Effect of ghrelin on glucose regulation in mice

Shaji K. Chacko, Morey W. Haymond, Yuxiang Sun, Juan C. Marini, Pieter J. J. Sauer, Xiaojun Ma, and Agneta L. Sunehag

1Department of Pediatrics, Baylor College of Medicine Children’s Nutrition Research Center, United States Department of Agriculture/Agricultural Research Service, Houston, Texas; and 2Department of Pediatrics, Beatrix Children’s Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Chacko SK, Haymond MW, Sun Y, Marini JC, Sauer PJJ, Ma X, Sunehag AL. Effect of ghrelin on glucose regulation in mice. Am J Physiol Endocrinol Metab 302: E1055–E1062, 2012. First published February 14, 2011; doi:10.1152/ajpendo.00445.2011.—Improvement of glucose metabolism after bariatric surgery appears to be from the composite effect of the alterations in multiple circulating gut hormone concentrations. However, their individual effect on glucose metabolism during different conditions is not clear. The objective of this study was to determine whether ghrelin has an impact on glycogenolysis, gluconeogenesis, and insulin sensitivity (using a mice model). Rate of appearance of glucose, glycogenolysis, and gluconeogenesis were measured in wild-type (WT), ghrelin knockout (ghrelin−/−), and growth hormone secretagogue receptor knockout (Ghsr−/−) mice in the postabsorptive state. The physiological nature of the fasting condition was ascertained by a short-term fast commenced immediately at the end of the dark cycle. Concentrations of glucose and insulin were measured, and insulin resistance and hepatic insulin sensitivity were calculated. Glucose concentrations were not different among the groups during the food-deprived period. However, plasma insulin concentrations were lower in the ghrelin−/− and Ghsr−/− than WT mice. The rates of gluconeogenesis, glycogenolysis, and indexes of insulin sensitivity were higher in the ghrelin−/− and Ghsr−/− than WT mice during the postabsorptive state. Insulin receptor substrate 1 and glucose transporter 2 gene expressions in hepatic tissues of the ghrelin−/− and Ghsr−/− were higher compared with that in WT mice. This study demonstrates that gluconeogenesis and glycogenolysis are increased and insulin sensitivity is improved by the ablation of the ghrelin or growth hormone secretagogue receptor in mice.

ghrelin; growth hormone secretagogue receptor; gluconeogenesis; bariatric surgery; insulin sensitivity

THE IMPROVEMENT IN INSULIN SENSITIVITY and in many cases reversal of type 2 diabetes after bariatric surgery procedures before any weight loss occurs are well established (6, 14, 16, 30, 46, 60). Improved glycemic control achieved immediately after surgery but before weight loss suggests a hormonal mechanism (10, 30). After bariatric surgery, fasting insulin concentrations dropped dramatically during the first week and persisted up to two years (30). The plasma ghrelin concentrations were decreased by ~70% following gastric bypass surgery compared with matched obese and normal-weight controls (10). Although beneficial effect of bariatric surgery on glucose metabolism has been associated with changes in several circulating gut hormone concentrations (26, 40, 61), their individual effect during different conditions remains to be elucidated. The relationship between the improvement of glucose metabolism and reduced ghrelin concentration as well as the effect of ghrelin on the constituents of glucose production remain unclear.

The stomach is the major source of circulating ghrelin. Furthermore, ghrelin-containing cells are particularly abundant in the fundus region of the stomach (1, 20, 53, 64). Sleeve gastrectomy, which involves the complete resection of the gastric fundus, resulted in better glycemic control compared with other forms of bariatric surgery (16).

Ghrelin stimulates the release of growth hormone via the growth hormone secretagogue receptor (24, 29, 51). Consequently, studying the effects of ghrelin on glucose metabolism using ghrelin infusion might be confounded by the effects of growth hormone secretion. In addition, ghrelin is produced by cells scattered in various tissues, and differential roles of acylated ghrelin on glucose metabolism were reported (11, 21, 22, 28, 43), limiting the prospect of studies in wild-type (WT) animals. Therefore, studies of glucose kinetics in the absence of ghrelin or its receptor using transgenic mice models (48, 51, 55, 62) provide a unique opportunity to investigate the effects of ghrelin on glucose metabolism independent of any action of growth hormone or isoforms of ghrelin.

Ghrelin infusion in growth hormone-deficient and normal mice (12) and in humans (59) provides evidence that ghrelin also affects glucose metabolism independent of growth hormone. Studies using glucose clamp demonstrated reduced insulin requirements for higher glucose disposal and a lower glucose-stimulated insulin release in the absence of ghrelin signaling (31, 42). Ghrelin reversed leptin’s inhibiting effect on insulin secretion, implying lower insulin in the absence of ghrelin (7, 25, 27, 39, 41, 47). These observations suggest lower insulin and enhanced glucose disposal in the absence of a ghrelin-mediated mechanism. Consequences of these hormonal changes on the constituents of glucose production during postabsorptive conditions are not yet known. Therefore, we hypothesized that, in the absence of ghrelin signaling in the postabsorptive state, insulin concentrations are lower, glucose production from glycogenolysis is higher, and insulin sensitivity is greater. To test this hypothesis, we compared glucose kinetics among postabsorptive WT, ghrelin knockout (ghrelin−/−), and growth hormone secretagogue receptor knockout (Ghsr−/−) mice.

MATERIALS AND METHODS

Animals and Housing

Four- to five-month-old adult male WT, ghrelin−/−, and Ghsr−/− mice were used for all experiments. The generation of ghrelin−/− and Ghsr−/− mice has been previously described (48, 51). All mice were on a pure C57BL/6J background and have been backcrossed to C57BL/6J for 13 generations. Mice were kept in a standard housing facility and had access to standard chow diet (Harlan Teklad rodent diet)
diet 2920x) with ad libitum access to autoclaved reverse-osmosis water. Mice were maintained under a 12-h light cycle (0600-1800) and constant temperature (75 ± 2°F). Glucose kinetic measurements were performed in a set of five animals (n = 5) in each group. Body composition was measured in another set of animals: WT (n = 15), ghrelin−/− (n = 9), and Ghsr−/− (n = 7) mice. Glucose, β-hydroxybutyrate, free fatty acid, and insulin concentrations were measured in identically fasted WT (n = 5), ghrelin−/− (n = 5), and Ghsr−/− (n = 5) mice. Gene expression levels in hepatic tissues of ghrelin−/− and Ghsr−/− mice were compared with their own WT controls in a set of six animals (n = 6) in each group using real-time RT-PCR. All procedures used in the animal experiments were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee.

Body Composition

Body composition parameters such as total body fat and total lean body mass were determined by magnetic resonance imaging (MRI) using EchoMRI-100 (QNMR Systems, Houston, TX).

Stable Isotopes

Sterile and pyrogen-free deuterium oxide (2H2O), 99 atom percent 2H, and [6,6-2H2]glucose, 99 atom% 2H, were purchased from Cambridge Isotopes Laboratories (Andover, MA). [6,6-2H2]glucose was dissolved in 0.5% 2H2O (made isotonic by addition of sodium chloride), filtered, and prepared for intravenous infusion.

Study Design

Rate of appearance of glucose and gluconeogenesis were measured in each group at the end of a short-term fast of 8 h commenced at the end of a 12-h dark cycle (0600 to 1400). On the day of the infusion, all mice were weighed and received an intraperitoneal dose of 99% 2H2O (4 mg/g body wt), resulting in a deuterium enrichment of ~0.5% in body water. After this 2H2O dose, the animals were given ad libitum access to water (0.5% 2H2O) for the rest of the study to maintain the body water deuterium enrichment at ~0.5%. The mice were then restrained in an infusion box, and a tail vein catheter was inserted as described previously (34). Two hours following the 2H2O dose, a primed constant-rate infusion of [6,6-2H2]glucose at ~0.75 mg·kg−1·min−1 (150 μl/h) was started and continued for 4 h. After 4 h of infusion, blood was drawn from the submandibular bundle and centrifuged for 15 min at 4°C. Plasma was separated and kept frozen at −80°C until analyzed. Blood samples from five mice were collected by 10.220.32.246 on October 30, 2017 http://ajpendo.physiology.org/ Downloaded from

Calculations

All kinetic measurements were performed under steady-state conditions. Total plasma glucose appearance rate (glucose Ra) was calculated from the M + 2 enrichment of [6,6-2H2]glucose in plasma using established isotope dilution equations (3). Under steady-state conditions, it is assumed that rate of appearance of glucose is equal to the rate of disappearance of glucose.

Rate of glucose production (mg·kg−1·min−1) (GPR) = glucose Ra − exogenous glucose

(i.e., only the tracer since the animals were fasting).

Fractional gluconeogenesis (i.e., gluconeogenesis as a fraction of glucose Ra) was calculated according to Chacko et al. (8, 9) as follows:

Fractional gluconeogenesis (GNG%Ra) = [(M + 1)(CH)nit(17/69) / 6]/E2H2O

Fig. 1. Steady-state enrichment was achieved between 3 and 4 h of continuous isotope infusion.
ROLE OF GHRELIN ON GLUCOSE REGULATION IN MICE

Table 1. Body composition measurements in WT, ghrelin−/−, and Ghsr−/− mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Ghsr−/−</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Lean body mass, g</td>
<td>23.0 ± 0.3</td>
<td>22.0 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Total body fat, g</td>
<td>6.7 ± 0.7</td>
<td>4.0 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.8 ± 1.5</td>
<td>13.7 ± 0.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild type; ghrelin−/−, ghrelin knockout; Ghsr−/−, growth hormone secretagogue receptor knockout; NS, not significant.

Table 2. Concentrations of glucose, insulin, β-hydroxybutyrate, and fatty acids in WT, ghrelin−/−, and Ghsr−/− mice during the postabsorptive state

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Ghsr−/−</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>124 ± 7</td>
<td>121 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.07 ± 0.25</td>
<td>0.98 ± 0.14</td>
<td>0.012</td>
</tr>
<tr>
<td>β-Hydroxybutyrate, mmol/l</td>
<td>3.85 ± 0.72</td>
<td>2.28 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>Free fatty acids, mmol/l</td>
<td>1.35 ± 0.22</td>
<td>1.29 ± 0.28</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. NS, not significant.
ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> mice compared with their own WT controls (Fig. 5).

**DISCUSSION**

Studies have shown that gut hormones are involved in the regulation of glucose metabolism (15, 23). However, the effect of ghrelin, an endogenous ligand for the growth hormone secretagogue receptor (28, 51), on endogenous glucose synthesis during in vivo conditions remains unclear. In the present study, we demonstrated that ablation of ghrelin or its receptor resulted in lower insulin concentration and increased glucose production via glycogenolysis and gluconeogenesis during postabsorptive conditions in mice (Fig. 2). Rates of glycogenolysis and gluconeogenesis were not different between ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> mice. These data demonstrate that ghrelin directly or indirectly plays a role in glucose homeostasis via the modulation of glycogenolysis and gluconeogenesis and that these effects most likely occur via the growth hormone secretagogue receptor.

As hypothesized, we observed that insulin concentrations were lower in both ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> mice compared with WT mice during the postabsorptive state (Table 1). To ascertain the physiological nature of the fasting condition in our studies, the fasting was commenced immediately at the end of the dark cycle. Lower insulin concentration during the postabsorptive condition could be related to higher insulin sensitivity reported in ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> than in WT mice (13, 31, 49, 50). Higher glucose disappearance rate observed in ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> than in WT during postabsorptive steady state despite any difference in blood glucose level supports the increased glucose disposal in these mice models. Measurement of whole body insulin sensitivity and insulin tolerance in ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> demonstrated that these mice models are less insulin resistant compared with WT (42, 49). This is in line with our present data of higher hepatic insulin sensitivity index and reduced insulin resistance in ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> compared with WT mice. These data might imply that absence of ghrelin signaling through mechanisms that are still not completely understood improves insulin sensitivity and thereby influences glucose uptake and glucose production. We observed that the IRS1 gene expression in hepatic tissues of the ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> was significantly higher (Fig. 3) compared with that in WT, which supports that the insulin action in the liver of the null mice is enhanced. It has been reported that ghrelin reverses leptin’s inhibiting effect on insulin secretion (7, 27). This suggests reduced insulin concentration in the absence of ghrelin signaling. Infusion of leptin has been demonstrated to increase both hepatic and peripheral insulin sensitivity in mice (25, 47).

In response to lower insulin concentration in the knockout groups than in the WT, glycogenolysis was significantly higher during the postabsorptive state (Fig. 1). This observation is consistent with the reports of increased glycogenolysis in

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**Table 3.**<sup>**R**</sup>, GPR, GNG, and fractional gluconeogenesis and indexes of HOMA-IR and HISI in WT, ghrelin<sup>−/−</sup>, and Ghsr<sup>−/−</sup> mice during the postabsorptive state

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Ghrelin&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Ghsr&lt;sup&gt;−/−&lt;/sup&gt;</th>
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<tr>
<td>Glucose R&lt;sub&gt;r&lt;/sub&gt;, mg·kg&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>12.89 ± 0.82</td>
<td>20.12 ± 1.89*</td>
<td>19.66 ± 0.82*</td>
</tr>
<tr>
<td>GPR, mg·kg&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>12.17 ± 0.81</td>
<td>19.34 ± 1.89*</td>
<td>18.93 ± 0.81*</td>
</tr>
<tr>
<td>GNG, mg·kg&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>9.30 ± 0.69</td>
<td>13.07 ± 0.99</td>
<td>13.15 ± 0.44</td>
</tr>
<tr>
<td>Fractional gluconeogenesis, %</td>
<td>0.72 ± 0.02</td>
<td>0.66 ± 0.03</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>14.8 ± 2.4</td>
<td>7.3 ± 1.3*</td>
<td>6.2 ± 1.9*</td>
</tr>
<tr>
<td>HISI</td>
<td>0.29 ± 0.01</td>
<td>0.40 ± 0.04*</td>
<td>0.44 ± 0.02*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05, ghrelin<sup>−/−</sup> vs. WT group and Ghsr<sup>−/−</sup> vs. WT group.

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**Fig. 2.** Rate of gluconeogenesis and glycogenolysis was significantly higher in ghrelin knockout (ghrelin<sup>−/−</sup>) and growth hormone secretagogue receptor knockout (Ghsr<sup>−/−</sup>) mice during the postabsorptive state compared with those in the wild-type (WT) group.

**Fig. 3.** The mRNA expression for insulin receptor substrate (IRS)-1 gene was significantly higher in ghrelin<sup>−/−</sup> and Ghsr<sup>−/−</sup> mice compared with those in the WT group, n = 6 mice.

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E1058 ROLE OF GHRELIN ON GLUCOSE REGULATION IN MICE

*H*ISI, hepatic insulin sensitivity index. *P < 0.05, ghrelin<sup>−/−</sup> vs. WT group and Ghsr<sup>−/−</sup> vs. WT group.
response to decreased insulin concentration (19, 44). Enhanced insulin sensitivity can potentially stimulate glycogen synthesis during glucose availability, resulting in increased glycogen stores. This might explain our observation of significantly higher rates of glycogenolysis in the ghrelin/H11002/H11002 and Ghsr/H11002/H11002 compared with WT mice (Fig. 2 and Table 3). We observed higher glycogen content in the liver slices of Ghsr/H11002/H11002 and ghrelin/H11002/H11002 mice compared with their own WT controls, although, to a lesser extent in ghrelin/H11002 mice (Fig. 5). Consistently, we found that Glut2 expression in hepatic tissues of ghrelin/H11002/H11002 and Ghsr/H11002/H11002 mice was significantly higher than that of WT (Fig. 4). In line with the more pronounced glycogen accumulation in the livers of the Ghsr/H11002 compared with ghrelin/H11002, the Ghsr/H11002 group had higher Glut2 gene expression than in the ghrelin/H11002 group. A previous report that ghrelin downregulates markers of glycogen synthesis is consistent with this observation (38).

Ghrelin has been reported to enhance gluconeogenic gene expression and glucose output during in vitro/vivo studies (2, 17). Thus the increased gluconeogenesis observed in the present study in the absence of ghrelin signaling might be mediated via an indirect mechanism. A potential mechanism for increased gluconeogenesis could be secondary to the opposing effects of ghrelin on leptin action (18, 27). Therefore, increased rate of gluconeogenesis might be because of the absence of the inhibiting effect of ghrelin on leptin.

Ghrelin infusion has been shown to induce insulin resistance and stimulate lipolysis (5, 58, 59). This indicates that absence of ghrelin action might be a reason for this increased insulin sensitivity. Furthermore, it has been reported that glucose production was more suppressed in ghrelin/H11002 than WT mice during a low-dose insulin clamp, suggesting increased hepatic insulin sensitivity in the absence of ghrelin (49). The ability of ghrelin to hamper insulin’s capacity to suppress endogenous glucose production is in line with the improved hepatic insulin sensitivity observed in ghrelin/H11002 and Ghsr/H11002 knockout mice (21).

There are studies demonstrating that short-term peripheral administration of ghrelin increased triglyceride content and
induced lipogenic gene expression in liver, whereas loss of ghrelin signaling was demonstrated to be protective against hepatic steatosis (2, 31). This might suggest that, in liver, ghrelin facilitates glucose disposal via de novo lipogenesis. Supporting this observation, ghrelin-induced adipogenesis has been previously reported (52, 54). This might also be a potential explanation for the higher glucose disposal observed during hyperinsulinemic clamp in mice treated with ghrelin (2, 21). Continuous ghrelin infusion was demonstrated to induce insulin resistance in muscle and to stimulate lipolysis (5, 58, 59). In addition, reduction of plasma ghrelin below physiological levels by insulin infusion during glucose clamp resulted in a sharp increase of insulin sensitivity in humans (32). In contrast low plasma ghrelin was reported in insulin-resistant obese adults compared with controls (37). The effect of insulin or glucose in suppressing plasma ghrelin and the ability of insulin to act as a physiological and dynamic modulator of plasma ghrelin has also been demonstrated (4, 45).

In agreement with other reports (31, 63, 65), the measurements of body composition (Table 2) revealed that both knock-out groups had significantly smaller fat mass but similar lean body mass compared with WT. Preferential metabolization of fat as an energy source was previously reported in ghrelin−/− and Ghsr−/− mice (50, 63, 65). Recently, it has been demonstrated that lower body fat during aging in Ghsr−/− compared with ghrelin−/− and WT mice is due to increased energy expenditure (33). Higher percent fat in the WT group is consistent with the reports of increased adiposity and lipogenic gene expression observed in studies involving ghrelin administration (2, 52, 54). Thus, lower fat accretion in the absence of ghrelin signaling might also be attributed to lower de novo lipogenesis.

Thus, our study demonstrates that glycogenolysis and gluconeogenesis are increased, and insulin sensitivity is improved, in mice by the absence of ghrelin action. The effect of reduced ghrelin concentration on glucose regulation could be a potential explanation for the improvement of glucose metabolism observed in patients after bariatric surgery, especially during postoperative/prandial conditions.

ACKNOWLEDGMENTS

This work is a publication of the United States Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX and had been funded in part with federal funds from the USDA/ARS under Cooperative Agreement nos. 58-6250-6-001 and 58-6250-0-008.

DISCLOSURES

The contents of this publication do not necessarily reflect the views of policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement from the U.S. Government.

AUTHOR CONTRIBUTIONS


REFERENCES

ROLE OF GHRELIN ON GLUCOSE REGULATION IN MICE


