Role of ghrelin in the regulation of energy balance in adult male albino rats

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Background
Ghrelin is a 28-amino acid acylated peptide that was recently identified as endogenous ligand for growth hormone secretagogue receptor. It is a potent orexigenic peptide that stimulates food intake, adipogenesis, and body weight gain. However, its physiological role in the regulation of energy homeostasis is still controversial.
Aim
The study was performed to show the physiological role of ghrelin in energy balance and body weight homeostasis through assessing the effect of obesity and undernutrition on the plasma ghrelin level.
Materials and methods
Twenty-four adult male albino rats weighing 140–190 g were divided into three groups: group I (control group): rats of this group were given free access to food and water, group II (induction of obesity): rats of this group were fed a high-caloric diet for induction of obesity, and group III (chronic food restriction): rats of this group were fed by 30% of the diet consumed by the control group.
Results
The body weight is significantly increased in rats, which fed a high-caloric diet for induction of obesity, whereas plasma ghrelin level was significantly decreased. Food-restricted rats showed significantly decreased body weight and significantly elevated plasma ghrelin level.
Conclusion
These findings suggest that ghrelin has a pivotal role in mediating the physiological responses to undernutrition and overnutrition. Changes in the circulating level of ghrelin can represent an adaptative response to prevent long-lasting alterations in the energy balance and body weight.

Keywords:
body weight, energy balance, ghrelin, rats

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Introduction

Obesity is one of the greatest public health challenges of the 21st century. It is linked to increased person's risk to develop diabetes, cardiovascular disease, osteoarthritis, and cancer [1]. Obesity is caused by interactions of several genetic and environmental factors [2]. The global increase in the prevalence of obesity and its associated comorbidities has stimulated researchers to better understand factors regulating energy homeostasis to prevent and/or treat obesity [3].

Several physiological mechanisms are involved in controlling food intake in mammals. The amount and composition of the eaten food varies considerably from person to person, meal to meal, and day to day [4]. Recently, gut peptide hormones have received growing attention because of their ability to regulate many gastrointestinal functions, especially food intake and digestive motility [5]. Ingested food stimulates the release of a variety of gastrointestinal hormones from enteroendocrine cells throughout the gastrointestinal tract. Gastrointestinal hormones play an important role in neuroendocrine regulation of food intake and postprandial satiety resulting in meal termination and orexigenic modulation [6]. Most of the gastrointestinal hormones with the exception of ghrelin increases satiety and decrease food intake. Ghrelin showed an opposite effect [4].

Ghrelin has been discovered by Kojima *et al.*[7] as a natural ligand for the growth hormone (GH) secretagogue receptor type 1a (GHS-R1a) [8,9]. After its discovery, it became evident that ghrelin is implicated in a variety of physiological processes, including cell protection, proliferation, metabolism, and reproduction via endocrine, autocrine, and/or paracrine pathways because of the widespread distribution of ghrelin and GHS-R expression in central and peripheral tissues [10,11]. The ghrelin receptor (GHS-R) is a typical G-protein coupled receptor. It has two forms: GHS-R1a, which binds

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ghrelin and leads to intracellular calcium mobilization, and GHS-R1b which is not able to bind ghrelin [12]. The GHS-R1a is expressed mainly in the hypothalamic–pituitary unit as well as in central nervous system, pancreas, lung, liver, kidney, small and large intestines, myocardium, spleen, ovary, testis, adrenal gland, adipose tissue, stomach, and the neuronal cells of the gut in both animals and humans, whereas GHS-R1b is expressed mainly in the peripheral organs, such as skin, myocardium, atria, immune cells, pituitary and thyroid glands, liver, breast, buccal mucosa, and placenta [13].

Ghrelin is a 28-amino acid peptide with an acyl side chain attached to the serine residue at position 3. This acyl group is crucial for ghrelin's orexigenic and GH-releasing actions [7,14,15]. Ghrelin stimulates food intake independent from its effect in stimulating GH secretion [16]. The orexigenic effect of ghrelin is mediated centrally through activating neurons in the hypothalamic arcuate and paraventricular nuclei [17,18]. Ghrelin also has an adipogenic effect in rodents *in vivo*. This may be induced directly through its action on adipose tissue or indirectly through stimulation of the appetite and food intake [19]. At present, ghrelin is the only potent peripheral orexigenic peptide; thus, it may be useful for treating disorders accompanied by chronic malnutrition due to decreased food intake, such as anorexia nervosa. Moreover, blocking or controlling the orexigenic effect of ghrelin could be a reasonable approach to decrease an excessive food intake in obesity [20,21].

Despite the intensive researches done on ghrelin hormone, it is still not completely clear which parts of digestive tract regulate ghrelin secretion [22] and the mechanisms controlling its secretion [23]. The secretion of ghrelin in stomach is stimulated by the combination of neural (vagus), mechanical (distension), chemical (osmolarity; caloric content and macronutrient composition of the meal), and hormonal (insulin) factors with unknown priority. However, the specific effects of respective nutrients and caloric content of the meal on ghrelin levels still need to be clarified. Impairment in ghrelin secretion in concert with other factors play an important role in the development of both obesity and anorexia nervosa, but factors regulating its physiological fasting and postprandial response in presence of obesity and anorexia nervosa are still partially understood [4].

The aim of the present study is to clarify the effect of change in caloric intake on the plasma ghrelin level and its possible role in the pathophysiology of obesity and malnutrition in adult male albino rats.

Materials and methods

Animals

Experimental protocol for the study was approved by the ethics of local committee on animal experiments. Twenty-four healthy adult male albino rats weighting 140–190 g with an average age of 8–10 weeks were obtained from Experimental Animal Breeding Farm, Helwan, Cairo, to be used in this study. They were housed in polypropylene cages under standard laboratory conditions (12 h light/dark cycle, 20–25°C, and relative humidity 55%). The animals were given commercial diet brought from El-Nasr Company (Cairo, Egypt) and tap water. All animals received care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences.

Experimental design

After the acclimatization period for 2 weeks, the rats were randomly divided into three equal groups (eight rats per each group) as follows:

(1) Group I (control group): rats of this group were given free access to food and water.
(2) Group II (induction of obesity): rats of this group were fed by high-caloric (HC) diet for 3 weeks to induce obesity in which 60% of calories were obtained from fats, 20% from proteins, and 20% from carbohydrates [24].
(3) Group III (chronic food restriction): rats of this group were fed by 30% of diet consumed by the control group for 3 weeks [25].

Assessment of body weight

The body weight of each rat in all groups was estimated at the first, 14th, and 21st days of the experiment.

Determination of plasma ghrelin level

Rat tail blood sample was drawn at the first, 14th, and 21st days of the experiment and centrifugated at 5000 *g* for 15 min. The red blood corpuscles were separated at the bottom of the tube leaving clear plasma above. Plasma ghrelin level was determined by radioimmunoassay using materials and protocols supplied by EK-031–31; Phoenix Pharmaceuticals Inc. (Belmont, CA, USA) [26].

Statistical analysis

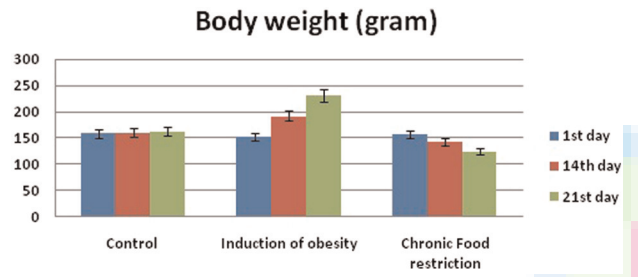
All analyses were performed using the program statistical package for social sciences, version 16

Table 1 Changes in body weight (g) in all studied group on the first, 14th, and 21st days of the study

Body weight (g)	Group I (control)	Group II (induction of obesity)	Group III (chronic food restriction)
First day	158.38±7.15	152.25±3.73	156.66±9.10
14th day	160.00±7.34	191.62±9.39†,§	143.12±4.79†,¶
21st day	162.50±7.28*	231.62±7.06‡,§,‖	124.62±6.84‡,¶,**

Data are expressed as mean±SD.
$P<0.05$ is significant tested by using Student's *t*-test.
*Significant difference versus the first day of the control group.
†Significant difference versus the 14th day of the control group.
‡Significant difference versus the 21st day of the control group.
§Significant difference versus the first day of group II (induction of obesity).
‖Significant difference versus the 14th day of group II (induction of obesity).
¶Significant difference versus the first day of group III (chronic food restriction).
**Significant difference versus the 14th day of group III (chronic food restriction).

Figure 1



Changes in body weight (g) in all studied group on the first, 14th, and 21st days of the study.

(SPSS Inc., Chicago, Illinois, USA). The data were presented as the mean±SD. Comparisons between two groups were analyzed by unpaired Student's *t*-test. *P* value less than 0.05 was considered statistically significant.

Results

Evaluation of the body weight on the first, 14th, and 21st days of the study

Table 1 and Fig. 1 show that in the control group (group I), there is nonsignificant increase in the body weight on the 14th day as compared with the first day of the study ($P>0.05$). On the 21st day, body weight is significant increased as compared with the first day of the study ($P<0.05$). However, this increase is nonsignificant as compared with the 14th day of the experimental study ($P>0.05$). In rats fed with a HC diet to induce obesity (group II), there is a significant increase in the body weight on the 14th day of the study as compared with the first day in the same group ($P<0.001$). The increase in body weight on the 21st day is significantly higher as compared with the first and the 14th day in the same group ($P<0.001$). With chronic food restriction (group III), rats show a significant decrease in the body weight on the 14th day of the study as compared with the first day in the same group ($P<0.01$). Also, on the 21st day, the body weight is significantly decreased as compared with the first and the 14th day in the same group ($P<0.001$).

On the 14th day of the study, the body weight of the group II (induction of obesity) is significantly increased as compared with group I (control group), whereas the body weight of group III (chronic food restriction) is significantly decreased as compared with the control group ($P<0.001$). On the 21st day of the study, the body weight of the group II (induction of obesity) is significantly increased as compared with group I (control group), but it is significantly decreased in rats of group III (chronic food restriction) as compared with the control group ($P<0.001$).

Evaluation of plasma ghrelin level on the first, 14th, and 21st days of the study

Table 2 and Fig. 2 show that there is nonsignificant change in the plasma ghrelin level in group I (control group) on the 14th day or on 21st day of the study as compared with the first day of the study in the same group ($P>0.05$). Also, there is nonsignificant change in the plasma ghrelin on the 21st of the study in group I (control group) as compared with the 14th day of the same group. In rats fed with a HC diet to induce obesity (group II), there is a significant decrease in the plasma ghrelin level on the 14th day of the study as compared with the first day in the same group ($P<0.001$). Also on the 21st day, there is a significant decrease in the plasma ghrelin level as compared with either the first ($P<0.001$) or the 14th day of the study ($P<0.05$) in the same group. With chronic food restriction (group III), rats show significant increase in the plasma ghrelin level on the 14th and the 21st day of the study as compared with the first day in the same group ($P<0.001$). This increase is significantly higher on the 21st day as compared with the 14th day in the same group ($P<0.001$).

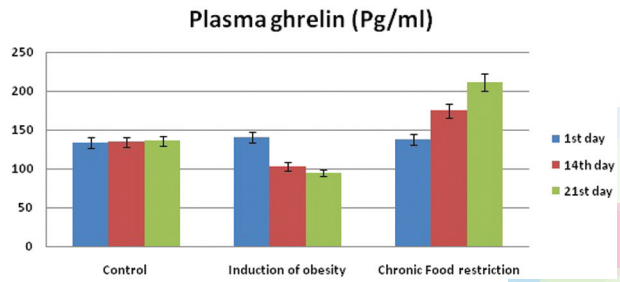
On the 14th day of the study, the plasma ghrelin level in rats of group II (induction of obesity) is significantly decreased as compared with group I (control group), but in rats of group III (chronic food restriction), it is significantly increased as compared with the control group ($P<0.001$). On the 21st day of the study, the plasma ghrelin level in rats of group II (induction of

Table 2 Changes in plasma ghrelin level (pg/ml) in all studied group on the first, 14th, and 21st days of the study

Plasma ghrelin level (pg/ml)	Group I (control)	Group II (induction of obesity)	Group III (chronic food restriction)
First day	133.96±6.13	140.00±11.18	137.62±5.04
14th day	134.27±5.80	103.12±8.14†,§	174.88±13.05†,¶
21st day	135.64±5.82	94.75±6.79‡,§,‖	211.38±13.13‡,¶,**

Data are expressed as mean±SD.
$P<0.05$ is significant tested by using Student's *t*-test.
*Significant difference versus the first day of the control group.
†Significant difference versus the 14th day of the control group.
‡Significant difference versus the 21st day of the control group.
§Significant difference versus the first day of group II (induction of obesity).
‖Significant difference versus the 14th day of group II (induction of obesity).
¶Significant difference versus the first day of group III (chronic food restriction).
**Significant difference versus the 14th day of group III (chronic food restriction).

Figure 2



Changes in plasma ghrelin level (pg/ml) in all studied group on the first, 14th, and 21st days of the study.

obesity) is significantly decreased as compared with group I (control group), whereas it is significantly increased in rats of group III (chronic food restriction) as compared with the control group ($P<0.001$).

Discussion

Ghrelin was discovered as an endogenous ligand for the GHS-R. It is synthesized as a preprohormone, which is then proteolytically processed into a 28-amino acid peptide [9]. Ghrelin is produced mainly by the stomach, but it is also widely expressed in different tissues, such as hypothalamus, pituitary gland, small intestine, large intestine, placenta, pancreas, kidney, testes, ovary, and lymphocytes [27]. GHS-R is highly expressed in the gastrointestinal tract, hypothalamus, brainstem, pituitary gland, adipose tissue, heart, lungs, pancreas, kidney, and other peripheral tissues [12,28].

Ghrelin was originally reported to induce GH release, but recently it was observed to be involved in many other physiological activities, including regulation of food intake, energy balance, and body weight as well as of lipid and glucose metabolism [1,8,9]. Ghrelin was found to produce a positive energy balance in rodents by promoting food intake [21] and decreasing energy expenditure and locomotor activity [20,29,30].

In the present study, the rats fed with normal control diet showed nonsignificant change in the plasma ghrelin even when body weight was significantly increased at the 21st day of the study, whereas rats fed with a HC diet showed significant increase in the body weight and significant decrease in the plasma ghrelin level on the 14th and 21st day of the study as compared with their corresponding values on the first day, and also as compared with the body weight and the plasma ghrelin level in the control group at the same days. These results were in agreement with the previous studies which postulated that ghrelin levels were found to be decreased in obese individuals [8,9,20,31–33]. Álvarez-Castro *et al.*[15] also postulated that the plasma ghrelin level was reduced in patients with obesity and metabolic syndrome and increased in obese patients, when they started losing weight. In humans, the level of postprandial ghrelin suppression is proportional to ingested caloric load. Postprandial suppression of ghrelin secretion and decreased ghrelin level could be explained by the effect of high levels of insulin or a combination of insulin and glucose [20,31,34–38]. In contrast to the results of the present study, Handjieva-Darlenska and Boyadjieva postulated that long-term intake of high-fat diet in rats, causes significant increase in the total body weight and hyperghrelinemia. This contrast may be due to differences in the percentage of fat and the composition of diet used in the study [39].

The present study also revealed a significant decrease in the body weight and significant increase in plasma ghrelin level in food-restricted rats on the 14th and 21st days of the study as compared with their corresponding values on the first day, and also as compared with the body weight and the plasma ghrelin level in the control group at the same days. These data were fairly consistent with previously published studies concluding that body weight was significantly decreased and the plasma ghrelin level was significantly increased by food and energy restriction [25,40]. Plasma ghrelin levels as well as ghrelin gastric mRNA, were upmodulated during undernutrition in normal rats and in pregnancy [8]. Central and peripheral administration of des-acyl ghrelin significantly decreases food intake and decreases gastric emptying in food-deprived mice [41,42]. Several previous studies found that fasting plasma ghrelin level is inversely related to BMI

[9,32,43,44]. Diet-induced weight loss increase baseline ghrelin level and improves plasma ghrelin response to carbohydrate meal in obese women [45,46]. Also, surgical induced weight loss as in gastric bypass surgery decreased plasma ghrelin level [47]. In contrast to our results, Hernandez *et al.*[48] found that food restriction in pigs did not significantly affect the plasma ghrelin level. This contrast may be due to differences in the animal species used in the study.

The results of the present study in addition to the overall previous results, indicate that changes in the plasma ghrelin level with either a HC diet or with a food-restricted diet seem to be a physiological adaptive response to the changes in the energy balance to prevent the long-lasting alterations in body weight [44,49,50], and could serve as an integrative signal reflecting changes in both fat and fat-free mass to hypothalamic centers controlling energy homeostasis [51].

Conclusions

Ghrelin is a key modulator and a pivotal link between the consumed calories and the neuroendocrine control of energy homeostasis as it plays critical roles in regulation of food intake, fuel substrate preference, and body weight. Thus, controlling the circulating plasma levels could be a useful therapeutic approach in the treatment of obesity, malnutrition, and anorexia nervosa. However, we recommend further studies on the factors affecting the circulating plasma level of ghrelin.

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Nil.

Conflict of interest

There are no conflicts of interest.

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