

# The endocrine effects of acylated and des-acylated ghrelin

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**Abstract:** Acylated ghrelin is one of the few peptides known whose isolation and characterization follow the description of its receptor and its basic biological functions. Characterized initially for its somatotrophic properties, ghrelin was shown later to exert various effects on other important physiological functions in mammals, such as appetite, gastric acid secretion, gut motility, insulin sensitivity, adiposity, and energy expenditure. Further, ghrelin influences cardiac function, reproduction, and the immune system as well. Here we present an overview of the discovery and subsequent development of ghrelin as an important peptide hormone involved in the control of energy metabolism in humans and other mammals. Recently reported effects of acylated ghrelin on glucose/lipid uptake, de novo lipogenesis, gluconeogenesis, lipid-droplet formation, fatty acid transport into mitochondria, and mitochondrial activity are particularly emphasized and discussed.

**Keywords:** Acylated ghrelin, des-acylated ghrelin, physiological functions, adipogenesis

## Introduction

The discovery of ghrelin originated from research by Cyril Bowers and collaborators on morphine addiction.<sup>1</sup> Indeed, the Met-enkephalin peptide analog Tyr-D-Trp-Gly-Phe-Met-NH<sub>2</sub> that they synthesized in 1976 was termed (D-Trp<sup>2</sup>)-GHRP because it displayed a weak growth hormone (GH)-releasing activity in vitro. On the other hand, the synthetic peptide was not active in vivo and showed no opiate activity. Through the following years, a large number of peptidic and peptidomimetic GH-releasing peptide (GHRP) analogs, also designated as growth-hormone secretagogues (GHS) were synthesized and tested. In 1989, Bowers' group developed an analog, His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (GHRP-6), that induced the release of GH both in vitro and in vivo, and most importantly was also active in man. Meanwhile, the discovery of GH-releasing factor (GRF) by Guillemin's<sup>2</sup> and Rivier's<sup>3</sup> groups in 1982 shadowed for a while the recognition of GHRPs as distinct GHS. However, an intensive research program undertaken by a GHRP believer, Roy Smith and his group at Merck Research Laboratories<sup>4</sup> led in 1992 to the development of a potent, orally active GHRP peptidomimetic analog, MK-0677, which was biologically specific and distinct from GRF in its GH-releasing activity. Shortly after, in 1996, the same group reported the cloning and characterization of a GTP-binding protein (G protein-coupled receptor or GPCR) in swine and humans that was shown to be the endogenous target for the GH secretagogues.<sup>5</sup> This GPCR, a typical G protein-coupled seven-transmembrane receptor, was named growth hormone-secretagogue receptor (GHS-R) and shown to be distinct from the receptor that binds GRF. Although it became another orphan GPCR with no identified recognized

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ligand, GHS-R was shown to be closely related to the motilin receptor GPR-38<sup>6</sup> and the neuromedin U (NMU) receptors NMU-R1 and NMU-R2.<sup>7</sup> The identification of these related receptors for gastrointestinal (GI) peptides led to the postulate that the ligand for GHS-R was also a peptide from the GI tract. This hypothesis was confirmed with the isolation and characterization of ghrelin by Kojima et al in 1999,<sup>8</sup> using the so-called orphan-receptor strategy.<sup>9</sup>

## Background

Kojima et al isolated ghrelin from rat stomach and determined its amino acid sequence. The name ghrelin comes from the word “ghre,” which means “grow” in the Proto-Indo-European language.<sup>8</sup> They found that ghrelin is a 28-amino acid peptide with a mass of 3371.9 daltons and that it is derived proteolytically from a 117-amino acid precursor. They also observed that human ghrelin is identical to rat ghrelin, other than two amino acids in positions 11 and 12:

Human	GSSFLSPEHQRVQQRKESKKPPAKLQPR
Rat	GSSFLSPEHQKAQQRKESKKPPAKLQPR

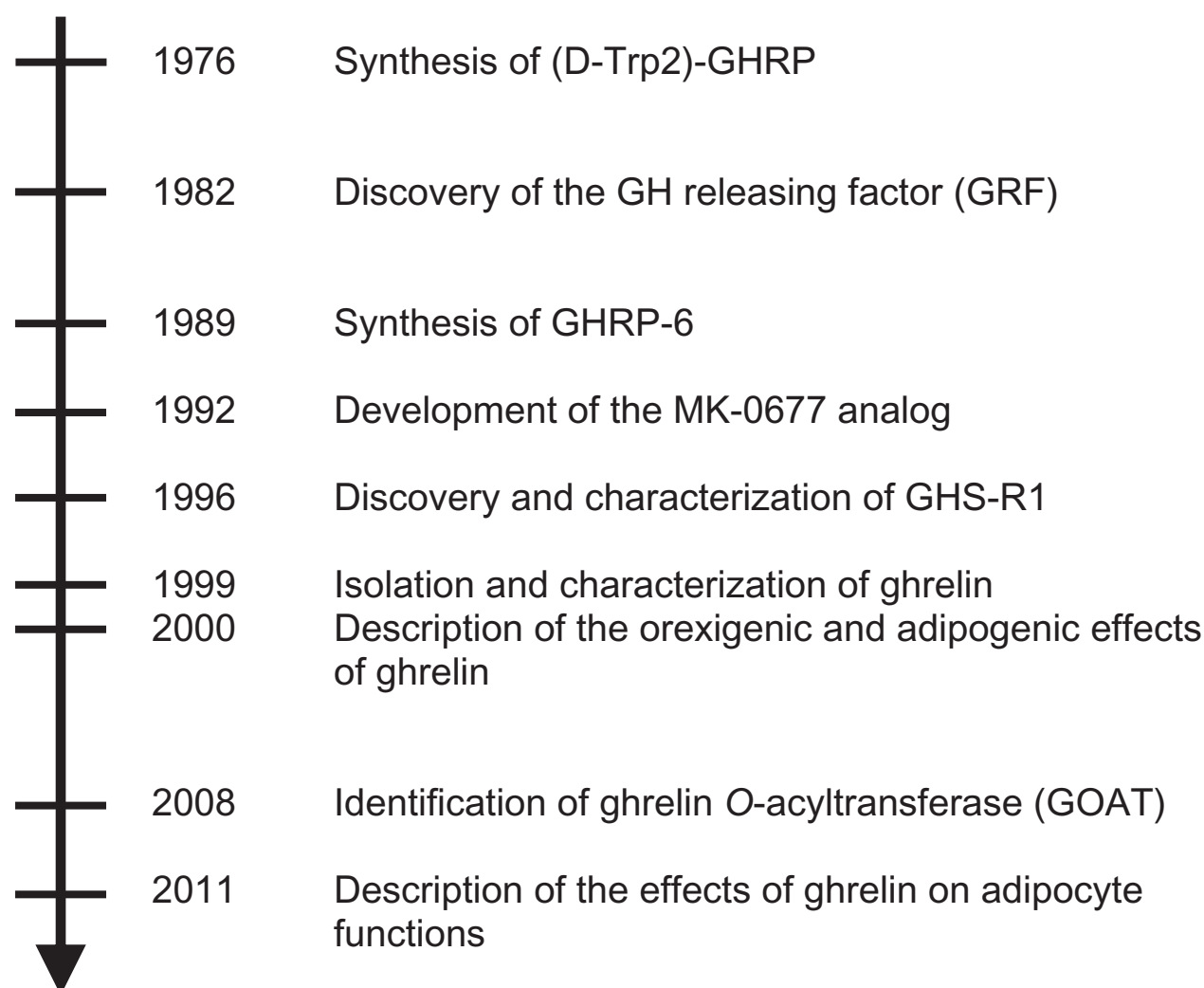
Uniquely among all other natural peptides isolated so far in animals, Kojima et al showed by electrospray mass spectrometry analysis that ghrelin is esterified on the hydroxyl group of its Ser<sup>3</sup> side chain with an octanoylated fatty acid function (acylated ghrelin or AG). They also demonstrated that this modification was required for GH-releasing activity. Later on, they reported that the ghrelin sequence, its *O*-octanoylation, as well as the structure of its receptor GHS-R1a, had been highly conserved in vertebrates over millions of years of evolution.<sup>10</sup> It was also found that threonine could replace serine in position 3 of ghrelin isolated from other species, and that other fatty-acid chains such as decanoic acid could substitute for octanoic acid without significant change in biological activity.<sup>11–13</sup> Ghrelin is predominantly secreted from X/A-like cells of the oxyntic mucosa in preprandial condition, but its expression was also detected in the gastrointestinal tract, pancreas, the brain, testis, thyroid gland, kidney, and placenta.<sup>14</sup> The mechanisms underlying the stimulation of AG release remain ill defined; however, GH, somatostatin, and specific nutrients were shown to inhibit its secretion in vitro or in vivo.<sup>15–19</sup>

More recently, in 2008, Yang et al<sup>20</sup> identified the enzyme that promotes the formation of an ester bond between octanoic acid and the Ser<sup>3</sup> hydroxyl group of ghrelin. This *O*-acyltransferase was termed ghrelin *O*-acyltransferase (GOAT) and is also known as membrane-bound *O*-acyltransferase 4 (MBOAT4). It is a membrane-bound enzyme belonging to a family of hydrophobic membrane-bound acyltransferases

of the endoplasmic reticulum that esterify long-chain fatty acids to target proteins. Yang et al demonstrated that GOAT is the only member of this family that octanoylates ghrelin when coexpressed in cultured endocrine cell lines with preproghrelin.<sup>20</sup> The activity of GOAT requires the presence of catalytic asparagine and histidine residues, which are remarkably conserved in this family. Consistent with its function, GOAT mRNA is mostly found in stomach and intestine, where most ghrelin-secreting tissues are located. It was also reported by Kangawa's group that a nonacylated form of ghrelin, termed des-acylated ghrelin (DAG), is also present in circulation.<sup>21</sup> Although DAG is believed to be the most abundant circulating form of ghrelin, it is well established that several biological actions of ghrelin, such as GH secretion and feeding behavior, require the presence of the acylated group on ghrelin. On the other hand, a number of studies published during recent years, including ours,<sup>22</sup> raise the possibility that DAG might also be at the origin of endocrine actions distinct from AG and possibly closely associated to specific pathological states, as further discussed in the following sections. For instance, we reported earlier that both increased AG concentrations and elevated AG/DAG ratios are modulated differentially in insulin-sensitive obese versus insulin-resistant obese postmenopausal women and might be associated with insulin resistance in that population.<sup>23</sup> More recently, we evaluated the direct effects of AG, DAG, and other peptides of the ghrelin family on preadipocyte proliferation, differentiation, and adipocyte lipid and glucose metabolism in 3T3-L1 cells.<sup>24</sup> Our results indicate that DAG might be acting through the GHS-R1<sup>25</sup> pathway in adipocytes and stimulating adipose-tissue hyperplasia and hypertrophy by mechanisms that remain to be elucidated. Altogether, our studies provide evidence that AG and DAG might simultaneously maintain and exacerbate an obese phenotype. It is expected that more endocrine manifestations of DAG will be revealed in the coming years.

## Biological activities of ghrelin Ghrelin and GH secretion

Although AG is recognized as a potent mediator of somatotroph activity in animal and human models, mice with a deletion of the ghrelin gene display no detectable growth defects.<sup>26</sup> In addition, no difference in ghrelin levels has been noted between normal and GH-deficient individuals.<sup>27–29</sup> Interestingly, AG levels were higher in children with poor weight gain than in those with short stature or chronic gastrointestinal dysfunctions,<sup>30</sup> indicating that AG would be released in response to an energy deficit rather than being



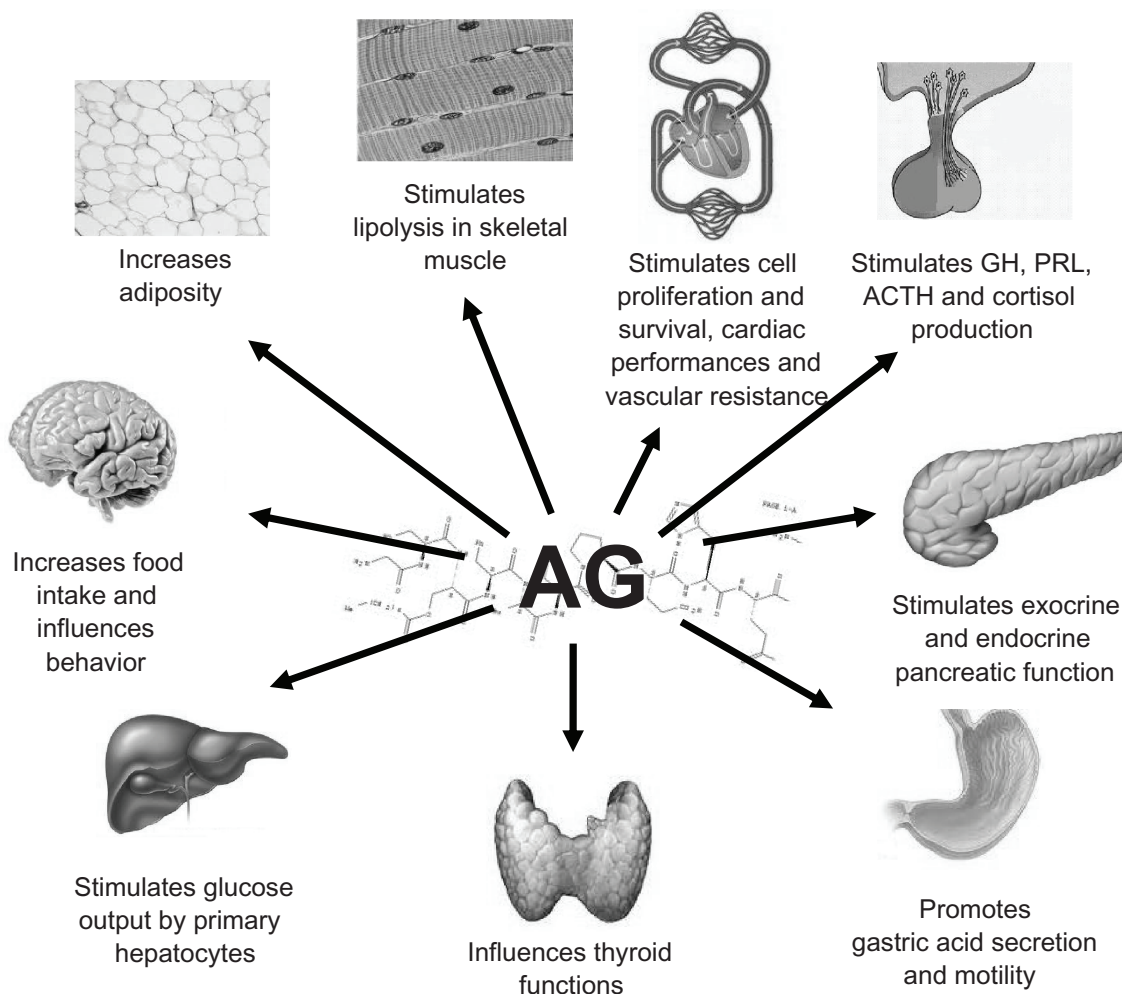
**Figure 1** Milestones for ghrelin, from the synthesis of (D-Trp2)-GHRP in 1976 to the recent description of ghrelin's effects on the regulation of adipocyte functions in 2011. **Abbreviations:** GHRP, growth hormone-releasing peptide; GHS-R, growth hormone-secretagogue receptor.

influenced by growth defects per se. Consequently, this either suggests that ghrelin is not essential for the regulation of normal growth functions or that other factors could compensate for its deficient secretion.

## Ghrelin and food intake

It is noteworthy that the first neuroendocrine effects of AG were reported using synthetic analogs of enkephalins before the genuine isolation and characterization of the endogenous peptide were performed.<sup>8</sup> In fact, synthetic GHS-R1a agonists, such as the GHRPs developed by Bowers, as well as other peptidomimetics such as MK-0677, were all shown to display somatotrophic activity.<sup>31</sup> Also, intravenous administration of synthetic AG stimulated ACTH, prolactin, and cortisol release in healthy humans,<sup>32</sup> and a number of other effects of AG on the hypothalamo–pituitary–adrenal axis were described. Tschöp and his colleagues were the first to report

that repeated injections of AG stimulated food intake and adiposity in rats.<sup>33</sup> Regulation of food intake requires sensing energy and modulation of behavior associated with appetite. The hypothalamus, the brain stem, and the limbic system are sensitive to blood levels of metabolic intermediates such as glucose, insulin, and fatty acids that allow the organism to attain nutrient and energy homeostasis. The description of the orexigenic effects of ghrelin emphasize the relevance of considering the peptide as a central and peripheral mediator of energy homeostasis. Further, central administration of AG was shown to stimulate neuronal activation in brain areas known to influence food intake and energy expenditure, such as the arcuate nucleus, the ventromedial nucleus, the dorsomedial nucleus, the paraventricular nucleus, the lateral hypothalamus, the central nucleus of the amygdala, the nucleus of the solitary tract of the brain stem, the ventral tegmental area (VTA), and the nucleus accumbens (NAC) of the



**Figure 2** Description of the different metabolic outcomes mediated by acylated ghrelin (AG) in different tissues and organs.  
**Abbreviations:** GH, growth hormone; PRL, prolactin; ACTH, adrenocorticotrophic hormone.

mesolimbic reward areas.<sup>34–43</sup> Injected in the hypothalamus, AG increased food intake by stimulating neuropeptide Y and agouti-related protein while inhibiting pro-opiomelanocortin neurons from the arcuate nucleus.<sup>44–46</sup> These orexigenic effects of AG could also be mediated through the inhibition of fatty-acid sensing in the hypothalamus.<sup>47</sup> Interestingly, it seems that AG not only affects appetite by promoting hunger but also through the stimulation of the reward system in dopaminergic and acetylcholine nicotinic neurons from VTA and NAC,<sup>48,49</sup> associating these effects with hedonism. However, AG could also be involved in the development of addictions such as alcoholism and chemical drug abuse.<sup>50–53</sup> Other central effects of AG include an increase in learning and memory capacities through the stimulation of serotonin reuptake in the dorsal raphe nucleus<sup>54–56</sup> and the neuroprotective effect observed through the restriction of dopaminergic neuron loss following the administration of the peptide in the substantia nigra.<sup>57–59</sup> In both pediatric and adult populations,

altered AG concentrations have been observed in pathological conditions associated with excessive or restrained feeding, such as obesity, Prader–Willi syndrome, diabetes mellitus, and anorexia nervosa.<sup>60</sup> In contrast to the overfeeding reported in children with Prader–Willi syndrome, potential defects in GHS-R1 signaling could explain the observation of elevated AG levels in patients with anorexia nervosa as well as in children with poor weight gain and infants with failure to thrive due to reduced appetite scores.<sup>61,62</sup> Furthermore, AG is currently considered as a clinical target for stimulating food intake in patients with cachexia.<sup>63</sup>

## Ghrelin and energy metabolism

### Ghrelin and thyroid

The expression of GHS-R1a has been detected in C cells and follicular cells of the thyroid gland in rats,<sup>64,65</sup> while GOAT expression was described in the thyroid gland.<sup>66</sup> In human subjects, it was observed that the administration

of AG induces an increase in free  $T_4$  while decreasing thyroid-stimulating hormone (TSH) concentrations, although free  $T_3$  levels were not affected.<sup>67</sup> In young patients with Graves' disease, total ghrelin levels were positively correlated with TSH, fasting insulin, glucose and homeostasis model assessment, but negatively associated with  $T_3$  and  $T_4$  levels.<sup>68</sup> Both DAG and AG levels were found to be significantly lower in hyperthyroid patients, and plasma concentrations were reestablished after return to a euthyroid state.<sup>69–72</sup> In the same studies, ghrelin levels were correlated with insulin-resistance parameters. Also, the AG-induced stimulation of GH was blunted, while ACTH release was twofold higher in patients with hyperthyroidism versus healthy individuals. These effects were no longer detectable after restoration of normal thyroid function.<sup>73,74</sup> In contrast, higher total ghrelin levels are observed in patients with hypothyroidism.<sup>70</sup> In rats, central administration of AG daily for 5 days was shown to increase the weight of the pituitary and to reduce the size of TSH-immunopositive cells in the pituitary.<sup>75</sup> These effects were associated with a reduction of TSH and an increase in  $T_4$  concentrations in the blood. In PC-C13 cells, AG stimulates TSH-induced expression of thyroglobulin, thyroperoxidase, and sodium-iodine symporter.<sup>64</sup> Treatment of rat primary pituitary cells with  $T_3$  prevents GHS-R1 mRNA degradation and consequently increases its translation.<sup>76</sup> Finally, AG treatment stimulates TSH-induced proliferation in FRTL-5 thyroid cells.<sup>77</sup> These results indicate that dysregulation of ghrelin levels can occur as a consequence of impaired thyroid function. In addition, results derived from cellular models indicate that, in turn, AG influences thyroid functions. As indicated by the divergent information reported in the literature, the influence of AG on the regulation of thyroid functions needs to be further investigated before determining its potential as a clinical target.

## Ghrelin and muscle

In healthy humans, total ghrelin levels are negatively associated with skeletal muscle mass.<sup>78</sup> We and others had previously observed that AG might have a detrimental role on insulin sensitivity in human, animal, and cellular models.<sup>23,79</sup> Overall, the influence of AG on skeletal muscle functions remains largely uninvestigated. In elderly individuals, basal and postprandial levels of ghrelin are negatively correlated with fat-free mass and appendicular skeletal muscle mass.<sup>80</sup> Furthermore, the administration of AG stimulates lipolysis in the skeletal muscle and decreases peripheral insulin sensitivity and energy expenditure.<sup>79,81,82</sup> Although the

results from studies of ghrelin on muscular functions are relatively consistent in humans, they are more ambiguous in rodent models. For instance, in normal young adult rats, the administration of AG twice daily for 4 days stimulates the phosphorylation of Akt and glycogen synthase kinase (GSK) as well as glucose transporter type 4 (GLUT4) mRNA expression in the soleus muscle, but not in the gastrocnemius.<sup>83</sup> The same treatment also prevents triglyceride accumulation in the muscle of rats submitted to a high-fat diet by reducing protein levels of inflammatory markers NF- $\kappa$ B and tumor necrosis factor (TNF)- $\alpha$ , while increasing the activity of mitochondrial enzymes cytochrome C oxidase and citrate synthase as well as peroxysome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) expression in the gastrocnemius (mainly glycolytic) muscle.<sup>84,85</sup> Interestingly, GHS-R1 expression increases in slow-twitch muscle fibers but not in the gastrocnemius muscle of rats submitted to food restriction versus rats fed ad libitum.<sup>86</sup> Further, treatment of extensor digitorum longus (EDL) muscle with AG reduces gCl and gK conductance in rats.<sup>87</sup> This effect is mediated through the protein kinase C pathway and abolished by cotreatment with (D-Lys<sup>3</sup>)-GHRP-6, an antagonist of GHS-R. In addition, lower ghrelin levels are observed in the circulation and in the soleus in trained versus untrained rats.<sup>88</sup> In nephrectomized rats, AG increases cytochrome C oxidase, citrate synthase, PGC-1 $\alpha$ , and PGC-1 $\beta$  activity or mRNA levels, and therefore prevents muscle wasting through a mechanism that involves the stimulation of Akt phosphorylation.<sup>89</sup> Furthermore, in rats submitted to severe burns, the administration of DAG for 24 hours increases EDL muscle mass and reduces TNF- $\alpha$  expression, and in combination with interferon (IFN)- $\gamma$  reduces protein synthesis in the gastrocnemius as well as in C2C12 myotubes.<sup>90,91</sup> These effects of DAG are mediated through the activation of PI3K and mammalian target of rapamycin pathways. DAG was also shown to restore Akt, GSK-3 $\beta$ , 4E-binding protein (BP)1, and forkhead box protein O1 phosphorylation while reducing muscle atrophy signals such as phospho-NF- $\kappa$ B levels as well as muscle atrophy F-box and muscle ring finger 1 mRNA expression after treatment with TNF- $\alpha$  and IFN- $\gamma$ . Treatment with AG also stimulates myoblast differentiation and myotube formation in vitro.<sup>92,93</sup> Taken together, the contrasting results that have been reported until now in human and rodent models, as well as in different types of muscle fibers (ie, glycolytic and oxidative), substantiate the need for further studies in which the degradation of AG into DAG, its half-life in the circulation, and its potential indirect neuroendocrine effects are also taken into consideration.



## Ghrelin and adipose tissue

Soon after the discovery of ghrelin, Tschöp et al were the first to report on its orexigenic and adipogenic properties.<sup>33</sup> In obese individuals, total ghrelin levels were shown to be lower in fasting conditions and were less reduced (or not reduced at all) in postprandial as compared to normal subjects.<sup>94</sup> Interestingly, in women with metabolic disturbances, AG concentrations are higher in individuals with morbid obesity than simple obesity. However, both fasting and postprandial reduction in total ghrelin and AG levels are greater in healthy lean women.<sup>95</sup> Higher AG and lower DAG concentrations are also observed in obese individuals with or without type 2 diabetes. It is presently debated whether higher or lower GHS-R1 expression is detected in human omental adipose tissues of obese individuals.<sup>96,97</sup> Meanwhile, the incubation of primary human omental primary adipocytes with either AG or DAG was shown to stimulate PPAR- $\gamma$ , SREBP-1, acetyl-coenzyme A (CoA) carboxylase (ACC), fatty-acid synthase (FAS), lipoprotein lipase (LPL), and perilipin expression as well as triglyceride accumulation in lipid droplets.<sup>96</sup> Also, it was reported that treatment of ex vivo human adipose tissue extracts with DAG decreases glycerol release from adipocytes as well as hormone-sensitive lipase expression, while either AG or DAG can increase LPL expression.<sup>98</sup>

For practical reasons, in vivo and in vitro models have so far provided the most important evidence of the influence of ghrelin on adipocyte functions. On the other hand, transgenic animals represent interesting tools to understand the physiological effects of ghrelin. In fact, the inactivation of the GHS-R1 gene reduces adiposity in mice.<sup>99</sup> This could be mediated through decreased glucose/lipid uptake, de novo lipogenesis, increased insulin sensitivity and thermogenesis in brown adipocytes (expression of uncoupling protein 1 [UCP-1]), and improvement of lipid profiles. Treatment of adipocytes with AG decreases UCP-1 expression, and this effect is reversed by a GHS-R1 antagonist. Like mice, rats with an inactive form of GHS-R1 display increased brown adipose tissue weight, UCP-1 expression, O<sub>2</sub> consumption, CO<sub>2</sub> production, rectal temperature, and dark-period locomotor activity as well as lower visceral adiposity.<sup>100</sup> Also, AG administration reduces noradrenaline release in brown adipocytes from wild-type but not from GHS-R1-deficient rats. Both in wild-type and GH-deficient rats, central administration of AG for 8 days increases food intake, body weight, energy efficiency, and percent omental and visceral adiposity.<sup>101</sup> With regard to the response to chronic central AG administration, there is no difference between wild-type and GH-deficient rats in adipose tissue. In addition, mRNA/protein expression as well as enzymatic

activities of ACC $\alpha$  and phosphorylated ACC $\alpha$ , LPL, FAS, stearoyl-CoA desaturase (SCD-1), malonyl-CoA decarboxylase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PGDH) are increased in response to acute or chronic central administrations of AG in rats.<sup>101,102</sup> However, an increase in carnitine palmitoyltransferase 1 m (CPT-1 m), combined with a decrease in malonyl-CoA levels, is observed only in wild-type rats, and the increased protein levels of AMP-activated protein kinase  $\alpha$ 1 (AMPK $\alpha$ 1) are detected exclusively in GH-deficient rats.<sup>102</sup> Central infusion of AG in rats reduces the expression of UCP-1 and UCP-3 in brown adipocytes.<sup>102</sup> Moreover, in rats submitted to a high-fat diet or overfeeding, decreased ghrelin levels are observed in response to liposuction.<sup>103</sup> Also, in mouse pups submitted to the same treatment, increased GHS-R1 mRNA expression is observed while AG plasma levels are reduced.<sup>104</sup> In this last report, white adipose tissue weight as well as protein content or phosphorylation of Akt, PI3K, AMPK, GLUT4 and CPT-1 increased while reduced peroxysome proliferator-activated receptor- $\gamma$  expression was also observed in overfed mouse pups.

The effects of peptides of the ghrelin family (ie, AG, DAG, GHRP-6, and obestatin) were recently investigated in pre- and mature adipocytes. We<sup>97</sup> and others<sup>105</sup> observed that both AG and DAG stimulated adipocyte differentiation. In mature adipocytes, DAG stimulates fatty-acid uptake in 3T3-L1 cells in a more potent manner than AG. These effects are antagonized by a GHS-R1 antagonist and by inhibitors of phospholipase C and PI3K. Also, DAG significantly decreases lipolysis (ie, glycerol and nonesterified fatty-acid release) in primary adipocytes and 3T3-L1 cells.<sup>97,105</sup> These results indicate that ghrelin influences preadipocyte proliferation and differentiation, as well as mature adipocyte functions. Although it is difficult to differentiate the specific effects of AG versus DAG in vivo, in vitro models provide a useful alternative. In fact, results indicate that DAG might influence the regulation of adipocyte functions more potently than AG per se. This highlights the relevance of taking into consideration the role of DAG on energy expenditure. It also suggests that further evaluation of the mechanisms responsible for AG des-acylation following its administration in humans and animals is warranted.<sup>106</sup> This last section suggests the significance of considering DAG as a mediator of pre-, differentiating, and mature adipocyte functions. Results obtained in vitro need to be confirmed in vivo in animal and human models, while future studies could also characterize long-term effects of DAG treatment on subcutaneous and visceral adipocyte physiology. This in turn could provide valuable clinical information regarding the mechanisms underlying the regulation of obesity and its related dysfunctions.

## Ghrelin and liver

Liver functions are important for the maintenance of glucose, lipid, and cholesterol homeostasis. Furthermore, nonalcoholic fatty liver disease (NAFLD) is closely associated with the development of metabolic dysfunctions such as insulin resistance, type 2 diabetes, and dyslipidemia.<sup>107</sup> In obese individuals, severe lipid accumulation in the liver is related to lower circulating ghrelin levels.<sup>108</sup> However, in patients with NAFLD and hepatitis C, DAG and AG plasma levels are shown to be higher than in normal individuals.<sup>109,110</sup> In addition, in humans, it was suggested that ghrelin levels should be reduced in response to the development of insulin resistance rather than being influenced by liver damage per se.<sup>111,112</sup>

In vivo, peripheral infusion of AG inhibits insulin-induced suppression of hepatic glucose production while stimulating overall glucose uptake in mice.<sup>113</sup> These results were confirmed with observation of an AG stimulatory effect on hepatic glucose release and its inhibition in response to a cotreatment with DAG in porcine primary hepatocytes in vitro.<sup>114</sup> In another study, peripheral administration of AG induced a lipogenic and glucogenic response in the liver, and this was mediated through reductions in AMPK activity as well as Akt and GSK phosphorylation.<sup>83,84</sup> Furthermore, hepatic mitochondrial activity was decreased by 44% following the administration of AG; however, this effect was not observed after treating primary hepatocytes with AG.<sup>115</sup> This suggests the influence of the des-acylated form of ghrelin – DAG. In response to the central administration of AG, protein/mRNA expression or enzymatic activity was increased for SCD-1, ACC $\alpha$ , pACC $\alpha$ , AMPK $\alpha$ 1, AMPK $\alpha$ 2, FAS, G6PDH, and 6PGDH, while the opposite effect was observed for CTP-1 and malonyl-CoA in wild-type rats.<sup>101</sup> Interestingly, all these effects are also shown to occur in wild-type and GH-deficient rats. However, the authors suggest that this decrease in CPT-1 expression and in malonyl-CoA concentrations could be GH-specific, since it could not be detected in GH-deficient rats. Studies on liver functions report that AG or DAG could increase de novo lipogenesis and fatty-acid desaturation while inhibiting mitochondrial transport. Although it remains speculative, this suggests a potential role of ghrelin in the regulation of lipid infiltration in the liver and could consequently lead to clinical applications.

## Conclusion

Other than stimulating GH release, ghrelin has also been shown to influence appetite, energy expenditure, adipocytes, myocytes, and hepatocytes as well as the reproductive and immune systems.<sup>116,117</sup> Nonetheless, important questions

remain regarding the tissue-specific activity of AG and its des-acylated counterpart DAG. For instance, convincing evidence indicates that although devoid of orexigenic activity, DAG rather than AG could modulate adipocyte functions such as preadipocyte proliferation, differentiation, energetic substrate uptake, lipogenesis, triglyceride synthesis, fatty-acid transport into mitochondria, and mitochondrial activity. The existence of such an array of ghrelin-supported phenomena highlights, for instance, the importance of evaluating the contribution of AG degradation to DAG following its administration in clinical and animal models. Also, the potential interaction between DAG and a receptor displaying a high degree of homology with GHS-R1, such as GPR38, deserves to be investigated.<sup>118</sup> Overall, although ghrelin was discovered less than 15 years ago, it has shed light on several key mechanisms that support its role in endocrine and neuroendocrine regulation of major metabolic functions.

## Disclosure

The authors report no conflicts of interest in this work.

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