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Physiological roles revealed by ghrelin and ghrelin receptor deficient mice

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Abstract

Ghrelin is a hormone made in the stomach and known primarily for its growth hormone releasing and orexigenic properties. Nevertheless, ghrelin through its receptor, the GHS-R1a, has been shown to exert many roles including regulation of glucose homeostasis, memory & learning, food addiction and neuroprotection. Furthermore, ghrelin could promote overall health and longevity by acting directly in the immune system and promoting an extended antigen repertoire. The development of mice lacking either ghrelin (*ghrelin*^{-/-}) or its receptor (*ghsr*^{-/-}) have provided a valuable tool for determining the relevance of ghrelin and its receptor in these multiple and diverse roles. In this review, we summarize the most important findings and lessons learned from the *ghrelin*^{-/-} and *ghsr*^{-/-} mice.

1. Introduction

Ghrelin is a 28 amino acid acylated peptide that is mainly released from the stomach and was first discovered as an endogenous ligand for the orphan growth hormone secretagogue receptor (GHS-R1a) [23]. This G protein-coupled receptor had been expression-cloned using a small synthetic molecule, MK-677, which was one of a family of synthetic compounds including GHRP-6, shown to stimulate the release of growth hormone (GH) *in vitro* from pituitary somatotrophs through the activation of a PLC-dependent pathway [3, 6, 7, 19, 31, 32]. Furthermore, MK-677 oral treatment restored the GH pulse amplitude in elderly subjects to a young adult profile, and increased total lean mass [5].

Ghrelin has been shown to exert many actions, of which regulation of the GH axis is but one. Ghrelin injections induced food intake in humans and rodents, and adiposity in rodents [28, 39, 43, 44]. In the last decade, countless studies have shown that acute pharmacologic doses of ghrelin increase feeding behavior. Upon ghrelin binding, activation of GHS-R1a in hypothalamic neuropeptide Y (NPY)/agouti-related peptide (AGRP) neurons leads to the

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release of orexigenic or appetite stimulating neurotransmitters into other hypothalamic nuclei, which results in increased food intake [10, 18].

The association of ghrelin with obesity is derived largely from pharmacology experiments in rodents, and clear interpretation of the results is confounded by the fact that acute administration of ghrelin causes a number of hormonal perturbations including release of GH, ACTH and glucocorticoids. The profile of plasma ghrelin concentrations epitomized by sharp peaks and valleys in these studies is quite different from what is observed *in vivo*. By contrast, the long-acting oral agonists of GHS-R1a administered chronically for up to 12 months provide a smoother agonist profile, and not cause elevations in ACTH or cortisol. Indeed, two-month treatment of obese subjects with MK-677 increased fat-free mass and energy expenditure without affecting fat mass [37].

The sights of expression of the GHS-R1a also indicate the significance of ghrelin in energy balance. The GHS-R1a is mainly expressed in β -cells of pancreatic islets, distinct areas of the hypothalamus, pituitary somatotrophs [17, 32], hippocampus, the ventral tegmental area (VTA), the substantia nigra (SN) and the dorsal raphe nucleus [17, 32] [1, 20]. Ghrelin actions include the regulation of memory and learning, activation of reward-related pathways and neuroprotection. An additional intriguing aspect of ghrelin biology is its link with immune function as illustrated by expression of GHS-R1a in the thymus, thymocytes and lymphocytes [13]; indeed, ghrelin treatment was shown to promote thymopoiesis in old mice [13]. In this review, we will examine the diverse physiological roles of ghrelin based on evidence collected from experimental studies in *ghrelin*^{-/-} and *ghsr*^{-/-} mice.

2. Ghrelin and energy balance

To characterize the physiological role of ghrelin in energy homeostasis, *ghsr*^{-/-} and *ghrelin*^{-/-} mice were generated [33, 36, 41, 47]. Although ghrelin regulates the amplitude of episodic GH release, *ghsr*^{-/-} mice were not dwarfs, and in fact appeared identical to their wild type (WT) littermates [36]. However, in these animals, ghrelin and MK-677 failed to induce GH release, whereas GH stimulated by growth hormone releasing hormone (GHRH) remained unaffected. These studies thus highlighted a selective ablation of ghrelin-induced GH release in *ghsr*^{-/-} mice, with the main GH regulatory pathway remaining intact. Importantly, these studies also provided the first evidence that ghrelin acts through the GHS-R1a *in vivo* [36].

In the *ghsr*^{-/-} mice created by our group, ghrelin treatment failed to induce increases in food intake, as was observed in WT littermates [36]. Insulin and leptin levels are reduced with fasting, and this response remained intact in mice lacking the GHS-R1a. In mice fed a normal chow diet, the body weights of congenic adult *ghsr*^{-/-} mice (C57BL6J) were modestly lower than WT controls. IGF-1 levels were also slightly lower, but food intake was unchanged [36]. In a second *ghsr*^{-/-} mouse line created by Zigman et al, *ghsr*^{-/-} mice of mixed genetic background (C57BL6J:129sv) fed a normal chow diet had similar body weights to WT mice, but were relatively resistant to diet induced obesity, at least when the high fat diet (HFD) was started immediately after weaning [47]. However, a caveat in this study was that the influence of the 129Sv genetic background in the knockout mice make them inherently more resistant to diet-induced obesity compared to congenic C57BL6J knockout mice. By the end of 19 weeks on a HFD, *ghsr*^{-/-} mice had consumed less food overall compared to WT, and had significant lower fat mass, as shown by a whole body dual X-ray absorptiometry (DEXA) [47]. These, *ghsr*^{-/-} mice also exhibited lower release of CO₂ over O₂ consumption ratio or respiratory quotient (RQ) and decreased total locomotor activity [47], suggesting ghrelin's role in energy metabolism may be more extensive and complex than merely increasing appetite.

The first *ghrelin*^{-/-} mice were characterized in 2003 by Sun et al [33]. This knockout was crucial in demonstrating ghrelin's role in energy balance. Originally it was thought that ablation of ghrelin would decrease food intake, mice weight/size and growth. However, these animals did not exhibit dwarfism or differences in their body composition (fat content), bone density, body weight or cumulative food intake over an 8 week period, when compared to WT littermates. These results were confirmed in *ghrelin*^{-/-} mice generated independently by Wortley et al. [41]. This group also showed that *ghrelin*^{-/-} mice had a normal circadian pattern of food intake, and that hypothalamic basal expression levels of AGRP, melanin-concentrating hormone (MCH), proopiomelanocortin, VGF peptide, and NPY were not distinct between *ghrelin*^{-/-} and WT mice [41]. Leptin and insulin levels responded as expected upon fasting, and both WT and *ghrelin*^{-/-} showed similar weight changes and food intake after a 24 hour fast and re-feed [33, 36, 41]. Together, these results indicate that hypothalamic regulatory feeding centers are intact in *ghrelin*^{-/-} mice.

Both male and female *ghrelin*^{-/-} mice are susceptible to a HFD over a 10 weeks period, resulting in increased fat deposition [33]. Wortley and co-workers confirmed these results, showing that *ghrelin*^{-/-} mice increased in body weight but this result did not correlated with an increase in food intake in response to a HFD [41]. Interestingly, in this study, RQ was also significantly reduced in *ghrelin*^{-/-} mice, as was reported in *ghsr*^{-/-} mice [35, 41]. The results suggest that in *ghrelin*^{-/-} mice most of the energy utilization comes from fat rather than carbohydrate, since the higher the RQ ratio the higher utilization of carbohydrates as the main source of energy. Interestingly, when animals were started on a HFD just 3 weeks post weaning, *ghrelin*^{-/-} mice showed increased energy expenditure, lower body weight, lower percentage of fat, but similar food intake, when compared to WT mice [42]. These results suggest that *ghrelin*^{-/-} mice on HFD have less efficient food utilization compared with WT mice [42], as shown in the *ghsr*^{-/-} mice.

Discrepancies in the reported results and conclusions from different groups regarding studies in *ghrelin*^{-/-} and *ghsr*^{-/-} mice are likely explained by the mixed background of the mutant mice used in the different studies [35]; although in the case of mice exposed to HFD, exposure to a HFD immediately after weaning, rather than during adulthood, may also have a role. The knockout mice were developed by targeting embryonic stem (ES) cells derived from 129Sv mice. Appropriately targeted ES cells are injected into the blastocyst of C57BL/6J mice to produce chimeric mice, and the chimeric mice are bred with C57BL/6J mice to generate germline heterozygotes. The homozygous knockout mice derived from these will be of mixed background with more traits from the 129Sv strain [35]. In contrast to the C57BL/6J mouse, the 129Sv mouse strain is resistant to diet-induced obesity, which compromises interpretation of results of metabolic studies done with these mice. To address this Sun et al backcrossed *ghrelin*^{-/-} and *ghsr*^{-/-} mice with C57BL/6J mice for 10 generations and tested them under positive and negative energy balance [35]. This study confirmed that *ghsr*^{-/-} mice have slightly lower body weights than WT mice, but their body weights increased similar to WT mice when they were exposed to a HFD. Neither the *ghsr*^{-/-} nor the *ghrelin*^{-/-} showed resistance to a HFD-induced obesity. Furthermore, changes in energy expenditure and RQ were the same in all WT, *ghrelin*^{-/-} and *ghsr*^{-/-} mice that were exposed to a HFD. Based on results from the collective studies, an important role for ghrelin in the development of obesity remains unclear.

3. Ghrelin and regulation of glucose homeostasis

Plasma levels of ghrelin and glucose are inversely related, such as under conditions of negative energy balance, ghrelin levels are high; and with positive energy balance, ghrelin levels are at a nadir. Indeed, fasting mice have higher endogenous ghrelin levels. As such, fasting mice are refractory to the inhibitory effects of exogenous ghrelin on glucose-

stimulated insulin secretion (GSIS) [34]. This result is hardly surprising, as in this context, ghrelin-binding sites on GHS-R1a would be saturated by endogenous ghrelin. Results such as these led Sun and colleagues to speculate that endogenous ghrelin has an important role in regulating glucose homeostasis.

Sun and colleagues investigated the role of ghrelin in leptin-deficient *ob/ob* mice to test whether the lack of ghrelin signaling would restore a lean phenotype and euglycemia in these genetically obese mice. The hormone leptin is released from adipose tissue and sends satiety signals to the brain. Mice that lack this hormone, *ob/ob* mice, become obese two weeks after birth, and present a clear type 2 diabetic profile with significant reduction in insulin sensitivity and glucose tolerance [25, 27]. Since ghrelin and leptin are mutual antagonists on hypothalamic feeding centers, it was hypothesized that the obese and diabetic phenotype of *ob/ob* mice was a consequence of unopposed ghrelin.

Ghrelin^{-/-} mice were crossed with the leptin knockout mouse (*ob/ob*) [34]. The ghrelin- and leptin-deficient mutant mice (*ghrelin*^{-/-}.*ob/ob*) double knockouts were not leaner than the *ob/ob*, but surprisingly ablation of ghrelin increased glucose-stimulated insulin secretion (GSIS) and lowered blood glucose [34] (Figure 1). Further investigations in *ghrelin*^{-/-} mice showed increased glucose disposal compared to wild-type mice, which was explained by increased GSIS coupled with improved insulin sensitivity. Increased glucose sensitivity of the β -cell in both (*ghrelin*^{-/-}.*ob/ob*) and *ghrelin*^{-/-} mice was at least partially explained by reduced expression of uncoupling protein-2 (*ucp-2*). The reduction in *ucp2* would improve efficiency of ATP production in response to glucose and increase activity of the ATP-sensitive K⁺ channel, resulting in increased influx of Ca²⁺ and augmentation of insulin release [34]. Thus, it was concluded that ghrelin does not lead to obesity, but is involved in the regulation of glucose homeostasis. Similarly, *ghsr*^{-/-} mice exposed to 16 weeks of 60% HFD had significantly lower plasma insulin levels, and a trend towards better glucose tolerance [24]. Since ghrelin inhibits insulin release from pancreatic β -cells, one way in which the lack of ghrelin could improve glucose homeostasis is by increasing insulin production and release [11]. Another explanation is that ghrelin ablation could improve insulin sensitivity in peripheral tissues. However, the GHS-R1a is not expressed in the majority of peripheral tissues, and the explanation for increased insulin sensitivity in *ghsr*^{-/-} remains to be determined.

4. Ghrelin actions in the Central Nervous System

4.1 Ghrelin regulates learning and memory processes

The first paper showing a connection between ghrelin and learning & memory was published in 2002 by Carlini et al [4]. In a step-down or avoidance-inhibitory task, rats injected icv with ghrelin just after training showed a dose-dependent increase in latency (i.e., time to move from the platform to the apparatus where the shock was delivered) [4]. This suggested that ghrelin may have a beneficial effect on memory retention. Additional studies have now shown that ghrelin can enhance memory formation, increase spine synapse density in the CA1 subregion of the murine hippocampus, and induced long-term potentiation (LTP) in mouse hippocampal slices [4, 12]. However, fewer studies have assessed learning and memory in *ghsr*^{-/-} or *ghrelin*^{-/-} mice.

Intriguingly, hippocampi from *ghrelin*^{-/-} mice reportedly show fewer dendritic spines than WT littermates, and this can be rescued with exogenous ghrelin treatment [12]. This was a fundamentally important observation because changes in synapse formation and dendritic spine number are necessary for formation and modulation of memory.

Long-term recognition memory was next tested in *ghrelin*^{-/-} and WT mice using a novel object recognition (NOR) test [12]. In this test, animals are exposed to a habituation period in which two novel objects are presented for 5 minutes in an open arena. The next day, one of the objects is exchanged for a novel object and localized to a new position. The time spent in exploring each object and the discrimination between the familiar object vs. the novel object are recorded as a measure of cognitive memory [12]. Wildtype mice behaved as expected and spent more time exploring the novel object, however *ghrelin*^{-/-} mice spent a similar amount of time exploring both objects at the time of the test [12]. But when these *ghrelin*^{-/-} mice were given exogenous ghrelin, they instead spent more time exploring the novel object. These intriguingly results are consistent with ghrelin promoting memory formation or cognitive ability[12], but additional studies are needed to resolve the cellular mechanisms involved. It will also be important to test whether or not *ghsr*^{-/-} mice share the impaired memory and hippocampal phenotype of *ghrelin*^{-/-} mice, and to determine the effects of administering ghrelin to *ghsr*^{-/-} mice. These experiments will test whether the beneficial effects of ghrelin on hippocampal function is regulated by GHS-R1a. While there remains no direct evidence for a ghrelin receptor other than GHS-R1a, indirect evidence for its existence is often cited.

4.2 Ghrelin protects neurons from MPTP-induced damage

Death of dopaminergic neurons or tyrosine hydroxylase (TH)-expressing cells due to apoptosis, mitochondrial dysfunction, and/or oxidative stress is the main cause of Parkinson's disease (PD)[15, 21, 29]. The first study to show that ghrelin has neuroprotective properties was published by Chung et al in 2007[8]. By measuring cell viability (MTT assay) and DNA fragmentation (TUNEL assay), they demonstrated that ghrelin prevented cell death in hypothalamic neurons exposed to oxygen-glucose deprivation (OGD). Additional studies showed that ghrelin had protective actions *in vivo* [21] [2, 26]. Indeed, ghrelin was also shown to protect dopamine neurons of the substantia nigra pars compacta (SNpc) in the 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced sub-acute mouse model of PD; neuroprotection was dependent upon *ucp2* expression [2].

Ghrelin^{-/-} mice are more sensitive to MPTP treatment than WT mice [2, 26]. Although MPTP treatment induced death of tyrosine hydroxylase-positive neurons in the SNpc of WT and *ghrelin*^{-/-} mice, cell death was significantly higher in *ghrelin*^{-/-} mice. MPTP treatment also reduced the amount of dopamine released into the striatum in WT and *ghrelin*^{-/-} mice; but *ghrelin*^{-/-} mice exhibited a larger decrease in striatal levels of dopamine and dopamine metabolite 3,4-Dihydroxyphenylacetic acid (DOPAC) [2]. *Ghsr*^{-/-} mice also showed exaggerated MPTP-induced neuronal cell loss when compared to WT animals. Importantly, selective reactivation of GHS-R1a expression in neurons from the VTA and SN of *ghsr*^{-/-} mice partly rescued MPTP-induced cell death [2]. It was concluded in this study that one of the possible mechanisms in which ghrelin exerts its neuroprotective actions against oxidative damage was by activating mitochondrial mechanisms dependent upon *ucp2*.

4.3 Ghrelin regulation of the dopaminergic system

Soon after ghrelin was discovered, it was demonstrated that icv administration of ghrelin in rats increases food intake and body weight [28]. Repeated overnight fasting 7 consecutive days significantly decreased food intake in *ghsr*^{-/-} and *ghrelin*^{-/-} mice compared to WT mice [1]. However, a potential caveat of repeated fasting is that during episodes of caloric restriction, the well-being of *ghrelin*^{-/-}, *ghsr*^{-/-} and *goat*^{-/-} mice is compromised[35, 38, 46].

Since the GHS-R1a is expressed in the VTA and SNpc [17], and GHS-R1a is co-expressed with DRD1 in subsets of these neurons[20], it was hypothesized that ghrelin may be

involved in release of dopamine in the ventral striatum and activation of reward/addiction pathways, including food addiction[1, 20]. In accordance with this hypothesis, Abizaid et al showed that ghrelin increased the frequency of action potentials in the VTA of fresh brain slices from WT, but not from *ghsr*^{-/-} mice[1]. In addition, ghrelin increased dopamine release in the striatum of WT, but not *ghsr*^{-/-} mice[1].

Intra-VTA ghrelin injections in mice produces behaviors characteristic of reward, such as increased locomotor activity and increased consumption of a high calorie food (peanut butter), without affecting intake of regular chow[14]. Both WT and *ghsr*^{-/-} prefer high calorie food to normal chow when both are available, but *ghsr*^{-/-} consume significantly less peanut butter compared to WT mice[14]. Interestingly, in this study it was shown that bilateral lesion of the VTA significantly attenuated ghrelin-induced ingestion of peanut butter, but not of regular chow. Multiple studies had demonstrated that icv injections of ghrelin increased regular chow intake, but this is the first study to show that intra-VTA ghrelin induces a preference for food with a high caloric content. This is important because foods with a high caloric content have been associated with dopamine release and reward, and, of course, lead to obesity

The studies summarized above provide important information regarding the potential of GHS-R1a agonists as therapeutic agents. Importantly, the use of *ghsr*^{-/-} mice in control experiments illustrates the specificity of the findings; nevertheless, it is important to exercise caution before concluding that the results are representative of the physiological function of endogenous ghrelin. In all of the studies cited, whether ghrelin is administered ip or intra-VTA, the doses of ghrelin are extraordinarily high and would far exceed endogenous ghrelin concentrations found in plasma and CSF. Indeed, there is no evidence that ghrelin is produced in the brain of rodents, other than from a small subset of neurons in the arcuate nucleus [10]. This is reinforced by a study in sheep where episodic changes in ghrelin were monitored in plasma and in cerebral spinal fluid (CSF). Concentrations of endogenous ghrelin in the CSF were 1000-fold lower than in plasma [16]. Tissue levels were also measured, and ghrelin was undetectable in the olfactory bulb, cortex, hippocampus and cerebellum. Very low concentrations were found in the hypothalamus (3-4 pg/mg protein), and as predicted high concentrations of endogenous ghrelin (112,000 pg/mg protein) were measured in the abomasum (corresponding to the stomach in monogastric animals) and in the small intestine (4,200 pg/mg protein). Hence, more studies are needed before extrapolating data derived from ghrelin pharmacology to ghrelin physiology.

Chronic ghrelin treatment augments acute cocaine-induced hyperlocomotion in rats, supporting the hypothesis that dopamine signaling is modulated by endogenous ghrelin actions [40]. However, cocaine-induced locomotor activity increases similarly in WT, *ghrelin*^{-/-} and *ghsr*^{-/-} mice during food restriction (i.e. when endogenous ghrelin levels are elevated) [9], suggesting that augmented locomotor activity associated with food restriction is not explained by increases in endogenous ghrelin.

5. Ghrelin induces thymopoeisis

T-cells produced in the thymus are essential for a robust immune system and overall health. During aging, thymic involution occurs, which is associated with marked reductions in the production of thymocytes and increased production of adipocytes. This leads to a decrease in the generation of T-cells that recognize new antigens. Although pre-existing T-cells are able to reproduce, the inability to develop immune defenses against new threats leads to an age-dependent decline in health. Thus, scientists have been seeking ways to increase thymopoeisis in the elderly population.

GHS-R1a is expressed in immune cells, including thymocytes and T-cells[13]. Infusion of ghrelin increases the number of thymocytes and thymus size in 14-, 20-, and 24-month old mice, and with decreased thymic adiposity at 14 month compared to vehicle infused animals [13] (Figures 2 and 3).

A comparison was made of the thymus from 2 mo and 24 mo WT, *ghrelin*^{-/-} and *ghsr*^{-/-} mice. Thymocyte counts in 2 mo mice were the same irrespective of genotype, but in 24 mo *ghrelin*^{-/-} and *ghsr*^{-/-} mice thymocytes were significantly reduced compared to age-matched WT mice (Fig. 4A). Age-dependent thymic involution is also more evident in *ghrelin*^{-/-} mice than in WT mice (Fig. 4B).

The accelerated age-dependent involution was partially reversed by ghrelin infusion in *ghrelin*^{-/-} mice, but not *ghsr*^{-/-} mice, indicating ghrelin is acting through GHS-R1a [13]. Based on observations that *ghsr*^{-/-} and *ghrelin*^{-/-} mice exhibit a decrease in progenitor cells when compared to WT mice at 20- and 24-months of age, it was concluded that ghrelin increases thymocyte counts by increasing progenitors from the lymph[13]. More recent studies have shown that compared to age-matched WT littermates, the thymi of 10-month old *ghsr*^{-/-} mice have fewer thymic epithelial cells necessary for T-cell development [45]. If confirmed in humans, this data suggest that activation of ghrelin signaling may have therapeutic benefit by enhancing immune responses in the elderly.

7. Summary

This review demonstrates the applicability studies utilizing *ghrelin*^{-/-}, *ghsr*^{-/-} and *goat*^{-/-} mice as a tool to help define multiple actions of ghrelin throughout the body. It was confirmed that GHS-R1a is the receptor involved in ghrelin modulation of GH pulse amplitude and food intake[36]. However, mechanisms explaining improved insulin sensitivity in congenic *ghrelin*^{-/-} and *Ghsr*^{-/-} mice remain a puzzle [24, 34, 35], but likely involve central rather than peripheral actions of ghrelin. Experiments in *ghrelin*^{-/-} and *goat*^{-/-} mice show that while these mice behave and appear identical to WT mice under standard stress-free housing conditions, in contrast to WT mice, their survival is threatened by acute reductions in ambient temperature or by markedly reducing food availability [38, 46]. In both contexts the negative impact on well-being is likely the failure to mount an appropriate counter-regulatory response to hypoglycemia [46].

It also remains unresolved how ghrelin modulates learning and memory and/or stimulates activation of dopamine signaling and reward. Studies by Abizaid et al and Andrew et al suggest that ghrelin can act directly on dopaminergic neurons to activate dopamine release in the ventral striatum [1, 2]. While this is one potential mechanism, it is unlikely to be the sole explanation. Hypothalamic neurons expressing GHS-R1a project to other areas of the brain, such as the amygdala, which is involved in the emotional component of drug abuse and addiction. In addition, Jiang et al showed that GHS-R1a and dopamine receptor type-1 (D1R) are co-expressed in subsets of neurons and can form heteromers[20]. Thus, ghrelin and the GHS-R1a may modulate signaling actions of dopamine and/or other neurotransmitters and neuropeptides.

The discovery of the orphan growth hormone secretagogue receptor, GHS-R1a in 1996, with its endogenous agonist ghrelin identified three years later, has led to an explosion of publications that contribute to our understanding of the biological importance of this signaling pathway that has been conserved for at least 400 million years[19, 22, 30]. The quest continues, particularly as it relates to elucidating the physiological role of endogenous ghrelin and the significance of ghrelin signaling during aging.

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Highlights

- Ghrelin and ghrelin receptor knockout mice exhibit improve insulin sensitivity.
- Ghrelin and ghrelin receptor knockout mice show accelerating aging of the immune system.
- Ghrelin is neuroprotective

Figure 1. Leptin deficient mice (*ob/ob*) are morbidly obese and diabetic. Mice lacking ghrelin and leptin (*ghrelin^{-/-}ob/ob*) are still obese, but have improved glucose tolerance.

Figure 2.

Ghrelin increases thymus size and thymocyte cell number in old mice. (A) Ghrelin infusion for 2 weeks increased thymus size in 14-month-old animals; (B) ghrelin increased total thymocyte numbers in 14-, 20- and 24-month-old mice. Reproduced with permission from the American Society for Clinical Investigation (Dixit, V.D., et al., *Ghrelin promotes thymopoiesis during aging*. J Clin

Figure 3.

Ghrelin infusion into 14-months old mice increases cellularity and reduces lipid deposits in the thymus. Vehicle (A, C, E); ghrelin infusion (B, D, F); Oil Red O staining illustrates decreased lipid deposits in response to ghrelin (E, F). Reproduced with permission from the American Society for Clinical Investigation (Dixit, V.D., et al., *Ghrelin promotes thymopoiesis during aging*. J Clin Invest, 2007. **117**(10): p. 2778-90.)

Figure 4.

Ghrelin and GHS-R-deficient mice exhibit accelerated age-associated thymic involution. (A) Thymocyte counts in 2 and 24 mo WT, *ghrelin*^{-/-} and *ghsr*^{-/-} mice. (B) Histology of thymus from 24 mo *ghrelin*^{-/-} and *ghsr*^{-/-} mice revealing loss of CMJs, reduced cellularity, and increased adipocytes. Reproduced with permission from the American Society for Clinical Investigation (Dixit, V.D., et al., *Ghrelin promotes thymopoiesis during aging*. J Clin Invest, 2007. **117**(10): p. 2778-90.)