

GHRELIN, LIPID METABOLISM, AND METABOLIC SYNDROME

PABLO B. MARTÍNEZ DE MORENTIN, SULAY TOVAR, RUBÉN NOGUEIRAS, MIGUEL LÓPEZ,
AND CARLOS DIÉGUEZ

Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela (IDIS) & CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), Spain

Abstract: Ghrelin is a 28-aminoacid peptide predominantly, but not exclusively, produced by stomach's X/A-like cells that acts through the growth hormone secretagogue receptor (GHS-R). It is the only peptidic hormone stimulating appetite and adiposity. Although ghrelin has a wide range of actions in the body, including the regulation of glucose homeostasis, regulation of gastric and pancreatic activity, and stimulation of growth hormone or cardiovascular activities, its main role is the regulation of energy balance. In fact, the ghrelin system has recently become an interesting target to design chemicals against obesity and related comorbidities. Fatty acids, which play a crucial role in the metabolic syndrome, are essential mediators for the actions of ghrelin on both food intake and adiposity. In this review we will summarize the current knowledge about ghrelin actions and signaling pathways on metabolic homeostasis and energy balance with special emphasis on those related to its effects at hypothalamic level.

29.1 INTRODUCTION

Ghrelin is a 28-aminoacid peptide predominantly, but not exclusively, produced by the stomach's X/A-like cells that acts through the growth hormone secretagogue receptor 1a (GHS-R1a). Ghrelin is the only peptidic hormone stimulating appetite and adiposity. Although ghrelin has a wide range of actions in the body, including the regulation of glucose homeostasis, regulation of gastric and pancreatic activity, and stimulation of growth hormone release and cardiovascular activities, its main role is the regula-

tion of energy balance. In fact, recently the ghrelin system has becoming an interesting target to design chemicals against obesity and related comorbidities. Fatty acids, which play a crucial role in metabolic syndrome, are essential mediators for the actions of ghrelin on both food intake and adiposity. In this chapter we will summarize the current knowledge about ghrelin actions and signaling pathways on metabolic homeostasis and energy balance with a special emphasis on those related to its effects at hypothalamic level.

29.2 GHRELIN: A STOMACH-DERIVED PEPTIDE MODULATING ENERGY BALANCE

Ghrelin, a hormone mainly produced in the stomach with orexigenic properties [1–6], has attracted great attention as a potential antiobesity therapeutic target [7, 8]. Chronic ghrelin administration promotes weight gain and adiposity in rodents [2], as well as increasing voluntary food-intake in humans [9]. Assessment of total circulating ghrelin levels (acylated and unacylated forms) have demonstrated an opposite relationship with body weight [10]. Contrary in obese patients diagnosed with Prader-Willi syndrome, increased levels of ghrelin are detected, which could explicate their hyperphagia and increased body weight [11]. Additionally, ghrelin levels are markedly increased before meals [12, 13]. Furthermore, ghrelin knockout (KO) mice [14] or ghrelin receptor KO (GHS-R KO) mice [15] are protected against diet-induced obesity (DIO). All this evidence suggests that ghrelin might be an important signal to get ready for meal initiation [12, 16, 17]. More recent data have demonstrated that meals inhibited secretion of both ghrelin and des-acyl ghrelin, and in contrast to previous findings assessing total ghrelin long-term fasting inhibited acylation but not total secretion [18, 19].

29.3 GHRELIN EFFECTS ON FOOD-INTAKE ARE MEDIATED BY THE OREXIGENIC NPY/AGRP NEURONS

The ghrelin receptor, namely the growth hormone secretagogue receptor 1a (GHS-R1a), is expressed in neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC) [5, 20–26], indicating that this set of neurons could be involved in ghrelin's orexigenic action. In keeping with this, adult male rats, fed or fasted, treated centrally (intracerebroventricularly, ICV) with ghrelin, showed increased AgRP and NPY expression in the ARC (Fig. 29.1)[3, 4, 27]. These changes are quite restricted to this hypothalamic region because no change was demonstrated in the mRNA levels of the other feeding-related neuropeptides (such as melanin concentrating hormone, MCH, and prepro-orexin) studied at any time evaluated [4]. The

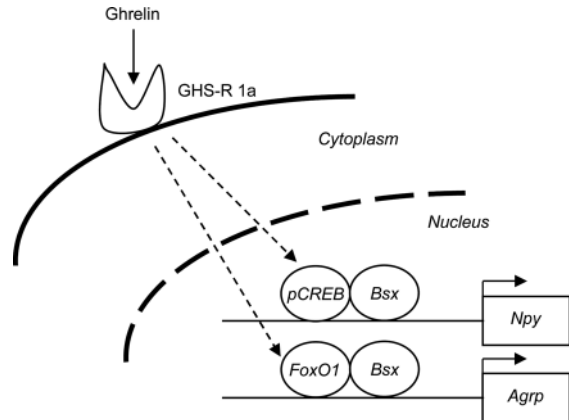


Fig. 29.1 The “classical” mechanism under ghrelin orexigenic effect. Ghrelin, acting on growth hormone secretagogue receptor 1a (GHS-R 1a), increased the expression of hypothalamic homeobox domain transcription factor (BSX), forkhead box O1 (FOXO1) and the phosphorylated cAMP response-element binding protein (pCREB). Subsequently, agouti-related peptide (AgRP) and neuropeptide Y (Npy) gene expression is stimulated in the arcuate nucleus of the hypothalamus (ARC).

physiological relevance of both neuropeptides as mediators of ghrelin effects was definitely established by assessing the response to ghrelin in KO mice. These experiments showed that although NPY KO or AgRP KO showed a normal response in terms of food intake to ghrelin, the double null NPY/AgRP mice failed to display any response, indicating the existence of redundancy among these two neuropeptides as mediators of ghrelin's orexigenic action [28].

29.4 TRANSCRIPTIONAL MACHINERY MEDIATING THE HYPOTHALAMIC ACTIONS OF GHRELIN

It has been reported that the hypothalamic homeobox domain transcription factor BSX, is highly expressed in AgRP/NPY neurons in the ARC and regulates ghrelin's stimulatory effect on AgRP and Npy gene expression [29–31]. Although both genes share BSX as a common part of the transcriptional system, BSX needs to interact with another two transcription factors to regulate AgRP and Npy mRNA expression: the forkhead box O1 (FOXO1) for AgRP gene and

the phosphorylated cAMP response-element binding protein (pCREB) for Npy gene [30–33]. We have shown that BSX, FOXO1 and pCREB protein content in the hypothalamus is increased after central ghrelin treatment (Fig. 29.1) [31]. Of note, the ghrelin-BSX-FOXO1/pCREB-AgRP/NPY pathway seems to exhibit a nucleus-specific pattern, because BSX expression in the dorsomedial nucleus of the hypothalamus (DMH) is unaffected by central ghrelin treatment [30].

29.5 HYPOTHALAMIC FATTY ACID METABOLISM AND AMPK MEDIATE GHRELIN'S ACTIONS ON FOOD INTAKE

Although the classic pathway involving AgRP/NPY neurons, and more recently BSX-FOXO1/pCREB, has shown some of the mechanisms underlying

the orexigenic effect of ghrelin, it was obvious that several key upstream and downstream factors involved in the transduction pathway of the activated GHS-R1a were still missing. Current evidence has demonstrated that nutrient-related metabolic pathways, such as lipid acid metabolism, may act as direct modulators of the hypothalamic control of feeding [6, 34–45], suggesting that maybe ghrelin could exert its orexigenic effect through these metabolic pathways.

This hypothesis was confirmed by data from several groups indicating that ghrelin modulates hypothalamic AMP-activated protein kinase (AMPK), a key upstream master regulator of lipid metabolism and its upstream kinase Ca²⁺/calmodulin (CaM)-dependent protein kinase kinase 2 (CaMKK2) (Fig. 29.2) [6, 37, 38, 42–45]. However, despite this evidence, the molecular events and anatomical aspects of this interaction have not been

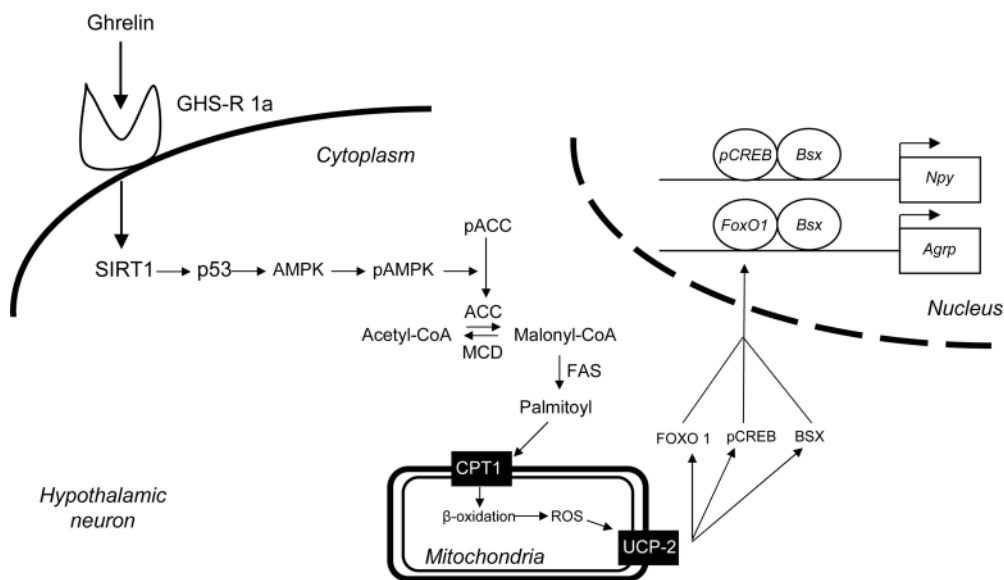


Fig. 29.2 Central ghrelin actions on hypothalamic lipid metabolism and AMPK. Ghrelin, acting on growth hormone secretagogue receptor 1a (GHS-R 1a), regulates hypothalamic AMP-activated protein kinase (AMPK), phosphorylating (pAMPK) and activating it, which in turn phosphorylates and inactivates acetyl-CoA carboxylase (ACC), decreasing the cytoplasmic pool of malonyl-CoA. The net result of this action is an increase in carnitine palmitoyltransferase 1 (CPT1) activity and subsequent fatty acid oxidation, which promotes the generation of reactive oxygen species (ROS) that are buffered by uncoupling protein 2 (UCP2). This mechanism is critical for ghrelin-induced electric activation of agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons, ghrelin-induced upregulation of AgRP and Npy gene expression in the ARC and ghrelin-induced feeding. MCD: malonyl-CoA decarboxylase; FAS: fatty acid synthase; BSX: homeobox domain transcription factor; FOXO1: forkhead box O1; pCREB: phosphorylated cAMP response-element binding protein.

fully identified and more importantly, there was no mechanistic data indicating that AMPK is required for ghrelin's orexigenic effects. Back in 2008, we investigated that possibility. By using a combination of pharmacological and genetic approaches, we first demonstrated that the physiological orexigenic response to ghrelin requires specific inhibition of fatty acid biosynthesis induced by AMPK resulting in decreased hypothalamic levels of malonyl-CoA and increased carnitine palmitoyltransferase 1 (CPT1, the key enzymatic activity modulating fatty acid oxidation into the mitochondria) activity (Fig. 29.2) [6, 43]. In addition, we also showed that fasting downregulates fatty acid synthase (FAS) in a region-specific manner, namely the ventromedial nucleus of the hypothalamus (VMH), and that this effect is regulated by an AMPK and ghrelin-dependent mechanisms (Fig. 29.2) [6, 43]. This mechanistic link was further explored by Diano and colleagues, who showed that hypothalamic fatty acid oxidation pathway, specifically involving AMPK and CPT1, elicited by ghrelin induced the production of reactive oxygen species (ROS), which are buffered by uncoupling protein 2 (UCP2) [44]. This mechanism involving UCP2 is critical for ghrelin-induced electric activation of AgRP/NPY neurons, ghrelin-triggered synaptic plasticity of POMC neurons and ghrelin-dependent gene transcription events, such as *AgRP* and *Npy*, in those neurons (Fig. 29.2) [44], integrating ghrelin signaling on GHS-R1a at the membrane with oxidative processes in the mitochondria and nuclear transcriptional events.

29.6 GHRELIN AND THE HYPOTHALAMIC SIRTUIN1 (SIRT1)/P53 AXIS

The sirtuins are a family of highly conserved NAD⁺-dependent deacetylases that act as cellular sensors to detect energy availability and modulate metabolic processes [46–48]. Two sirtuins that are central to the control of metabolic processes are mammalian SIRT1 and SIRT3, which are localized to the nucleus and mitochondria, respectively [46–48]. Both are activated by high NAD⁺ levels, a condition caused by low cellular energy status [46–48]. By deacetylating a variety of proteins that induce catabolic

processes while inhibiting anabolic processes, SIRT1 and SIRT3 coordinately increase cellular energy stores and ultimately maintain cellular energy homeostasis [46–48].

One of the most important cellular targets of SIRT1 is p53, a central tumor suppressing protein that regulates many cellular activities, such as cell cycle regulation, DNA repair, and programmed cell death. As SIRT1 binds and deacetylates p53 to decrease its transcriptional activity, p53 is suggested to play a central role in SIRT1-mediated functions in tumorigenesis and senescence [48–52].

Several lines of evidence have linked AMPK to SIRT1. AMPK controls the expression of genes involved in energy metabolism in mouse skeletal muscle by acting in coordination with SIRT1 [46–48]. AMPK increases SIRT1 activity by increasing cellular NAD⁺ levels [53]. Interestingly, this interaction between SIRT1 and AMPK seems to be reciprocal, as SIRT1 activation stimulates fatty acid oxidation and indirectly activates AMPK [54]. Besides this functional evidence, morphological data have demonstrated that SIRT1 is present in metabolically important areas of the brain, including the ARC, VMH, DMH, and paraventricular hypothalamic (PVH) nuclei [55, 56], where it is modulated by nutritional status, leptin, and melanocortin tone [55–59].

On the basis of these data, we have recently explored the possible relationship between ghrelin and SIRT1/p53 pathway at hypothalamic level. Our data showed that pharmacological blockade of hypothalamic SIRT1 (by using the specific antagonist Ex527) prevents the orexigenic action of ghrelin [58, 60]. Consistent with these pharmacological results, the selective disruption of SIRT1 in AgRP neurons in mice blunted electric responses of AgRP neurons to ghrelin and decreased food intake [58]. Also in keeping with this evidence, p53 null mice are insensitive to ghrelin in terms of feeding behavior. Of note, the link ghrelin-SIRT1/p53 is upstream of hypothalamic AMPK signaling because ghrelin failed to phosphorylate AMPK either when rats were pre-treated with Ex527 or in p53 KO mice. Overall, these data indicate that ghrelin specifically triggers a central SIRT1/p53 pathway that is essential for its orexigenic action (Fig. 29.2).

29.7 DOES GHRELIN ACT ON ALTERNATIVE CANONICAL ENERGY SENSORS BESIDES AMPK AND SIRT1?

So far, we have shown that central ghrelin exerts its action through modulation of AMPK and SIRT1, which act as cell and whole-body energy sensors [3, 4, 6, 31, 42, 44, 60, 61]. The key question is whether that is an exclusive relationship, or whether ghrelin may act on additional energy gauges.

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine-threonine kinase that acts sensing cellular changes in energy balance, growth factors, nutrients and oxygen [62–66]. mTOR is a component of at least two multi-protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [62, 63, 66]. mTORC1 phosphorylates and modulates the activity of the serine/threonine ribosomal protein S6 kinase 1 (S6K1). In turn, S6K1 phosphorylates and activates S6, a ribosomal protein involved in translation [62, 63, 66].

Recent evidence has shown that hypothalamic mTOR signaling plays a major role in modulating energy balance by responding to nutrient availability and the hormonal milieu [67–75]. Thus, central administration of hormones (i.e., leptin; thyroid hormone; ciliary neurotrophic factor, CNTF; bone morphogenetic protein 7, BMP7) or metabolites (i.e., leucine, α -lipoic acid) regulates feeding behavior through modulation of hypothalamic mTOR [67, 69, 70, 75, 76]. On the basis of these data, we have recently investigated whether ghrelin actions may be modulated by hypothalamic mTOR signaling. Our data show that central ghrelin administration promotes a marked increase in the phosphorylated (active) form of mTOR and its downstream targets, pS6K1 and pS6 in the ARC. Importantly, inhibition of mTOR signaling following inhibition of mTOR (by using rapamycin) reverses the orexigenic action of ghrelin treatment [77]. Of note, this action is associated with normalization of AgRP and NPY expression in the ARC, as well as pCREB and FOXO1. These data indicate that activation of hypothalamic mTOR signaling as a mediator of food intake might be of potential importance for the understanding and treatment of obesity.

29.8 CENTRAL GHRELIN ACTIONS ON PERIPHERAL LIPID METABOLISM

In addition to its role as main modulator of hypothalamic lipid metabolism, recent data have also highlighted the role of ghrelin as a main modulator of peripheral lipid metabolism [78]. Central administration of ghrelin promotes adiposity by the stimulation of the lipogenic program in the white adipose tissue (WAT) in a food intake-independent fashion [61, 79–81]. In particular, central ghrelin administration stimulates AgRP/NPY neurons, which promotes the blockade of the melanocortin receptors 3 and 4 (MC3R and MC4R) and regulation of peripheral lipid metabolism through the sympathetic nervous system (SNS) [79, 80]. As a result of these events, mRNA expression of various key fat storage enzymes such as lipoprotein lipase (LPL), ACC α , FAS, and stearoyl-CoA desaturase 1 (SCD1) is induced in WAT; on the other hand, the rate-limiting step in fat oxidation, CPT1, was decreased [61, 79, 80]. Altogether, this evidence indicates that central ghrelin's action is physiologically relevant in the control of adipocyte metabolism and that ghrelin could elicit processes in the central nervous system (CNS) in preparation for the ingestion of food [78].

Interestingly, besides the actions of ghrelin on WAT lipid metabolism, central ghrelin also affects energy expenditure. Thus, central administration of ghrelin decreased the expression of uncoupling proteins 1 and 3 (UCP1 and UCP3) in brown adipose tissue (BAT) [79] (Fig. 29.3), suggesting that, in addition to increased lipogenesis, decreased thermogenesis and energy expenditure might contribute to increased adiposity. In this sense, a recent and elegant report from Horvath and colleagues demonstrated that the feeding-independent lipogenic actions of ghrelin are enhanced in UCP2 null mice [81]. The molecular mechanisms under this effect are not totally clarified, but UCP2 KO mice display high expressions of lipogenic enzymes, such as FAS, SCD1, and LPL and decreased the expression of CPT1a in WAT [81]. These data also highlight the key role of UCP2 as mediator of ghrelin actions at central [44] and peripheral levels [81].

One important caveat is whether the role of ghrelin as growth hormone (GH) secretagogue [1, 82–84]

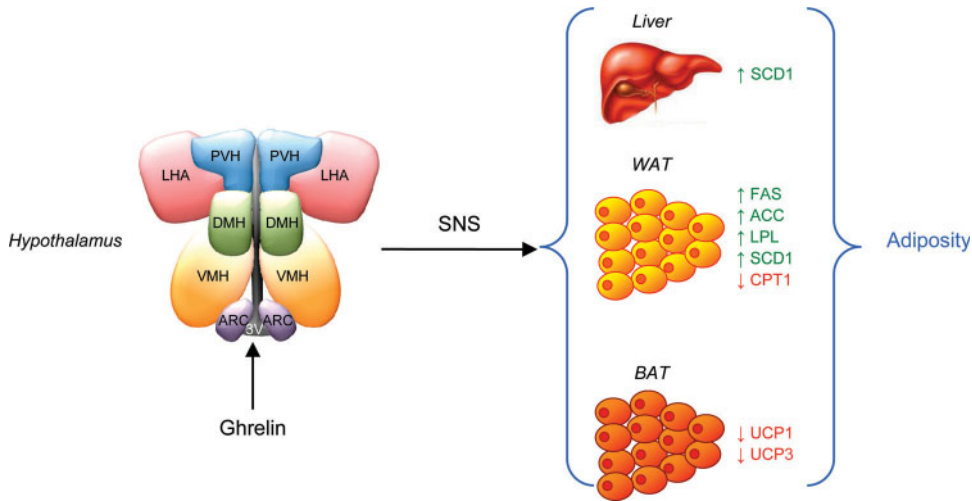


Fig. 29.3 Central ghrelin actions on peripheral lipid metabolism. Ghrelin stimulates agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons in the ARC. This action leads to subsequent modulation of lipid metabolism in liver and white adipose tissue (WAT) through the sympathetic nervous system (SNS). Central ghrelin also decreases the expression of uncoupling proteins 1 and 3 (UCP1 and UCP3) in brown adipose tissue (BAT), which decreases thermogenesis and energy expenditure. Subsequently, the overall effect of central ghrelin contributes to increased adiposity. 3V: third ventricle; ACC: acetyl CoA carboxylase; ARC: arcuate nucleus of the hypothalamus; CPT1: carnitine palmitoyltransferase; LPL: lipoprotein lipase; SCD1: stearoyl-CoA desaturase 1; DMH: dorsomedial nucleus of the hypothalamus; FAS: fatty acid synthase; LHA: lateral hypothalamic area; PVH: paraventricular nucleus of the hypothalamus; VMH: ventromedial nucleus of the hypothalamus.

may be relevant in its actions on energy balance. This question is of relevance, bearing in mind the important actions of GH on peripheral lipid metabolism [85–87]. To address this issue, we have recently examined the effects of chronic central ghrelin administration on liver and adipose lipid metabolism in dwarf (GH-deficient) rats. Our results demonstrate that central chronic ghrelin administration regulates adipose lipid metabolism, mainly in a GH-independent fashion, as a result of increased mRNA, protein expression, and activity levels of ACC α , FAS, and SCD1 [61]. Alternatively, central ghrelin regulates hepatic de novo lipogenesis in a GH-independent manner but fatty acid oxidation in a GH-dependent manner, because CPT1 was inhibited only in normal rats [61]. Moreover, and quite the opposite to the hypothalamus [6, 43, 44], we have showed that in peripheral tissues, increased total levels of ghrelin during food deprivation do not mediate the effects of fasting. In these tissues, complete food deprivation downregulates the expression of lipogenic enzymes, and activates (in liver) or downregulates (in WAT)

CPT1, which are opposite effects to those observed after the ghrelin treatment [61].

29.9 CONCLUSION

Compelling evidence over the last decade has demonstrated that ghrelin exerts a deep effect in hypothalamic networks modulating feeding behavior and peripheral metabolism. Of note, lipid metabolism seems to be a canonical downstream pathway modulating ghrelin's effects at central and peripheral level [6, 31, 37, 38, 42–44, 61, 79, 81, 88].

On the basis of these data, it is appealing to speculate that ghrelin favors energy stores in order to diminish negative effects in periods of food deficiency [78, 89]. During fasting, increased ghrelin levels stimulate food intake and make possible anabolic processes when food becomes available by triggering biological responses that modulate the efficiency of energy storage, i.e., increasing lipogenesis and inducing UCP2 in WAT, which shifts the organism from a negative

energy balance state to a neutral energy balance state, preventing overweight and obesity [6, 44, 61, 78, 81, 90]. However, this mechanism, which was primarily designed as a response to fasting under obesogenic conditions, such as high fat diet (HFD) [14, 15] or GH deficiency [61], seems to increase excessively positive energy balance and fat mass, which ultimately may lead to harmful pro-obese and diabetic states.

Otherwise, a new and crucial link between ghrelin and lipids has been recently exposed by studying ghrelin O-acyl transferase (GOAT), the enzyme responsible for ghrelin acylation [91, 92]. Tschöp and colleagues have gracefully demonstrated that GOAT is regulated by nutrient availability and depends on precise dietary lipids, such as medium chain fatty acids, which act as acylation substrates [93]. This evidence links dietary lipids to ghrelin action and indicates that to get optimal nutrient partitioning, the accessibility to high-caloric food is signaled to the hypothalamus through readily absorbable medium-chain fatty acids originating from the GOAT-ghrelin system working as a nutrient sensor.

Overall, these data recognize the ghrelin–lipid metabolism interaction as a key homeostatic mechanism modulating energy balance. Further work will be necessary to investigate the therapeutic implication of the ghrelin–lipid metabolism partnership for the treatment of obesity and metabolic syndrome.

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