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REVIEW

Ghrelin-ghrelin *O*-acyltransferase system in the pathogenesis of nonalcoholic fatty liver disease

Shao-Ren Zhang, Xiao-Ming Fan

Shao-Ren Zhang, Xiao-Ming Fan, Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, Shanghai 201508, China

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Correspondence to: Xiao-Ming Fan, MD, PhD, Professor, Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, No. 1508 Longhang Road, Shanghai 201508, China. xiaomingfan57@sina.com
Telephone: +86-21-34189990
Fax: +86-21-57943141

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is currently considered as the most common liver disease in Western countries, and is rapidly becoming a serious threat to public health worldwide. However, the underlying mechanisms leading to the development of NAFLD are still not fully understood. The ghrelin-ghrelin O-acyltransferase (GOAT) system has recently been found to play a crucial role in both the development of steatosis and its progression to nonalcoholic steatohepatitis. Ghrelin, the natural ligand of the growth hormone secretagogue receptor, is a 28-amino acid peptide possessing a unique acylation on the serine in position 3 catalyzed by GOAT. The ghrelin-GOAT system is involved in insulin resistance, lipid metabolism dysfunction, and inflammation, all of which play important roles in the pathogenesis of NAFLD. A better understanding of ghrelin-GOAT system biology led to the identification of its potential roles in NAFLD. Molecular targets modulating ghrelin-GOAT levels and the biologic effects are being studied, which provide a new insight into the pathogenesis of NAFLD. This review probes into the possible relationship between the ghrelin-GOAT system and NAFLD, and considers the potential mechanisms by which the ghrelin-GOAT system brings about insulin resistance and other aspects concerning NAFLD.

Key words: Energy homeostasis; Ghrelin-ghrelin *O*-acyltransferase system; Insulin resistance; Lipid metabolism; Nonalcoholic fatty liver disease

Core tip: Nonalcoholic fatty liver disease (NAFLD) is a progressive disorder that can lead to impaired liver function and, ultimately, liver failure. The ghrelin-ghrelin *O*-acyltransferase (GOAT) system has recently been found to play a crucial role in both the development of steatosis and its progression to nonalcoholic steatohepatitis. This review probes into the possible relationship between ghrelin-GOAT system and NAFLD, and considers the potential mechanisms by which the ghrelin-GOAT system brings about insulin resistance and other aspects concerning NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a metabolic disorder syndrome that is not due to the abuse of alcohol. The term NAFLD encompasses a spectrum of histologically defined liver disorders. The disease can progress from macrovesicular lipid accumulation in the hepatocytes (termed steatosis) to nonalcoholic steatohepatitis (NASH) to outright fibrosis, cirrhosis, and even hepatocellular carcinoma[1-3] (Figure 1). The occurrence of NAFLD is strongly linked to obesity, insulin resistance (IR) and other aspects of the metabolic syndrome.

The reported prevalence of NAFLD in the United States and other Western countries ranges from 30% to 46%[4-6]. This disease has also become prevalent in Eastern countries where it has become a significant public health concern[7,8]. However, patients with NAFLD often have normal liver aminotransferases and the potential presence of NAFLD may be neglected by clinicians[9-12]. Patients with NAFLD are always at high risk for cardiometabolic complications, such as type 2 diabetes (T2DM) and cardiovascular disease[13-16].

The exact pathogenesis of NAFLD remains unknown. A number of environmental and genetic factors are involved in the NAFLD development and progression (Figure 1). The "two-hits hypothesis" is currently the most recognized theory to explain NAFLD development and progression[17]. Fat accumulation in hepatocytes is considered as the primary insult, while the following events, including mitochondrial dysfunction, lipid peroxidation, IR, and oxidative stress, result in liver cell inflammation and apoptosis, which eventually progress from simple steatosis to NASH[18-20]. Although the "two-hits hypothesis" of NAFLD pathogenesis is currently the most recognized theory, the "multi-hits hypothesis" that involves lipotoxicity, oxidative stress, mitochondrial dysfunction, a chronic inflammatory state, and endoplasmic reticulum stress, is getting more and more attention. The "multi-hits hypothesis" summarizes the complex factors and interactions between cytokines, free fatty acids (FFAs) metabolism, inflammation, and IR in NAFLD[21,22].

As oxidative stress and inflammation are key events in the progression from simple steatosis to NASH, retardation of these processes may reverse the development of NAFLD[18,20]. Thus, use of low side-effect agents that ameliorate those key events of NAFLD may provide important therapeutic evidence for the development of NAFLD. In recent years, a number of chemical agents have been found to have protective efficacy against NAFLD-induced liver injury, oxidative stress, and inflammation[23-26].

Recently, several studies have shown some advances in the pathogenesis of NAFLD. Advances in the understanding of autophagy have provided insights into the relationship between autophagy and NAFLD. Autophagy might stimulate lipid metabolism and have therapeutic potential in NAFLD[27]. *Helicobacter pylori* infection is involved in the pathogenesis of IR, which is closely linked with NAFLD[28]. The role of *H. pylori* infection in the development of NAFLD is gaining attention because its eradication is easy and much less expensive than long-term treatment of the other risk factors. Besides, overexpression of miR-185, an endogenous non-protein coding small RNA molecule, improved insulin sensitivity and reduced liver steatosis in an NAFLD animal model, and thus may be a therapeutic target[29]. In recent years, several adipocytokines and proinflammatory cytokines, which decrease or enhance IR, were also found to be involved in the pathogenesis of NAFLD[30-32]. Nevertheless, the complicated mechanisms of NAFLD are not entirely clear at present. Future better-designed research will provide more insights into the pathogenesis and therapeutic strategies for NAFLD.

The ghrelin-ghrelin *O*-acyltransferase (GOAT) system has recently been reported to play a crucial role in both the development of steatosis and progression to NASH. The ghrelin-GOAT system is involved in IR, lipid metabolism dysfunction, and inflammation, all of which play important roles in the pathogenesis of NAFLD[33-36]. Therefore, there is an urgent need to better understand the mechanisms of ghrelin-GOAT system involvement in NAFLD. This review will illuminate the relationship between the ghrelin-GOAT system and pathogenesis of NAFLD.

OVERVIEW OF THE GHRELIN-GOAT SYSTEM

Ghrelin

Ghrelin is a small peptide and hormone comprised of 28 amino acids[37] that is mainly produced by the stomach and the pancreas, which stimulates appetite and is a potent stimulator of growth hormone through the action of its receptor, the growth horomone secretagogue receptor[38,39]. A number of studies have shown that exogenous administration of ghrelin produces multiple physiologic effects, including the ability to increase food intake and decrease energy expenditure[38,40]. Ghrelin undergoes a post-translational modification, in which the third serine residue is covalently linked to a medium-chain fatty acid, typically octanoate[38]. The *O*-n-octanoylation of ghrelin is unique[41], and only the octanoylated form, which represents 10%-15% of circulating ghrelin, is able to stimulate body weight gain and food intake[42,43].

There are two forms of ghrelin: acylated and des-acyl ghrelin (DAG). Without food intake, both forms rise gradually in the plasma. Although some effects of DAG are still controversial and its receptor has not been identified, the biologic activities of DAG have

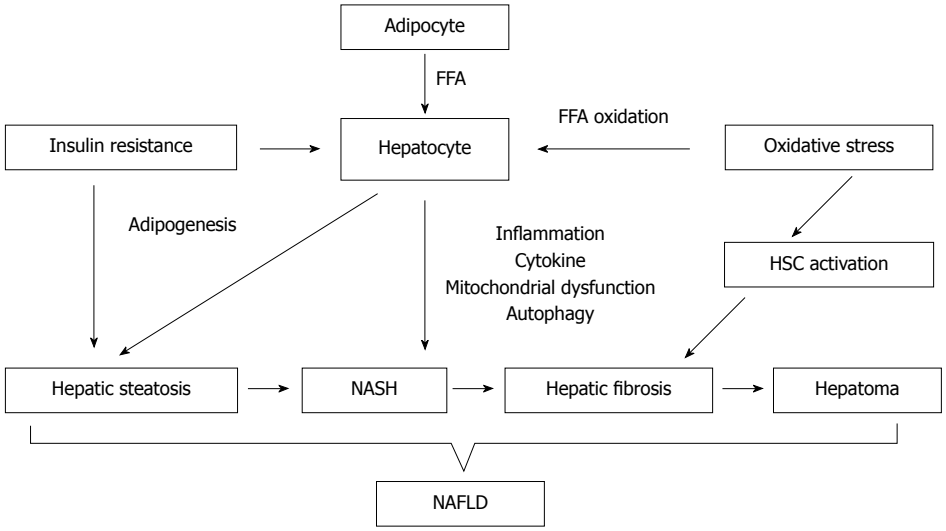

Figure 1 Pathogenesis and natural history of nonalcoholic fatty liver disease. FFA: Free fatty acid; HSC: Hepatic stellate cell; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

been reported, including gastric motility[44,45], adiposity, and glucose metabolism[46]. Further evidence for metabolic function of ghrelin has been provided by phenotypic analysis of rodents with genetic deletions of either ghrelin[47] or its receptor, GHS-R1a[48].

Ghrelin O-acyltransferase

Ghrelin is acylated on the serine in position 3. Both forms of ghrelin may result from the processing of preproghrelin[49]. The acylation is catalyzed by GOAT during the processing of the peptide[29,50]. Ingestion of either medium-chain fatty acids or medium-chain triglycerides can enhance acylation of ghrelin[51].

Lim *et al*[52] found that GOAT is expressed in all human tissues studied (stomach, adrenal cortex, breast, and right and left colon). The widespread expression of GOAT corresponds to the widespread distribution of ghrelin expression. GOAT expression was high in the stomach and gut, the major ghrelin-secreting tissues, and in the pituitary, ghrelin showed autocrine and paracrine effects. In addition to the important endocrine effects of acylated ghrelin, the paracrine effects of locally synthesized and acylated ghrelin may also be important. The concept was supported by the identification of GOAT expression in various tissues. It will be helpful to search for GOAT inhibitors as an alternative approach to reduce the actions of ghrelin, such as feeding and adiposity. As the action of GOAT and its inhibition are very specific to ghrelin, this may be a promising therapeutic target.

The activity of GOAT is modulated by fasting and satiation[53-55]. Although feeding suppresses both acylated ghrelin and DAG, long-term fasting inhibits ghrelin acylation but not total ghrelin secretion[56]. The exact effect of fasting and feeding on *GOAT* mRNA expression remains vague[57,58]. GOAT has been confirmed as a leptin-regulated gene[57], and González *et al*[58] found that exogenous leptin administration markedly increased *GOAT* mRNA levels in the gastric mucosa of fasted rats. It has been indicated that fasting low-leptin levels prevent an increase in *GOAT* mRNA levels, and therefore, GOAT can be added to the list of leptin-regulated genes under this specific condition. Leptin is the primary signal through which the hypothalamus senses the nutritional state and modulates food intake and energy balance. Leptin plays an opposite functional role of ghrelin in food intake and it also regulates ghrelin receptor GHS-R1a[59,60]. Increased *GOAT* mRNA levels relevant to chronic malnutrition may elucidate the potential mechanism responsible for increased acylated ghrelin levels in anorexia nervosa[61].

Alimentary lipids are important for the activation of GOAT. In fact, *GOAT* knockout mice subjected to a diet containing 10% medium-chain triglycerides exhibited lower body weights, possibly due to lower fat mass, compared to wild-type mice[57]. In addition, large amounts of acyl ghrelin were produced by *GOAT* transgenic mice[57]. An important function of ghrelin is the maintenance of viability during famine. The study of wild-type and *GOAT* knockout mice subjected to a 60% calorie-restricted diet showed 30% and 75% body weight loss, respectively, which could explain this hypothesis[62].

GHRELIN-GOAT SYSTEM AND NAFLD

The ghrelin-GOAT system is linked to energy and lipid metabolism, IR, inflammation, and apoptotic cell death, which are common to both obesity and NAFLD. Therefore, the role of the ghrelin-GOAT system in NAFLD has become a subject of considerable interest in recent years.

The relation of the ghrelin gene products and their involvement in metabolic and inflammatory pathways linked with the development of NAFLD were recently

reported[34]. It was found that patients with NASH had a twofold higher concentration of DAG than patients with non-NASH. Ghrelin concentration positively correlated with fibrosis stage. Apparently, products of the ghrelin gene may be important for the pathogenesis of NASH and fibrosis. The report by Li *et al*[33] showed that both administration of ghrelin during the induction of NAFLD and after the establishment of NAFLD could improve liver injury *via* attenuating alanine aminotransferase/aspartate transaminase, oxidative stress, inflammation, apoptosis, and restoring hepatic lipid metabolism. Such effects might partly act through targeting the PI3K/Akt and LKB1/AMPK pathways. Therefore, ghrelin can be a critical therapeutic agent against NAFLD. However, other research yielded different results. One study reported that the plasma levels of ghrelin in obese individuals were lower than those in normal-weight people, indicating that ghrelin may not be related to the progression of obesity[63].

The role of ghrelin in appetite regulation and energy metabolism is well established and it is now recognized as a very promising target for the treatment of NAFLD[64]. GHS-R antagonists and ghrelin antibodies are being studied in these systems[65]. An anti-obesity vaccine that prevents ghrelin from reaching the central nervous system has been developed[66]. A glucagon-like peptide-1 receptor agonist, exendin-4, has shown the effect of inhibiting ghrelin secretion[67].

Besides ghrelin and GHS-R, GOAT has also been implicated as a potential target for anti-NAFLD treatment[35,36]. GOAT inhibition will lead to decreased ghrelin acylation and increased levels of DAG, which is suggested to be beneficial for glucose homeostasis[68,69]. At the moment, there are no anti-NAFLD drugs on the market that target the ghrelin system. This is mainly due to the variation or lack of efficacy, potency, non-selectivity, poor bioavailability, sustained weight loss, and/or adverse side effects. However, specific antagonists are being developed and their relevance to clinical practice is being studied.

Role of the ghrelin-GOAT system in IR

IR is a disorder in insulin signaling in many organs, including the liver, fat, and muscle, and is a major characteristic of obesity, T2DM, and NAFLD. IR is an essential requirement for NAFLD and is believed to influence "the first hit" in NAFLD. Some research has illuminated that IR is a typical character of NAFLD[70-73]. NAFLD is highly prevalent among patients with T2DM[74]. By addressing NAFLD both as a consequence and as a cause of IR through lessons learned from the liver of patients with T2DM, Takamura *et al*[75] presented the remarkable changes in the liver in NAFLD. The development of NAFLD appears to be associated with food intake, as diet is an important contributor to its pathogenesis[76].

In recent years, ghrelin has been found to play a direct role in glucose homeostasis. A number of reports have demonstrated ghrelin expression in pancreatic islets[77-80] and the ability of ghrelin to regulate insulin secretion and promote β-cell proliferation and survival[81,82]. However, the role of ghrelin in the secretion and action of insulin remains controversial. Some studies show that ghrelin increases insulin secretion[79,81,83-85], whereas other reports show that it inhibits it[86-89].

Mice genetically deficient in the GOAT enzyme lack acylated ghrelin and exhibit a modest decrease in body weight and fat mass when fed a diet rich in medium-chain triglycerides[57]. When these mice were subjected to a period of severe caloric restriction, they were unable to maintain normal blood glucose levels, resulting in eventual death, unless either ghrelin or growth hormone was provided[62]. A recent report showed that a GOAT-specific acyltransferase inhibitor could improve glucose tolerance and reduce weight gain, indicating the effect of the ghrelin-GOAT system on glucose homeostasis[90].

IR patients with NAFLD show decreased insulin sensitivity not only in muscle, but also in liver and adipose tissue[73,91]. The adipose tissue becomes resistant to the anti-lipolytic effect of insulin, and the release of fatty acids is increased in IR[92]. An important source of FFAs is the increased spillover from chylomicrons under postprandial conditions[93]. It was supposed that ectopic fat may be a defense mechanism against lipotoxicity[94,95], and that patients with NAFLD develop NASH and cirrhosis only after a second hit. Therefore, it is not surprising that gut hormones that are known to control the uptake of nutrients by organs are now increasingly investigated in NAFLD.

Role of the ghrelin-GOAT system in lipid and energy metabolism

The first step in the development of NAFLD is hepatic steatosis, which is characterized by macrovesicular accumulation of triglycerides in the cytoplasm of hepatocytes. Sources of increased hepatic lipids in NAFLD include excess dietary chylomicron remnants, increased new lipogenesis, or excess FFAs released from the lipolysis of adipose tissue[96-98]. Saturated fat seems to stimulate hepatic lipid accumulation and progression into NASH, whereas unsaturated fat, choline, antioxidants, and high-protein diets rich in isoflavones seem to have a more preventive effect. Li *et al*[99] recently found that ghrelin activated hepatocyte lipogenesis *via* the mTOR-PPARγ signaling pathway; ghrelin-induced lipogenesis was mediated by mTOR, and the effect was significantly attenuated by PPARγ antagonism in cultured hepatocytes and in *PPAR*γ-deficient mice.

Lipid accumulation within the liver represents an equilibrium between synthesis and utilization. The indiscriminate lipid metabolism and increased lipid flux through the liver result in intracellular stress, apoptosis, and consequent liver damage[100]. IR in adipose tissue

with uncontrolled lipolysis resulting in enhanced FFA delivery to the liver has been postulated to be a critical factor in development of NAFLD. IR in adipose tissue has been shown to correlate with severity of liver biopsy findings in NASH[101]. Adipose tissue tumor necrosis factor (TNF)-α and circulating interleukin 6 are associated with IR and circulating FFA levels, and both are increased in patients with NAFLD[102-104]. It is entirely plausible that endocrine abnormalities with hormonal excess and deficiencies may be implicated in the pathogenesis of NAFLD.

Ghrelin stimulates food intake and decreases energy expenditure in rats[48,105-108]. Ghrelin also increases appetite and stimulates food intake in humans[105]. A recent study evaluated ghrelin levels and their relationship with NAFLD and IR in obese adolescents, and found that ghrelin was negatively correlated with weight[109]. Ghrelin concentrations decrease with weight gain resulting from overfeeding, pregnancy, or olanzapine treatment[110-113]. Indeed, ghrelin stimulates the gene expression of lipogenic enzymes, such as acetyl CoA carboxylase, stearoyl CoA desaturase, and fatty acid synthase in white adipose tissue[114].

In order to determine *GOAT* expression and functional regulation, measurement of its protein levels and activity will be critical. Recently, genetic variation in *GOAT* was found in association with anorexia nervosa[115], though whether it may also be involved in NAFLD remains unknown. Inhibition of GOAT by a peptide-based bisubstrate analog (GO-CoA-Tat) reduced weight gain and improved glucose tolerance in wild-type mice[84]. In fact, GOAT is the only enzyme responsible for ghrelin acylation and its alteration will only affect the physiologic process of ghrelin acylation. In the future, special medicine targeting GOAT may be designed as a novel therapeutic approach for NAFLD.

Role of the ghrelin-GOAT system in inflammation

A small portion of patients with NAFLD will develop inflammation and fibrosis, termed NASH, which is a more progressive, inflammatory disease phenotype of NAFLD[18]. In recent years, the roles of ghrelin in immunity regulation under inflammatory conditions and liver protection have been being clarified. In the gastrointestinal tract, administration of exogenous ghrelin ameliorates the release of proinflammatory cytokines, promotes cell proliferation, and reduces apoptosis after TNF-α- or lipopolysaccharide-induced inflammation[116]. Administration of ghrelin has therapeutic effects for several inflammatory diseases in rodent models, including sepsis[117], intestinal ischemia and reperfusion injury[118], pancreatic disease[119], cardiovascular disease[120], and gastrointestinal disease[121]. In addition, a recent study demonstrated that pretreatment with ghrelin prior to carbon tetrachloride intoxication attenuated liver injury and oxidative stress[122]. Thus, inflammation represents an important mechanism for the development of NAFLD to NASH. Now that recent research has suggested the anti-inflammatory role of ghrelin in many organs, it is possible that future study will identify its pharmacologic role in the development of NAFLD.

CONCLUSION

NAFLD is becoming a serious threat to public health worldwide. However, the underlying mechanisms leading to the development of NAFLD are not fully understood. The involvement of the ghrelin-GOAT system in NAFLD and a better understanding of its biology have led to the identification of pharmacologic targets and the development of pharmacologic compounds for the treatment of NAFLD and related diseases. Thus, the ghrelin-GOAT system represents a promising target for the treatment of NAFLD.

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