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Stress-related alterations of acyl and desacyl ghrelin circulating levels: mechanisms and functional implications

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Abstract

Ghrelin is the only known peripherally produced and centrally acting peptide hormone that stimulates food intake and digestive functions. Ghrelin circulates as acylated and desacylated forms and recently the acylating enzyme, ghrelin-*O*-acyltransferase (GOAT) and the de-acylating enzyme, thioesterase 1/lysophospholipase 1 have been identified adding new layers of complexity to the regulation of ghrelin. Stress is known to alter gastrointestinal motility and food intake and was recently shown to modify circulating ghrelin and GOAT levels with differential responses related to the type of stressors including a reduction induced by physical stressors (abdominal surgery and immunological/endotoxin injection, exercise) and elevation by metabolic (cold exposure, fasting and caloric restriction) and psychological stressors. However, the pathways underlying the alterations of ghrelin under these various stress conditions are still largely to be defined and may relate to stress-associated autonomic changes. There is evidence that alterations of circulating ghrelin may contribute to the neuroendocrine and behavioral responses along with sustaining the energetic requirement needed upon repeated exposure to stressors. A better understanding of these mechanisms will allow targeting components of ghrelin signaling that may improve food intake and gastric motility alterations induced by stress.

Keywords

abdominal surgery; food intake; ghrelin-*O*-acyltransferase (GOAT); motility; somatostatin 2 receptor

1. Introduction

The stress response is induced by exposure to stressors of either physical/immunological, emotional/psychological or of mixed origin that threaten the homeostasis [20]. Hallmarks of the neuroendocrine and autonomic efferent components of the stress response are the

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activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system. This occurs largely through the activation of cortical limbic and pontine pathways involved in the release of corticotropin releasing factor (CRF) from parvocellular neurons in the paraventricular nucleus of the hypothalamus [20, 57]. Among functional alterations induced by acute or chronic stress exposure are those occurring at the level of gastrointestinal propulsive motor functions along with the reduction of caloric intake which can be recapitulated by central and peripheral injection of CRF [111, 112].

Ghrelin is a 28-amino acid peptide hormone that was identified more than a decade ago and shown to be the endogenous ligand of the growth hormone (GH) secretagogue receptor 1a (GHS-R1a) [62] presently renamed ghrelin (GRLN) receptor [27]. Ghrelin is unique among peptides due to its acylation with medium-chain fatty acids at the serine-3 residue which is essential for many of its biological actions to stimulate GH release, food intake, gut motility and to elicit anti-inflammatory responses in rodents and humans [63]. This esterification process has only recently been deciphered and involves the enzyme ghrelin-*O*-acyltransferase (GOAT), a member of the superfamily of membrane-bound *O*-acyltransferases (MBOATs), namely MBOAT4 [44, 134]. In human and rodent tissues, expression of GOAT mRNA is the highest in stomach and pancreas followed by the intestine (duodenum, jejunum, ileum and colon) [1, 44, 134]. GOAT mRNA expression is also observed in other endocrine tissues such as the pituitary gland [34] and testis [134] and there is *in vitro* evidence for cellular expression of GOAT in chondrocytes [41] and circulating monocytes [54]. At the subcellular level, MBOATs, and possibly also GOAT, is localized in the endoplasmic reticulum [44, 134].

The unacylated peptide, desacyl ghrelin represents by far the most abundant form of circulating ghrelin as indicated by an acyl/total ghrelin ratio that varies from 1:55 to 1:5 depending upon the method of blood processing to prevent the rapid degradation of the acylated form in tissues and the circulation by endogenous esterase activity [29, 50, 92, 99, 110]. Desacyl ghrelin does not activate the GRLN receptor, therefore it was initially presumed to be inactive [8]. However, there is evidence *in vitro* and *in vivo* that desacyl ghrelin plays a role in the development of adipocytes and maintenance of energy homeostasis [115, 137]. Moreover, recent convergent pharmacological studies indicate the unique activity of desacyl ghrelin to oppose acyl ghrelin's stimulatory effects on food intake [51, 106], gut motility [47] and pancreatic polypeptide secretion [67], while alone several reports showed either no [47, 51, 67, 106] and few studies an inhibitory effect on these functions [3, 16]. These findings point towards a relevant implication of the acyl/desacyl ghrelin ratio in determining the overall physiological response [65] and the importance of specific assays measuring both forms simultaneously [72] using stringent conditions of sample collection to prevent acyl ghrelin cleavage [72, 110].

The majority of circulating forms of ghrelin is produced by ghrelin positive X/A-like cells that are located in the epithelium of both oxyntic and antral mucosa of the rodent stomach and P/D1 enteroendocrine cells of the gastric fundus in humans [25, 84] as indicated by the pronounced reduction of circulating ghrelin after gastrectomy [2, 56]. Using specific antibodies against acyl and desacyl ghrelin, immunofluorescent double staining showed that acyl and desacyl ghrelin overlap in closed-type round cells, whereas desacyl ghrelin is also found in open-type cells which extend cytoplasmatic processes toward the gastric lumen and are positive for somatostatin and negative for acyl ghrelin [84]. Limited amounts of ghrelin are also produced in the small and large intestine [25], pancreas [26] and other peripheral viscera such as kidney, liver, heart, testis, adipose tissue and skin [6, 39]. In addition, ghrelin is synthesized locally in the hypothalamus where ghrelin immunoreactive neurons have been described in the arcuate nucleus [58, 73] and adjacent to the third ventricle [22].

The regulation of gastric and circulating forms of ghrelin has been initially documented largely in relation with the nutritional status (for review see [64]). Recently, alterations of circulating ghrelin by environmental and visceral stressors [104] and the potential role of ghrelin in the stress response have received growing attention [21, 88]. Therefore, in the present review we provide the state-of-knowledge on changes of the ghrelin signaling system induced by exposure to various stressors of either physical/immunological, metabolic or psychological origin in rodents and the clinical setting, the potential underlying mechanisms involved and functional implications of an altered ghrelin signaling system in the stress response.

2. Influence of various stressors on ghrelin expression

2.1. Changes of gastric ghrelin synthesis

Early on, the acute metabolic stress of fasting was established to increase gastric ghrelin mRNA expression in mice [131] and rats [60, 117]. This was associated with decreased gastric ghrelin peptide content linked with increased ghrelin secretion resulting in elevated circulating ghrelin levels and reversal of all these changes by feeding [60, 117]. The mechanisms that drive the fasting-induced increased ghrelin synthesis and inhibition upon re-feeding may be linked to changes in insulin status under these conditions as shown by the inverse correlation between changes in circulating levels of insulin and those of circulating and gastric ghrelin and ghrelin changes in response to insulin [83, 125].

Exposure to prolonged stressors such as daily 90-min restraint stress for 5 days in rats [140] or tail pinch stress (10 min every 4 h for 24 h) in fasted mice [4] also increased gastric ghrelin mRNA expression while no change was observed on day 1 in rats [140]. These data clearly indicate that repeated and sustained stressors such as fasting, restraint and tail pinch are associated with up-regulation of ghrelin synthesis in the rodent stomach which may account for the elevated circulating ghrelin levels under these stress conditions (Table 1).

2.2. Changes of circulating ghrelin levels

Several stressors have been described to influence plasma levels of ghrelin and response patterns are largely specific to the category of stressors (Table 1).

Endotoxin—Intraperitoneal (ip) injection of lipopolysaccharide (LPS) is widely used to study the innate defense response to Gram-negative bacterial infection [70] and a well established stress model to activate the HPA axis [38, 122]. We used LPS as an immunological stressor and injected LPS (*Escherichia coli*, serotype 055:B5) intraperitoneally at 100 µg/kg, which is a dose mimicking some of the clinical features of acute Gram-negative infections such as mild increase in body temperature, reduction of appetite, delayed gastric transit and metabolic alterations (decreased insulin and increased glucose plasma levels) without inducing severe sickness found in endotoxin shock [69, 70, 125]. Under these conditions, LPS strongly reduces fasting plasma levels of total ghrelin levels in conscious rats [7, 105, 125]. Time course studies in fasted rats showed that LPS induces a rapid onset (within one hour) and linear time-related decrease of total ghrelin (acyl plus desacyl ghrelin) and acyl ghrelin plasma levels reaching a peak inhibition at 5 h with a return to basal after 24 h post injection [105, 125]. Of interest was the fact that fasting acylated ghrelin levels decreased more rapidly than desacyl ghrelin mainly at 2 h post LPS injection leading to an acyl/desacyl ghrelin ratio of 1:5 instead of 1:3 [105]. Subsequently, other investigators likewise reported that ip injection of LPS at 100 µg/kg reduced fasting plasma levels of total ghrelin to those observed in the fed state as monitored at 3–12 h post injection in rats [36, 45, 77]. Studies in healthy human subjects fasted overnight showed that intravenous injection of LPS at 2 ng/kg induces a biphasic response with a rapid surge at 2 h

post injection followed by a continuous decline reaching significance at 5–6 h post injection [124]. Likewise, under conditions of a Gram-negative bacterial infection by *Helicobacter pylori*, levels of circulating ghrelin are lowered in infected individuals compared to healthy human subjects and eradication of the bacteria restores ghrelin [55].

By contrast, under conditions of septic shock there was an elevation of ghrelin levels as monitored throughout the 1 – 48 h period post injection of endotoxin at 1 mg/kg in dogs fasted overnight [135], at 18 h post cecal ligation and perforation in fasted rats [14] and during the 1–3 day period after clinical signs of sepsis in humans [81]. Since sepsis reduces ghrelin clearance [129] this may contribute to the elevated levels of ghrelin, although specific mechanisms stimulating ghrelin synthesis and release or alterations in ghrelin esterase activity [29] under these septic conditions cannot be ruled out.

Abdominal surgery—With regard to visceral stressors, abdominal surgery (laparotomy and 1 min cecal palpation), induced a rapid and long-lasting decrease in fasted plasma acyl and desacyl ghrelin levels observed at 0.5, 2 and 5 h post surgery with levels partly recovered at 7 h and fully restored at 24 h post procedure in fasted rats [105, 109]. In humans, in contrast, one group of investigators reported that patients undergoing surgery showed no changes in total ghrelin plasma levels during the first 6 h and a transient increase at 24 h post resection of the colon compared to preoperative levels [82]. However, in this study it is difficult to dissect whether the changes or lack of change reflect the metabolic and pathologic conditions of the subjects and/or species differences. Further clinical studies are also needed to ascertain whether surgery impacts on the acyl form of ghrelin levels since there is evidence in human subjects that stress can significantly influence circulating acyl ghrelin levels which are not reflected when only total ghrelin is monitored [100].

Physical exercise—Recent studies showed that strenuous physical cycling exercise for 60 min at 50% of $\text{VO}_{2\text{max}}$ in healthy subjects results in a linear time-related decline in fasted levels of acyl ghrelin while the change of desacyl and total ghrelin plasma levels did not reach significance as measured at various time points during the 60-min exercise period [100]. The dissociation between changes in magnitude or patterns of acyl and desacyl ghrelin plasma levels induced at 2 h after abdominal surgery in fasted rats and exercise in fasted human subjects along with the differential response to intragastric luminal pH from *ex vivo* perfused rat stomach [84] supports the assumption of distinct mechanisms regulating acyl *versus* desacyl ghrelin secretion and/or acylation under these conditions and the importance of measuring both ghrelin forms in addition to total ghrelin as recently recognized [72].

Environmental factors—There is consistent evidence that - in contrast to immune, visceral or strenuous physical stressors - exposure to various modalities of psychological/ environmental stressors elevates ghrelin plasma levels in experimental animals and humans (Table 1). An acute (60 min) or continuous (over 5 days) psychological stressor of water avoidance stress or acute exposure to cold (physical stressor) increases plasma acyl [87, 104] and total [66, 87, 104] ghrelin levels in rats fed *ad libitum* or in a fasted state. Likewise, repeated 90-min restraint stress/day over 5 days increased circulating acyl ghrelin levels on days 3 and 5 compared to non-stressed controls in rats while a non-significant trend was observed on day 1 [140]. In addition, subjecting mice to 10 daily bouts of social defeat by aggressive CD1 male mice for a 10-day period, as a model of induced depression, increased circulating acyl ghrelin levels at day 11 which persisted for at least four weeks after the last defeat [76]. Other modalities of chronic unpredictable heterotypic stressors by exposing mice to two 15-min daily sessions of either restraint, predator scent, novel aversive environment, aggressive male, noise or open field for 14 days results in a significant elevation of acyl ghrelin monitored at 5 min after the last exposure to restraint stress [88].

Likewise, in humans the Trier Social Stress Test consisting of an interview in front of three investigators and giving a speech which is videotaped, moderately increased plasma total ghrelin levels [93, 96]. In addition, a 30-min cold exposure in healthy 12-h fasted subjects induces a significant rise in plasma ghrelin levels [116].

2.3. Changes of gastric and circulating GOAT expression

Since GOAT is the only enzyme that octanoylates ghrelin and largely restricted to the stomach [44, 134, 138], the regulation of gastric GOAT mRNA expression by various stressors has been recently investigated to get insight into the relationship with changes in the acyl/desacyl ratio. So far, data are available mainly in relation with the influence of metabolic status, immunological challenge (LPS) or surgical stress on GOAT expression in rodents.

It is well established that the anticipation of food [24, 121] and overnight fasting elevates circulating ghrelin levels in rodents and humans [72, 120, 128]. When blood is processed using a method that increases the recovery of acyl ghrelin by 80% compared with the standard method, overnight fasting induced a similar rise in acylated and desacyl ghrelin resulting in an unchanged acyl/desacyl ghrelin ratio in rats [110] and human subjects [72]. However, studies on the regulation of GOAT under conditions of fasting have resulted in divergent data in rats. In one study, 48-h fasting did not change gastric GOAT mRNA levels but 21 days of 70-% caloric restriction led to a marked increase of gastric GOAT mRNA expression [43]. Another study showed a decreased gastric GOAT mRNA expression along with reduced stomach ghrelin mRNA levels following 35% caloric restriction for five weeks [94]. Reports in mice indicate a significant increase of gastric [34, 131] as well as pituitary and hypothalamic GOAT mRNA expression following 24 – 48 h fasting [34], whereas others demonstrated a decrease under conditions of fasting for 13, 24 and 36 h [61]. One factor contributing to these divergent results may be the different experimental conditions of caloric deprivation leading to differential loss of body fat stores and liver glycogen. Indeed, a recent study in GOAT knockout mice indicates that the key function of acyl ghrelin under conditions of sustained caloric restriction is to elevate GH secretion to maintain normal blood glucose levels [138]. Therefore, acylation of ghrelin by GOAT may be tightly regulated by the extent of caloric restriction impacting on glucose and fat stores [138]. In addition, another study described activation of the GOAT-ghrelin signaling system by fatty acid availability leading to the hypothesis of GOAT being a nutrient, especially lipid, sensor [61]. At the protein level, we recently reported that plasma GOAT protein concentration increased after 24-h fasting in rats and more prominently in mice [107]. However, the role of circulating GOAT and mechanisms of action involved in acylation of desacyl ghrelin are still largely unknown.

With regards to immunological stress, LPS injected intraperitoneally at 100 µg/kg in fasted rats induces a decrease in the acyl/desacyl ghrelin ratio at 2 h post injection that was associated with a 38% decrease in plasma GOAT protein concentrations and a slight (10%) increase in gastric GOAT protein levels, whereas gastric GOAT mRNA levels were not altered [105]. These results suggest that the inhibition of GOAT protein by LPS may contribute to the faster drop of acylated ghrelin. Interestingly, LPS also decreased GOAT mRNA expression *in vitro* in murine chondrogenic ATDC-5 cells as well as in immortalized human juvenile costal chondrocyte cells T/C-28a2 [41] indicating a direct effect of LPS on GOAT expressing cells. In addition to the reduced acylation of desacyl ghrelin that may have a bearing with the reduction of circulating GOAT protein, an increased de-acylation may contribute to the fast drop of acyl ghrelin. Recently, the ghrelin de-acylating enzyme acyl-protein thioesterase 1/lysophospholipase 1 has been identified and the release of this enzyme from macrophage-like cells was increased *in vitro* after stimulation with LPS [98].

This finding is in line with increased ghrelin de-acylase activity in sera obtained from rats treated with LPS [98].

With regards to surgical stress, abdominal surgery (laparotomy plus 1 min cecal palpation) that induced a more pronounced drop of acylated than desacyl ghrelin at 2 h post surgery associated with a decreased acyl/desacyl ghrelin ratio, also reduced gastric and plasma GOAT protein concentrations [109]. Taken together, these results suggest that decreased gastric and plasma GOAT levels under conditions of an acute physical/immune stressor may also be part of the mechanisms involved in the reduction of acyl ghrelin plasma concentrations under these conditions. However, additional studies are needed to examine the regulation of GOAT expression under different metabolic and stress conditions, preferably at the protein level. It will also be relevant to investigate whether GOAT activity that can be assessed by the use of catalytic assay based on enzyme-linked click-chemistry (cat-ELCCA) [35] is also altered under conditions of increased or decreased acyl ghrelin expression induced by different stressors.

3. Underlying mechanisms of stress-related alterations of circulating ghrelin

Circulating ghrelin can be regulated by both the central nervous system as well as by direct mechanisms occurring at the level of the gastric mucosa. These pathways provide potential mechanisms through which various stressors alter circulating ghrelin although they remain largely to be explored.

3.1. Autonomic nervous system

Electrical stimulation of gut sympathetic nerves not only elevates portal noradrenalin levels but also robustly increases portal ghrelin concentration in anesthetized, overnight fasted rats [85]. Similarly, intravenous injection of the catecholamine releasing agent, tyramine also elevated circulating ghrelin levels in rats [85]. However, when infused systemically, adrenalin did not alter circulating ghrelin levels [85] suggesting a direct neural mode of action in the sympathetically mediated ghrelin secretion. By contrast, in isolated rat stomach preparations, submucosal microinfusion of adrenaline and noradrenaline increased ghrelin secretion as well [28] which may point towards direct activation of ghrelin-containing cells by hormones released from sympathetic nerves. These results were corroborated by another study showing that a β -adrenergic agonist, isoproterenol increased plasma ghrelin levels in rats [49]. A direct effect on ghrelin cells is supported by recent *in vitro* data showing that cultured ghrelinoma cells express β_1 -adrenergic receptor mRNA and treatment with noradrenalin or adrenalin stimulates ghrelin secretion, an effect blocked by the β_1 -adrenergic receptor antagonist, atenolol [139]. Moreover, when mice were treated with reserpine, an agent to deplete adrenergic neurotransmitters from sympathetic neurons, ghrelin plasma levels did not show the expected rise after fasting [139]. These data point to a physiological role of the sympathetic nervous system in the stimulation of ghrelin release under fasted conditions.

In addition, ghrelin secretion is under the positive control of cholinergic mechanisms. Bethanechol, a muscarinic receptor agonist injected subcutaneously increased plasma ghrelin levels in rats [49] and humans [11], whereas cholinergic withdrawal by vagotomy acutely reduces circulating ghrelin levels in rats [49]. Similarly, the muscarinic receptor antagonist atropine reduces fasting plasma ghrelin levels in rats [49], a finding that was confirmed in humans [78, 79]. We recently used acute cold exposure in rats that activates neurons of the dorsal motor nucleus of the vagus and gastric vagal efferent activity through brainstem thyrotropin-releasing hormone (TRH) signaling, a pathway which is also involved

in the cephalic phase of gastric secretion [113]. Exposure to cold ambient temperature for 1–3 h induces an increased TRH mRNA expression in the medulla oblongata in TRH-synthesizing neurons of the raphe pallidus, raphe obscurus and parapyramidal region compared to animals kept at normal ambient temperature [132, 133]. Cold ambient temperature also increased the expression of the neuronal marker Fos [97] in TRH-containing neurons of the raphe pallidus, raphe obscurus and the parapyramidal region along with their projection sites including the nucleus of the solitary tract and dorsal motor nucleus of the vagus nerve compared to animals kept at regular room temperature [10, 80, 126]. In addition, functional studies showed that blockade of brain medullary TRH signaling pathways blocked the vagally mediated stimulation of gastric secretory and motor function [113]. Using this model of brain-medullary TRH gastric vagal activation, we found that cold exposure or intracisternal injection of a stable TRH analog increased plasma acyl ghrelin levels and prevented the abdominal surgery-induced lowering of fasted ghrelin levels [104]. Likewise, in healthy humans sham feeding or cold exposure increased circulating levels of ghrelin [46, 103, 116] albeit the response to sham feeding was less than the established elevation of pancreatic polypeptide [103].

These experimental and clinical data support that ghrelin release is under the positive control of both vagal and sympathetic efferent pathways. As the autonomic nervous system is a major effector system that serves to maintain homeostasis during exposure to stressors [40], it can be postulated that stressor-specific alterations in the autonomic nerve efferent activity within the stomach may represent an important brain-gut pathway contributing to the alterations of circulating ghrelin levels induced by different stressors which, however, remains to be delineated.

3.2. Inflammatory mediators

Convergent data show the involvement of interleukin-1 signaling in LPS-inhibitory effects using pharmacological and genetic approaches in rodents. First, interleukin-1 β injected intravenously induces a sustained lowering of total ghrelin levels in fasted rats [125]. Second, the peripheral injection of interleukin-1 receptor antagonist blocks the LPS-induced reduction of plasma total ghrelin levels in fasted rats [125] and lastly, in fasted mice with deletion of the interleukin-1 receptor, ip injected LPS failed to suppress circulating ghrelin while wild type littermates showed a decrease [77]. However, since the interleukin-1 receptor is not expressed in X/A-like cells but other cells of the gastric oxyntic mucosa [77], an indirect mechanism can be assumed. We and others previously showed that a prostaglandin dependent mechanism is involved in the early phase of the ghrelin inhibition induced by LPS injected intraperitoneally at a low dose [77, 125]. This was supported by the abolition of the LPS-induced reduction of circulating total ghrelin at 3 h by the non-selective cyclooxygenase 1/2 inhibitors, indomethacin or ketorolac, whereas the sustained decrease at 5 h was not prevented [77, 125]. Characterization of receptor subtype(s) was established by showing that peripheral injection of prostacyclin reduced plasma total ghrelin levels while prostaglandin E₂ or carbocyclic thromboxane A₂ did not and that prostacyclin receptors (PGI₂) are expressed on gastric cells containing ghrelin mRNA [77]. These findings support a direct action of prostacyclin on X/Alike cells downstream of activated interleukin-1 β signaling. Interestingly, the ghrelin receptor is able to form heterodimers with the prostacyclin receptor which seems to be of biological relevance resulting in attenuation of the constitutive activity of phospholipase 2 whereas the affinity for ghrelin is not altered [19].

3.3. Somatostatin

It is well established that peripheral administration of somatostatin suppresses ghrelin release and circulating levels in rats [28, 101, 102]. We recently observed that gastric ghrelin

positive X/A-like cells express the somatostatin receptor 2 (sst₂) and that peripheral injection of the selective sst₂ agonist, S-346-011 reduced fasting ghrelin levels similarly to abdominal surgery in rats [109]. In addition, the sst₂ antagonist, S-406-028 prevented the abdominal surgery-induced reduction of circulating ghrelin [109]. Since abdominal surgery activates capsaicin sensitive afferents and calcitonin gene-related peptide (CGRP) signaling [48, 89, 141] and stimulation of capsaicin sensitive afferents containing CGRP increases gastric somatostatin release [12, 53, 95], the decrease of ghrelin under conditions of surgery is likely to be sst₂ mediated, possibly *via* a paracrine effect of gastric somatostatin containing D cells. A regulatory role of the endogenous somatostatin tone in ghrelin secretion is further supported by the robust increase in circulating levels of ghrelin in somatostatin knockout mice compared to wild type in both fed and fasted state associated with an up-regulation of gastric ghrelin mRNA levels [75]. Whether gastric GOAT gene expression may also be negatively regulated by somatostatin, remains to be investigated. However, in mouse primary pituitary cell cultures *in vitro*, somatostatin decreased GOAT mRNA levels and somatostatin knockout mice display an increased pituitary GOAT mRNA expression [34]. Based on these findings, blocking somatostatin-sst₂ signaling to increase acyl ghrelin levels may be a promising approach to promote appetite after abdominal surgery. This pathway established in rodents may have clinical relevance as the sst_{2a} is also expressed on ghrelin cells in the human gastric mucosa [32] and the preferential sst₂ agonist octreotide inhibits ghrelin release in humans [5].

3.4. Mammalian Target of Rapamycin (mTOR)

At the cellular level, convergent evidence supports an inverse relationship between gastric mTOR activity and gastric ghrelin mRNA as well as GOAT mRNA expression [131]. The protein mTOR is a highly conserved serine-threonine kinase which serves as an intracellular ATP sensor activated by positive energy balance (elevated ATP/AMP ratio) [31]. Increased mTOR activity is linked with the phosphorylation of S6 ribosomal protein [52]. In the mouse stomach, an antibody directed against phospho-mTOR, the active form of mTOR, stained 100% of ghrelin positive cells in the corpus mucosa [131]. Repeated administration of the mTOR activator, L-leucine over a period of six days increased gastric phospho-S6 levels in the corpus mucosa which was associated with a decrease in gastric expression of ghrelin mRNA and GOAT mRNA, gastric preproghrelin and plasma ghrelin in mice [131]. Conversely, repeated injections of the mTOR inhibitor, rapamycin or 48-h fasting decreased gastric phospho-S6 and was associated with a significantly increased expression of gastric ghrelin mRNA, GOAT mRNA, preproghrelin and circulating ghrelin in the mouse stomach [131]. Further *in vitro* studies established that the increased activity of mTOR decreases ghrelin promoter activity. The functional relevance of mTOR was further supported by the demonstration that rapamycin increases dark phase feeding in mice, an effect blocked by the GRLN receptor antagonist, D-Lys-3-GHRP-6 [131]. Collectively, this report supports the involvement of mTOR in the gastric mucosa as a fuel sensing pathway that regulates food intake through direct modulation of ghrelin and GOAT expression. As these studies have been generated under conditions of severe metabolic stress induced by a 48-h fast in mice, the relevance of this pathway under other stress conditions not linked with drastic caloric restriction warrants further investigations.

4. Functional implications of altered ghrelin signaling induced by stress

Various stressors are well established to impact on food intake, gut propulsive motility and immune functions [20, 112]. Stress-related changes of circulating ghrelin levels can play a double role either contributing to the behavioral, visceral and immune alterations or conversely play an adaptive role to minimize these stress manifestations and contribute to the adaptive response to stress [21, 88].

4.1. Food intake

We previously showed that the acute injection of LPS (100 µg/kg, ip) reduced the food intake response to a fast in rats [7, 105] which was completely reversed by ip injection of ghrelin [125]. In addition, repeated ghrelin injection over a period of five days increased body weight gain in LPS treated rats compared to LPS alone [45]. Similarly to an acute injection of LPS, abdominal surgery as a physical stressor decreases ghrelin levels in rats associated with a robust reduction of re-feeding food intake in fasted rats [104, 108]. Restoration of ghrelin levels by brain activation of somatostatin signaling prevented the reduction of circulating acyl ghrelin and the anorexigenic response [108]. These findings point towards a role of reduced circulating ghrelin levels induced by LPS or abdominal surgery in the associated anorexigenic response under these conditions.

In contrast to these acute immune/physical stressors, chronic social defeat stress by subjecting C57BL/6/J mice to aggressive CD1 male mice 10 times per day increases circulating acyl ghrelin levels assessed on day 11 that was still present three weeks after the end of the stress exposure compared to non-stressed controls [76]. This chronic stress regimen resulted in an increased daily food intake in wild type mice which was not observed in GRLN receptor knockout mice indicative of a key role played by the activation of GRLN receptors in the orexigenic response to this chronic psychological stress paradigm [76]. Other recent studies also using GRLN receptor knockout mice showed a role of increased acyl ghrelin in the metabolic response to chronic unpredictable stress for 14 days [88]. These experimental reports support a link between ghrelin alterations, chronic stress and food intake that may have implications in the underlying mechanisms of stress-related disruptions of food intake. However, in humans the elevation of circulating ghrelin levels following an acute Trier Social Stress Test did not correlate with the urge to eat after the stress [96]. Although these differences may reflect rodent *versus* human studies, it is likely that they may relate to the importance of the chronicity of the psychological stressor to develop an association with altered feeding pattern.

4.2. Gastrointestinal motility

Postoperative gastric ileus develops after gastrointestinal surgery and is associated with delayed gastric emptying and intestinal transit [74]. Prolonged postoperative ileus causes increased hospitalization times associated with higher healthcare costs [136]. Therefore, effective management strategies to prevent this condition are needed. Due to its gastroprokinetic effects, ghrelin has received much attention as a treatment strategy in the management of postoperative ileus [13]. Several studies reported that ghrelin and the ghrelin receptor agonists, RC-1139 and TZP-101 injected intravenously at the end of the abdominal surgery reverse the surgery-induced delay of gastric emptying in dogs [118] and rats [54, 90, 119, 123]. In addition, TZP-101 also shortened the time to the first bowel movement and accelerated recovery associated with earlier hospital discharge in patients following major abdominal surgery [91]. We established that surgical stress induced a rapid and sustained drop in acyl ghrelin in rats [104, 109]. However, the peripheral injection of an sst₂ receptor antagonist to block sst₂ receptor signaling during surgery restored the inhibited fasting levels of circulating ghrelin to normal but did not prevent the delayed gastric emptying in rats [109]. In contrast to the peripheral sst₂ receptor mediated inhibition of circulating ghrelin during surgery [109], central activation of sst₂ receptors by intracisternal injection of an sst₂ agonist restores the inhibited circulating acyl ghrelin levels under these conditions [108]. This is associated with the restoration of the feeding response to an overnight fast that is inhibited post abdominal surgery while the delayed gastric emptying post surgery is maintained [108]. These data suggest that acute normalization of inhibited fasting levels of ghrelin to physiological levels is not sufficient to promote gastroprokinetic effects under conditions of postoperative gastric ileus. This hypothesis is further corroborated by several

clinical reports showing that pharmacological doses of ghrelin are needed to exert gastroprokinetic effects [86, 114], whereas lower doses able to increase GH release do not alter gastric emptying [23]. Similarly, ghrelin injected peripherally at supraphysiological doses also reverses the LPS-induced delay of gastric emptying in rats [125] and mice [18, 30].

In other studies, in rats exposed to an acute restraint stress, gastric emptying was delayed while after repeated restraint stress over five consecutive days, gastric emptying normalized to values seen in the non-stressed group that was associated with a significant increase of gastric ghrelin mRNA [140]. The restoration of gastric emptying was blocked by treatment with a GRLN receptor antagonist [140]. Similarly, under conditions of repeated psychological stress, water avoidance stressed rats showed increased endogenous acyl ghrelin levels associated with accelerated gastric emptying of a liquid non-nutrient viscous solution on days 3 and 5 [87]. These data indicate a primary role of increased ghrelin in the adaptive response to a homotypic stressor with the recruitment of physiological ghrelin levels by psychological stress exerting a gastroprokinetic effect. These divergent results may be related to the distinct underlying mechanisms of visceral *versus* psychological stressors with additional local inhibitory mechanisms recruited under conditions of postoperative ileus [9] that interfere with the gastroprokinetic action of ghrelin.

4.3. Effects on immune functions

Several studies demonstrated the beneficial effects of peripheral ghrelin or ghrelin agonist treatment (intravenous or subcutaneous injection, respectively) under conditions of acute endotoxemia as shown by the attenuation of pulmonary infiltration [17, 71] and reduction of mortality following high doses of LPS [15]. These effects could be exerted *via* direct modulation of cytokines as ghrelin reduces the production of the pro-inflammatory cytokines, interleukin-1 β and tumor necrosis factor α (TNF- α) and increases the release of the anti-inflammatory cytokine, interleukin-10 from LPS-stimulated murine macrophages [127] and increases glucocorticoid release [37]. Similarly, ghrelin also reduces TNF- α and interleukin-6 levels *in vivo* after induction of sepsis by cecal ligation and puncture in rats, an effect blocked by vagotomy indicating a vagal mode of ghrelin's anti-inflammatory action. In mice with trinitrobenzene sulfonic acid (TNBS) induced colitis, ghrelin was reported to modulate the anti-inflammatory interleukin-10/transforming growth factor- β -1 leading to the improvement of clinical symptoms and histological signs of colitis and preventing its recurrence [42, 54]. Conversely, intracerebroventricular injection of the GRLN receptor antagonist, [D-Arg¹,D-Phe⁵,D-Trp^{9,11},Leu¹¹]substance P increased both, circulating noradrenalin and TNF- α levels in naïve animals [130] indirectly highlighting the potential benefits conferred by increasing ghrelin signaling to dampen inflammation.

4.4. Coping functions

A recent review attempted to clarify the role of ghrelin as part of coping mechanisms in the behavioral response to stress [21]. Mice kept under conditions of caloric restriction develop increased circulating acyl ghrelin levels and display anxiolytic and antidepressant behavior as tested with the elevated plus maze test (more time spent in open arms) and forced swim test (longer latency to immobility, less time spent in total immobility) [76]. These effects were absent in GRLN receptor knockout mice but recapitulated by injection of ghrelin subcutaneously in wild type mice [76]. In a model of chronic defeat stress, wild type mice show an elevation of circulating ghrelin levels and develop depressive-like symptoms such as reduced social interaction [76]. These depressive-like symptoms are more pronounced in GRLN receptor KO mice [76] giving rise to a role for ghrelin in stress coping by inducing anxiolytic and antidepressant-like effects. Consistent with an anxiolytic effect of ghrelin, Wistar Kyoto rats known to display more anxiety than Sprague Dawley rats have 2-fold

lower fasting ghrelin levels than Sprague Dawley rats [33]. In addition, circulating levels of ghrelin are increased in rats subjected to restraint stress, which is associated with decreased corticosterone levels [140]. In a recent human study, an intravenous infusion of ghrelin blunted the mental stress-induced increase in blood pressure and sympathetic nerve activity [68].

However, one early study showed that ghrelin injected either intraperitoneally or into the third brain ventricle of mice induced an anxiogenic response assessed in the plus maze test which was mediated by the activation of CRF signaling since the non-selective CRF_{1/2} receptor antagonist, α -helical CRF₉₋₄₁ co-injected with ghrelin into the brain prevented this anxiogenic effect [4]. These data are consistent with recent evidence that ghrelin stimulates CRF release in a rat hypothalamic cell line [59]. Clearly, additional studies are required to elucidate the contrasting effect of ghrelin's action on the behavioral response to stress and the differential actions in acute *versus* chronic experimental models of behavioral responses to stress.

5. Summary

Ghrelin has attracted much attention over the past decade and thereby its role as physiological regulator of food intake and its implication in the regulation of gastrointestinal motility were established. The identification of GOAT, the enzyme involved in the acylation of ghrelin, provided a very relevant target to modulate ghrelin-sensitive physiological actions mediated by acyl ghrelin, the only form activating the GRLN receptor to induce stimulation of GH secretion, food intake and gastrointestinal motility. Both, circulating forms of ghrelin including acylated and desacyl ghrelin as well as GOAT are sensitive to various stressors including mixed/physical (abdominal surgery, endotoxin injection, exercise), metabolic (fasting, caloric restriction) and psychological stressors in rodents and healthy subjects. Exposure to acute physical stressors (abdominal surgery and immunological/endotoxin injection, exercise) reduced, whereas metabolic (cold exposure, fasting and caloric restriction) and psychological stressors increased ghrelin levels. Investigating the regulation of the different components of the ghrelin signaling system under acute *versus* chronic stress conditions may have implications in the identification of mechanisms underlying or contributing to the stress-related alterations of food intake and gastrointestinal motility in animal models and translational applications in humans.

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References

1. An W, Li Y, Xu G, Zhao J, Xiang X, Ding L, et al. Modulation of ghrelin O-acyltransferase expression in pancreatic islets. *Cell Physiol Biochem*. 2010; 26:707–16. [PubMed: 21063108]
2. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab*. 2001; 86:4753–8. [PubMed: 11600536]
3. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, et al. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut*. 2005; 54:18–24. [PubMed: 15591499]
4. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, et al. A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology*. 2001; 74:143–7. [PubMed: 11528215]

5. Barkan AL, Dimaraki EV, Jessup SK, Symons KV, Ermolenko M, Jaffe CA. Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *J Clin Endocrinol Metab.* 2003; 88:2180–4. [PubMed: 12727973]
6. Barreiro ML, Gaytan F, Caminos JE, Pinilla L, Casanueva FF, Aguilar E, et al. Cellular location and hormonal regulation of ghrelin expression in rat testis. *Biol Reprod.* 2002; 67:1768–76. [PubMed: 12444052]
7. Basa NR, Wang L, Arteaga JR, Heber D, Livingston EH, Taché Y. Bacterial lipopolysaccharide shifts fasted plasma ghrelin to postprandial levels in rats. *Neurosci Lett.* 2003; 343:25–8. [PubMed: 12749989]
8. Bednarek MA, Feighner SD, Pong SS, McKee KK, Hreniuk DL, Silva MV, et al. Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J Med Chem.* 2000; 43:4370–6. [PubMed: 11087562]
9. Boeckxstaens GE, de Jonge WJ. Neuroimmune mechanisms in postoperative ileus. *Gut.* 2009; 58:1300–11. [PubMed: 19671558]
10. Bonaz B, Taché Y. Induction of Fos immunoreactivity in the rat brain after cold-restraint induced gastric lesions and fecal excretion. *Brain Res.* 1994; 652:56–64. [PubMed: 7953723]
11. Broglio F, Gottero C, Van Koetsveld P, Prodham F, Destefanis S, Benso A, et al. Acetylcholine regulates ghrelin secretion in humans. *J Clin Endocrinol Metab.* 2004; 89:2429–33. [PubMed: 15126574]
12. Bunnett NW, Helton WS, Debas HT, Ensink JW. CGRP stimulates the release of pro-somatostatin-derived peptides from the gastric fundus. *Am J Physiol.* 1990; 258:G316–9. [PubMed: 1968318]
13. Camilleri M, Papanthanasopoulos A, Odunsi ST. Actions and therapeutic pathways of ghrelin for gastrointestinal disorders. *Nat Rev Gastroenterol Hepatol.* 2009; 6:343–52. [PubMed: 19434096]
14. Chang L, Du JB, Gao LR, Pang YZ, Tang CS. Effect of ghrelin on septic shock in rats. *Acta Pharmacol Sin.* 2003; 24:45–9. [PubMed: 12511228]
15. Chang L, Zhao J, Yang J, Zhang Z, Du J, Tang C. Therapeutic effects of ghrelin on endotoxic shock in rats. *Eur J Pharmacol.* 2003; 473:171–6. [PubMed: 12892835]
16. Chen CY, Chao Y, Chang FY, Chien EJ, Lee SD, Doong ML. Intracisternal des-acyl ghrelin inhibits food intake and non-nutrient gastric emptying in conscious rats. *Int J Mol Med.* 2005; 16:695–9. [PubMed: 16142407]
17. Chen J, Liu X, Shu Q, Li S, Luo F. Ghrelin attenuates lipopolysaccharide-induced acute lung injury through NO pathway. *Med Sci Monit.* 2008; 14:BR141–6. [PubMed: 18591913]
18. Chen YT, Tsai SH, Sheu SY, Tsai LH. Ghrelin improves LPS-induced gastrointestinal motility disturbances: roles of NO and prostaglandin E2. *Shock.* 2010; 33:205–12. [PubMed: 19503023]
19. Chow KB, Leung PK, Cheng CH, Cheung WT, Wise H. The constitutive activity of ghrelin receptors is decreased by co-expression with vasoactive prostanoid receptors when over-expressed in human embryonic kidney 293 cells. *Int J Biochem Cell Biol.* 2008; 40:2627–37. [PubMed: 18573679]
20. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol.* 2009; 5:374–81. [PubMed: 19488073]
21. Chuang JC, Zigman JM. Ghrelin's Roles in Stress, Mood, and Anxiety Regulation. *Int J Pept.* 2010
22. Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron.* 2003; 37:649–61. [PubMed: 12597862]
23. Cremonini F, Camilleri M, Vazquez Roque M, McKinzie S, Burton D, Baxter K, et al. Obesity does not increase effects of synthetic ghrelin on human gastric motor functions. *Gastroenterology.* 2006; 131:1431–9. [PubMed: 17101319]
24. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes.* 2001; 50:1714–9. [PubMed: 11473029]
25. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the

- gastrointestinal tracts of rats and humans. *Endocrinology*. 2000; 141:4255–61. [PubMed: 11089560]
26. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, et al. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes*. 2002; 51:124–9. [PubMed: 11756331]
 27. Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, et al. International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol Rev*. 2005; 57:541–6. [PubMed: 16382107]
 28. de la Cour CD, Norlen P, Hakanson R. Secretion of ghrelin from rat stomach ghrelin cells in response to local microinfusion of candidate messenger compounds: a microdialysis study. *Regul Pept*. 2007; 143:118–26. [PubMed: 17573135]
 29. De Vriese C, Gregoire F, Lema-Kisoka R, Waelbroeck M, Robberecht P, Delporte C. Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. *Endocrinology*. 2004; 145:4997–5005. [PubMed: 15256494]
 30. De Winter BY, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, et al. Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. *Neurogastroenterol Motil*. 2004; 16:439–46. [PubMed: 15305999]
 31. Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G. Mammalian TOR: a homeostatic ATP sensor. *Science*. 2001; 294:1102–5. [PubMed: 11691993]
 32. Fischer T, Doll C, Jacobs S, Kolodziej A, Stumm R, Schulz S. Reassessment of sst2 somatostatin receptor expression in human normal and neoplastic tissues using the novel rabbit monoclonal antibody UMB-1. *J Clin Endocrinol Metab*. 2008; 93:4519–24. [PubMed: 18697876]
 33. Florentzson M, Svensson K, Astin-Nielsen M, Andersson K, Hakanson R, Lindstrom E. Low gastric acid and high plasma gastrin in high-anxiety Wistar Kyoto rats. *Scand J Gastroenterol*. 2009; 44:401–7. [PubMed: 19089754]
 34. Gahete MD, Cordoba-Chacon J, Salvatori R, Castano JP, Kineman RD, Luque RM. Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. *Mol Cell Endocrinol*. 2010; 317:154–60. [PubMed: 20035826]
 35. Garner AL, Janda KD. cat-ELCCA: a robust method to monitor the fatty acid acyltransferase activity of ghrelin O-acyltransferase (GOAT). *Angew Chem Int Ed Engl*. 2010; 49:9630–4. [PubMed: 20845345]
 36. Gayle DA, Desai M, Casillas E, Beloosesky R, Ross MG. Gender-specific orexigenic and anorexigenic mechanisms in rats. *Life Sci*. 2006; 79:1531–6. [PubMed: 16697419]
 37. Giordano R, Picu A, Broglio F, Bonelli L, Baldi M, Berardelli R, et al. Ghrelin, hypothalamus-pituitary-adrenal (HPA) axis and Cushing's syndrome. *Pituitary*. 2004; 7:243–8. [PubMed: 16132204]
 38. Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, et al. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. *Am J Physiol*. 1994; 267:R164–70. [PubMed: 8048620]
 39. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab*. 2002; 87:2988. [PubMed: 12050285]
 40. Goldstein DS. Adrenal responses to stress. *Cell Mol Neurobiol*. 2010; 30:1433–40. [PubMed: 21061156]
 41. Gomez R, Lago F, Gomez-Reino JJ, Dieguez C, Gualillo O. Expression and modulation of ghrelin O-acyltransferase in cultured chondrocytes. *Arthritis Rheum*. 2009; 60:1704–9. [PubMed: 19479831]
 42. Gonzalez-Rey E, Chorny A, Delgado M. Therapeutic action of ghrelin in a mouse model of colitis. *Gastroenterology*. 2006; 130:1707–20. [PubMed: 16697735]
 43. Gonzalez CR, Vazquez MJ, Lopez M, Dieguez C. Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *J Mol Endocrinol*. 2008; 41:415–21. [PubMed: 18835978]

44. Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A*. 2008; 105:6320–5. [PubMed: 18443287]
45. Hataya Y, Akamizu T, Hosoda H, Kanamoto N, Moriyama K, Kangawa K, et al. Alterations of plasma ghrelin levels in rats with lipopolysaccharide-induced wasting syndrome and effects of ghrelin treatment on the syndrome. *Endocrinology*. 2003; 144:5365–71. [PubMed: 12960078]
46. Heath RB, Jones R, Frayn KN, Robertson MD. Vagal stimulation exaggerates the inhibitory ghrelin response to oral fat in humans. *J Endocrinol*. 2004; 180:273–81. [PubMed: 14765979]
47. Hirayama H, Shiina T, Shima T, Kuramoto H, Takewaki T, J BF, et al. Contrasting effects of ghrelin and des-acyl ghrelin on the lumbo-sacral defecation center and regulation of colorectal motility in rats. *Neurogastroenterol Motil*. 2011; 22:1124–31. [PubMed: 20584261]
48. Holzer P, Lippe IT, Holzer-Petsche U. Inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. *Gastroenterology*. 1986; 91:360–3. [PubMed: 3721122]
49. Hosoda H, Kangawa K. The autonomic nervous system regulates gastric ghrelin secretion in rats. *Regul Pept*. 2008; 146:12–8. [PubMed: 17720259]
50. Hosoda H, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun*. 2000; 279:909–13. [PubMed: 11162448]
51. Inhoff T, Mönnikes H, Noetzel S, Stengel A, Goebel M, Dinh QT, et al. Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats. *Peptides*. 2008; 29:2159–68. [PubMed: 18938204]
52. Inoki K, Conradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet*. 2005; 37:19–24. [PubMed: 15624019]
53. Inui T, Kinoshita Y, Yamaguchi A, Yamatani T, Chiba T. Linkage between capsaicin-stimulated calcitonin gene-related peptide and somatostatin release in rat stomach. *Am J Physiol*. 1991; 261:G770–4. [PubMed: 1683166]
54. Jeffery P, McDonald V, Tippet E, McGuckin M. Ghrelin in gastrointestinal disease. *Mol Cell Endocrinol*. 2011
55. Jeffery PL, McGuckin MA, Linden SK. Endocrine impact of *Helicobacter pylori*: Focus on ghrelin and ghrelin o-acyltransferase. *World J Gastroenterol*. 2011; 17:1249–60. [PubMed: 21455323]
56. Jeon TY, Lee S, Kim HH, Kim YJ, Son HC, Kim DH, et al. Changes in plasma ghrelin concentration immediately after gastrectomy in patients with early gastric cancer. *J Clin Endocrinol Metab*. 2004; 89:5392–6. [PubMed: 15531487]
57. Joels M, Baram TZ. The neuro-symphony of stress. *Nat Rev Neurosci*. 2009; 10:459–66. [PubMed: 19339973]
58. Kageyama H, Kitamura Y, Hosono T, Kintaka Y, Seki M, Takenoya F, et al. Visualization of ghrelin-producing neurons in the hypothalamic arcuate nucleus using ghrelin-EGFP transgenic mice. *Regul Pept*. 2008; 145:116–21. [PubMed: 17945362]
59. Kageyama K, Kumata Y, Akimoto K, Takayasu S, Tamasawa N, Suda T. Ghrelin stimulates corticotropin-releasing factor and vasopressin gene expression in rat hypothalamic 4B cells. *Stress*. 2011
60. Kim MS, Yoon CY, Park KH, Shin CS, Park KS, Kim SY, et al. Changes in ghrelin and ghrelin receptor expression according to feeding status. *Neuroreport*. 2003; 14:1317–20. [PubMed: 12876464]
61. Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, et al. GOAT links dietary lipids with the endocrine control of energy balance. *Nat Med*. 2009; 15:741–5. [PubMed: 19503064]
62. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999; 402:656–60. [PubMed: 10604470]
63. Kojima M, Kangawa K. Ghrelin: more than endogenous growth hormone secretagogue. *Ann N Y Acad Sci*. 2010; 1200:140–8. [PubMed: 20633142]
64. Kojima M, Kangawa K. Ghrelin: structure and function. *Physiol Rev*. 2005; 85:495–522. [PubMed: 15788704]

65. Koyama KI, Yasuhara D, Nakahara T, Harada T, Uehara M, Ushikai M, et al. Changes in acyl ghrelin, des-acyl ghrelin, and ratio of acyl ghrelin to total ghrelin with short-term refeeding in female inpatients with restricting-type anorexia nervosa. *Horm Metab Res.* 2010; 42:595–8. [PubMed: 20411479]
66. Kristensson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville de la Cour C, et al. Acute psychological stress raises plasma ghrelin in the rat. *Regul Pept.* 2006; 134:114–7. [PubMed: 16540188]
67. Kumar R, Salehi A, Rehfeld JF, Hoglund P, Lindstrom E, Hakanson R. Proghrelin peptides: Desacyl ghrelin is a powerful inhibitor of acylated ghrelin, likely to impair physiological effects of acyl ghrelin but not of obestatin A study of pancreatic polypeptide secretion from mouse islets. *Regul Pept.* 2010; 164:65–70. [PubMed: 20619300]
68. Lambert E, Lambert G, Ika-Sari C, Dawood T, Lee K, Chopra R, et al. Ghrelin Modulates Sympathetic Nervous System Activity and Stress Response in Lean and Overweight Men. *Hypertension.* 2011
69. Langhans W. Anorexia of infection: current prospects. *Nutrition.* 2000; 16:996–1005. [PubMed: 11054606]
70. Langhans W. Bacterial products and the control of ingestive behavior: clinical implications. *Nutrition.* 1996; 12:303–15. [PubMed: 8875513]
71. Li G, Li J, Zhou Q, Song X, Liang H, Huang L. Growth hormone releasing peptide-2, a ghrelin agonist, attenuates lipopolysaccharide-induced acute lung injury in rats. *Tohoku J Exp Med.* 2010; 222:7–13. [PubMed: 20805679]
72. Liu J, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, Johnson ML, et al. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab.* 2008; 93:1980–7. [PubMed: 18349056]
73. Lu S, Guan JL, Wang QP, Uehara K, Yamada S, Goto N, et al. Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. *Neurosci Lett.* 2002; 321:157–60. [PubMed: 11880196]
74. Luckey A, Livingston E, Taché Y. Mechanisms and treatment of postoperative ileus. *Arch Surg.* 2003; 138:206–14. [PubMed: 12578422]
75. Luque RM, Gahete MD, Hochgeschwender U, Kineman RD. Evidence that endogenous SST inhibits ACTH and ghrelin expression by independent pathways. *Am J Physiol Endocrinol Metab.* 2006; 291:E395–403. [PubMed: 16825606]
76. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci.* 2008; 11:752–3. [PubMed: 18552842]
77. Madison LD, Scarlett JM, Levasseur P, Zhu X, Newcomb K, Batra A, et al. Prostacyclin signaling regulates circulating ghrelin during acute inflammation. *J Endocrinol.* 2008; 196:263–73. [PubMed: 18252949]
78. Maier C, Riedl M, Vila G, Wolzt M, Clodi M, Ludvik B, et al. Differential regulation of plasma obestatin and ghrelin by meal intake and the cholinergic system in lean, but not obese individuals. *J Clin Endocrinol Metab.* 2010; 95:E214–8. [PubMed: 20660037]
79. Maier C, Schaller G, Buranyi B, Nowotny P, Geyer G, Wolzt M, et al. The cholinergic system controls ghrelin release and ghrelin-induced growth hormone release in humans. *J Clin Endocrinol Metab.* 2004; 89:4729–33. [PubMed: 15356087]
80. Martinez V, Wang L, Taché Y. Central TRH receptor 1 antisense blocks cold-induced gastric emptying but not brain c-Fos induction. *Peptides.* 2001; 22:81–90. [PubMed: 11179601]
81. Maruna P, Gurlich R, Frasko R, Rosicka M. Ghrelin and leptin elevation in postoperative intra-abdominal sepsis. *Eur Surg Res.* 2005; 37:354–9. [PubMed: 16465060]
82. Maruna P, Gurlich R, Rosicka M. Ghrelin as an acute-phase reactant during postoperative stress response. *Horm Metab Res.* 2008; 40:404–9. [PubMed: 18404598]
83. McCowen KC, Maykel JA, Bistrian BR, Ling PR. Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *J Endocrinol.* 2002; 175:R7–11. [PubMed: 12429053]

84. Mizutani M, Atsuchi K, Asakawa A, Matsuda N, Fujimura M, Inui A, et al. Localization of acyl ghrelin- and des-acyl ghrelin-immunoreactive cells in the rat stomach and their responses to intragastric pH. *Am J Physiol Gastrointest Liver Physiol*. 2009; 297:G974–80. [PubMed: 20501445]
85. Mundinger TO, Cummings DE, Taborsky GJ Jr. Direct stimulation of ghrelin secretion by sympathetic nerves. *Endocrinology*. 2006; 147:2893–901. [PubMed: 16527847]
86. Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, et al. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut*. 2005; 54:1693–8. [PubMed: 16085693]
87. Ochi M, Tominaga K, Tanaka F, Tanigawa T, Shiba M, Watanabe T, et al. Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. *Life Sci*. 2008; 82:862–8. [PubMed: 18343456]
88. Patterson ZR, Ducharme R, Anisman H, Abizaid A. Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *Eur J Neurosci*. 2010; 32:632–9. [PubMed: 20597975]
89. Plourde V, Wong HC, Walsh JH, Raybould HE, Taché Y. CGRP antagonists and capsaicin on celiac ganglia partly prevent postoperative gastric ileus. *Peptides*. 1993; 14:1225–9. [PubMed: 7510881]
90. Poitras P, Polvino WJ, Rocheleau B. Gastrokinetic effect of ghrelin analog RC-1139 in the rat. Effect on post-operative and on morphine induced ileus. *Peptides*. 2005; 26:1598–601. [PubMed: 16112398]
91. Popescu I, Fleshner PR, Pezzullo JC, Charlton PA, Kosutic G, Senagore AJ. The Ghrelin agonist TZP-101 for management of postoperative ileus after partial colectomy: a randomized, dose-ranging, placebo-controlled clinical trial. *Dis Colon Rectum*. 2010; 53:126–34. [PubMed: 20087086]
92. Raff H. Total and active ghrelin in developing rats during hypoxia. *Endocrine*. 2003; 21:159–61. [PubMed: 12897380]
93. Raspopow K, Abizaid A, Matheson K, Anisman H. Psychosocial stressor effects on cortisol and ghrelin in emotional and non-emotional eaters: influence of anger and shame. *Horm Behav*. 2010; 58:677–84. [PubMed: 20540943]
94. Reimer RA, Maurer AD, Lau DC, Auer RN. Long-term dietary restriction influences plasma ghrelin and GOAT mRNA level in rats. *Physiol Behav*. 2010; 99:605–10. [PubMed: 20149910]
95. Ren J, Young RL, Lassiter DC, Harty RF. Calcitonin gene-related peptide mediates capsaicin-induced neuroendocrine responses in rat antrum. *Gastroenterology*. 1993; 104:485–91. [PubMed: 8093880]
96. Rouach V, Bloch M, Rosenberg N, Gilad S, Limor R, Stern N, et al. The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. *Psychoneuroendocrinology*. 2007; 32:693–702. [PubMed: 17560728]
97. Sagar SM, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science*. 1988; 240:1328–31. [PubMed: 3131879]
98. Satou M, Nishi Y, Yoh J, Hattori Y, Sugimoto H. Identification and characterization of acyl-protein thioesterase 1/lysophospholipase I as a ghrelin deacylation/lysophospholipid hydrolyzing enzyme in fetal bovine serum and conditioned medium. *Endocrinology*. 2010; 151:4765–75. [PubMed: 20685872]
99. Shanado Y, Kometani M, Uchiyama H, Koizumi S, Teno N. Lysophospholipase I identified as a ghrelin deacylation enzyme in rat stomach. *Biochem Biophys Res Commun*. 2004; 325:1487–94. [PubMed: 15555596]
100. Shiiya T, Ueno H, Toshinai K, Kawagoe T, Naito S, Tobina T, et al. Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocr J*. 2011
101. Shimada M, Date Y, Mondal MS, Toshinai K, Shimbara T, Fukunaga K, et al. Somatostatin suppresses ghrelin secretion from the rat stomach. *Biochem Biophys Res Commun*. 2003; 302:520–5. [PubMed: 12615065]
102. Silva AP, Bethmann K, Raulf F, Schmid HA. Regulation of ghrelin secretion by somatostatin analogs in rats. *Eur J Endocrinol*. 2005; 152:887–94. [PubMed: 15941929]

103. Simonian HP, Kresge KM, Boden GH, Parkman HP. Differential effects of sham feeding and meal ingestion on ghrelin and pancreatic polypeptide levels: evidence for vagal efferent stimulation mediating ghrelin release. *Neurogastroenterol Motil.* 2005; 17:348–54. [PubMed: 15916622]
104. Stengel A, Goebel M, Luckey A, Yuan PQ, Wang L, Taché Y. Cold ambient temperature reverses abdominal surgery-induced delayed gastric emptying and decreased plasma ghrelin levels in rats. *Peptides.* 2010; 31:2229–35. [PubMed: 20817059]
105. Stengel A, Goebel M, Wang L, Reeve JR Jr, Taché Y, Lambrecht NW. Lipopolysaccharide differentially decreases plasma acyl and desacyl ghrelin levels in rats: Potential role of the circulating ghrelin-acylating enzyme GOAT. *Peptides.* 2010; 31:1689–96. [PubMed: 20599577]
106. Stengel A, Goebel M, Wang L, Taché Y. Ghrelin, des-acyl ghrelin and nesfatin-1 in gastric X/A-like cells: role as regulators of food intake and body weight. *Peptides.* 2010; 31:357–69. [PubMed: 19944123]
107. Stengel A, Goebel M, Wang L, Taché Y, Sachs G, Lambrecht NW. Differential distribution of ghrelin-O-acyltransferase (GOAT) immunoreactive cells in the mouse and rat gastric oxyntic mucosa. *Biochem Biophys Res Commun.* 2010; 392:67–71. [PubMed: 20059966]
108. Stengel A, Goebel-Stengel M, Wang L, Luckey A, Hu E, Rivier J, et al. Central administration of pansomatostatin agonist ODT-8 prevents abdominal surgery-induced inhibition of circulating ghrelin, food intake and gastric emptying. *Neurogastroenterol Motil.* 2011
109. Stengel A, Goebel-Stengel M, Wang L, Shaikh A, Lambrecht NW, Rivier J, et al. Abdominal surgery inhibits circulating acyl ghrelin and ghrelin-O-acyltransferase levels in rats: role of the somatostatin receptor 2. *Am J Physiol Gastrointest Liver Physiol.* 2011
110. Stengel A, Keire D, Goebel M, Evilevitch L, Wiggins B, Taché Y, et al. The RAPID method for blood processing yields new insight in plasma concentrations and molecular forms of circulating gut peptides. *Endocrinology.* 2009; 150:5113–8. [PubMed: 19819958]
111. Stengel A, Taché Y. Corticotropin-releasing factor signaling and visceral response to stress. *Exp Biol Med (Maywood).* 2010; 235:1168–78. [PubMed: 20881321]
112. Stengel A, Taché Y. Neuroendocrine Control of the Gut During Stress: Corticotropin-Releasing Factor Signaling Pathways in the Spotlight. *Annu Rev Physiol.* 2009; 71:219–39. [PubMed: 18928406]
113. Taché Y, Yang H, Miampamba M, Martinez V, Yuan PQ. Role of brainstem TRH/TRH-R1 receptors in the vagal gastric cholinergic response to various stimuli including sham-feeding. *Auton Neurosci.* 2006; 125:42–52. [PubMed: 16520096]
114. Tack J, Depoortere I, Bisschops R, Verbeke K, Janssens J, Peeters T. Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther.* 2005; 22:847–53. [PubMed: 16225494]
115. Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, et al. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology.* 2004; 145:234–42. [PubMed: 14551228]
116. Tomasik PJ, Sztefko K, Pizon M. The effect of short-term cold and hot exposure on total plasma ghrelin concentrations in humans. *Horm Metab Res.* 2005; 37:189–90. [PubMed: 15824975]
117. Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M, et al. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res Commun.* 2001; 281:1220–5. [PubMed: 11243865]
118. Trudel L, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannon P, et al. Two new peptides to improve post-operative gastric ileus in dog. *Peptides.* 2003; 24:531–4. [PubMed: 12860196]
119. Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P, et al. Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. *Am J Physiol Gastrointest Liver Physiol.* 2002; 282:G948–52. [PubMed: 12016119]
120. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature.* 2000; 407:908–13. [PubMed: 11057670]

121. Tschöp M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, et al. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest*. 2001; 24:RC19–21. [PubMed: 11434675]
122. Turnbull AV, Lee S, Rivier C. Mechanisms of hypothalamic-pituitary-adrenal axis stimulation by immune signals in the adult rat. *Ann N Y Acad Sci*. 1998; 840:434–43. [PubMed: 9629270]
123. Venkova K, Fraser G, Hoveyda HR, Greenwood-Van Meerveld B. Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. *Dig Dis Sci*. 2007; 52:2241–8. [PubMed: 17436082]
124. Vila G, Maier C, Riedl M, Nowotny P, Ludvik B, Luger A, et al. Bacterial endotoxin induces biphasic changes in plasma ghrelin in healthy humans. *J Clin Endocrinol Metab*. 2007; 92:3930–4. [PubMed: 17666475]
125. Wang L, Basa NR, Shaikh A, Luckey A, Heber D, St-Pierre DH, et al. LPS inhibits fasted plasma ghrelin levels in rats: role of IL-1 and PGs and functional implications. *Am J Physiol Gastrointest Liver Physiol*. 2006; 291:G611–20. [PubMed: 16959954]
126. Wang L, Cardin S, Martinez V, Taché Y. Intracerebroventricular CRF inhibits cold restraint-induced c-fos expression in the dorsal motor nucleus of the vagus and gastric erosions in rats. *Brain Res*. 1996; 736:44–53. [PubMed: 8930307]
127. Waseem T, Duxbury M, Ito H, Ashley SW, Robinson MK. Exogenous ghrelin modulates release of pro-inflammatory and anti-inflammatory cytokines in LPS-stimulated macrophages through distinct signaling pathways. *Surgery*. 2008; 143:334–42. [PubMed: 18291254]
128. Wren AM, Small CJ, Abbott CR, Dhillon WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. *Diabetes*. 2001; 50:2540–7. [PubMed: 11679432]
129. Wu R, Zhou M, Cui X, Simms HH, Wang P. Ghrelin clearance is reduced at the late stage of polymicrobial sepsis. *Int J Mol Med*. 2003; 12:777–81. [PubMed: 14533009]
130. Wu R, Zhou M, Das P, Dong W, Ji Y, Yang D, et al. Ghrelin inhibits sympathetic nervous activity in sepsis. *Am J Physiol Endocrinol Metab*. 2007; 293:E1697–702. [PubMed: 17911350]
131. Xu G, Li Y, An W, Li S, Guan Y, Wang N, et al. Gastric mammalian target of rapamycin signaling regulates ghrelin production and food intake. *Endocrinology*. 2009; 150:3637–44. [PubMed: 19406939]
132. Yang H, Wu SV, Ishikawa T, Taché Y. Cold exposure elevates thyrotropin-releasing hormone gene expression in medullary raphe nuclei: relationship with vagally mediated gastric erosions. *Neuroscience*. 1994; 61:655–63. [PubMed: 7969936]
133. Yang H, Yuan PQ, Wang L, Taché Y. Activation of the parapyramidal region in the ventral medulla stimulates gastric acid secretion through vagal pathways in rats. *Neuroscience*. 2000; 95:773–9. [PubMed: 10670444]
134. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell*. 2008; 132:387–96. [PubMed: 18267071]
135. Yilmaz Z, Ilcol YO, Ulus IH. Endotoxin increases plasma leptin and ghrelin levels in dogs. *Crit Care Med*. 2008; 36:828–33. [PubMed: 18431270]
136. Zeinali F, Stulberg JJ, Delaney CP. Pharmacological management of postoperative ileus. *Can J Surg*. 2009; 52:153–7. [PubMed: 19399212]
137. Zhang W, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology*. 2008; 149:4710–6. [PubMed: 18535105]
138. Zhao TJ, Liang G, Li RL, Xie X, Sleeman MW, Murphy AJ, et al. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci U S A*. 2010; 107:7467–72. [PubMed: 20231469]
139. Zhao TJ, Sakata I, Li RL, Liang G, Richardson JA, Brown MS, et al. Ghrelin secretion stimulated by {beta}1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. *Proc Natl Acad Sci U S A*. 2010; 107:15868–73. [PubMed: 20713709]
140. Zheng J, Dobner A, Babygirija R, Ludwig K, Takahashi T. Effects of repeated restraint stress on gastric motility in rats. *Am J Physiol Regul Integr Comp Physiol*. 2009; 296:R1358–65. [PubMed: 19261914]

141. Zittel TT, Reddy SN, Plourde V, Raybould HE. Role of spinal afferents and calcitonin gene-related peptide in the postoperative gastric ileus in anesthetized rats. *Ann Surg.* 1994; 219:79–87. [PubMed: 8297181]

Highlights

- The major circulating forms of ghrelin are acyl and desacyl ghrelin.
- Recently, the ghrelin acylating and de-acylating enzymes have been identified.
- The stress-induced alteration of gastrointestinal motility and food intake strongly depends on the stressor.
- The stress-induced alterations of ghrelin signaling are reviewed here.
- A better understanding of these mechanisms could lead to new treatment strategies for e.g. improving food intake and motility under conditions of stress.

Table 1

Effects of different stressors on circulating ghrelin levels

Stressor	Procedure	Species	Condition	Plasma ghrelin	Reference
Immune	100 µg/kg LPS ip	Rat	Overnight fasting	↓ (total)	[7, 36, 45, 77, 105, 125]
Immune	100 µg/kg LPS ip	Rat	Overnight fasting	↓ (acyl, desacyl)	[105]
Immune	Septic shock by 1 mg/kg LPS iv	Dog	14-h fasting	↑ (total)	[135]
Immune	2 ng/kg LPS iv	Human	Overnight fasting	↑ at 2 h, ↓ at 5–6 h (total)	[124]
Physical	Abdominal surgery (laparotomy and 1 min cecal palpation)	Rat	Overnight fasting	↓ (acyl, desacyl)	[109]
Physical	Large abdominal surgery (e.g. colon resection)	Human	Overnight fasting	↑ at 24 h (total)	[81, 82]
Physical	Physical exercise at 50% of $\dot{V}O_{2max}$	Human	Overnight fasting	↓ (acyl), = (desacyl, total)	[100]
Physical	Exposure to cold (4–6 °C) ambient temperature for 90 min	Rat	Ad libitum fed	↑ (acyl, desacyl)	[104]
Physical	Exposure to cold (2 °C) ambient temperature for 30 min	Human	12-h fast	↑ (total)	[116]
Psychological	Water avoidance (1 h or 5 days)	Rat	Ad libitum fed	↑ at 90 min (acyl) ↑ at days 3 and 5 (acyl)	[66, 87]
Psychological	Restraint (90 min/day for 5 days)	Rat	Ad libitum fed	↑ days 3 and 5 (acyl)	[140]
Psychological	High anxiety	Wistar Kyoto Rat	16-h fasted	↑ (acyl)	[33]
Psychological	Social defeat (10 bouts/day for 10 days)	Mouse	Ad libitum fed	↑ at days 11 and 39 (acyl)	[76]
Psychological	chronic un- predictable heterotypic stress for 14 days	Mouse	Ad libitum fed	↑ (acyl)	[88]
Psychological	Trier Social Stress Test	Human	1-h fast	↑ (total)	[93, 96]

↑ increase, ↓ decrease, = no change