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Chronic administration of ghrelin regulates plasma glucose and normalizes insulin levels following fasting hyperglycemia and hyperinsulinemia ☆

Fatemeh Goshadrou [a,*], Faranak Kazerouni [b], Nasrin Mehranfard [a], Bahman Sadeghi [a,c]

[a] Department of Physiology, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Laboratory Medicine, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor. The majority of the previous studies have shown that the short-term ghrelin treatment induces hyperglycemia and hypoinsulinemia in healthy humans and rodents. However, the results obtained from long-term treatment with ghrelin are not clear enough. In this study, we assessed acute (1 day) and chronic (21 days) effects of intraperitoneally administered ghrelin (at different doses of 1, 10 and 20 μg/kg) during a 12-h fasting period in rats using glucose oxidase method and direct sandwich ELISA (the Enzyme-Linked Immunosorbent Assay) and then compared the effects of exogenous ghrelin on blood glucose and insulin levels on day 21 with those on day 1. The results showed that acute ghrelin administration markedly increased fasting plasma glucose at doses of 1 and 10 μg/kg as well as insulin levels at 1 μg/kg in comparison to control values. Ghrelin (at 1 μg/kg) altered plasma glucose but not insulin levels on the 21st day compared to control values. In addition, the comparison of the influence of ghrelin administration on plasma glucose and insulin levels on day 21 with those on the first day revealed that the chronic administration of ghrelin notably decreased plasma glucose and insulin levels relative to the acute ghrelin treatment. These findings indicate that hyperglycemia and hyperinsulinemia caused by the exogenous ghrelin during acute treatment are temporary and prolonged treatment with ghrelin regulates plasma glucose and restores insulin to normal levels, suggesting a possible role for ghrelin in improving insulin resistance.

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1. Introduction

Ghrelin is an acylated 28-amino acid peptide that was discovered as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima et al., 1999). Also, ghrelin is a gastric hormone that influences energy balance (Choi et al., 2003; Tschöp et al., 2000; Wren et al., 2000, 2001) and gastrointestinal motility (De La Cour et al., 2004; Fujino et al., 2003; Masuda et al., 2000). The effects on energy balance are represented as adiposity and body weight gain, possibly because ghrelin stimulates food intake and suppresses fat metabolism and energy consumption (Choi et al., 2003; Tschöp et al., 2000; Wren et al., 2000, 2001). Therefore, this peptide significantly increases food intake and induces obesity in rats, suggesting that ghrelin is involved in the central regulation of feeding behavior (Tschöp et al., 2000; Wren et al., 2000). Furthermore, an up-regulation has been observed in ghrelin mRNA levels in the murine stomach during fasting. In addition, circulating ghrelin levels were increased in fasted animals (De La Cour et al., 2001; Toshinai et al., 2001) but decreased in cases of obesity (Tschöp et al., 2001). These studies suggest a role for ghrelin in glucose homeostasis and insulin release. The first investigations on ghrelin indicated that acute ghrelin administration induced an increase in glucose levels and reduced insulin secretion in humans (Broglio et al., 2001). Moreover, in another study, intraperitoneal administration of ghrelin in a dose-dependent manner, at concentrations of 1 and 10 nmol/kg, significantly elevated blood glucose levels at the 30th min after the injection in fasted mice, overnight. In this study also, ghrelin injection at concentrations of 1 and 10 nmol/kg along with simultaneous administration of 1 g/kg glucose induced an increase in glucose levels at the 30th and 60th min and remarkably reduced the insulin levels at the 5th and 10th min compared with

☆ This research was carried out at the Department of Physiology, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

* Corresponding author.
E-mail addresses: fgoshadrou@sbmu.ac.ir (F. Goshadrou), fk_kazerouni@yahoo.com (F. Kazerouni), nasrin_mehranfar@yahoo.com (N. Mehranfard), bahman-s1983@yahoo.com (B. Sadeghi).

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control values (Dezaki et al., 2004). Comparable results also obtained in other studies in humans and rodents (Arosio et al., 2003; Broglio et al., 2003, 2004; Cui et al., 2008; Nieminen and Mustonen, 2004; Vestergaard et al., 2008a). In general, the majority of previous studies have indicated that in healthy humans and rodents, short-term treatment with ghrelin induces hyperglycemia and hypoinsulinism. However, long-term treatment with ghrelin has not still yielded clear enough results. In an investigation, intraperitoneal injection of ghrelin at a dose of 10 μg/kg/day for 4 days caused an elevation in the plasma glucose concentrations (Nieminen and Mustonen, 2004). Additionally, repeated intraperitoneal injections of ghrelin for 5 days in mice under a high fat diet significantly increased insulin levels. In this study, blood glucose concentration also displayed a moderate increase under these conditions but it was not significant (Asakawa et al., 2003). In another research, subcutaneous administration of ghrelin for 4 days to normal rats resulted in hyperglycemia without any significant changes in plasma insulin levels (Barazzoni et al., 2005, 2007). In addition, Theander-Carrillo et al. reported that intracerebroventricular (ICV) administration of ghrelin for 6 days during euglycemic hyperinsulinemic clamp did not change plasma glucose or insulin levels (Theander-Carrillo et al., 2006). Kamegai et al. also obtained similar results, demonstrating that repeated chronic injection of ghrelin into the lateral ventricle of rats (1 μg/rat every 12 h for 72 h) increased food intake and body weight, without any changes in plasma glucose and insulin levels (Kamegai et al., 2001). Besides, another research showed that daily ICV administration of ghrelin for 5 days to adult male rats increased insulin levels without any significant changes in plasma glucose levels (Stevanović et al., 2008). This information suggests that the influence of ghrelin on glucose and insulin levels depends on a combination of factors including the dose of ghrelin used, species differences, as well as the glycemic condition under which experiments are performed. It is therefore essential to determine the (1) optimal doses of ghrelin and (2) glycemic state under which ghrelin treatment is carried out. In the present study, for the first time to our knowledge, we investigated the acute (short-term) and chronic (long-term) effects of ghrelin on plasma glucose and insulin levels at several different doses and compared the obtained results from the acute and chronic ghrelin treatments during a 12-h fasting period in rats on days 1 and 21 following intraperitoneal injection of ghrelin.

2. Materials and methods

2.1. Animals

In the current study, twenty-five Adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 240–290 g were used. The animals were maintained under standard housing conditions with a 12/12 h light/dark cycle (lights on at 7 AM) and temperature-controlled (22 ± 1 °C) environment. During the whole period of the experiment, animals were given standard laboratory chow and water *ad libitum*. However, 12 h prior to collecting blood samples, rats were just restricted from food. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were confirmed by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.2. Materials

In the present study, Ghrelin (acylated form) was bought from Tocris Bioscience (Bristol, UK) and was diluted with saline to the final concentration of 3×10^{-7} mol l^{-1}. Also, Polypropylene tubes containing EDTA-2Na (1 mg/ml blood) and aprotinin (Ohkura Pharmaceutical, Inc., Kyoto, Japan; 1000 kallikrein inactivator IU/ml) were used to maintain the blood samples. Glucose oxidase reagent (Glu/C II; Wako Pure Chemicals, Osaka, Japan) was employed in the glucose oxidase method to assay the plasma glucose levels. Additionally, Plasma insulin levels were estimated by direct sandwich ELISA method with an enzyme immunoassay (EIA) using rat insulin kit (Rat Insulin ELISA kit, Mercodia AB, Uppsala, Sweden) along with employing two monoclonal antibodies.

2.3. Methods

Rats were given intraperitoneal injection of 250 μl ghrelin (AG) at doses of 1, 10 and 20 μg/kg for 1 day (short-term period) and 21 days (long-term period) after a 12-h fasting period. The control group received saline for 1 and 21 days. Retro-orbital blood

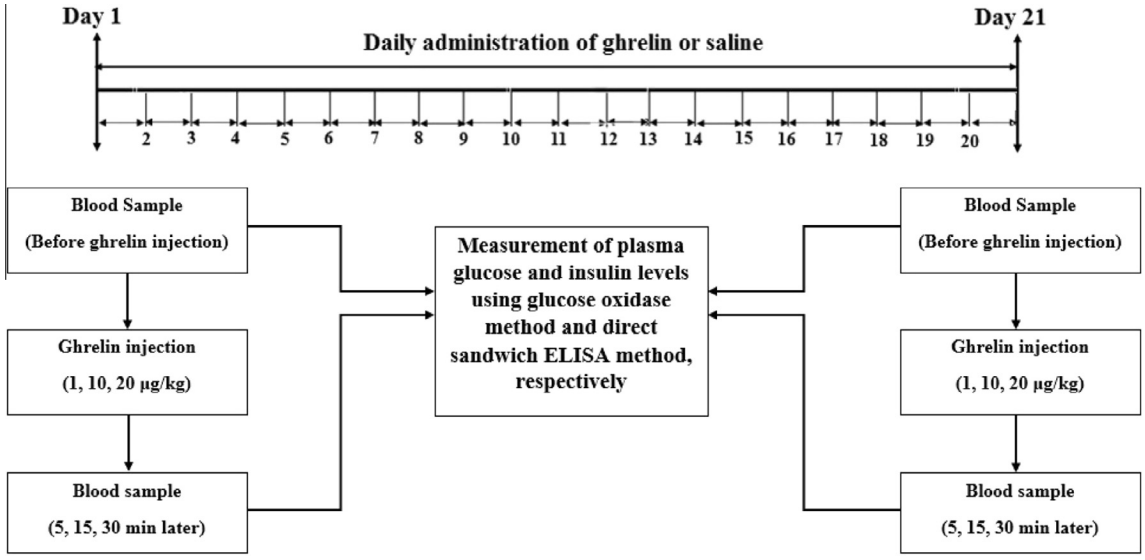

Fig. 1. The overall procedure of the acute (1 day) and chronic (21 days) experiments of the effects of ghrelin on plasma glucose and insulin levels in rats. Blood samples were collected on day 1 and day 21 before ghrelin injection while the rats were fasted for 12 h. 5, 15 and 30 min after the administration of ghrelin at three different doses (1, 10 and 20 μg/kg) blood was collected again. Afterwards, plasma glucose and insulin levels were measured by glucose oxidase and direct sandwich ELISA methods, respectively.

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