Ghrelin

from neuroendocrine to metabolic actions

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Ghrelin is a GH-releasing acylated peptide from stomach

M Kojima, H Hosoda, Y Date, M Nakazato, H Matsuo, K Kangawa

The endogenous ligand specific for GHS-R is a peptide of 28 amino acids, where the Ser3 is n-octanoylated.

The acylated peptide specifically releases GH both in vivo and in vitro, and O-n-octanoylation at serine 3 is essential for the activity.

GH release from the pituitary may be regulated not only by hypothalamic GHRH, but also by ghrelin.

Fig. 5. Effects of ghrelin on pituitary hormone secretion, in vitro and in vivo.
a, Dose-response relationships in GH release from rat pituitary cells induced by ghrelin (left panel) and GHRH (right panel) (in vitro).
b, Effects of high-dose (10^-6M) ghrelin on hormone secretions from rat primary pituitary cells, in vitro.
c, Time courses of plasma hormone concentrations after intravenous injections of ghrelin into male rats. Synthetic ghrelin (10 μg) was injected iv in male rats.

Nature 1999; 402: 656-660
### Orally active, Synthetic GH Secretagogues

<table>
<thead>
<tr>
<th>Peptidyl GHS (GHRPs)</th>
<th>Non-Peptidyl GHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D-Trp2)-metENKH</td>
<td>1977</td>
</tr>
<tr>
<td>GHRP-6</td>
<td>1984</td>
</tr>
<tr>
<td>GHRP-1</td>
<td>1991</td>
</tr>
<tr>
<td>hexarelin</td>
<td>1992</td>
</tr>
<tr>
<td>GHRP-2</td>
<td>1993</td>
</tr>
<tr>
<td></td>
<td>1994</td>
</tr>
<tr>
<td></td>
<td>1995</td>
</tr>
<tr>
<td>EP-51389</td>
<td>1996</td>
</tr>
<tr>
<td>ipamorelin</td>
<td>1998</td>
</tr>
<tr>
<td></td>
<td>1999</td>
</tr>
</tbody>
</table>

**Ghrelin discovery**
Stimulation of the GH / IGF-I axis by daily oral administration of a GH secretagogue (MK-677) in healthy elderly subjects

IM Chapman, MA Bach, E Van Cauter, M Farmer, D Krupa, AM Taylor, LM Schilling, KY Cole, EH Skiles, SS Pezzoli, ML Hartman, JD Veldhuis, GJ Gormley, MO Thorner

Fig. 2. Mean (±se) serum GH concentrations (mcg/L) in older subjects after 2 weeks of treatment with placebo (O; n=10), 10mg/day MK-677(●; n=12) and 25 mg/day MK-677 (●; n=10). Evening treatment time (between 2200-2300h) is indicated by an arrow.

Fig. 3. Percent changes from baseline of serum IGF-I concentrations (mcg/L) after 2 and 4 weeks of treatment with placebo (n=10) and once daily oral evening MK-677 doses of 2 mg (n=10), 10 mg (n=12) and 25 mg (n=10)

JCEM, 1996
Synthetic GHS are unlikely candidate to replace rhGH for treatment of idiopathic GHD

Treatment with GH is efficacious but non-physiologic and administered parenterally. MK-0677 is an orally available mimic of the GH releasing peptides which stimulates pulsatile GH secretion. Ninety-four previously untreated, prepubertal GHD (height < 5th percentile, growth velocity (GV) < 25th percentile, peak GH < 10 ng/ml on 2 tests) children were given a single oral dose of MK-0677. Of these, 75 children (52 boys, 23 girls) exhibited a peak GH response ≥ 1.9 ng/ml and, after 6 months of observation for baseline GV, were randomly assigned to receive MK-0677 0.4 or 0.8 mg/kg/day or placebo for another 6 months. Seventy-two patients completed the study. A preliminary analysis of mean GV pretreatment and at Month 6 demonstrates ≥ 3 cm/year mean increase with MK-0677 treatment (Table), although five of 48 patients receiving MK-0677 had < 1 cm/yr change in GV compared to baseline. The mean GV standard deviation score (GV SDS) was positive on MK-0677 treatment. There were no serious drug-related adverse experiences. In summary, in GH-naive GHD prepubertal children, MK-0677 was generally well tolerated and resulted in increases in GV in most patients compared to pretreatment. MK-0677 may present an orally active alternative for treatment of GHD children.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Pre-Rx</th>
<th>Month 6</th>
<th>GV</th>
<th>GV SDS</th>
<th>GV SDS</th>
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<tr>
<td>Placebo</td>
<td>24</td>
<td>4.2 ± 1.8</td>
<td>4.6 ± 1.4</td>
<td>0.4 ± 2.3</td>
<td>-1.7 ± 1.8</td>
<td>-1.0 ± 2.0</td>
</tr>
<tr>
<td>MK-0677 0.4 mg/kg</td>
<td>23</td>
<td>3.4 ± 1.4</td>
<td>6.4 ± 2.7</td>
<td>3.0 ± 2.6*</td>
<td>-2.5 ± 1.9</td>
<td>0.8 ± 3.3*</td>
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<tr>
<td>MK-0677 0.8 mg/kg</td>
<td>25</td>
<td>3.4 ± 1.7</td>
<td>6.8 ± 2.0</td>
<td>3.4 ± 2.1*</td>
<td>-2.6 ± 1.8</td>
<td>1.3 ± 2.4*</td>
</tr>
</tbody>
</table>

* p < 0.001 vs. baseline and vs. placebo
Mean fat-free mass decreased in the placebo group but increased in the MK-677 group ($P \ 0.001$) as did body cell mass, as reflected by intracellular water ($P \ 0.021$).

Increased fat-free mass did not result in changes in strength or function.

No differences were observed in abdominal visceral fat or total fat mass; however, the increase in limb fat was greater in the MK-677 group ($P \ 0.001$).

Body weight increased 0.8 kg in the placebo group and 2.7 kg in the MK-677 group ($P \ 0.003$).

Fasting blood glucose level increased an average of 0.3 mmol/L in the MK-677 group ($P \ 0.015$), and insulin sensitivity decreased.

Cortisol levels increased 47 nmol/L in MK-677 recipients ($P \ 0.020$).

The study power (duration and participant number) was insufficient to evaluate functional end points in healthy elderly persons.
Ghrelin: discovery of the natural endogenous ligand for the GH secretagogue receptor

M. Kojima, H. Hosoda, H. Matsuo, K. Kangawa

Ghrelin (28 aa)
and also des-Gln14-ghrelin (27 aa),
another natural ligand of GHS-R,
belong to a family of homologous enterohormones including motilin (MTL),
natural ligand of the MTL-R (but not of GHS-R)

Ghrelin is the only protein with an O-linked octanoyl side group on its 3rd serine residue. This modification is crucial for ghrelin’s physiological effects.

We characterized human GOAT, the ghrelin O Acyl transferase that specifically octanoylates GRLN in Ser3. Transcripts for both GOAT and ghrelin occur mostly in stomach and pancreas.

GOAT is conserved across vertebrates and genetic disruption of the GOAT gene in mice leads to complete absence of acylated ghrelin in circulation.

The occurrence of ghrelin and GOAT in stomach and pancreas tissues demonstrates the relevance of GOAT in the acylation of ghrelin and further implicates acylated ghrelin in pancreatic function.
Purification and distribution of ghrelin: the natural endogenous ligand for the Growth Hormone Secretagogue Receptor

Kojima M, Hosoda H, Kangawa K.

RT-PCR analyses of rat ghrelin and GHS-R.
PCR templates were synthesized from 1 mg of total RNAs
Biological actions of ghrelin, a new GEP hormone

- Influence on appetite, food intake, energy balance, sleep and behaviour
- Stimulation of GH, PRL, ACTH and AVP secretion
- Inhibition of gonadotropin secretion
- Influence on gastric acid secretion and GE motility
- Modulation of cell proliferation and survival
- Influence on exocrine and endocrine pancreatic function
- Influence on cardiac cell proliferation and survival, cardiac performances and vascular resistance
- Influence on energy metabolism
- Influence on glucose and lipid metabolism
- Influence on carbohydrate metabolism
- Influence on exonuclease and endocrine pancreatic function
- Influence on energy metabolism
- Influence on glucose and lipid metabolism
- Influence on carbohydrate metabolism
- Influence on exonuclease and endocrine pancreatic function
Ghrelin and GHS action is not specific for GH

GH, PRL, ACTH and cortisol responses
to (1.0 µg/kg i.v.) acylated or unacylated ghrelin or HEX in normal young adults

- ghrelin
- des-octanoyl-ghrelin
- HEX

Time (min)
Influence of Acylated-Ghrelin on anterior pituitary hormones

**Stimulation of GH secretion**

**Stimulation of PRL secretion**

**Stimulation of ACTH secretion**

**Inhibition of gonadotropin secretion**

*(Cellular and hormonal actions in the gonads)*
The stimulatory effect of ghrelin on GH secretion is mostly mediated by actions at the hypothalamic level.

Ghrelin synergizes with GHRH and requires endogenous hypothalamic GHRH to stimulate GH secretion.

The GH-releasing action of ghrelin and synthetic GHS is refractory to either the inhibitory action of glucose, lipids, muscarinic antagonists and even somatostatin or the stimulatory action of cholinergic agonists, arginine.
Acylated-Ghrelin plays some physiological role in the control of GH secretion allowing amplification of GH pulsatility.
Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie restricted mice


PNAS, 2010

Here, we eliminate the GOAT gene in mice, thereby eliminating all octanoylated ghrelin from blood. On normal or high fat diets, Goat−/− mice grew and maintained the same weights as wild-type (WT) littermates. When subjected to 60% calorie restriction, WT and Goat−/− mice both lost 30% of body weight and 75% of body fat within 4 days. In both lines, fasting blood glucose initially declined equally. After 4 days, glucose stabilized in WT mice at 58–76 mg/dL. In Goat−/− mice, glucose continued to decline, reaching 12–36 mg/dL on day 7. At this point, WT mice showed normal physical activity, whereas Goat−/− mice were moribund. GH rose progressively in calorierestricted WT mice and less in Goat−/− mice. Infusion of either ghrelin or GH normalized blood glucose in Goat−/− mice and prevented death. Thus, an essential function of ghrelin in mice is elevation of GH levels during severe calorie restriction, thereby preserving blood glucose and preventing death.

GHSR-null mice are not anorectic dwarfs
PNAS, 2004

Deletion of Ghrelin impairs neither Growth nor Appetite
Sun Y, Ahmed S, Smith RG.
Mol Cell Biol. 2003
Ghrelin induces adiposity in rodents

M Tschöp, DL Smiley, ML Heiman

The discovery of the peptide hormone ghrelin, an endogenous ligand for the growth hormone secretagogues (GHS) receptor, yielded the surprising result that the principal site of ghrelin synthesis is the stomach and not the hypothalamus. Although ghrelin is likely to regulate pituitary growth hormone (GH) secretion along with GH-releasing hormone and somatostatin, GHS receptors have also been identified on hypothalamic neurons and in the brainstem. Apart from potential paracrine effects, ghrelin may thus offer an endocrine link between stomach, hypothalamus and pituitary, suggesting an involvement in regulation of energy balance.

We show that peripheral daily administration of ghrelin caused weight gain by reducing fat utilization in mice and rats. Intracerebroventricular administration of ghrelin generated a dose-dependent increase in food intake and body weight. Rat serum ghrelin concentrations were increased by fasting were reduced by re-feeding or oral glucose administration, but not by water ingestion.

We propose that ghrelin, in addition to its role in regulating GH secretion, signals the hypothalamus when an increase in metabolic efficiency is necessary.

Nature 2000; 407: 908-913
The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis.


Ghrelin is expressed in a previously uncharacterized group of neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei.

These neurons send efferents onto key hypothalamic circuits, including those producing NPY, AGRP, POMC products and CRH.

Within the hypothalamus, ghrelin bound mostly on presynaptic terminals of NPY neurons. Ghrelin stimulated the activity of arcuate NPY neurons and mimicked the effect of NPY in the paraventricular nucleus of the hypothalamus (PVH).

At the hypothalamic level, the release of ghrelin may stimulate the release of orexigenic peptides and neurotransmitters, thus representing a novel regulatory circuit controlling energy homeostasis.

Orexigenic Action of Peripheral Ghrelin is Mediated by NPY and Agouti-Related Protein (AgRP)

Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van Der Ploeg LH, Howard AD, MacNeil DJ, Qian S

Ghrelin, a stomach-derived orexigenic hormone, has stimulated great interest as a potential target for obesity control. Pharmacological evidence indicates that ghrelin's effects on food intake are mediated by NPY and AgRP in the CNS. These include intracerebroventricular (icv) application of antibodies to neutralize NPY and AgRP, and the application of an NPY Y1 receptor antagonist, which blocks some of the orexigenic effects of ghrelin.

Here we describe treatment of Agrp(−/−);Npy(−/−) and Mc3r(−/−);Mc4r(−/−) double knockout mice as well as Npy(−/−) and Agrp(−/−) single knockout mice with either ghrelin or an orally-active non-peptide ghrelin agonist. The data demonstrate that NPY and AgRP are required for the orexigenic effects of ghrelin, as well as the involvement of the melanocortin pathway in ghrelin signaling. Our results outline a functional interaction between the NPY and AgRP pathways.

While deletion of either NPY or AgRP caused only modest effect, ablation of both ligands abolished the orexigenic action of ghrelin.

This evidence establishes an in vivo orexigenic function for NPY and AgRP mediating the effect of ghrelin.
The gut-derived hormone ghrelin exerts its effect on the brain by regulating neuronal activity. Ghrelin-induced feeding behaviour is controlled by arcuate nucleus neurons that co-express neuropeptide Y and agouti-related protein (NPY/AgRP neurons). However, the intracellular mechanisms triggered by ghrelin to alter NPY/AgRP neuronal activity are poorly understood.

Ghrelin initiates robust changes in hypothalamic mitochondrial respiration in mice that are dependent on uncoupling protein 2 (UCP2). Activation of this mitochondrial mechanism is critical for ghrelin-induced mitochondrial proliferation and electric activation of NPY/AgRP neurons, for ghrelin-triggered synaptic plasticity of POMC-expressing neurons, and for ghrelin-induced food intake.

The UCP2-dependent action of ghrelin on NPY/AgRP neurons is driven by a hypothalamic fatty acid oxidation pathway involving AMPK, CPT1 and free radicals that are scavenged by UCP2.

These results reveal a signalling modality connecting mitochondria-mediated effects of GPC receptors on neuronal function and associated behaviour.
**GHSR-null mice are not anorectic dwarfs**
*PNAS, 2004*

**Deletion of Ghrelin impairs neither Growth nor Appetite**
Sun Y, Ahmed S, Smith RG.
*Mol Cell Biol. 2003*

**Mice lacking ghrelin receptors resist the development of diet-induced obesity**
*JCI 2005*

**Deletion of ghrelin reveals no effect on food intake but a primary role in energy balance**
*Obes Res. 2004*

**Simultaneous deletion of ghrelin and its receptor increases motor activity and energy expenditure**
P. Pfluger, H. Kirchner, S. Gündel, B. Schrott, D. Perez-Tilve, S. Fu, S. Benoit, T. Horvath, K. Wortley, M. Sleeman, M. Tschöp
*Am J Physiol, 2007*
The brain integrates long-term energy balance. Peripheral signals relating to long-term energy stores are produced by adipose tissue (leptin) and the pancreas (insulin). Feedback relating to recent nutritional state takes the form of absorbed nutrients, neuronal signals, and gut peptides. Neuronal pathways, primarily by way of the vagus nerve, relate information about stomach distention and chemical and hormonal milieu in the upper small bowel to the NTS within the dorsal vagal complex (DVC). Hormones released by the gut have incretin-, hunger-, and satiety-stimulating actions. The incretin hormones GLP-1, GIP, and potentially OXM improve the response of the endocrine pancreas to absorbed nutrients. GLP-1 and OXM also reduce food intake. Ghrelin is released by the stomach and stimulates appetite. Gut hormones stimulating satiety include CCK released from the gut to feedback by way of vagus nerves. OXM and PYY are released from the lower gastrointestinal tract and PP is released from the islets of Langerhans.
Ghrelin Treatment Causes Increased Food Intake and Retention of Lean Body Mass in a Rat Model of Cancer Cachexia


Ghrelin agonism would represent an effective anabolic tool to treat cachexia.

Endocrinology, 2007
Ghrelin enhances appetite and increases food intake in humans

Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillon WS, Ghatei MA, Bloom SR.

One major dream is still that Ghrelin antagonism would represent an effective tool to treat obesity
Ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion

Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S.

FIG. 2.

A, Comparison of plasma ghrelin concentrations in anorexia nervosa patients (BMI 18.5), healthy controls (18.5 BMI 25), and simple obesity patients (BMI 25).

B, Negative correlation between plasma ghrelin concentration and BMI.
Ghrelin secretion is mostly under metabolic control

Ghrelin levels are increased by
- energy restriction
- acetylcholine

Ghrelin levels are decreased by
- feeding
- glucose load
- insulin
- hypoglycemia
- somatostatin
Ghrelin, the only identified circulating orexigenic signal, is unique in structure in which a specific acyl-modification of its third serine occurs. This acylation is necessary for ghrelin to bind to its receptor and to exert its biologic activity, which is catalyzed by ghrelin GOAT. Although ghrelin is mainly secreted from gastric X/A like endocrine cells, it is also expressed in pancreatic islet cells and regulates insulin secretion. We studied the expression and regulation of GOAT in pancreas.

**Insulin inhibits the expression of GOAT mRNA and GOAT promoter activity in a dose and time-dependent manner.**

The mammalian target of rapamycin (mTOR) is activated by insulin. Blocking mTOR signaling by either rapamycin or overexpression of its negative regulator tuberous sclerosis complex 1 (TSC1) or TSC2 attenuates the inhibitory effect of insulin on the transcription and translation of GOAT.

**GOAT is present in pancreatic islet cells and insulin inhibits the GOAT expression via mTOR signaling.**
The orexigenic effect of (acylated) ghrelin and its ability to induce adiposity obviously predict, at least indirect, metabolic actions

... however, ghrelin exerts also relevant direct metabolic actions!!

Evidence for a “diabetogenic” action of synthetic GHS and acylated ghrelin

Synthetic GHS stimulate the HPA axis and are diabetogenic in the Zucker diabetic fatty rat (Clark et al, Endocrinology 1997)

Prolonged daily oral administration of a GH secretogogue (MK-677) in healthy elderly subjects produced significant increases in fasting glucose and worsened insulin sensitivity (Chapman et al, JCEM 1996)

Ghrelin counteracts the inhibitory effect of insulin on gluconeogenesis from hepatoma cells (Murata et al, JBC 2002)

Ghrelin inhibits insulin secretion stimulated by glucose, arginine and carbachol from the perifused rat pancreas (Egido et al, EJE 2002)

Ghrelin induces hyperglycemia in humans (Broglio et al, JCEM 2002)
Octanoyl-ghrelin (1.0 µg/kg i.v.) increases glucose and reduces insulin levels in humans.

Acylated-GHRELIN
Non Acylated-GHRELIN
Saline
Blockade of Pancreatic Islet–Derived Ghrelin Enhances Insulin Secretion to Prevent High-Fat Diet–Induced Glucose Intolerance


**FIG. 2.** Insulinostatic effects of endogenous ghrelin in perfused pancreas.

A: Blockade of GHSR by [D-Lys3]GHRP-6 (1 mol/l) enhanced glucose (8.3 mmol/l)-induced insulin release in perfused rat pancreas, whereas exogenous ghrelin (10 nmol/l) administration inhibited it (n = 6–9).

B: Immunoneutralization of endogenous ghrelin using an anti-ghrelin antiserum (0.1%) enhanced glucose (8.3 mmol/l)-induced insulin release in perfused rat pancreas (n = 3–4).

Diabetes, 2006
Ghrelin Suppresses Glucose-Stimulated Insulin Secretion and Deteriorates Glucose Tolerance in Healthy Humans


The orexigenic gut hormone ghrelin and its receptor are present in pancreatic islets. Although ghrelin reduces insulin secretion in rodents, its effect on insulin secretion in humans has not been established. The goal of this study was to test the hypothesis that circulating ghrelin suppresses glucose-stimulated insulin secretion in healthy subjects.

The three ghrelin infusions raised plasma total ghrelin concentrations to 4-, 15-, and 23-fold above the fasting level, respectively. Ghrelin infusion did not alter fasting plasma insulin or glucose, but compared with saline, the 0.3, 0.9, and 1.5 nmol/kg/h doses decreased AIRg (2,152 ± 448 vs. 1,478 ± 2,889, 1,419 ± 275, and 1,120 ± 174 pmol/l) and Kg (0.3 and 1.5 nmol/kg/h doses only) significantly (P < 0.05 for all). Ghrelin infusion raised plasma growth hormone and serum cortisol concentrations significantly (P < 0.001 for both), but had no effect on glucagon, epinephrine, or norepinephrine levels (P = 0.44, 0.74, and 0.48, respectively).

Exogenous ghrelin reduces glucose-stimulated insulin secretion and glucose disappearance in healthy humans. Our findings suggest that ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve Beta-cell function.
Effects of acylated ghrelin (AG, 0.5 µg/Kg/h i.v. over 16 h) on GH, insulin, glucose and FFA profiles in normal subjects

The continuous infusion of acylated ghrelin is followed by:

- Increase in GH pulsatility
- Increase in the glucose response to meals
- Reduction in the early but increase in the late insulin response to meals
- Reduction in overnight FFA levels
The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas


GRLN expression occurs in a separate population of islet cells in human fetal, neonatal, and adult pancreas. In fetuses, GRLN cells in pancreas outnumber those in stomach. GRLN mRNA in pancreas precedes by far that in stomach. The pancreas is the major ghrelin source in fetal life.

Ghrelin cells replace insulin-producing β-cells in mouse models of pancreas development


Normal mouse pancreas contains a population of ghrelin-producing cells, a new islet "epsilon" cell population.

Nkx2.2 mutant endocrine cells have been replaced by cells that produce ghrelin. The expansion of ghrelin-producing cells at the expense of beta cells may be a general phenomenon, because Pax4 mutant mice display similar phenotype. It is proposed that insulin and ghrelin cells share a common progenitor and that Nkx2.2 and Pax4 are required to specify or maintain differentiation of the beta cell fate.

There is a genetic component underlying the balance between insulin and ghrelin in regulating glucose metabolism.
We generated Tg mice overexpressing a ghrelin analog, which possessed ghrelin-like activity in the absence of acylation at Ser3 and could be synthesized in vivo.

As the replacement of Ser3 of ghrelin with Trp3 (Trp3-ghrelin) preserves a low level of ghrelin activity and Trp3-ghrelin can be synthesized in vivo, we generated mice overexpressing Trp3-ghrelin by using the hSAP (human serum-amyloid-P) promoter. Plasma Trp3-ghrelin concentrations in the Tg mice were approximately 85-fold higher than plasma ghrelin concentrations in non-Tg littermates. Because Trp3-ghrelin is approximately 1/10–1/20 less potent than ghrelin in vivo, plasma Trp3-ghrelin concentrations in Tg mice were calculated to have an activity approximately 6-fold greater than that of acylated ghrelin seen in non-Tg mice (85-fold x 1/10–1/20).

Tg mice exhibited normal growth and glucose metabolism in their early life stage. However, 1-yr-old Tg mice showed impaired glucose tolerance and reduced insulin sensitivity.

FIG. 3. Analysis of Trp3-ghrelin Tg mice.
A, Changes of body weight. B, Body fat percentage and lean body mass. C, Glucose tolerance test (0.75 g/kg). D, Serum insulin levels at baseline, 2 min, and 30 min after ip glucose injection. E, Insulin tolerance test after treatment with 1.5 U/kg regular insulin. F, Insulin 1 mRNA levels in the pancreases.
Mice lacking ghrelin receptors resist the development of diet-induced obesity


Table 3
Blood glucose levels and corresponding serum insulin levels of male GHSR-null mice and wild-type mice after 19 weeks on a standard chow diet

<table>
<thead>
<tr>
<th>Study group</th>
<th>During light cycle</th>
<th>2–5 h after end of dark cycle</th>
<th>5–8 h after end of dark cycle</th>
<th>8–11 h after end of dark cycle</th>
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<tr>
<td><strong>Blood glucose (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT mice</td>
<td>156 ± 5 (18)^A</td>
<td>159 ± 8 (6)</td>
<td>150 ± 6 (9)</td>
<td>171 ± 10 (3)</td>
</tr>
<tr>
<td>GHSR-null mice</td>
<td>140 ± 4 (21)^B</td>
<td>147 ± 6 (10)</td>
<td>132 ± 7 (6)^C</td>
<td>137 ± 8 (5)^B</td>
</tr>
<tr>
<td><strong>Serum insulin (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT mice</td>
<td>0.778 ± 0.115</td>
<td>1.002 ± 0.190</td>
<td>0.648 ± 0.167</td>
<td>0.797 ± 0.33</td>
</tr>
<tr>
<td>GHSR-null mice</td>
<td>0.523 ± 0.106</td>
<td>0.474 ± 0.135^B</td>
<td>0.476 ± 0.205</td>
<td>0.715 ± 0.29</td>
</tr>
</tbody>
</table>

^AThe number of samples tested for each study group are noted in parentheses. ^BStatistically lower value than for wild-type controls; P < 0.05. ^CStatistically lower value (trend) than that for wild-type controls; 0.05 ≤ P < 0.1.
Characterization of the insulin sensitivity of GRLN-R KO mice using glycemic clamps


The improved glucose homeostasis of GhrR KO mice is characterized by improvements of glucose disposal relative to WT controls.

GhrR KO mice have intact 1st phase insulin response but require less insulin for glucose disposal.

Our experiments reveal that the insulin sensitivity of GhrR KO mice is due to both BW independent and dependent factors. We also provide several lines of evidence that a key feature of the GhrR KO mouse is maintenance of hepatic insulin sensitivity during metabolic challenge.
Ablation of ghrelin improves the diabetic but NOT the obese phenotype of ob/ob mice

That ghrelin KO has positive impact on glucose metabolism and insulin sensitivity indicates that the predominant influence of the ghrelin system is “diabetogenic”

Blockade of Pancreatic Islet–Derived Ghrelin Enhances Insulin Secretion to Prevent High-Fat Diet–Induced Glucose Intolerance


FIG. 4. Ghrelin knockout (Ghr-KO) mice display increased insulin and decreased glucose levels

A: Acylated ghrelin was absent in ghrelin KO
B and C: The number and the size of islets on pancreatic sections were not different between WT and ghrelin KO mice

D: Glucose-induced insulin release was enhanced in ghrelin KO islets

E and F: Islet insulin (E) contents and mRNAs expressions (F) of insulin were not different between wild-type and ghrelin KO mice

G and H: In GTTs ghrelin KO mice exhibited attenuated elevations of blood glucose (G) and enhanced insulin levels (H) in comparison to WT

I: Glucose levels during the ITT did not differ between ghrelin KO and WT mice

Diabetes, 2006
Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite and promote weight loss


Figure 3: In vivo characterization of the effects of YIL-870 on glucose tolerance and weight loss.

a) Structure of YIL-870.

b) Effect of YIL-870 in rat IP glucose tolerance test. YIL-870 (10 mg/kg) or vehicle was dosed orally. 5 hours later, a 2 g/kg glucose challenge was administered by I.P. injection. Relative to vehicle, YIL-870 produced a 17% decrease in glucose AUC over the course of the experiment.

c) Effect of YIL-870 (10 mg/kg) on body weight in DIO mice. Rimonabant (3 mg/kg) served as a positive control.

d) Effect on food intake from experiment in panel.

e) Effect on body composition from experiment in panel c).

f) Effect of YIL-870 dose-response on body weight in DIO mice.
Circulating ghrelin levels

Is it reasonable that Nature is so stupid to produce so much of something useless?
Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans

Insulin and glucose variations after acylated ghrelin (AG), Non-acylated ghrelin (UAG) and AG+UAG administration (1.0 µg/kg each)
Unacylated ghrelin decreases glucose output from primary pig hepatocytes and even counteracts the increase of glucose output induced by acylated-ghrelin and glucagon.

- Control vs UAG: p<0.001
- AG vs control: p<0.001
- AG + UAG vs AG: p<0.001
- Glucagon vs control: p<0.001
- Glucagon + UAG vs glucagon: p<0.05
Des-acyl ghrelin and acyl ghrelin prevent either serum starvation- or cytokine-induced apoptosis in human pancreatic islet cells

Granata R et al., Endocrinology 2007
Ghrelin prevents β-cell destruction in the pancreas of STZ-treated rats

In streptozotocin treated rats, the pretreatment with ghrelin either acylated or not:

- increases pancreatic synthesis and secretion of insulin
- by saving the β-cells, antagonizes the diabetogenic action of STZ i.e. normalizes glucose levels

Granata R et al., 2008
The metabolic actions of non-acylated ghrelin would be mediated by a GRLN receptor still unknown.

This is a NON GHS-R1a receptor !!!

Effects of unacylated ghrelin (UAG, 1.0 µg/Kg/h i.v. over 16 h) on GH, insulin, glucose and FFA profiles in normal subjects

The continuous infusion of Non-acylated ghrelin is followed by:

- Decrease in overnight glucose levels
- Quicker insulin response to meals
- Reduction in overnight FFA levels
Effect of des-acyl ghrelin on adiposity and glucose metabolism

W. Zhang, B. Chai1, J. Li, H. Wang, M.W. Mulholland
Endocrinology, 2008

Epididymal and perirenal fat masses decreased 35±9% and 52±9% respectively in FABP4-ghrelin transgenic mice.

FABP4-ghrelin transgenic mice are resistant to obesity induced by high fat diet. Brown fat mass was not affected by over-expression of ghrelin in adipose tissue.

Glucose tolerance tests showed glucose levels to be significantly lower in FABP4-ghrelin transgenic mice than in controls after glucose administration.

Insulin sensitivity testing showed that FABP4-ghrelin transgenic mice had a 28±5% greater hypoglycemic response to insulin.

Our study demonstrates that over-expression of des-acyl ghrelin from the FABP4 promoter impairs the development of white adipose tissues and improves glucose tolerance and insulin sensitivity in mice.
We determined the cell expression profile of GRLN and GOAT in stomach and pancreas; also we analyzed the metabolic phenotype of GOAT KO mice.

GOAT is expressed mainly in GRLN+ cells indicating that GRLN is a critical substrate for GOAT and that des-acyl GRLN in circulation likely does not result from GRLN positive cells lacking GOAT. We also observed an increase in the number and a change in the distribution of GRLN and GOAT+ cells in a subset of islets from diabetics, further implicating locally acylated GRLN in pancreatic function.

Metabolic phenotyping of GOAT deficient mice and WT littermates after chronic exposure to a medium chain triglyceride rich diet that triggers GRLN acylation in WT led to a significantly lower body weight mostly reflecting diminished fat mass of GOAT KO mice compared to WT.

GOAT KO had positive impact on glucose metabolism and insulin sensitivity indicating a major role for non acylated ghrelin in the control of glucose metabolism.
Ghrelin is a gastric peptide hormone that stimulates weight gain in vertebrates. The biological activities of ghrelin require octanoylation of the peptide on Ser3, an unusual posttranslational modification that is catalyzed by the enzyme ghrelin O-acyltransferase (GOAT).

We show the design, synthesis, and characterization of GO-CoA-Tat, a peptide-based bisubstrate analog that antagonizes GOAT.

GO-CoA-Tat potently inhibits GOAT in vitro, in cultured cells, and in mice.

I.P. administration of GO-CoA-Tat improves glucose tolerance and reduces weight in wild-type mice but not in ghrelin-deficient mice, suggesting that its beneficial effects are specifically due to GOAT inhibition.

WT mice treated with GO-CoA-Tat showed (A) increase in insulin secretion and (B) decrease in blood glucose after intraperitoneal glucose challenge.
The relative excess of acylated GRLN (or non acylated GRLN deficit ?) would contribute to obesity-associated insulin resistance in metabolic syndrome and diabetes type 2.

Acylated ghrelin levels were increased, whereas desacyl ghrelin levels were decreased, in obesity and obesity-associated T2D.

BMI, WHR, insulin and HOMA index correlated positively with acylated but negatively with non acylated ghrelin levels.

Given the lipogenic effect of acylated ghrelin, the elevation of its concentrations in obese individuals may play a role in excessive fat accumulation in obesity.

Int J Obesity, 2009
Acylated ghrelin exerts strong, negative impact on glucose metabolism that is, in turn, positively modulated by Non Acylated ghrelin

Inhibition of acylated ghrelin by GRLN antagonists or GOAT inhibitors or non acylated ghrelin (or its fragments or analogues) improves insulin secretion and sensitivity, glucose and lipid metabolism and would have clinical perspectives for the treatment of diabetes mellitus and metabolic syndrome
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