et al.*, 2006). Exciting prospects lie ahead for an anti-ghrelin vaccine to treat obesity (Carlson & Cummings, 2006).

Ghrelin is acylated on its serine 3 residue with octanoate, an eight-carbon fatty acid. This post-translational modification is mediated by ghrelin O-acyltransferase (GOAT) (Yang *et al.*, 2008) and is essential for GHS-R1a activation and the endocrine actions of ghrelin (Kojima & Kangawa, 2005). Consistent with its function, GOAT mRNA is primarily limited to the stomach; the main ghrelin-secreting tissue (Yang *et al.*, 2008). Acylated ghrelin induces body weight gain and adiposity by promoting food intake and decreasing energy expenditure (Asakawa *et al.*, 2005; Tschop *et al.*, 2000). GOAT has been implicated as another potential target for the development of anti-obesity treatment (Gualillo *et al.*, 2008; Yang *et al.*, 2008). Chronic treatment of mice on a high fat diet with GO-CoA-Tat, a peptide-based GOAT inhibitor, prevented the significant weight gain observed in vehicle-treated controls. Moreover, quantitative magnetic resonance (QMR) measurements showed that, relative to controls, GO-CoA-Tat treated animals displayed significantly lower fat mass, but not lean mass (Barnett *et al.*, 2010).

1.5. Ghrelin and Prader-Willi syndrome

Prader-Willi syndrome (PWS) is a complex genetic disorder characterised by childhood-onset hyperphagia-associated obesity, mental retardation, GH deficiency (short stature), hypogonadism, sleep and thermoregulation disturbance (Goldstone, 2004). The PWS genotype is characterized by loss of several paternal genes in the region q11–13 on chromosome 15 (Nicholls & Knepper, 2001). PWS is a human genetic obesity syndrome and the most common cause of syndromic obesity.

In contrast to other forms of obesity, patients with PWS display significantly reduced visceral adiposity, and are protected from the metabolic complications of obesity, namely insulin resistance and hypertriglyceridemia (Goldstone *et al.*, 2001). Patients with PWS have high ghrelin levels relative to their obesity, with fasting plasma concentrations up to three- to four-fold higher in PWS than in non-PWS obese controls. (Bizzarri *et al.*, 2010; Cummings *et al.*, 2002b; Gimenez-Palop *et al.*, 2007; Goldstone *et al.*, 2005; Haqq *et al.*, 2003a; Paik *et al.*, 2004). Levels of fasting ghrelin may also even be higher in PWS patients than in lean controls (Bizzarri *et al.*, 2010; Choe *et al.*, 2005a; Cummings *et al.*, 2002b; Delparigi *et al.*, 2002; Gimenez-Palop *et al.*, 2007). Post-prandial levels of ghrelin do fall in patients with PWS though they remain elevated compared to non-PWS obese controls, with conflicting results as to whether the magnitude of the decrease in ghrelin is blunted (Gimenez-Palop *et al.*, 2007; Goldstone *et al.*, 2005; Haqq *et al.*, 2003b). In addition, the age-related decline in ghrelin is blunted in PWS infants and children (Haqq *et al.*, 2008).

Hypothalamic dysfunction has been suggested to underlie the obesity that is typical of PWS however fasting and post-prandial ghrelin levels are not elevated in patients with hypothalamic obesity (Goldstone *et al.*, 2005; Kanumakala *et al.*, 2005). Two- to three-fold increases in the numbers of ghrelin-expressing cells were noted in the gastric body and fundus of PWS patients as compared to lean and obese controls (Choe *et al.*, 2005a). Imprinting of paternal genes in region q11–13 on chromosome 15 may induce the production of excessive amounts of transcription factors that increase ghrelin expression or, loss of a transcription inhibitory factor that normally suppresses ghrelin expression (Kojima & Kangawa, 2005). Hyperghrelinemia of PWS appears to be partially, but not solely, explained by reduced visceral adiposity (Goldstone *et al.*, 2004) and relative hypoinsulinemia (Goldstone *et al.*, 2004; Goldstone *et al.*, 2005). Other possible causes of the elevated ghrelin levels in PWS include abnormal parasympathetic vagal innervation of the stomach (Goldstone, 2004; Higgins *et al.*, 2007) and abnormal sympathetic tone (Higgins *et al.*, 2007).

Some studies have shown elevated ghrelin levels in PWS infants and children and thus it appears that hyperghrelinemia may precede hyperphagia in PWS, though this has not been universally seen in all studies (Erdie-Lalena *et al.*, 2006; Feigerlova *et al.*, 2008; Haqq *et al.*, 2008). Neonatal transgenic deletion PWS mice die from severe neonatal hypoglycemia due to depletion of liver glycogen stores, and ghrelin levels increase reciprocally with the falling glucose levels (Stefan *et al.*, 2005)

Although it has been hypothesised that hyperphagia in PWS may be partly explained by rapid gastric emptying due to increased gastric motility caused by elevated ghrelin levels, in fact patients with PWS have delayed gastric emptying (Choe *et al.*,

2005b). High circulating levels of ghrelin can be hypothesised as a potential cause for hyperphagia and obesity in PWS (Cummings *et al.*, 2002b; Delparigi *et al.*, 2002; Haqq *et al.*, 2003a). However, not all PWS patients have elevated ghrelin levels, and pharmacological reduction of ghrelin levels to within normal levels using a somatostatin infusion was unable to reduce acute food intake in PWS adults (Tan *et al.*, 2004). Furthermore, chronic treatment with a long-acting somatostatin analogue led to a sustained decrease in ghrelin concentrations but did not affect weight, eating behaviour or appetite in adolescents with PWS (De *et al.*, 2008). However such interventions may also lower circulating anorexigenic gastroenteropancreatic hormones which may complicate their interpretation (De *et al.*, 2008; Tan *et al.*, 2004). Thus although other pathological defects are felt to cause or contribute to hyperphagia in PWS, including reduced circulating anorexigenic pancreatic polypeptide levels (Zipf *et al.*, 1983), hypothalamic dysfunction (Goldstone *et al.*, 2003; Goldstone, 2004), defects in brain reward and satiety systems (Hinton *et al.*, 2006; Holsen *et al.*, 2006; Miller *et al.*, 2007), and decreased brain-derived neurotrophic factor (BDNF) which functions downstream of leptin and POMC/MC4R signalling (Han *et al.*, 2010), it will need the clinical development of ghrelin antagonists or GOAT inhibitors to be able to definitively assess the role of hyperghrelinemia in the hyperphagia of PWS.

1.6.1 Common genetic variations of *GHRL* and *GHS-R* genes in human obesity

Relationships have been described between obesity and genomic variants of the ghrelin (*GHRL*) (Gueorguiev *et al.*, 2009; Korbonits *et al.*, 2002; Kuzuya *et al.*, 2006; Leskela *et al.*, 2009; Miraglia Del *et al.*, 2004; Ukkola *et al.*, 2001; Ukkola *et al.*, 2002)

and ghrelin receptor (*GHS-R*) (Baessler *et al.*, 2005; Gueorguiev *et al.*, 2009; Liu *et al.*, 2007) genes.

The Leu72Met (C247A) polymorphism of the ghrelin gene was detected in 15.6% (12.5% heterozygote and 3.1% homozygote) of obese subjects and in 12.5% (all heterozygotes) of non-obese control subjects. This polymorphism has been associated with an earlier onset of obesity (Ukkola *et al.*, 2001). We studied the ghrelin gene in obese French children and found a positive correlation between the Leu72Met heterozygote variant with higher BMI and early-onset obesity (Korbonits *et al.*, 2002) and a similar significant association between the Leu72Met polymorphism and early-onset obesity was observed in Italian children (Miraglia Del *et al.*, 2004). In addition, the Leu72Met polymorphism has been suggested to contribute to weight gain in middle-aged Japanese men (Kuzuya *et al.*, 2006). However, the above findings could not be reproduced by other studies (Bing *et al.*, 2005; Hinney *et al.*, 2002; Jo *et al.*, 2005; Zhu *et al.*, 2010). Furthermore, one study of 3004 subjects even reported the Leu72Met polymorphism to be protective against fat accumulation; the polymorphism was related to lower BMI, fat mass and reduced visceral fat (Ukkola *et al.*, 2002). Another study suggests that Leu72 allele carriers are more prone to weight variability in response to environmental factors given the reduced frequency of Leu72Met polymorphism in monozygotic twins discordant for obesity (Leskela *et al.*, 2009).

The Arg51Gln (G185A) variant of the ghrelin gene was found in six (all heterozygotes) obese subjects (6.3%) but was not found in non-obese controls (Ukkola *et al.*, 2001). Obese subjects with the Arg51Gln variant had lower body weights than obese subjects with the wild-type Arg51Arg variant (Ukkola *et al.*, 2001). Although studies

have showed correlation between Arg51Gln and lower ghrelin levels (Poykko *et al.*, 2003; Ukkola *et al.*, 2002), another study was unable to confirm this finding (Vivenza *et al.*, 2004).

Direct sequencing identified 12 *GHRL* single-nucleotide polymorphisms (SNPs) and 8 *GHS-R* SNPs (Gueorguiev *et al.*, 2009). The *GHRL* polymorphism A265T and the *GHS-R* polymorphism G477A have been associated with obesity in a French cohort of 263 families, while the *GHRL* variant Leu72Met (C247A) and *GHS-R* variant C60T may modulate eating patterns. However, after correction for multiple comparisons and testing in a German cohort with early-onset extreme obesity, it appears that these variants of *GHRL* and *GHS-R* genes are not strong genetic factors determining the susceptibility to obesity (Gueorguiev *et al.*, 2009).

Linkage and association of SNPs and haplotypes within the *GHS-R* gene region and human obesity has been demonstrated. Baessler *et al.* performed analysis of the entire *GHS-R* gene region (99.3 kb) in 178 pedigrees with multiple obese members (1,095 Caucasian individuals) and in an independent sample of the general population (1,418 Caucasians individuals). The linkage disequilibrium analysis revealed a disequilibrium block consisting of five SNPs, which showed significant association with obesity in both study cohorts (Baessler *et al.*, 2005). However, Wang *et al.* studied SNPs of the *GHS-R* in German children and adolescents and failed to find conclusive evidence for the involvement of the *GHS-R* gene in body weight regulation (Wang *et al.*, 2004). This is supported by a systematic study of common *GHS-R* variants in three large population-based UK cohort studies comprising 5807 adults and 843 children, which found no association with body weight or BMI (Garcia *et al.*, 2008). In addition, a

population-based study of 15,854 Danish subjects, in which seven common *GHS-R* SNPs were genotyped, failed to show association between the *GHS-R* variants and obesity (Gjesing *et al.*, 2010). Further clinical studies are required to better define the frequency and the aetiological role of *GHS-R* isoforms in obesity (Liu *et al.*, 2007).

1.6.2. *GHS-R* mutations in patients

Ala204Glu (Pantel *et al.*, 2006; Wang *et al.*, 2004) and Phe279Leu (Wang *et al.*, 2004) are rare *GHS-R* mutations which lead to loss of constitutive activity of GHS-R and subsequent short stature and obesity (Holst & Schwartz, 2006). Obesity would be unexpected in loss-of-function mutations of GHS-R, however compensatory changes in related pathways may account for the obese phenotype in subjects with the inactivating mutations (Holst & Schwartz, 2006). Four missense mutations of the *GHS-R*: Ala204Glu, Phe279Leu, Ile134Thr and Val160Met, have been reported and Liu *et al.* have shown that all these mutations affect the constitutive activity of the GHS-R, thus may have physiological consequences and/or pharmacogenomic implications (Liu *et al.*, 2007).

Wang *et al.* studied 746 obese German children and adolescents, 232 underweight students, 96 normal-weight students and 43 children with short stature, and identified two individuals with novel missense mutations of the *GHS-R*. The Ala204Glu mutation was detected in a child with obesity and the Phe279Leu mutation was found in a child with short stature (Wang *et al.*, 2004). Pantel *et al.* studied 2 unrelated families of Moroccan origin and identified a GHS-R missense mutation that segregates with short stature. The Ala204Glu mutation was not observed in a control group of Moroccan individuals with normal stature, indicating that it is not a frequent polymorphism. In family 1, the 13 year

old female proband with significant height reduction (3.7SD below the mean, according to French reference values) was found to carry the Ala204Glu mutation in the homozygous state; whilst the parents were heterozygous for this mutation (maternal and paternal height was noted to be 2.7SD and 3.7SD below the mean, respectively). In addition, several members of family 1 were overweight or obese – BMI of the 13 year old proband was 26.2kg/m^2 (Pantel *et al.*, 2006). In family 2, the 11 year old female proband was found to carry the same mutation in the heterozygous state, and the affected father (height 2SD below mean) was also heterozygous for the mutation (Pantel *et al.*, 2006).

In contrast to the Ala204Glu mutation which has been implicated in short stature with dominant transmission; recessive GHS-R mutations have also been reported (Pantel *et al.*, 2009). The proband was born to unrelated parents of normal phenotype, presented with episodes of abdominal pain, vomiting, hypoglycemia, and ketosis and required glucose infusions. He showed reduced height, weight, BMI and delayed bone age. Endocrine investigations revealed markedly low insulin-like growth factor 1 (IGF-1) levels and the patient displayed a low growth hormone (GH) response to provocative tests. *GHS-R* sequencing revealed that the patient was compound heterozygous for two new defects: a mutation predicting a premature stop codon (Trp2X) inherited from his unaffected father, therefore strongly arguing against haploinsufficiency as a disease mechanism, and a missense mutation (Arg237Trp), inherited from his healthy mother (Pantel *et al.*, 2009).

In a study of 82 Danish obese probands and 28 lean subjects, a rare mutation in the promoter region (-151 C/T) of *GHS-R* was identified in one obese individual. The -151 C/T mutation results in increased *GHS-R* transcriptional activity thereby increased

expression of *GHS-R* and increased potential for constitutive activity. This mutation co-segregated with obesity in the pedigree of the identified proband. The proband, her son, and sister had all been overweight or obese since the onset of puberty and her mother since her early twenties. Screening for the -151C/T *GHS-R* mutation was also performed in 289 obese Czech children, and an additional heterozygous carrier of the -151C/T mutation was identified. Co-segregation was seen for all obese individuals in the Czech pedigree; however two members of this pedigree were carriers of this mutation but not obese, possibly due to incomplete penetrance. The -151C/T mutation was subsequently genotyped in a population-based study of 15,854 Danish subjects; however in this study no association was found between the -151C/T mutation and obesity (Gjesing *et al.*, 2010).

1.7. Ghrelin and bariatric surgery

Gastric bypass operations, the most common and most effective form of bariatric surgery, provide patients with morbid obesity an option of long-term sustained weight loss (Buchwald & Williams, 2004; Fobi, 2004). Data regarding the alteration of ghrelin levels following gastric bypass surgery are inconsistent (Higgins *et al.*, 2007; Pournaras & le Roux, 2009; Pournaras & le Roux, 2010; Vincent & le Roux, 2008).

Many studies have shown gastric bypass operations to be associated with significant reduction of ghrelin levels (Cummings *et al.*, 2002a; Geloneze *et al.*, 2003; Lin *et al.*, 2004; Roth *et al.*, 2009; Stylopoulos *et al.*, 2005; Suzuki *et al.*, 2005). In addition, the normal, meal-related fluctuation and the diurnal rhythm of ghrelin level was found to be absent after gastric bypass surgery (Cummings *et al.*, 2002a). Ghrelin levels

were significantly suppressed in diet-induced obese rats post-gastric bypass as compared with both sham-operated obese and lean control rats (Shin *et al.*, 2010). GHS-R1a protein expression in the hypothalamus was found to be much lower in rats following gastric bypass as compared to sham-operated controls (Wang & Liu, 2010).

The suppression of ghrelin levels following gastric bypass surgery may contribute to reduced hunger and the weight-reducing effect of the procedure. However, the mechanism for the decrease in plasma ghrelin levels following gastric bypass is not yet known. It may be explained by major loss of gastric mucosa, thus ghrelin-producing endocrine cells. Alternatively, direct contact between gastric mucosa with food may be important for the production and secretion of ghrelin (Beckman *et al.*, 2010; Kojima & Kangawa, 2005).

In contrast, ghrelin levels have been found to remain unchanged after gastric bypass surgery in several studies (Pournaras & le Roux, 2010; Vincent & le Roux, 2008). Increases in ghrelin levels post-gastric bypass have also been seen (Garcia-Fuentes *et al.*, 2008; Holdstock *et al.*, 2003; Perez-Romero *et al.*, 2010; Ybarra *et al.*, 2009); however this may be associated with active weight loss rather than the surgery itself (Faraj *et al.*, 2003). Several explanations have been proposed for the conflicting findings regarding ghrelin levels following gastric bypass surgery including differences in pre-operative insulin resistance, surgical methods and post-operative vagal nerve dysfunction (Pournaras & le Roux, 2010; Vincent & le Roux, 2008).

Weight loss following gastric banding is independent of circulating plasma ghrelin, as an increase in fasting ghrelin is accompanied by a paradoxical decrease in hunger, but relies on changes in eating behaviour induced by gastric restriction (Schindler

et al., 2004). In sleeve gastrectomy, the fundus of the stomach, the main location of ghrelin-producing cells, is excluded and studies have confirmed a decrease in fasting ghrelin levels after the procedure (Cohen *et al.*, 2005; Langer *et al.*, 2005; Wang & Liu, 2009). In a recent review, Beckman *et al.* suggest that the stronger, better designed studies indicate greatly reduced ghrelin levels in the post-gastric bypass state as compared to the obese or lean state, or when weight loss is achieved through procedures other than gastric bypass or through lifestyle modification (Beckman *et al.*, 2010).

2. Ghrelin and endocrine diseases

The role of ghrelin in growth hormone deficiency, acromegaly, diabetes mellitus as well as in gastrointestinal neuroendocrine tumours will be discussed elsewhere in this special issue, but we will summarise data on Cushing's syndrome, thyroid disease, pheochromocytoma and insulinoma.

2.1.1. Ghrelin and hypothalamic-pituitary-adrenal axis

Ghrelin shows stimulatory effects on the corticotropic system (Giordano *et al.*, 2004). Systemic administration of ghrelin, or the synthetic hexarelin, has been shown to increase ACTH and cortisol levels in healthy subjects (Arvat *et al.*, 2001; Korbonits *et al.*, 1999a). A hypothalamic site of action of ghrelin on the hypothalamic-pituitary-adrenal (HPA) axis has been proposed, as transection of the hypophyseal stalk dramatically decreased cortisol responses to synthetic growth hormone secretagogue (GHS) treatment (Hickey *et al.*, 1996). Furthermore these compounds fail to increase ACTH release from pituitary tissue *in vitro* (Shimon *et al.*, 1998). There are data to

suggest that ghrelin stimulates the HPA axis at a hypothalamic level, at least in part via arginine vasopressin (AVP) and CRH (Coiro *et al.*, 2005; Korbonits *et al.*, 1999a; Mozid *et al.*, 2003). It has been shown that NPY can stimulate CRH and AVP release from the rat hypothalamus (Korbonits *et al.*, 1999b), therefore the effect of ghrelin on CRH and AVP could occur through direct action on CRH and AVP neurons in the paraventricular nucleus, or may occur indirectly via NPY (Mozid *et al.*, 2003). Ghrelin does not have direct stimulatory effects on adrenal hormone secretion (Andreis *et al.*, 2003; Barreiro *et al.*, 2002). It appears that a feedback loop may exist between ghrelin levels and the HPA axis. While ghrelin stimulates the HPA axis, hypercortisolemia is associated with reduced ghrelin levels (Otto *et al.*, 2004).

2.1.2 Ghrelin and Cushing's syndrome

The complications of Cushing's syndrome include central obesity, impaired glucose tolerance, insulin resistance, dyslipidemia, hepatic steatosis and systemic arterial hypertension (Arnaldi *et al.*, 2003a) and eventually the metabolic syndrome (Anagnostis *et al.*, 2009; Arnaldi *et al.*, 2003a). Ghrelin levels are decreased in patients with Cushing's syndrome (Libe *et al.*, 2005; Otto *et al.*, 2004). This may be explained by the associated hyperinsulinemia and insulin resistance (Goldstone *et al.*, 2004; Goldstone *et al.*, 2005; Poykko *et al.*, 2003). In Cushing's syndrome, the decrease in plasma ghrelin concentration is not associated with ACTH levels. Both endogenous hypercortisolemia, in which ACTH levels are reduced, and Cushing's disease which results from elevated ACTH secretion, lead to reduced ghrelin levels (Otto *et al.*, 2004). Ghrelin levels significantly increased after successful surgical treatment of Cushing's disease (Libe *et*

al., 2005; Otto *et al.*, 2004). These findings make it unlikely that ghrelin has a causative role in the unfavourable energy balance and characteristic body composition of Cushing's syndrome (Otto *et al.*, 2004), but rather it appears that ghrelin levels in Cushing's syndrome are altered as a consequence of the metabolic disturbance.

The ghrelin-induced secretion of ACTH and cortisol is enhanced in Cushing's disease patients as compared to healthy controls (Correa-Silva *et al.*, 2006; Leal-Cerro *et al.*, 2002; Miljic *et al.*, 2010). GHS-R overexpression has been demonstrated in pituitary and ectopic ACTH-secreting tumours and in other neuroendocrine tumours (Arnaldi *et al.*, 2003b; Korbonits *et al.*, 2001). In patients with an ACTH-secreting tumour, ghrelin may further stimulate ACTH levels, and subsequently cortisol levels, through direct action on tumoral GHS-R (Correa-Silva *et al.*, 2006). Ghrelin has been shown to act directly on corticotrope tumour cells derived from patients with Cushing's disease and this supports the suggestion of a cellular basis for the exaggerated ACTH response to ghrelin in Cushing's disease (Martinez-Fuentes *et al.*, 2006). In addition, ghrelin has been shown *in vitro* to stimulate ACTH secretion by human ACTH-secreting pituitary adenomas (Pecori *et al.*, 2007). After one month of ketoconazole treatment and potent inhibition of steroidogenesis, patients with Cushing's disease showed significant reduction in cortisol responses to ghrelin (Correa-Silva *et al.*, 2007).

2.2. Ghrelin and thyroid disease

Graves' disease patients have been reported to display increased appetite and food intake as compared to healthy subjects and this hyperphagia may be a response to the excessive energy expenditure which characterises hyperthyroidism. Interestingly,

normalisation of appetite occurs after pharmacological therapy (Cugini *et al.*, 1999). It appears that the hyperphagia of hyperthyroidism is not mediated by circulating ghrelin (Katargari *et al.*, 2008). Circulating plasma ghrelin levels are reduced in hyperthyroidism as compared to the euthyroid state (Altinova *et al.*, 2006a), exhibit a positive correlation with thyroid stimulating hormone (TSH) levels (Sawicka *et al.*, 2010) and rise to normal levels with pharmacological therapy of hyperthyroidism (Braclik *et al.*, 2008; Gimenez-Palop *et al.*, 2005; Riis *et al.*, 2003; Rojdmarm *et al.*, 2005; Theodoropoulou *et al.*, 2009). Total ghrelin levels were found to be significantly lower in overt than in subclinical hyperthyroid patients, with a trend to a reduction also in acylated ghrelin levels (Tanda *et al.*, 2009). Insulin resistance is increased in hyperthyroidism (Gimenez-Palop *et al.*, 2005) and hyperinsulinaemia has been shown to suppress ghrelin levels (Saad *et al.*, 2002) regardless of thyroid status (Riis *et al.*, 2003).

In hyperthyroid rats increased ghrelin-immunoreactivity in gastric mucosa has been observed along with the decreased plasma ghrelin concentration and this suggests that thyroxine may inhibit ghrelin secretion (Dadan *et al.*, 2008).

There are variable data on ghrelin in hypothyroidism. A rat model of hypothyroidism showed increased circulating ghrelin and gastric ghrelin mRNA levels (Caminos *et al.*, 2002). Following thyroxine replacement in hypothyroid patients, serum ghrelin levels showed a significant reduction (Braclik *et al.*, 2008; Gjedde *et al.*, 2008). In contrast, other studies have found ghrelin levels in hypothyroid patients to be similar to those of healthy subjects and there was no significant change in ghrelin levels after medical treatment of hypothyroidism (Gimenez-Palop *et al.*, 2005; Sadegholvad *et al.*, 2007; Tanda *et al.*, 2009). Anti-parietal cell antibodies and atrophic gastritis has been

detected in patients with autoimmune thyroid disease (Centanni *et al.*, 1999). Furthermore, patients with Hashimoto's thyroiditis have displayed reduced ghrelin levels as compared to euthyroid subjects, and those with high thyroid peroxidase antibody titres showed greater reduction in ghrelin levels (Altinova *et al.*, 2006b). Thus, thyroid peroxidase antibodies may have a ghrelin lowering effect. In autoimmune thyroid disease there may be reduced production of ghrelin due to destructive antibody-mediated action on the gastric mucosa.

Ghrelin and GHS-R1a mRNA expression has been demonstrated in normal human thyroid by RT-PCR (Gnanapavan *et al.*, 2002). Another study found expression of ghrelin in fetal but not in infant or adult thyroid (Volante *et al.*, 2003), and ghrelin may operate as a regulator of thyroid cell differentiation during normal development. Ghrelin appears to be re-expressed in thyroid tumours (Volante *et al.*, 2003) and ghrelin immunoreactivity has been demonstrated in medullary, follicular and papillary thyroid carcinomas (Raghuay *et al.*, 2006). Human medullary thyroid carcinoma surgical specimens showed higher ghrelin content than normal thyroid tissues (Kanamoto *et al.*, 2001). However, ghrelin levels were reduced in thyroid tissue of patients with papillary thyroid carcinoma, as compared with normal thyroid tissue (Karaoglu *et al.*, 2009). Ghrelin (Volante *et al.*, 2003) and growth hormone secretagogues (Cassoni *et al.*, 2000) have been shown to inhibit *in vitro* cell proliferation of thyroid carcinoma cell lines, and may have a role in regulating thyroid tumour growth. Thus, high levels of ghrelin in thyroid tumours may be a mechanism by which there is an attempt to prevent tumour expansion.

2.3. Pheochromocytoma

Pheochromocytomas are rare catecholamine-secreting tumours that arise from chromaffin cells of the adrenal medulla. Ghrelin immunoreactivity has been demonstrated in the medulla of human adrenal glands and in human pheochromocytomas (Raghu *et al.*, 2008). Ghrelin has been shown to inhibit basal dopamine release and may have an effect in reducing the catecholamine secretion of pheochromocytomas (Nanmoku *et al.*, 2003). The expression of ghrelin mRNA was found to be significantly less in pheochromocytomas as compared to normal adrenal tissue (Ueberberg *et al.*, 2008), thus reduced ghrelin levels may have a role in the increased catecholamine secretion of pheochromocytomas.

2.4. Insulinoma

The role of ghrelin in beta-cell function is discussed elsewhere in this special issue. Expression of ghrelin and GHS-R1a mRNA has been detected in human insulinomas (Ekeblad *et al.*, 2007; Volante *et al.*, 2002). Acylated ghrelin, via the GHS-R1a, was found to stimulate insulin secretion in INS-1E rat insulinoma cells. Unacylated ghrelin also stimulated insulin secretion but independently of GHS-R1a (Gauna *et al.*, 2006). In contrast, ghrelin has recently been shown to inhibit insulin secretion in MIN6 insulinoma cells partly via the AMPK-UCP pathway (Wang *et al.*, 2010).

3. Conclusions

There is currently much research into the numerous physiological and pathophysiological roles of ghrelin. Ghrelin plays a crucial function in appetite

regulation, as well as short- and long-term energy homeostasis. The current obesity pandemic is a serious public health challenge. Antagonism of ghrelin and its signalling pathway may in future have a powerful role to play in the management of obesity. Continued research into PWS, genetic variations of *ghrelin* and *GHS-R* genes and gastric bypass and other forms of bariatric surgery will provide further insight into the role of ghrelin in obesity. Most likely as a result of secondary insulin resistance, altered ghrelin levels have also been observed in Cushing's syndrome and thyroid disease.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- Altinova, A.E., Toruner, F.B., Akturk, M., Elbeg, S., Yetkin, I., Cakir, N., Arslan, M. 2006a. Reduced serum acylated ghrelin levels in patients with hyperthyroidism. *Horm. Res.* 65, 295-299.
- Altinova, A.E., Toruner, F., Karakoc, A., Yetkin, I., Ayvaz, G., Cakir, N., Arslan, M. 2006b. Serum Ghrelin Levels in patients with Hashimoto's thyroiditis. *Thyroid* 16, 1259-1264.
- Anagnostis, P., Athyros, V.G., Tziomalos, K., Karagiannis, A., Mikhailidis, D.P. 2009. Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J. Clin. Endocrinol. Metab* 94, 2692-2701.
- Anderson, K.A., Ribar, T.J., Lin, F., Noeldner, P.K., Green, M.F., Muehlbauer, M.J., Witters, L.A., Kemp, B.E., Means, A.R. 2008. Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab* 7, 377-388.
- Andersson, U., Filipsson, K., Abbott, C.R., Woods, A., Smith, K., Bloom, S.R., Carling, D., Small, C.J. 2004. AMP-activated protein kinase plays a role in the control of food intake. *J. Biol. Chem.* 279, 12005-12008.
- Andreis, P.G., Malendowicz, L.K., Trejter, M., Neri, G., Spinazzi, R., Rossi, G.P., Nussdorfer, G.G. 2003. Ghrelin and growth hormone secretagogue receptor are expressed in the rat adrenal cortex: Evidence that ghrelin stimulates the growth, but not the secretory activity of adrenal cells. *FEBS Lett.* 536, 173-179.
- Andrews, Z.B., Liu, Z.W., Wallingford, N., Erion, D.M., Borok, E., Friedman, J.M., Tschop, M.H., Shanabrough, M., Cline, G., Shulman, G.I., Coppola, A., Gao, X.B., Horvath, T.L., Diano, S. 2008. UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature* 454, 846-851.
- Arnaldi, G., Angeli, A., Atkinson, A.B., Bertagna, X., Cavagnini, F., Chrousos, G.P., Fava, G.A., Findling, J.W., Gaillard, R.C., Grossman, A.B., Kola, B., Lacroix, A., Mancini, T., Mantero, F., Newell-Price, J., Nieman, L.K., Sonino, N., Vance, M.L., Giustina, A., Boscaro, M. 2003a. Diagnosis and complications of Cushing's syndrome: a consensus statement. *J. Clin. Endocrinol. Metab* 88, 5593-5602.
- Arnaldi, G., Mancini, T., Kola, B., Appolloni, G., Freddi, S., Concettoni, C., Bearzi, I., Masini, A., Boscaro, M., Mantero, F. 2003b. Cyclical Cushing's syndrome in a patient with a bronchial neuroendocrine tumor (typical carcinoid) expressing ghrelin and growth hormone secretagogue receptors. *J. Clin. Endocrinol Metab* 88, 5834-5840.
- Arvat, E., Maccario, M., Di, V.L., Broglio, F., Benso, A., Gottero, C., Papotti, M., Muccioli, G., Dieguez, C., Casanueva, F.F., Deghenghi, R., Camanni, F., Ghigo, E. 2001. Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J. Clin. Endocrinol Metab* 86, 1169-1174.

- 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, 947-952.
- Asakawa, A., Inui, A., Fujimiya, M., Sakamaki, R., Shinfuku, N., Ueta, Y., Meguid, M.M., Kasuga, M. 2005. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 54, 18-24.
- Baessler, A., Hasinoff, M.J., Fischer, M., Reinhard, W., Sonnenberg, G.E., Olivier, M., Erdmann, J., Schunkert, H., Doering, A., Jacob, H.J., Comuzzie, A.G., Kissebah, A.H., Kwitek, A.E. 2005. Genetic linkage and association of the growth hormone secretagogue receptor (ghrelin receptor) gene in human obesity. *Diabetes* 54, 259-267.
- Barnett, B.P., Hwang, Y., Taylor, M.S., Kirchner, H., Pfluger, P.T., Bernard, V., Lin, Y.Y., Bowers, E.M., Mukherjee, C., Song, W.J., Longo, P.A., Leahy, D.J., Hussain, M.A., Tschop, M.H., Boeke, J.D., Cole, P.A. 2010. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. *Science* 330, 1689-1692.
- Barreiro, M.L., Pinilla, L., Aguilar, E., Tena-Sempere, M. 2002. Expression and homologous regulation of GH secretagogue receptor mRNA in rat adrenal gland. *Eur. J. Endocrinol* 147, 677-688.
- Beckman, L.M., Beckman, T.R., Earthman, C.P. 2010. Changes in gastrointestinal hormones and leptin after Roux-en-Y gastric bypass procedure: a review. *J. Am. Diet. Assoc.* 110, 571-584.
- Bing, C., Ambye, L., Fenger, M., Jorgensen, T., Borch-Johnsen, K., Madsbad, S., Urhammer, S.A. 2005. Large-scale studies of the Leu72Met polymorphism of the ghrelin gene in relation to the metabolic syndrome and associated quantitative traits. *Diabet. Med.* 22, 1157-1160.
- Bizzarri, C., Rigamonti, A.E., Luce, A., Cappa, M., Cella, S.G., Berini, J., Sartorio, A., Muller, E.E., Salvatoni, A. 2010. Children with Prader-Willi syndrome exhibit more evident meal-induced responses in plasma ghrelin and peptide YY levels than obese and lean children. *Eur. J. Endocrinol* 162, 499-505.
- Braclik, M., Marcisz, C., Giebel, S., Orzel, A. 2008. Serum leptin and ghrelin levels in premenopausal women with stable body mass index during treatment of thyroid dysfunction. *Thyroid* 18, 545-550.
- Briggs, D.I., Enriori, P.J., Lemus, M.B., Cowley, M.A., Andrews, Z.B. 2010. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. *Endocrinology* 151, 4745-4755.
- Buchwald, H., Williams, S.E. 2004. Bariatric surgery worldwide 2003. *Obes. Surg.* 14, 1157-1164.
- Callahan, H.S., Cummings, D.E., Pepe, M.S., Breen, P.A., Matthys, C.C., Weigle, D.S. 2004. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J. Clin. Endocrinol Metab* 89, 1319-1324.

- Caminos, J.E., Seoane, L.M., Tovar, S.A., Casanueva, F.F., Dieguez, C. 2002. Influence of thyroid status and growth hormone deficiency on ghrelin. *Eur. J. Endocrinol* 147, 159-163.
- Carlson, M.J., Cummings, D.E. 2006. Prospects for an anti-ghrelin vaccine to treat obesity. *Mol. Interv.* 6, 249-252.
- Cassoni, P., Papotti, M., Catapano, F., Ghe, C., Deghenghi, R., Ghigo, E., Muccioli, G. 2000. Specific binding sites for synthetic growth hormone secretagogues in non-tumoral and neoplastic human thyroid tissue. *J. Endocrinol* 165, 139-146.
- Centanni, M., Marignani, M., Gargano, L., Corleto, V.D., Casini, A., Delle, F.G., Andreoli, M., Annibale, B. 1999. Atrophic body gastritis in patients with autoimmune thyroid disease: an underdiagnosed association. *Arch. Intern. Med.* 159, 1726-1730.
- Chen, H.Y., Trumbauer, M.E., Chen, A.S., Weingarth, D.T., Adams, J.R., Frazier, E.G., Shen, Z., Marsh, D.J., Feighner, S.D., Guan, X.M., Ye, Z., Nargund, R.P., Smith, R.G., Van der Ploeg, L.H., Howard, A.D., MacNeil, D.J., Qian, S. 2004. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* 145, 2607-2612.
- Choe, Y.H., Song, S.Y., Paik, K.H., Oh, Y.J., Chu, S.H., Yeo, S.H., Kwon, E.K., Kim, E.M., Rha, M.Y., Jin, D.K. 2005a. Increased density of ghrelin-expressing cells in the gastric fundus and body in Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 90, 5441-5445.
- Choe, Y.H., Jin, D.K., Kim, S.E., Song, S.Y., Paik, K.H., Park, H.Y., Oh, Y.J., Kim, A.H., Kim, J.S., Kim, C.W., Chu, S.H., Kwon, E.K., Lee, K.H. 2005b. Hyperghrelinemia does not accelerate gastric emptying in Prader-Willi syndrome patients. *J. Clin. Endocrinol Metab* 90, 3367-3370.
- Christ-Crain, M., Kola, B., Lolli, F., Fekete, C., Seboek, D., Wittmann, G., Feltrin, D., Igreja, S.C., Ajodha, S., Harvey-White, J., Kunos, G., Muller, B., Pralong, F., Aubert, G., Arnaldi, G., Giacchetti, G., Boscaro, M., Grossman, A.B., Korbonits, M. 2008. AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. *FASEB J.* 22, 1672-1683.
- Cohen, R., Uzzan, B., Bihan, H., Khochtali, I., Reach, G., Catheline, J.M. 2005. Ghrelin levels and sleeve gastrectomy in super-super-obesity. *Obes. Surg.* 15, 1501-1502.
- Coiro, V., Saccani-Jotti, G., Minelli, R., Melani, A., Milli, B., Manfredi, G., Volpi, R., Chiodera, P. 2005. Adrenocorticotropin/cortisol and arginine-vasopressin secretory patterns in response to ghrelin in normal men. *Neuroendocrinology* 81, 103-106.
- Correa-Silva, S.R., Nascif, S.O., Lengyel, A.M. 2006. Decreased GH secretion and enhanced ACTH and cortisol release after ghrelin administration in Cushing's disease: comparison with GH-releasing peptide-6 (GHRP-6) and GHRH. *Pituitary.* 9, 101-107.
- Correa-Silva, S.R., Nascif, S.O., Silva, M.R., Molica, P., Lengyel, A.M. 2007. Effect of one month ketoconazole treatment on GH, cortisol and ACTH release after ghrelin, GHRP-6 and GHRH administration in patients with Cushing's disease. *Arq Bras. Endocrinol Metabol.* 51, 1110-1117.

- Cowley, M.A., Smith, R.G., Diano, S., Tschop, M., Pronchuk, N., Grove, K.L., Strasburger, C.J., Bidlingmaier, M., Esterman, M., Heiman, M.L., Garcia-Segura, L.M., Nillni, E.A., Mendez, P., Low, M.J., Sotonyi, P., Friedman, J.M., Liu, H., Pinto, S., Colmers, W.F., Cone, R.D., Horvath, T.L. 2003. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649-661.
- Cugini, P., Paggi, A., Cristina, G., Ceccotti, P., Pellegrino, A.M., Fontana, S., Di, M.A., Vacca, K., Di, S.G. 1999. Hunger sensation in Graves' disease before and after pharmacological therapy. *Clin. Ter.* 150, 115-119.
- Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S. 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714-1719.
- Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P., Purnell, J.Q. 2002a. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N. Engl. J. Med.* 346, 1623-1630.
- Cummings, D.E., Clement, K., Purnell, J.Q., Vaisse, C., Foster, K.E., Frayo, R.S., Schwartz, M.W., Basdevant, A., Weigle, D.S. 2002b. Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat. Med.* 8, 643-644.
- Cummings, D.E., Frayo, R.S., Marmonier, C., Aubert, R., Chapelot, D. 2004. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am. J. Physiol Endocrinol Metab* 287, E297-E304
- Cummings, D.E. 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav.* 89, 71-84.
- Dadan, J., Zbucki, R.L., Sawicki, B., Winnicka, M.M. 2008. Estimation of gastric ghrelin-positive cells activity in hyperthyroid rats. *Folia Histochem. Cytobiol.* 46, 511-517.
- Davies, J.S., Kotokorpi, P., Eccles, S.R., Barnes, S.K., Tokarczuk, P.F., Allen, S.K., Whitworth, H.S., Guschina, I.A., Evans, B.A., Mode, A., Zigman, J.M., Wells, T. 2009. Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. *Mol. Endocrinol* 23, 914-924.
- De, W.K., Ishkanian, S.L., Bogarin, R., Miranda, C.A., Ghatei, M.A., Bloom, S.R., Pacaud, D., Chanoine, J.P. 2008. Long-acting octreotide treatment causes a sustained decrease in ghrelin concentrations but does not affect weight, behaviour and appetite in subjects with Prader-Willi syndrome. *Eur. J. Endocrinol* 159, 381-388.
- Delparigi, A., Tschop, M., Heiman, M.L., Salbe, A.D., Vozarova, B., Sell, S.M., Bunt, J.C., Tataranni, P.A. 2002. High circulating ghrelin: a potential cause for hyperphagia and obesity in prader-willi syndrome. *J. Clin. Endocrinol Metab* 87, 5461-5464.
- Druce, M., Bloom, S.R. 2006. The regulation of appetite. *Arch. Dis. Child* 91, 183-187.

- Egecioglu, E., Stenstrom, B., Pinnock, S.B., Tung, L.Y., Dornonville, d.l.C., Lindqvist, A., Hakanson, R., Syversen, U., Chen, D., Dickson, S.L. 2008. Hypothalamic gene expression following ghrelin therapy to gastrectomized rodents. *Regul. Pept.* 146, 176-182.
- Ekeblad, S., Lejonklou, M.H., Grimfjard, P., Johansson, T., Eriksson, B., Grimelius, L., Stridsberg, M., Stalberg, P., Skogseid, B. 2007. Co-expression of ghrelin and its receptor in pancreatic endocrine tumours. *Clin. Endocrinol (Oxf)* 66, 115-122.
- English, P.J., Ghati, M.A., Malik, I.A., Bloom, S.R., Wilding, J.P. 2002. Food fails to suppress ghrelin levels in obese humans. *J. Clin. Endocrinol Metab* 87, 2984
- Erdie-Lalena, C.R., Holm, V.A., Kelly, P.C., Frayo, R.S., Cummings, D.E. 2006. Ghrelin levels in young children with Prader-Willi syndrome. *J. Pediatr.* 149, 199-204.
- Faraj, M., Havel, P.J., Phelis, S., Blank, D., Sniderman, A.D., Cianflone, K. 2003. Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. *J. Clin. Endocrinol Metab* 88, 1594-1602.
- Feigerlova, E., Diene, G., Conte-Auriol, F., Molinas, C., Gennero, I., Salles, J.P., Arnaud, C., Tauber, M. 2008. Hyperghrelinemia precedes obesity in Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 93, 2800-2805.
- Fobi, M.A. 2004. Surgical treatment of obesity: a review. *J. Natl. Med. Assoc.* 96, 61-75.
- Garcia, E.A., Heude, B., Petry, C.J., Gueorguiev, M., Hassan-Smith, Z.K., Spanou, A., Ring, S.M., Dunger, D.B., Wareham, N., Sandhu, M.S., Ong, K.K., Korbonits, M. 2008. Ghrelin receptor gene polymorphisms and body size in children and adults. *J. Clin. Endocrinol Metab* 93, 4158-4161.
- Garcia-Fuentes, E., Garrido-Sanchez, L., Garcia-Almeida, J.M., Garcia-Arnes, J., Gallego-Perales, J.L., Rivas-Marin, J., Morcillo, S., Cardona, I., Soriguer, F. 2008. Different effect of laparoscopic Roux-en-Y gastric bypass and open biliopancreatic diversion of Scopinaro on serum PYY and ghrelin levels. *Obes. Surg.* 18, 1424-1429.
- Gauna, C., Delhanty, P.J., van Aken, M.O., Janssen, J.A., Themmen, A.P., Hofland, L.J., Culler, M., Broglio, F., Ghigo, E., van der Lely, A.J. 2006. Unacylated ghrelin is active on the INS-1E rat insulinoma cell line independently of the growth hormone secretagogue receptor type 1a and the corticotropin releasing factor 2 receptor. *Mol. Cell Endocrinol* 251, 103-111.
- Geloneze, B., Tambascia, M.A., Pilla, V.F., Geloneze, S.R., Repetto, E.M., Pareja, J.C. 2003. Ghrelin: a gut-brain hormone: effect of gastric bypass surgery. *Obes. Surg.* 13, 17-22.
- Gimenez-Palop, O., Gimenez-Perez, G., Mauricio, D., Berlanga, E., Potau, N., Vilardell, C., Arroyo, J., Gonzalez-Clemente, J.M., Caixas, A. 2005. Circulating ghrelin in thyroid dysfunction is related to insulin resistance and not to hunger, food intake or anthropometric changes. *Eur. J. Endocrinol* 153, 73-79.

- Gimenez-Palop, O., Gimenez-Perez, G., Mauricio, D., Gonzalez-Clemente, J.M., Potau, N., Berlanga, E., Trallero, R., Laferrere, B., Caixas, A. 2007. A lesser postprandial suppression of plasma ghrelin in Prader-Willi syndrome is associated with low fasting and a blunted postprandial PYY response. *Clin. Endocrinol (Oxf)* 66, 198-204.
- Giordano, R., Picu, A., Broglio, F., Bonelli, L., Baldi, M., Berardelli, R., Ghigo, E., Arvat, E. 2004. Ghrelin, hypothalamus-pituitary-adrenal (HPA) axis and Cushing's syndrome. *Pituitary*. 7, 243-248.
- Gjedde, S., Vestergaard, E.T., Gormsen, L.C., Riis, A.L., Rungby, J., Moller, N., Weeke, J., Jorgensen, J.O. 2008. Serum ghrelin levels are increased in hypothyroid patients and become normalized by L-thyroxine treatment. *J. Clin. Endocrinol Metab* 93, 2277-2280.
- Gjesing, A.P., Larsen, L.H., Torekov, S.S., Hainerova, I.A., Kapur, R., Johansen, A., Albrechtsen, A., Boj, S., Holst, B., Harper, A., Urhammer, S.A., Borch-Johnsen, K., Pisinger, C., Echwald, S.M., Eiberg, H., Astrup, A., Lebl, J., Ferrer, J., Schwartz, T.W., Hansen, T., Pedersen, O. 2010. Family and population-based studies of variation within the ghrelin receptor locus in relation to measures of obesity. *PLoS. One*. 5, e10084
- Gnanapavan, S., Kola, B., Bustin, S.A., Morris, D.G., McGee, P., Fairclough, P., Bhattacharya, S., Carpenter, R., Grossman, A.B., Korbonits, M. 2002. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J. Clin. Endocrinol Metab* 87, 2988
- Goldstone, A.P., Thomas, E.L., Brynes, A.E., Bell, J.D., Frost, G., Saeed, N., Hajnal, J.V., Howard, J.K., Holland, A., Bloom, S.R. 2001. Visceral adipose tissue and metabolic complications of obesity are reduced in Prader-Willi syndrome female adults: evidence for novel influences on body fat distribution. *J. Clin. Endocrinol Metab* 86, 4330-4338.
- Goldstone, A.P., Unmehopa, U.A., Thomas, E.L., Brynes, A.E., Bell, J.D., Frost, G., Ghatei, M.A., Holland, A., Bloom, S.R., Swaab, D.F. 2003. Hypothalamic neuropeptides and regulation of fat mass in Prader-Willi syndrome. In: U Eiholzer, D l'Allemand, W Zipf (eds.) *Prader-Willi Syndrome as a Model for Obesity*. Basel, Switzerland: Karger Publishers, pp.31-43.
- Goldstone, A.P. 2004. Prader-Willi syndrome: advances in genetics, pathophysiology and treatment. *Trends Endocrinol Metab* 15, 12-20.
- Goldstone, A.P., Thomas, E.L., Brynes, A.E., Castroman, G., Edwards, R., Ghatei, M.A., Frost, G., Holland, A.J., Grossman, A.B., Korbonits, M., Bloom, S.R., Bell, J.D. 2004. Elevated fasting plasma ghrelin in prader-willi syndrome adults is not solely explained by their reduced visceral adiposity and insulin resistance. *J. Clin. Endocrinol Metab* 89, 1718-1726.
- Goldstone, A.P., Patterson, M., Kalingag, N., Ghatei, M.A., Brynes, A.E., Bloom, S.R., Grossman, A.B., Korbonits, M. 2005. Fasting and postprandial hyperghrelinemia in Prader-Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma. *J. Clin. Endocrinol Metab* 90, 2681-2690.

- Gualillo, O., Lago, F., Dieguez, C. 2008. Introducing GOAT: a target for obesity and anti-diabetic drugs? *Trends Pharmacol. Sci.* 29, 398-401.
- Gueorguiev, M., Lecoœur, C., Meyre, D., Benzinou, M., Mein, C.A., Hinney, A., Vatin, V., Weill, J., Heude, B., Hebebrand, J., Grossman, A.B., Korbonits, M., Froguel, P. 2009. Association studies on ghrelin and ghrelin receptor gene polymorphisms with obesity. *Obesity. (Silver. Spring)* 17, 745-754.
- Han, J.C., Muehlbauer, M.J., Cui, H.N., Newgard, C.B., Haqq, A.M. 2010. Lower brain-derived neurotrophic factor in patients with prader-willi syndrome compared to obese and lean control subjects. *J. Clin. Endocrinol Metab* 95, 3532-3536.
- Hansen, T.K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J.S., Jorgensen, J.O. 2002. Weight loss increases circulating levels of ghrelin in human obesity. *Clin. Endocrinol (Oxf)* 56, 203-206.
- Haqq, A.M., Farooqi, I.S., O'Rahilly, S., Stadler, D.D., Rosenfeld, R.G., Pratt, K.L., LaFranchi, S.H., Purnell, J.Q. 2003a. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 88, 174-178.
- Haqq, A.M., Stadler, D.D., Rosenfeld, R.G., Pratt, K.L., Weigle, D.S., Frayo, R.S., LaFranchi, S.H., Cummings, D.E., Purnell, J.Q. 2003b. Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 88, 3573-3576.
- Haqq, A.M., Grambow, S.C., Muehlbauer, M., Newgard, C.B., Svetkey, L.P., Carrel, A.L., Yanovski, J.A., Purnell, J.Q., Freemark, M. 2008. Ghrelin concentrations in Prader-Willi syndrome (PWS) infants and children: changes during development. *Clin. Endocrinol (Oxf)* 69, 911-920.
- Helmling, S., Maasch, C., Eulberg, D., Buchner, K., Schroder, W., Lange, C., Vonhoff, S., Wlotzka, B., Tschop, M.H., Rosewicz, S., Klusmann, S. 2004. Inhibition of ghrelin action in vitro and in vivo by an RNA-Spiegelmer. *Proc. Natl. Acad. Sci. U. S. A* 101, 13174-13179.
- Hickey, G.J., Drisko, J., Faidley, T., Chang, C., Anderson, L.L., Nicolich, S., McGuire, L., Rickes, E., Krupa, D., Feeney, W., Friscino, B., Cunningham, P., Frazier, E., Chen, H., Laroque, P., Smith, R.G. 1996. Mediation by the central nervous system is critical to the in vivo activity of the GH secretagogue L-692,585. *J. Endocrinol* 148, 371-380.
- Higgins, S.C., Gueorguiev, M., Korbonits, M. 2007. Ghrelin, the peripheral hunger hormone. *Ann. Med.* 39, 116-136.
- Hill, J.O. 2006. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr. Rev.* 27, 750-761.
- Hinney, A., Hoch, A., Geller, F., Schafer, H., Siegfried, W., Goldschmidt, H., Remschmidt, H., Hebebrand, J. 2002. Ghrelin gene: identification of missense variants and a frameshift mutation in extremely obese children and adolescents and healthy normal weight students. *J. Clin. Endocrinol Metab* 87, 2716

- Hinton, E.C., Holland, A.J., Gellatly, M.S., Soni, S., Patterson, M., Ghatei, M.A., Owen, A.M. 2006. Neural representations of hunger and satiety in Prader-Willi syndrome. *Int. J. Obes. (Lond)* 30, 313-321.
- Holdstock, C., Engstrom, B.E., Ohrvall, M., Lind, L., Sundbom, M., Karlsson, F.A. 2003. Ghrelin and adipose tissue regulatory peptides: effect of gastric bypass surgery in obese humans. *J. Clin. Endocrinol Metab* 88, 3177-3183.
- Holsen, L.M., Zarccone, J.R., Brooks, W.M., Butler, M.G., Thompson, T.I., Ahluwalia, J.S., Nollen, N.L., Savage, C.R. 2006. Neural mechanisms underlying hyperphagia in Prader-Willi syndrome. *Obesity. (Silver. Spring)* 14, 1028-1037.
- Holst, B., Schwartz, T.W. 2006. Ghrelin receptor mutations--too little height and too much hunger. *J. Clin. Invest* 116, 637-641.
- Jo, D.S., Kim, S.L., Kim, S.Y., Hwang, P.H., Lee, K.H., Lee, D.Y. 2005. Preproghrelin Leu72Met polymorphism in obese Korean children. *J. Pediatr. Endocrinol Metab* 18, 1083-1086.
- Kadowaki, T., Yamauchi, T., Kubota, N. 2008. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett.* 582, 74-80.
- Kanamoto, N., Akamizu, T., Hosoda, H., Hataya, Y., Ariyasu, H., Takaya, K., Hosoda, K., Saijo, M., Moriyama, K., Shimatsu, A., Kojima, M., Kangawa, K., Nakao, K. 2001. Substantial production of ghrelin by a human medullary thyroid carcinoma cell line. *J. Clin. Endocrinol Metab* 86, 4984-4990.
- Kanumakala, S., Greaves, R., Pedreira, C.C., Donath, S., Warne, G.L., Zacharin, M.R., Harris, M. 2005. Fasting ghrelin levels are not elevated in children with hypothalamic obesity. *J. Clin. Endocrinol Metab* 90, 2691-2695.
- Karaoglu, A., Aydin, S., Dagli, A.F., Cummings, D.E., Ozercan, I.H., Canatan, H., Ozkan, Y. 2009. Expression of obestatin and ghrelin in papillary thyroid carcinoma. *Mol. Cell Biochem.* 323, 113-118.
- Katargari, S.A., Milousis, A., Pagonopoulou, O., Asimakopoulos, B., Nikolettos, N.K. 2008. Ghrelin in pathological conditions. *Endocr. J.* 55, 439-453.
- Kojima, M., Kangawa, K. 2005. Ghrelin: structure and function. *Physiol Rev.* 85, 495-522.
- Kola, B., Hubina, E., Tucci, S.A., Kirkham, T.C., Garcia, E.A., Mitchell, S.E., Williams, L.M., Hawley, S.A., Hardie, D.G., Grossman, A.B., Korbonits, M. 2005. Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J. Biol. Chem.* 280, 25196-25201.
- Kola, B., Boscaro, M., Rutter, G.A., Grossman, A.B., Korbonits, M. 2006. Expanding role of AMPK in endocrinology. *Trends Endocrinol Metab* 17, 205-215.

- Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Harvey-White, J., Liposits, Z., Kunos, G., Grossman, A.B., Fekete, C., Korbonits, M. 2008. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS. One.* 3, e1797
- Kola, B., Korbonits, M. 2009. Shedding light on the intricate puzzle of ghrelin's effects on appetite regulation. *J. Endocrinol* 202, 191-198.
- Kopelman, P.G. 2000. Obesity as a medical problem. *Nature* 404, 635-643.
- Korbonits, M., Kaltsas, G., Perry, L.A., Putignano, P., Grossman, A.B., Besser, G.M., Trainer, P.J. 1999a. The growth hormone secretagogue hexarelin stimulates the hypothalamo-pituitary-adrenal axis via arginine vasopressin. *J. Clin. Endocrinol Metab* 84, 2489-2495.
- Korbonits, M., Little, J.A., Forsling, M.L., Tringali, G., Costa, A., Navarra, P., Trainer, P.J., Grossman, A.B. 1999b. The effect of growth hormone secretagogues and neuropeptide Y on hypothalamic hormone release from acute rat hypothalamic explants. *J. Neuroendocrinol.* 11, 521-528.
- Korbonits, M., Bustin, S.A., Kojima, M., Jordan, S., Adams, E.F., Lowe, D.G., Kangawa, K., Grossman, A.B. 2001. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. *J. Clin. Endocrinol Metab* 86, 881-887.
- Korbonits, M., Gueorguiev, M., O'Grady, E., Lecoecur, C., Swan, D.C., Mein, C.A., Weill, J., Grossman, A.B., Froguel, P. 2002. A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children. *J. Clin. Endocrinol Metab* 87, 4005-4008.
- Korbonits, M., Grossman, A.B. 2004. Ghrelin: update on a novel hormonal system. *Eur. J. Endocrinol* 151 Suppl 1, S67-S70
- Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., Kozono, H., Takamoto, I., Okamoto, S., Shiuchi, T., Suzuki, R., Satoh, H., Tsuchida, A., Moroi, M., Sugi, K., Noda, T., Ebinuma, H., Ueta, Y., Kondo, T., Araki, E., Ezaki, O., Nagai, R., Tobe, K., Terauchi, Y., Ueki, K., Minokoshi, Y., Kadowaki, T. 2007. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab* 6, 55-68.
- Kuzuya, M., Ando, F., Iguchi, A., Shimokata, H. 2006. Preproghrelin Leu72Met variant contributes to overweight in middle-aged men of a Japanese large cohort. *Int. J. Obes. (Lond)* 30, 1609-1614.
- Langer, F.B., Reza Hoda, M.A., Bohdjalian, A., Felberbauer, F.X., Zacherl, J., Wenzl, E., Schindler, K., Luger, A., Ludvik, B., Prager, G. 2005. Sleeve gastrectomy and gastric banding: effects on plasma ghrelin levels. *Obes. Surg.* 15, 1024-1029.
- le Roux, C.W., Patterson, M., Vincent, R.P., Hunt, C., Ghatei, M.A., Bloom, S.R. 2005. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. *J. Clin. Endocrinol Metab* 90, 1068-1071.

- Leal-Cerro, A., Torres, E., Soto, A., Dios, E., Deghenghi, R., Arvat, E., Ghigo, E., Dieguez, C., Casanueva, F.F. 2002. Ghrelin is no longer able to stimulate growth hormone secretion in patients with Cushing's syndrome but instead induces exaggerated corticotropin and cortisol responses. *Neuroendocrinology* 76, 390-396.
- Leskela, P., Ukkola, O., Vartiainen, J., Ronnema, T., Kaprio, J., Bouchard, C., Kesaniemi, Y.A. 2009. Fasting plasma total ghrelin concentrations in monozygotic twins discordant for obesity. *Metabolism* 58, 174-179.
- Libe, R., Morpurgo, P.S., Cappiello, V., Maffini, A., Bondioni, S., Locatelli, M., Zavanone, M., Beck-Peccoz, P., Spada, A. 2005. Ghrelin and adiponectin in patients with Cushing's disease before and after successful transsphenoidal surgery. *Clin. Endocrinol (Oxf)* 62, 30-36.
- Lim, C.T., Kola, B., Korbonits, M. 2010. AMPK as a mediator of hormonal signalling. *J. Mol. Endocrinol* 44, 87-97.
- Lin, E., Gletsu, N., Fugate, K., McClusky, D., Gu, L.H., Zhu, J.L., Ramshaw, B.J., Papanicolaou, D.A., Ziegler, T.R., Smith, C.D. 2004. The effects of gastric surgery on systemic ghrelin levels in the morbidly obese. *Arch. Surg.* 139, 780-784.
- Liu, G., Fortin, J.P., Beinborn, M., Kopin, A.S. 2007. Four missense mutations in the ghrelin receptor result in distinct pharmacological abnormalities. *J. Pharmacol. Exp. Ther.* 322, 1036-1043.
- Lopez, M., Lage, R., Saha, A.K., Perez-Tilve, D., Vazquez, M.J., Varela, L., Sangiao-Alvarellos, S., Tovar, S., Raghay, K., Rodriguez-Cuenca, S., Deoliveira, R.M., Castaneda, T., Datta, R., Dong, J.Z., Culler, M., Sleeman, M.W., Alvarez, C.V., Gallego, R., Lelliott, C.J., Carling, D., Tschop, M.H., Dieguez, C., Vidal-Puig, A. 2008. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab* 7, 389-399.
- Martinez-Fuentes, A.J., Moreno-Fernandez, J., Vazquez-Martinez, R., Duran-Prado, M., de la Riva, A., Tena-Sempere, M., Dieguez, C., Jimenez-Reina, L., Webb, S.M., Pumar, A., Leal-Cerro, A., Benito-Lopez, P., Malagon, M.M., Castano, J.P. 2006. Ghrelin is produced by and directly activates corticotrope cells from adrenocorticotropin-secreting adenomas. *J. Clin. Endocrinol Metab* 91, 2225-2231.
- Miljic, D., Joksimovic, M., Doknic, M., Iovic, M., Djurovic, M., Pekic, S., Tancic, M., Soldatovic, I., Stojanovic, M., Nale, D., Macut, D., Damjanovic, S., Popovic, V. 2010. ACTH and cortisol responses to ghrelin and DDAVP in patients with Cushing's disease (CD) and adrenal enlargement. *J. Endocrinol Invest* 33, 526-529.
- Miller, J.L., James, G.A., Goldstone, A.P., Couch, J.A., He, G., Driscoll, D.J., Liu, Y. 2007. Enhanced activation of reward mediating prefrontal regions in response to food stimuli in Prader-Willi syndrome. *J. Neurol. Neurosurg. Psychiatry* 78, 615-619.
- Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.B., Lee, A., Xue, B., Mu, J., Fofelle, F., Ferre, P., Birnbaum, M.J., Stuck, B.J., Kahn, B.B. 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 428, 569-574.

- Miraglia Del, G.E., Santoro, N., Cirillo, G., Raimondo, P., Grandone, A., D'Aniello, A., Di, N.M., Perrone, L. 2004. Molecular screening of the ghrelin gene in Italian obese children: the Leu72Met variant is associated with an earlier onset of obesity. *Int. J. Obes. Relat Metab Disord.* 28, 447-450.
- Mozid, A.M., Tringali, G., Forsling, M.L., Hendricks, M.S., Ajodha, S., Edwards, R., Navarra, P., Grossman, A.B., Korbonsits, M. 2003. Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vasopressin. *Horm. Metab Res.* 35, 455-459.
- Murata, M., Okimura, Y., Iida, K., Matsumoto, M., Sowa, H., Kaji, H., Kojima, M., Kangawa, K., Chihara, K. 2002. Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. *J. Biol. Chem.* 277, 5667-5674.
- 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, 194-198.
- Nanmoku, T., Takekoshi, K., Ishi, K., Kawakami, Y., Isobe, K., Shibuya, S., Okuda, Y., Nakai, T. 2003. Effect of Ghrelin on catecholamine secretion in rat pheochromocytoma PC12 cells. *Endocr. Res.* 29, 17-21.
- Nicholls, R.D., Knepper, J.L. 2001. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu. Rev. Genomics Hum. Genet.* 2, 153-175.
- Olszewski, P.K., Schioth, H.B., Levine, A.S. 2008. Ghrelin in the CNS: from hunger to a rewarding and memorable meal? *Brain Res. Rev.* 58, 160-170.
- Otto, B., Tschop, M., Heldwein, W., Pfeiffer, A.F., Diederich, S. 2004. Endogenous and exogenous glucocorticoids decrease plasma ghrelin in humans. *Eur. J. Endocrinol* 151, 113-117.
- Paik, K.H., Jin, D.K., Song, S.Y., Lee, J.E., Ko, S.H., Song, S.M., Kim, J.S., Oh, Y.J., Kim, S.W., Lee, S.H., Kim, S.H., Kwon, E.K., Choe, Y.H. 2004. Correlation between fasting plasma ghrelin levels and age, body mass index (BMI), BMI percentiles, and 24-hour plasma ghrelin profiles in Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 89, 3885-3889.
- Pantel, J., Legendre, M., Cabrol, S., Hilal, L., Hajaji, Y., Morisset, S., Nivot, S., Vie-Luton, M.P., Grouselle, D., de, K.M., Kadiri, A., Epelbaum, J., le, B.Y., Amselem, S. 2006. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J. Clin. Invest* 116, 760-768.
- Pantel, J., Legendre, M., Nivot, S., Morisset, S., Vie-Luton, M.P., le, B.Y., Epelbaum, J., Amselem, S. 2009. Recessive isolated growth hormone deficiency and mutations in the ghrelin receptor. *J. Clin. Endocrinol Metab* 94, 4334-4341.
- Pecori, G.F., Bucciarelli, L.G., Saccani, A., Scacchi, M., Pesce, S., Losa, M., Cavagnini, F. 2007. Ghrelin stimulates adrenocorticotrophic hormone (ACTH) secretion by human ACTH-secreting pituitary adenomas in vitro. *J. Neuroendocrinol.* 19, 208-212.

- Perez-Romero, N., Serra, A., Granada, M.L., Rull, M., Alastrue, A., Navarro-Diaz, M., Romero, R., Fernandez-Llamazares, J. 2010. Effects of two variants of Roux-en-Y Gastric bypass on metabolism behaviour: focus on plasma ghrelin concentrations over a 2-year follow-up. *Obes. Surg.* 20, 600-609.
- Pournaras, D.J., le Roux, C.W. 2009. The effect of bariatric surgery on gut hormones that alter appetite. *Diabetes Metab* 35, 508-512.
- Pournaras, D.J., le Roux, C.W. 2010. Ghrelin and metabolic surgery. *Int. J. Pept.* 2010, Article ID 217267, 1-5.
- Poykko, S.M., Kellokoski, E., Horkko, S., Kauma, H., Kesaniemi, Y.A., Ukkola, O. 2003. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. *Diabetes* 52, 2546-2553.
- Purnell, J.Q., Weigle, D.S., Breen, P., Cummings, D.E. 2003. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *J. Clin. Endocrinol Metab* 88, 5747-5752.
- Raghu, K., Garcia-Caballero, T., Nogueiras, R., Morel, G., Beiras, A., Dieguez, C., Gallego, R. 2006. Ghrelin localization in rat and human thyroid and parathyroid glands and tumours. *Histochem. Cell Biol.* 125, 239-246.
- Raghu, K., Garcia-Caballero, T., Bravo, S., Alvarez, C.V., Gonzalez, R., Dieguez, C., Beiras, A., Fraga, M., Gallego, R. 2008. Ghrelin localization in the medulla of rat and human adrenal gland and in pheochromocytomas. *Histol. Histopathol.* 23, 57-65.
- Riis, A.L., Hansen, T.K., Moller, N., Weeke, J., Jorgensen, J.O. 2003. Hyperthyroidism is associated with suppressed circulating ghrelin levels. *J. Clin. Endocrinol Metab* 88, 853-857.
- Rodriguez, A., Gomez-Ambrosi, J., Catalan, V., Gil, M.J., Becerril, S., Sainz, N., Silva, C., Salvador, J., Colina, I., Fruhbeck, G. 2009. Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. *Int. J. Obes. (Lond)* 33, 541-552.
- Rojdmark, S., Calissendorff, J., Danielsson, O., Brismar, K. 2005. Hunger-satiety signals in patients with Graves' thyrotoxicosis before, during, and after long-term pharmacological treatment. *Endocrine.* 27, 55-61.
- Roth, C.L., Reinehr, T., Scherthaner, G.H., Kopp, H.P., Kriwanek, S., Scherthaner, G. 2009. Ghrelin and obestatin levels in severely obese women before and after weight loss after Roux-en-Y gastric bypass surgery. *Obes. Surg.* 19, 29-35.
- Saad, M.F., Bernaba, B., Hwu, C.M., Jinagouda, S., Fahmi, S., Kogosov, E., Boyadjian, R. 2002. Insulin regulates plasma ghrelin concentration. *J. Clin. Endocrinol Metab* 87, 3997-4000.
- Sadegholvad, A., Afkhamizadeh, M., Ranjbar-Omrani, G. 2007. Serum ghrelin changes in thyroid dysfunction. *Arch. Iran Med.* 10, 168-170.

- Salome, N., Hansson, C., Taube, M., Gustafsson-Ericson, L., Egcioglu, E., Karlsson-Lindahl, L., Fehrentz, J.A., Martinez, J., Perrissoud, D., Dickson, S.L. 2009. On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: new insights from studies exploiting a potent ghrelin receptor (GHS-R1A) antagonist. *J. Neuroendocrinol.* 21, 777-785.
- Sawicka, B., Bossowski, A., Szalecki, M., Wysoka, J., Koput, A., Zelazowska-Rutkowska, B., Tobolczyk, J., Rogowski, F., Luba, M. 2010. Relationship between metabolic parameters and thyroid hormones and the level of gastric peptides in children with autoimmune thyroid diseases. *J. Pediatr. Endocrinol Metab* 23, 345-354.
- Schellekens, H., Dinan, T.G., Cryan, J.F. 2010. Lean mean fat reducing "ghrelin" machine: hypothalamic ghrelin and ghrelin receptors as therapeutic targets in obesity. *Neuropharmacology* 58, 2-16.
- Schindler, K., Prager, G., Ballaban, T., Kretschmer, S., Riener, R., Buranyi, B., Maier, C., Luger, A., Ludvik, B. 2004. Impact of laparoscopic adjustable gastric banding on plasma ghrelin, eating behaviour and body weight. *Eur. J. Clin. Invest* 34, 549-554.
- Seoane, L.M., Lopez, M., Tovar, S., Casanueva, F.F., Senaris, R., Dieguez, C. 2003. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* 144, 544-551.
- Shearman, L.P., Wang, S.P., Helmling, S., Stribling, D.S., Mazur, P., Ge, L., Wang, L., Klussmann, S., Macintyre, D.E., Howard, A.D., Strack, A.M. 2006. Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. *Endocrinology* 147, 1517-1526.
- Shimon, I., Yan, X., Melmed, S. 1998. Human fetal pituitary expresses functional growth hormone-releasing peptide receptors. *J. Clin. Endocrinol Metab* 83, 174-178.
- Shin, A.C., Zheng, H., Townsend, R.L., Sigalet, D.L., Berthoud, H.R. 2010. Meal-induced hormone responses in a rat model of Roux-en-Y gastric bypass surgery. *Endocrinology* 151, 1588-1597.
- Stefan, M., Ji, H., Simmons, R.A., Cummings, D.E., Ahima, R.S., Friedman, M.I., Nicholls, R.D. 2005. Hormonal and metabolic defects in a prader-willi syndrome mouse model with neonatal failure to thrive. *Endocrinology* 146, 4377-4385.
- Stylopoulos, N., Davis, P., Pettit, J.D., Rattner, D.W., Kaplan, L.M. 2005. Changes in serum ghrelin predict weight loss after Roux-en-Y gastric bypass in rats. *Surg. Endosc.* 19, 942-946.
- Sun, Y., Butte, N.F., Garcia, J.M., Smith, R.G. 2008. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology* 149, 843-850.
- Suzuki, S., Ramos, E.J., Goncalves, C.G., Chen, C., Meguid, M.M. 2005. Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model. *Surgery* 138, 283-290.

- Tan, T.M., Vanderpump, M., Khoo, B., Patterson, M., Ghatei, M.A., Goldstone, A.P. 2004. Somatostatin infusion lowers plasma ghrelin without reducing appetite in adults with Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 89, 4162-4165.
- Tanda, M.L., Lombardi, V., Genovesi, M., Ultimieri, F., Lai, A., Gandolfo, M., Dalle, M., I, Grasso, L., Bogazzi, F., Broglio, F., Ghigo, E., Martino, E., Bartalena, L. 2009. Plasma total and acylated Ghrelin concentrations in patients with clinical and subclinical thyroid dysfunction. *J. Endocrinol Invest* 32, 74-78.
- Theodoropoulou, A., Psyrogiannis, A., Metallinos, I.C., Habeos, I., Vgenakis, A.G., Kyriazopoulou, V. 2009. Ghrelin response to oral glucose load in hyperthyroidism, before and after treatment with antithyroid drugs. *J. Endocrinol Invest* 32, 94-97.
- Tong, J., Prigeon, R.L., Davis, H.W., Bidlingmaier, M., Kahn, S.E., Cummings, D.E., Tschop, M.H., D'Alessio, D. 2010. Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes* 59, 2145-2151.
- Toshinai, K., Date, Y., Murakami, N., Shimada, M., Mondal, M.S., Shimbara, T., Guan, J.L., Wang, Q.P., Funahashi, H., Sakurai, T., Shioda, S., Matsukura, S., Kangawa, K., Nakazato, M. 2003. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 144, 1506-1512.
- Tschop, M., Smiley, D.L., Heiman, M.L. 2000. Ghrelin induces adiposity in rodents. *Nature* 407, 908-913.
- Tschop, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E., Heiman, M.L. 2001. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 50, 707-709.
- Ueberberg, B., Unger, N., Sheu, S.Y., Walz, M.K., Schmid, K.W., Saeger, W., Mann, K., Petersenn, S. 2008. Differential expression of ghrelin and its receptor (GHS-R1a) in various adrenal tumors and normal adrenal gland. *Horm. Metab Res.* 40, 181-188.
- Ukkola, O., Ravussin, E., Jacobson, P., Snyder, E.E., Chagnon, M., Sjostrom, L., Bouchard, C. 2001. Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. *J. Clin. Endocrinol Metab* 86, 3996-3999.
- Ukkola, O., Ravussin, E., Jacobson, P., Perusse, L., Rankinen, T., Tschop, M., Heiman, M.L., Leon, A.S., Rao, D.C., Skinner, J.S., Wilmore, J.H., Sjostrom, L., Bouchard, C. 2002. Role of ghrelin polymorphisms in obesity based on three different studies. *Obes. Res.* 10, 782-791.
- Vincent, R.P., le Roux, C.W. 2008. Changes in gut hormones after bariatric surgery. *Clin. Endocrinol (Oxf)* 69, 173-179.
- Vivenza, D., Rapa, A., Castellino, N., Bellone, S., Petri, A., Vacca, G., Aimaretti, G., Broglio, F., Bona, G. 2004. Ghrelin gene polymorphisms and ghrelin, insulin, IGF-I, leptin and anthropometric data in children and adolescents. *Eur. J. Endocrinol* 151, 127-133.

- Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., Muccioli, G., Ghigo, E., Papotti, M. 2002. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J. Clin. Endocrinol Metab* 87, 1300-1308.
- Volante, M., Allia, E., Fulcheri, E., Cassoni, P., Ghigo, E., Muccioli, G., Papotti, M. 2003. Ghrelin in fetal thyroid and follicular tumors and cell lines: expression and effects on tumor growth. *Am. J. Pathol.* 162, 645-654.
- Wang, H.J., Geller, F., Dempfle, A., Schauble, N., Friedel, S., Lichtner, P., Fontenla-Horro, F., Wudy, S., Hagemann, S., Gortner, L., Huse, K., Remschmidt, H., Bettecken, T., Meitinger, T., Schafer, H., Hebebrand, J., Hinney, A. 2004. Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J. Clin. Endocrinol Metab* 89, 157-162.
- Wang, Y., Liu, J. 2009. Plasma ghrelin modulation in gastric band operation and sleeve gastrectomy. *Obes. Surg.* 19, 357-362.
- Wang, Y., Liu, J. 2010. Combination of bypassing stomach and vagus dissection in high-fat diet-induced obese rats-a long-term investigation. *Obes. Surg.* 20, 375-379.
- Wang, Y., Nishi, M., Doi, A., Shono, T., Furukawa, Y., Shimada, T., Furuta, H., Sasaki, H., Nanjo, K. 2010. Ghrelin inhibits insulin secretion through the AMPK-UCP2 pathway in beta cells. *FEBS Lett.* 584, 1503-1508.
- Wen, J.P., Liu, C.E., Hu, Y.T., Chen, G., Lin, L.X. 2010. Globular adiponectin regulates energy homeostasis through AMP-activated protein kinase-acetyl-CoA carboxylase (AMPK/ACC) pathway in the hypothalamus. *Mol. Cell Biochem.* 344, 109-115.
- Williams, D.L., Grill, H.J., Cummings, D.E., Kaplan, J.M. 2006. Overfeeding-induced weight gain suppresses plasma ghrelin levels in rats. *J. Endocrinol Invest* 29, 863-868.
- Wortley, K.E., del Rincon, J.P., Murray, J.D., Garcia, K., Iida, K., Thorner, M.O., Sleeman, M.W. 2005. Absence of ghrelin protects against early-onset obesity. *J. Clin. Invest* 115, 3573-3578.
- Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillon, W.S., Ghatei, M.A., Bloom, S.R. 2001a. Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol Metab* 86, 5992
- Wren, A.M., Small, C.J., Abbott, C.R., Dhillon, W.S., Seal, L.J., Cohen, M.A., Batterham, R.L., Taheri, S., Stanley, S.A., Ghatei, M.A., Bloom, S.R. 2001b. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 50, 2540-2547.
- Xiao, B., Heath, R., Saiu, P., Leiper, F.C., Leone, P., Jing, C., Walker, P.A., Haire, L., Eccleston, J.F., Davis, C.T., Martin, S.R., Carling, D., Gamblin, S.J. 2007. Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature* 449, 496-500.

- Yang, J., Brown, M.S., Liang, G., Grishin, N.V., Goldstein, J.L. 2008. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132, 387-396.
- Ybarra, J., Bobbioni-Harsch, E., Chassot, G., Huber, O., Morel, P., Assimacopoulos-Jeannet, F., Golay, A. 2009. Persistent correlation of ghrelin plasma levels with body mass index both in stable weight conditions and during gastric-bypass-induced weight loss. *Obes. Surg.* 19, 327-331.
- Zhu, J.F., Liang, L., Zou, C.C., Fu, J.F. 2010. Plasma ghrelin levels and polymorphisms of ghrelin gene in Chinese obese children and adolescents. *Ir. J. Med. Sci.* 179, 345-349.
- Zigman, J.M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J.N., Lee, C.E., Jones, J.E., Deysher, A.E., Waxman, A.R., White, R.D., Williams, T.D., Lachey, J.L., Seeley, R.J., Lowell, B.B., Elmquist, J.K. 2005. Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J. Clin. Invest* 115, 3564-3572.
- Zipf, W.B., O'Dorisio, T.M., Cataland, S., Dixon, K. 1983. Pancreatic polypeptide responses to protein meal challenges in obese but otherwise normal children and obese children with Prader-Willi syndrome. *J. Clin. Endocrinol Metab* 57, 1074-1080.
- 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. *Proc. Natl. Acad. Sci. U. S. A* 103, 13226-13231.

Figure 1

Figure 1: Flow diagram showing the proposed molecules involved in the appetite-inducing effect of ghrelin (Kola & Korbonits, 2009).

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Figure 2

Figure 2: Ghrelin has tissue-specific effects on AMPK activity. $\rightarrow$, stimulation; $\neg$, inhibition (Kola *et al.*, 2005; Kola *et al.*, 2006).

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