

Ghrelin Suppresses Glucose-Stimulated Insulin Secretion and Deteriorates Glucose Tolerance in Healthy Humans

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OBJECTIVE—The orexigenic gut hormone ghrelin and its receptor are present in pancreatic islets. Although ghrelin reduces insulin secretion in rodents, its effect on insulin secretion in humans has not been established. The goal of this study was to test the hypothesis that circulating ghrelin suppresses glucose-stimulated insulin secretion in healthy subjects.

RESEARCH DESIGN AND METHODS—Ghrelin (0.3, 0.9 and 1.5 nmol/kg/h) or saline was infused for more than 65 min in 12 healthy patients (8 male/4 female) on 4 separate occasions in a counterbalanced fashion. An intravenous glucose tolerance test was performed during steady state plasma ghrelin levels. The acute insulin response to intravenous glucose (AIRg) was calculated from plasma insulin concentrations between 2 and 10 min after the glucose bolus. Intravenous glucose tolerance was measured as the glucose disappearance constant (Kg) from 10 to 30 min.

RESULTS—The three ghrelin infusions raised plasma total ghrelin concentrations to 4-, 15-, and 23-fold above the fasting level, respectively. Ghrelin infusion did not alter fasting plasma insulin or glucose, but compared with saline, the 0.3, 0.9, and 1.5 nmol/kg/h doses decreased AIRg ($2,152 \pm 448$ vs. $1,478 \pm 2,889$, $1,419 \pm 275$, and $1,120 \pm 174$ pmol/l) and Kg (0.3 and 1.5 nmol/kg/h doses only) significantly ($P < 0.05$ for all). Ghrelin infusion raised plasma growth hormone and serum cortisol concentrations significantly ($P < 0.001$ for both), but had no effect on glucagon, epinephrine, or norepinephrine levels ($P = 0.44$, 0.74 , and 0.48 , respectively).

CONCLUSIONS—This is a robust proof-of-concept study showing that exogenous ghrelin reduces glucose-stimulated insulin secretion and glucose disappearance in healthy humans. Our findings raise the possibility that endogenous ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve β -cell function. *Diabetes* 59:2145–2151, 2010

Ghrelin has gained considerable attention over the last decade for its unique role in regulating mealtime hunger and lipid metabolism, as well as short- and long-term energy homeostasis (1–3). It is the only known circulating factor that promotes food intake and increases fat mass. Ghrelin is secreted mainly from the stomach and proximal small bowel, and stimulates growth hormone (GH) secretion (4–6), in addition to its effect on energy balance. In healthy subjects, plasma ghrelin levels rise progressively before meals and fall to a nadir within 1 hour after eating, with changes in plasma levels during meals varying two- to threefold (7–8). Under pathologic conditions associated with severe malnutrition and weight loss, such as anorexia nervosa (9), cancer, or cardiac cachexia (10–11), plasma total ghrelin levels are increased up to threefold compared with healthy individuals. Besides its well known effects on feeding behavior, fat mass, and GH secretion, ghrelin has recently been implicated in the regulation of glucose homeostasis (12–13).

The GH secretagogue receptor (GHSR)-1a, also known as the ghrelin receptor, is widely distributed and has been localized to the hypothalamus, pituitary, liver, adipocyte, and pancreas (14–15). Both ghrelin and GHSR are expressed in human and rat pancreatic islets on both α - (16–17) and β -cells (18–19), and ghrelin is produced in a novel endocrine islet cell type that shares lineage with glucagon-secreting cells (20–21). Pancreatic ghrelin cells exist as the predominant cell type in fetal human islets, and expression in the pancreas during development significantly precedes its occurrence in the stomach (20). In animal mutant models, an early block in the differentiation of insulin-producing β cells leads to an enormous increase in ghrelin-producing ϵ cells, suggesting a developmental link between ghrelin and insulin (22). In vitro, ghrelin inhibits glucose-stimulated insulin secretion in a dose-dependent manner from cultured pancreata (23), isolated pancreatic islets (19,24), and immortalized β -cell lines (19,21), suggesting that it acts directly on β cells to achieve this effect. In experimental animals, both ghrelin released from pancreatic islets and exogenous ghrelin inhibit glucose-stimulated insulin secretion (16,24–26). Targeted gene deletion of ghrelin improves glucose tolerance and augments insulin secretion in *ob/ob* mice, suggesting a possible physiologic role which could be mediated by effects on islet function (27). Consistent with these findings, ghrelin gene deletion was shown to prevent glucose intolerance induced by a high-fat diet, an environmentally-induced model of hyperglycemia (26). Together, these findings indicate the potential of ghrelin blockade to prevent both genetically (*ob* gene)- and environmentally (high-fat diet)-induced glucose intolerance.

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The effect of ghrelin on insulin secretion in humans is controversial. Intravenous injection of ghrelin decreases plasma insulin and increases blood glucose in some studies, suggesting inhibition of insulin secretion (12,28). However, this finding has not been universally observed (29), and it is unclear whether such effects occur at physiologic or only pharmacologic doses of ghrelin. Prior studies performed in humans primarily assessed the impact of ghrelin on β -cell function in the fasting state, and there is little information on the effect of the peptide on stimulated insulin release. Therefore, the role of ghrelin in the regulation of glucose homeostasis in humans remains poorly understood.

In this study, we determined the effect of ghrelin on glucose-stimulated insulin secretion and glucose tolerance. We infused acyl-ghrelin, the bioactive endogenous ligand of the GHSR-1a, at variable doses with the aim of raising plasma total ghrelin level to physiologic (less than twofold), supraphysiologic (two- to threefold) and pharmacologic (more than threefold) levels. An intravenous glucose tolerance test (IVGTT) was performed at steady state plasma ghrelin levels to determine the effect on glucose-stimulated insulin secretion and glucose tolerance in healthy, nonobese subjects.

RESEARCH DESIGN AND METHODS

Subjects. Healthy volunteers between the ages of 18 and 55 years with a BMI between 18 and 29 kg/m² were recruited from the greater Cincinnati area. Subjects with a history or clinical evidence of impaired fasting glucose or diabetes, recent myocardial infarction, congestive heart failure, active liver or kidney disease, growth hormone deficiency or excess, neuroendocrine tumor, anemia, or who were on medications known to alter insulin sensitivity were excluded.

All study procedures were conducted at the Cincinnati Veteran Affairs Medical Center General Clinical Research Center. All study participants gave informed consent for the study by signing a form approved by University of Cincinnati Institutional Review Board.

Experimental protocol. Subjects arrived at the Clinical Research Center between 0700 and 0730 after a 10–12 h fast for four separate experiments. Intravenous catheters were placed in the veins of both forearms for blood sampling and infusion of test substances. The arm with the sampling catheter was heated to 55°C to arterialized venous blood.

Synthetic human acylated ghrelin was obtained from Bachem AG (Rubendorf, Switzerland). The authenticity of the peptide was verified by mass spectrometry, the purity was >95%, and reconstituted material was sterile and free of detectable pyrogens. On the morning of the 4 study days, either saline (as a control) or synthetic ghrelin dissolved in sterile saline solution was infused at doses of 0.3, 0.9, or 1.5 nmol/kg/h (equivalent to 1, 3, or 5 μ g/kg/h) for a total of 65 min. The order of infusions was randomized, and study visits separated by at least 5 days. The use of synthetic human ghrelin was approved under the U.S. Food and Drug Administration Investigational New Drug 79,009.

After 55 min of ghrelin infusion, ~ 6 plasma half-lives of acyl-ghrelin (28), subjects received an intravenous bolus of glucose (11.4 g/m² body surface area) over 60 s as the initiation of an IVGTT (time 0). Blood samples were removed at 2, 3, 4, 5, 6, 8, and 10 min after intravenous glucose bolus for the estimation of acute insulin response to glucose (AIRg) and acute C-peptide response to glucose (ACRg). Another seven blood samples were taken at 12, 14, 16, 20, 22, 25, and 30 min for the calculation of glucose disappearance and ghrelin pharmacokinetics. Blood was placed on ice and plasma separated by centrifugation within 1 hour, with the plasma stored at -80°C until used for assay. Blood pressure and heart rate were monitored every 15 min during the study procedure. A complete blood count, liver and kidney function tests, and an electrocardiogram were obtained as part of the safety monitoring of ghrelin use at the end of the last visit.

Assays. Blood glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (YSI 2,300 STAT Plus; Yellow Springs Instruments, Yellow Springs, OH). Plasma immunoreactive insulin levels were measured using a double-antibody radioimmunoassay (RIA) as described previously (30). C-peptide levels were measured using a commercial RIA kit (Millipore). Total immunoreactive ghrelin was measured by RIA (Millipore, Billerica, MA). The lower and upper limits of detection were 27 and 1,765

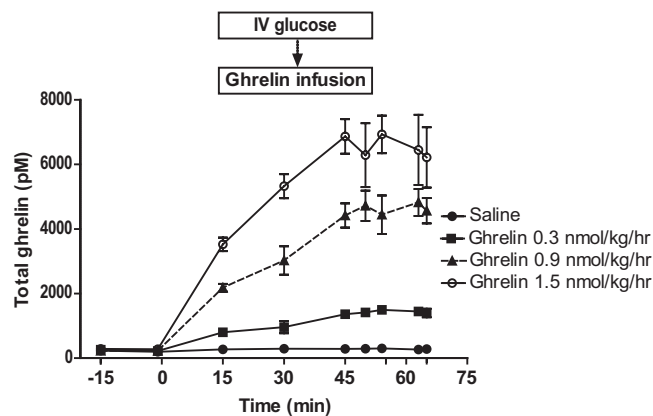


FIG. 1. Plasma total ghrelin levels during continuous intravenous infusions (minus 15 to 65 min) of saline, 0.3, 0.9, or 1.5 nmol/kg/h of acyl ghrelin in healthy men and women. A bolus intravenous dose of glucose (11.4 g/m² body surface area) was infused more than 1 min after plasma ghrelin had reached a steady state (55 min). The acyl ghrelin infusions resulted in a dose-dependent increase in plasma ghrelin.

pmol/l (93 and 6,000 pg/ml), respectively, and the intra-assay and interassay coefficients of variation (CV) were 6.4 and 16.3%, respectively. The ghrelin antibody used in the assay was directed toward the C-terminus of the molecule and binds both acyl- and desacyl-ghrelin, as well as truncated ghrelin species. Serum concentrations of human GH (hGH) were measured using the automated Immulite 2000 chemiluminescent assay system (Siemens, Bad Nauheim, Germany). This sandwich immunoassay uses a monoclonal mouse-anti-hGH capture- and a polyclonal rabbit-anti-hGH detection antibody. The intra-assay CV was 3%, and interassay variability ranged from 7%. Samples for glucagon were collected with benzamidine and heparin and were measured by RIA (Millipore, Billerica, MA). Cortisol levels were measured using the Corti-Cote RIA kit (MP Biomedicals, Orangeburg, NY). Plasma epinephrine and norepinephrine were measured using the CatCombi ELISA kit (IBL International; Hamburg, Germany). All samples were run in duplicate, and all specimens from a given participant were processed in the same assay.

Calculations. AIRg and ACRg were calculated as the average plasma insulin and C-peptide increment above baseline from 2–10 min after intravenous glucose administration, respectively. The glucose disappearance constant (31) was computed for each IVGTT as the slope of the natural logarithm of glucose from 10 to 30 min. The rate of ghrelin disappearance was calculated as the slope of the natural logarithm of ghrelin after cessation of the ghrelin infusion at 65 min (10 min after the glucose bolus was given).

Statistical analysis. The data were analyzed using ANOVA with 4 treatment levels (control, and ghrelin infusion rates of 0.3, 0.9, and 1.5 nmol/kg/h) and time of sampling being the repeated measure. Dependent variables included insulin, glucose, GH, cortisol, and glucagon concentrations. AIRg and ACRg for the 4 treatment levels were compared using a single-factor ANOVA. Post hoc analysis to compare control with each of the ghrelin infusion levels was performed using a Dunnett test. Data were analyzed using GraphPad Prism version 5.0 (GraphPad Software). All results are expressed as mean $\pm$ SEM unless otherwise noted.

RESULTS

Subject characteristics. Twelve healthy subjects (8 male and 4 female) age 26.0 ± 3.8 years with a BMI of 24.1 ± 1.4 kg/m² were enrolled in the study. No subject had a fasting blood glucose of >5.5 mmol/l. Mean fasting blood glucose for the group was 4.9 ± 0.2 mmol/l, and mean fasting plasma insulin was 37.8 ± 6.2 pmol/l.

Ghrelin pharmacokinetics. Steady-state levels were reached after ~ 45 min for all 3 doses of acyl-ghrelin infusion. The average total ghrelin concentration during the time period between 45 and 54 min (10, 5, and 1 min before to intravenous glucose administration) for saline and the 3 acyl-ghrelin infusions were 304 ± 18 , $1,429 \pm 49$, $4,629 \pm 194$, and $7,045 \pm 295$ pmol/l. The 0.3, 0.9 and 1.5 nmol/kg/h infusions raised the total ghrelin immunoreactivity 4.5-, 15.4-, and 22.6-fold above an average basal level of 308 ± 30 pmol/l for the three infusions (Fig. 1;

TABLE 1

Basal plasma glucose and insulin levels during continuous intravenous infusions of saline, 0.3, 0.9, or 1.5 nmol/kg/h acyl ghrelin (0–54 min) before an IVGTT

Infusion rate	Plasma glucose (mg/dl)		Plasma insulin (pM)	
	Baseline	Ghrelin steady state ($t = 45\text{--}54\text{ min}$)	Baseline	Ghrelin steady state ($t = 45\text{--}54\text{ min}$)
Saline	83.8 $\pm$ 4.6	86.9 $\pm$ 1.0	34.1 $\pm$ 4.8	36.5 $\pm$ 5.8
Ghrelin (0.3 nmol/kg/h)	86.5 $\pm$ 2.4	96.7 $\pm$ 6.7	36.3 $\pm$ 4.8	30.4 $\pm$ 5.0
Ghrelin (0.9 nmol/kg/h)	91.4 $\pm$ 2.1	93.5 $\pm$ 2.7	42.0 $\pm$ 6.6	36.1 $\pm$ 6.9
Ghrelin (1.5 nmol/kg/h)	87.6 $\pm$ 1.6	91.5 $\pm$ 2.4	39.1 $\pm$ 5.8	25.6 $\pm$ 3.9

Data for baseline plasma glucose and insulin concentration were calculated as the average of the -15 - and -1 -min values. Data for plasma glucose and insulin at steady-state ghrelin concentration were calculated as the average of 45, 50, and 54 min values.

supplementary Table 1 is available in an online appendix at <http://diabetes.diabetesjournals.org/cgi/content/full/db10-0504/DC1>. The intrasubject CV percentage for the saline, 0.3, 0.9, and 1.5 nmol/kg/h ghrelin infusions were 13.8, 7.7, 6.8, and 7.0%, respectively. The intersubject CV percentage for the steady-state total ghrelin measurement with different ghrelin infusion rates were 23.7, 20.1, 14.6, and 24.1%, respectively. After cessation of the ghrelin infusion at 65 min, total ghrelin levels declined after a first-order (exponential) decrease with an overall elimination rate constant (K_{el}) of 0.023 min^{-1} , corresponding to an elimination half-life of 30 min.

Effects of exogenous ghrelin on plasma insulin and glucose. The average fasting plasma glucose and insulin values at baseline and at times when ghrelin concentration reached a steady state (45 to 54 min) are shown in Table 1. Infusion of exogenous ghrelin did not alter fasting plasma concentrations of insulin and glucose from baseline ($P > 0.05$ for all comparisons).

Compared with saline, the doses of 0.3, 0.9, and 1.5 nmol/kg/h ghrelin each resulted in a significant reduction of AIRg ($2,152 \pm 448$ to $1,478 \pm 288$, $1,419 \pm 2,751$ and $1,210 \pm 188\text{ pmol/l}$, $P < 0.05$, < 0.05 , and < 0.01 , respectively) during an IVGTT (Fig. 2A and B). The magnitude of suppression in AIRg increased with higher doses of ghrelin administration, suggesting a dose-dependent relationship between circulating ghrelin concentration and insulin secretion. Similar to AIRg, a significant suppression of C-peptide release in response to intravenous glucose was also seen with all three doses of ghrelin infusions (5.8 ± 0.9 to 4.1 ± 0.4 , 4.2 ± 0.5 , and $3.6 \pm 0.6\text{ nmol/l}$, $P < 0.05$, < 0.05 , and < 0.01 , respectively) (Fig. 2C). In addition, ghrelin infusion at the 0.3 and 1.5 nmol/kg/h doses also significantly decreased the rate of glucose disappearance ($P < 0.05$ for both comparisons; Fig. 3).

Effects of exogenous ghrelin on counterregulatory hormones. The three doses of ghrelin raised peak plasma GH levels by 12-, 114-, and 75-fold above baseline, respectively (Fig. 4). The 0.9 and 1.5 nmol/kg/h rates of ghrelin infusion also raised plasma cortisol levels significantly as compared with baseline at 30, 54, and 65 min ($P < 0.01$) (Fig. 5). Ghrelin infusion, regardless of dose, had no effect on glucagon secretion ($P = 0.44$) (supplementary Fig. 1). Plasma epinephrine and norepinephrine levels did not differ between baseline and 54 min when ghrelin in the circulation reached a steady state, regardless of the type of infusion the subjects received (supplementary Fig. 2).

Side effects. Ghrelin infusion was generally well tolerated. The most common complaints during infusion of ghrelin were hunger and “warm sensation.” These symptoms were transient and resolved spontaneous after ces-

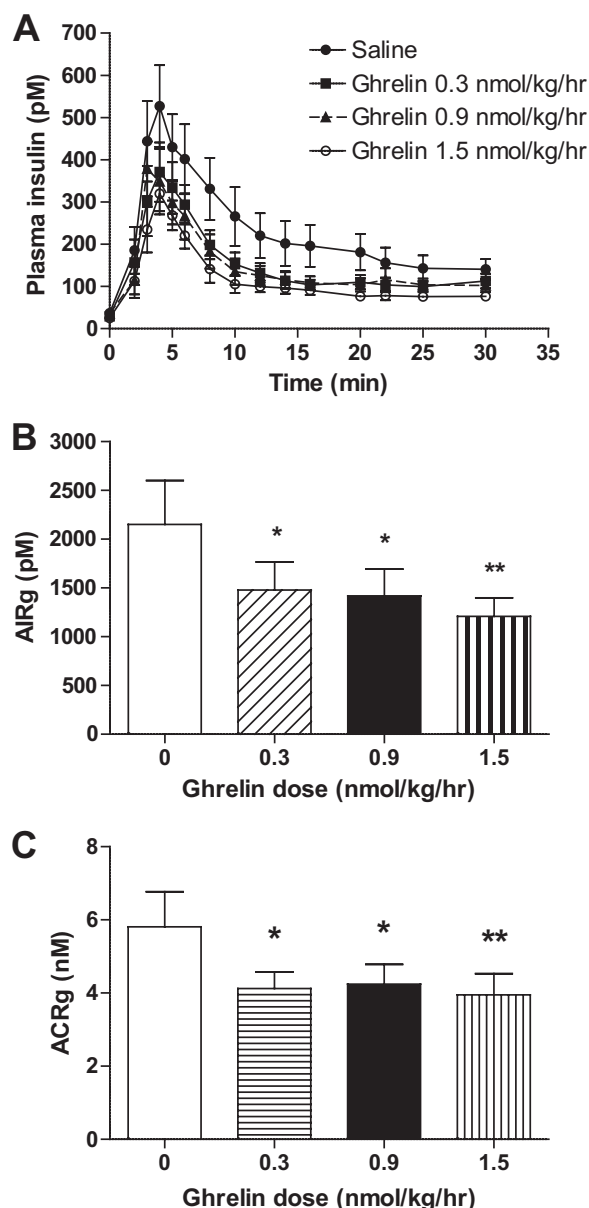


FIG. 2. A: Plasma insulin concentrations during an IVGTT after 55-min infusions of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h, or saline. B: The acute insulin response to intravenous glucose (AIRg) determined during infusions of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h dose, or saline. * $P < 0.05$, ** $P < 0.01$. C: The acute C-peptide response to intravenous glucose (ACRg) determined during infusions of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h dose, or saline. * $P < 0.05$, ** $P < 0.01$.

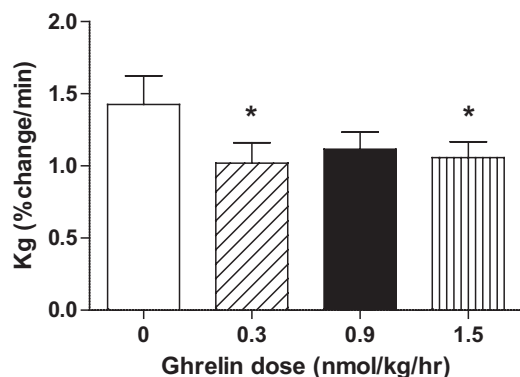


FIG. 3. Glucose disappearance constant (Kg) determined during infusions of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h, or saline. * $P < 0.05$.

sation of the infusion. One subject, while receiving the 1.5 nmol/kg per hour ghrelin infusion, experienced a 23-mmHg decrease in mean arterial blood pressure without a significant change in heart rate. The subject was asymptomatic except for feeling “warm and hungry” during the event. The blood pressure returned to baseline within minutes after the ghrelin infusion was discontinued prematurely. This blood pressure change was not observed in any other subject or with any other dose.

DISCUSSION

Preclinical studies support a role for ghrelin to regulate glucose metabolism as well as energy balance and GH secretion. However, the effect of ghrelin on insulin secretion and glucose tolerance in humans has not been clearly established in the limited number of studies reported previously. In the present study, we examined the effect of a range of ghrelin doses on dynamic insulin secretion and glucose metabolism and demonstrated that acyl-ghrelin suppresses glucose-stimulated insulin secretion and worsens intravenous glucose tolerance in healthy humans. These effects appear to be present at concentrations of ghrelin above the usual physiologic range, and in a pattern consistent with dose-dependence. Our findings extend to humans the effects previously best demonstrated in mice, and suggest that ghrelin has a role in systemic glucose

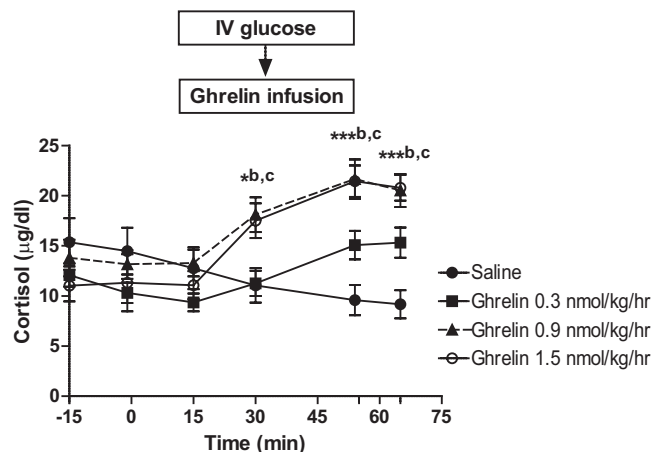


FIG. 5. Plasma cortisol concentrations during a 65-min infusion of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h, or saline. Glucose was administered as an intravenous bolus after 55 min of the infusion. *b* and *c* are saline vs. 0.9 and 1.5 nmol/kg/h ghrelin, respectively; * $P < 0.05$, *** $P < 0.001$.

homeostasis. Moreover, our results raise possibilities for targeting the human ghrelin system as a means to improve disorders of glucose metabolism.

Several studies have examined the effect of ghrelin on insulin secretion in humans. In a study of healthy young males by Broglio et al. (12), an intravenous bolus injection of ghrelin (0.3 nmol/kg or 1.0 μg/kg) significantly increased fasting plasma glucose levels followed by a reduction in serum insulin levels beginning at 15 and 30 min after ghrelin administration, respectively, suggesting inhibition of insulin secretion. When the same dose of ghrelin was given as a continuous intravenous infusion to subjects who had undergone total gastrectomy, by necessity reducing the production of most endogenous ghrelin, C-peptide levels were suppressed when compared with saline infusion (32). In contrast, Lucidi et al. (29) infused acyl ghrelin at a rate of 7.5 or 15 pmol/kg/min for 2 hours in 8 healthy subjects and failed to observe a significant change in fasting plasma glucose and insulin levels. However, all previous studies in humans used fasting insulin as the marker of ghrelin effects on the β-cell, with no examination of stimulated insulin secretion. In the present study, we examined the effect of continuous infusions of low, medium, and high doses of acyl ghrelin on AIRg, a well established measure of insulin secretion that we think provides a more sensitive measure of β-cell function. The measure of C-peptide levels during the IVGTT confirms the changes in AIRg, and supports an effect of ghrelin on insulin secretion rather than insulin clearance. Moreover, the continuous infusion of ghrelin during the IVGTT also eliminated any potential bias in the β-cell response introduced by rapid changes in plasma ghrelin levels as occur with a bolus injection of the peptide. Based on these design advantages, we believe that ours is the most robust proof-of-concept study yet of the effect of ghrelin on insulin secretion in humans.

Similar to Lucidi et al. (29), we did not observe a significant change in fasting insulin or glucose levels with any of the 3 doses of ghrelin. On the other hand, we did find a clear suppressive effect of ghrelin on the first-phase insulin response in an apparent dose-dependent fashion, with the greatest effect seen with the highest dose ghrelin (Fig. 2). In addition, the decrease in intravenous glucose tolerance is consistent with a reduction of insulin secre-

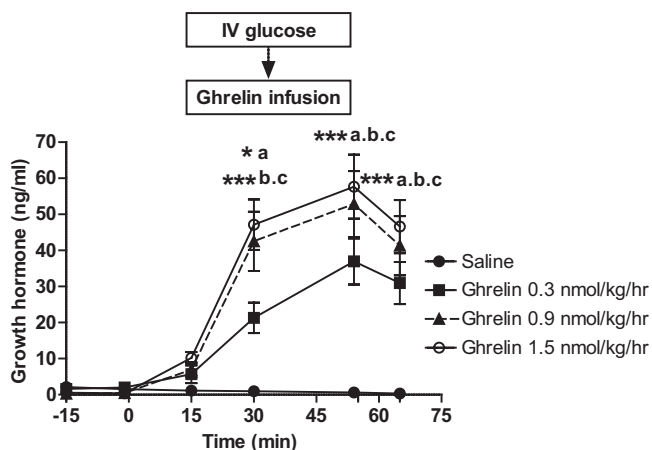


FIG. 4. Plasma growth hormone concentrations during a 65-min infusion of acyl ghrelin at 0.3, 0.9, or 1.5 nmol/kg/h, or saline. Glucose was administered as an intravenous bolus after 55 min of the infusion. *a*, *b*, and *c* are saline vs. 0.3, 0.9, or 1.5 nmol/kg/h of ghrelin, respectively; * $P < 0.05$, *** $P < 0.001$.

tion. Our observations are in keeping with several *in vitro* studies that have provided evidence that ghrelin has an inhibitory effect on stimulated insulin secretion from pancreatic β -cells (16,19,21,24–26) and *in vivo* studies that have shown a deteriorating effect on glucose tolerance (25,27). The mechanisms by which ghrelin could inhibit insulin secretion are unknown. Ghrelin may exert a direct effect on the β -cell or act indirectly by stimulating the secretion of counter-regulatory hormones that affect insulin secretion, or activating neural pathways that regulate islet function (33–38). The signaling mechanisms for insulinostatic ghrelin action in islet β -cells have been explored. Both endogenous and exogenous ghrelin has been shown to attenuate glucose-induced insulin release via $G_{\alpha_{12}}$ -mediated activation of Kv channels, and suppression of action potential firing and $[Ca^{2+}]_i$ increases in β -cells (39). Furthermore, both ghrelin and its receptor are expressed in human and rat pancreatic islets (on α -, β -, and ϵ -cells) (16,18,20,40), and normal mouse pancreas contains a small population of ghrelin producing ϵ -cells which appear to be distinct from α - and β -cells (22,41). Ghrelin-immunoreactive cells are abundant in human islets during development, outnumbering those in the stomach, but few are present in adults (20). It is interesting to note that mice lacking the homeodomain protein Nkx2.2, which is essential for the differentiation of insulin-producing β -cells, have islets in which the β -cells are almost completely replaced by ϵ -cells (22). These findings raise the possibility of a shared common progenitor for both β - and ϵ -cells and suggest a role of ghrelin in the pancreatic islet, perhaps as a regulator of glucose homeostasis. Lastly, gut-brain crosstalk has been well described, and it is possible that ghrelin achieves its metabolic actions in the pancreas, muscle, adipose tissue, and liver via central ghrelin and insulin signaling (42–44).

As for possible indirect mechanisms of ghrelin action on the islet, previous studies in animals and humans have shown that both epinephrine and cortisol exhibit inhibitory effects on insulin secretion (33–36). We have shown here that cortisol levels were significantly elevated when higher doses of acyl ghrelin were given (0.9 and 1.5 nmol/kg/h). However, since steroid hormone action is thought to be mediated primarily by changes in gene transcription (45), it seems unlikely that the acute effect of ghrelin on AIRg can be explained by glucocorticoid activity. In contrast to a previous observation (46), we did not observe an increase in epinephrine levels with ghrelin administration. This could be caused by the difference in assay reproducibility (or perhaps sensitivity) or the method of ghrelin administration. As expected, GH levels were significantly elevated during ghrelin infusion (Fig. 5). Acutely, infusion of GH to levels within the physiologic range (27 ± 2 ng/ml) decrease insulin-mediated glucose uptake in the periphery within 2 to 12 h (37). In this study, the plasma insulin response to hyperglycemia was not altered by GH, and other investigators have noted increased plasma insulin concentration after 12-h infusion of GH to healthy volunteers (38). Therefore, we do not think the effects of ghrelin to reduce AIRg can be explained by changes in plasma GH.

Theoretically, the decrease in insulin secretion with ghrelin administration could be an adaptation to an increase in peripheral insulin sensitivity. However, previous studies in humans and animals seem to suggest that ghrelin consistently reduces, rather than improves, peripheral insulin sensitivity (12,28,32,47). The length of the

IVGTT was limited by the total dose of ghrelin we could administer to each individual based on FDA requirements. For this reason, we do not have insulin sensitivity measures from IVGTT in this study. Overall, our data do not support indirect actions of counter-regulatory hormones or systemic insulin sensitivity to mediate the effects of ghrelin on insulin secretion.

Although in this study the effects of ghrelin on β -cell function occurred at supraphysiologic concentrations, it is important to consider that since ghrelin is produced in the islet ϵ cells (20–21), intra-islet ghrelin concentrations may reach very high levels, raising the possibility that ghrelin could act locally on β cells via paracrine mechanisms (48–49), similarly to what has been demonstrated in adult rat islets (16). It is generally accepted that the level of hormone working in a paracrine/autocrine manner is higher than that working in an endocrine manner. Therefore, our observation of a suppressive effect of ghrelin on insulin secretion while the circulating level is in the supraphysiologic range does not exclude the possibility of a physiologic function of this hormone. Further studies will be necessary to delineate mechanisms by which endogenous ghrelin may affect islet function. Based on our results, endocrine, paracrine, and neural mechanisms are all plausible possibilities.

The role of ghrelin on α -cell function in humans has not been well established. Glucagon secretion is enhanced by ghrelin *in vitro* (25), but the effect of ghrelin on its release is less impressive *in vivo*, with levels largely unchanged or mildly increased after ghrelin administration (23,25,29,32). In our hands, no relevant change in glucagon level was seen with pharmacologic level ghrelin administration during fasting or IVGTT. Future studies that employ measurement of dynamic changes of glucagon level using more sensitive methods should be done to confirm this finding.

Conclusion. Our study demonstrates that exogenous ghrelin markedly reduces the first-phase insulin and C-peptide responses to intravenous glucose in healthy humans. These findings raise the possibility that endogenous ghrelin has a role in physiologic insulin secretion, and that ghrelin antagonists could improve β -cell function and serve as a novel drug target for the treatment of type 2 diabetes.

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No other potential conflicts of interest relevant to this article were reported.

J.T. researched data and wrote the manuscript. R.L.P. and H.W.D. researched data, contributed to discussion, and reviewed/edited the manuscript. M.B. researched data and reviewed/edited the manuscript. S.E.K. contributed to discussion and reviewed/edited the manuscript. D.E.C. reviewed/edited the manuscript. M.H.T. and D.D. contributed to discussion and reviewed/edited the manuscript.

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REFERENCES

1. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908–913
2. Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav* 2006;89:71–84
3. Castaneda TR, Tong J, Datta R, Culler M, Tschop MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010;31:44–60
4. Bowers CY, Momany FA, Reynolds GA, Hong A. On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 1984;114:1537–1545
5. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;402:656–660
6. Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *J Endocrinol Invest* 2000;23:493–495
7. Tschop M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, Folwaczny C. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 2001;24:RC19–21
8. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001;50:1714–1719
9. Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* 2001;145:669–673
10. Shimizu Y, Nagaya N, Isobe T, Imazu M, Okumura H, Hosoda H, Kojima M, Kangawa K, Kohno N. Increased plasma ghrelin level in lung cancer cachexia. *Clin Cancer Res* 2003;9:774–778
11. Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, Hosoda H, Shimizu W, Yamagishi M, Oya H, Koh H, Yutani C, Kangawa K. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* 2001;104:2034–2038
12. Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 2001;86:5083–5086
13. Sun Y, Asnicar M, Smith RG. Central and peripheral roles of ghrelin on glucose homeostasis. *Neuroendocrinology* 2007;86:215–228
14. Howard AD, Feighner SD, Cully DF, Arena JP, Liberators PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevich M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996;273:974–977
15. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* 1997;48:23–29
16. Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in beta-cells: implication in the glycemic control in rodents. *Diabetes* 2004;53:3142–3151
17. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002;51:124–129
18. Volante M, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, Muccioli G, Ghigo E, Papotti M. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J Clin Endocrinol Metab* 2002;87:1300–1308
19. Colombo M, Gregersen S, Xiao J, Hermansen K. Effects of ghrelin and other neuropeptides (CART, MCH, orexin A and B, and GLP-1) on the release of insulin from isolated rat islets. *Pancreas* 2003;27:161–166
20. Wierup N, Svensson H, Mulder H, Sundler F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002;107:63–69
21. Wierup N, Yang S, McEvilly RJ, Mulder H, Sundler F. Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *J Histochem Cytochem* 2004;52:301–310
22. Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proc Natl Acad Sci U S A* 2004;101:2924–2929
23. Egido EM, Rodriguez-Gallardo J, Silvestre RA, Marco J. Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J Endocrinol* 2002;146:241–244
24. Reimer MK, Pacini G, Ahren B. Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology* 2003;144:916–921
25. Salehi A, Dornonville de la Cour C, Hakanson R, Lundquist I. Effects of ghrelin on insulin and glucagon secretion: a study of isolated pancreatic islets and intact mice. *Regul Pept* 2004;118:143–150
26. Dezaki K, Sone H, Koizumi M, Nakata M, Kakei M, Nagai H, Hosoda H, Kangawa K, Yada T. Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 2006;55:3486–3493
27. Sun Y, Asnicar M, Saha PK, Chan L, Smith RG. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metab* 2006;3:379–386
28. Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K, Kangawa K. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J Endocrinol* 2004;150:447–455
29. Lucidi P, Murdolo G, Di Loreto C, Parlanti N, De Cicco A, Fatone C, Taglioni C, Fanelli C, Broglio F, Ghigo E, Bolli GB, Santeusano F, De Feo P. Metabolic and endocrine effects of physiological increments in plasma ghrelin concentrations. *Nutr Metab Cardiovasc Dis* 2005;15:410–417
30. Elder DA, Prigeon RL, Wadwa RP, Dolan LM, D'Alessio DA. Beta-cell function, insulin sensitivity, and glucose tolerance in obese diabetic and nondiabetic adolescents and young adults. *J Clin Endocrinol Metab* 2006;91:185–191
31. Kahn SE, Montgomery B, Howell W, Ligueros-Saylan M, Hsu CH, Devineni D, McLeod JF, Horowitz A, Foley JE. Importance of early phase insulin secretion to intravenous glucose tolerance in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2001;86:5824–5829
32. Damjanovic SS, Lalic NM, Pesko PM, Petakov MS, Jotic A, Miljic D, Lalic KS, Lukic L, Djurovic M, Djukic VB. Acute effects of ghrelin on insulin secretion and glucose disposal rate in gastrectomized patients. *J Clin Endocrinol Metab* 2006;91:2574–2581
33. Tamagawa T, Henquin JC. Epinephrine modifications of insulin release and of $86Rb^{+}$ or $45Ca^{2+}$ fluxes in rat islets. *Am J Physiol* 1983;244:E245–E252
34. Sherwin RS, Sacca L. Effect of epinephrine on glucose metabolism in humans: contribution of the liver. *Am J Physiol* 1984;247:E157–E165
35. Barseghian G, Levine R. Effect of corticosterone on insulin and glucagon secretion by the isolated perfused rat pancreas. *Endocrinology* 1980;106:547–552
36. Kalhan SC, Adam PA. Inhibitory effect of prednisone on insulin secretion in man: model for duplication of blood glucose concentration. *J Clin Endocrinol Metab* 1975;41:600–610
37. Bratusch-Marrain PR, Smith D, DeFronzo RA. The effect of growth hormone on glucose metabolism and insulin secretion in man. *J Clin Endocrinol Metab* 1982;55:973–982
38. Rizza RA, Mandarino LJ, Gerich JE. Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes* 1982;31:663–669
39. Dezaki K, Kakei M, Yada T. Ghrelin uses $Galphai2$ and activates voltage-dependent K^{+} channels to attenuate glucose-induced Ca^{2+} signaling and insulin release in islet β -cells: novel signal transduction of ghrelin. *Diabetes* 2007;56:2319–2327
40. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2002;87:2988
41. Andralojc KM, Mercalli A, Nowak KW, Albarello L, Calcagno R, Luzi L, Bonifacio E, Doglioni C, Piemonti L. Ghrelin-producing epsilon cells in the developing and adult human pancreas. *Diabetologia* 2009;52:486–493
42. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 2002;8:1376–1382
43. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci* 2002;5:566–572
44. Pocai A, Morgan K, Buettner C, Gutierrez-Juarez R, Obici S, Rossetti L. Central leptin acutely reverses diet-induced hepatic insulin resistance. *Diabetes* 2005;54:3182–3189
45. Gronemeyer H. Control of transcription activation by steroid hormone receptors. *FASEB J* 1992;6:2524–2529

46. Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, Hayashi Y, Kangawa K. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol* 2001;280: R1483–R1487
47. Vestergaard ET, Gormsen LC, Jessen N, Lund S, Hansen TK, Moller N, Jorgensen JO. Ghrelin infusion in humans induces acute insulin resistance and lipolysis independent of growth hormone signaling. *Diabetes* 2008;57: 3205–3210
48. Ahima RS. Ghrelin—a new player in glucose homeostasis? *Cell Metab* 2006;3:301–302
49. van der Lely AJ. Ghrelin and new metabolic frontiers. *Horm Res 71 Suppl* 2009;1:129–133

ONLINE APPENDIX: Supplemental Material:

Table 1: Plasma total ghrelin levels during saline, 0.3, 0.9, or 1.5 nmol/kg/hr dose acyl ghrelin continuous IV infusion prior to IVGTT (0-54 minutes) in healthy men (n = 8) and women (n = 4).

Plasma total ghrelin (pM)	Ghrelin infusion rate			
Infusion time (min)	Saline	0.3 nmol/kg/hr	0.9 nmol/kg/hr	1.5 nmol/kg/hr
0	292.3 ± 31.8	314.6 ± 26.6	300.1 ± 31.4	311.3 ± 36.2
45	299.3 ± 33.5	1362.6 ± 85.2	4356.7 ± 341.0	6956.4 ± 487.4
50	294.2 ± 32.2	1432.6 ± 80.6	4673.5 ± 422.8	7144.8 ± 604.3
54	345.1 ± 27.3	1495.7 ± 95.0	4894.0 ± 226.7	7061.8 ± 538.1

Figure 1: Administration of acyl ghrelin at doses of 0.3, 0.9, or 1.5 nmol/kg/hr did not alter plasma glucagon levels.

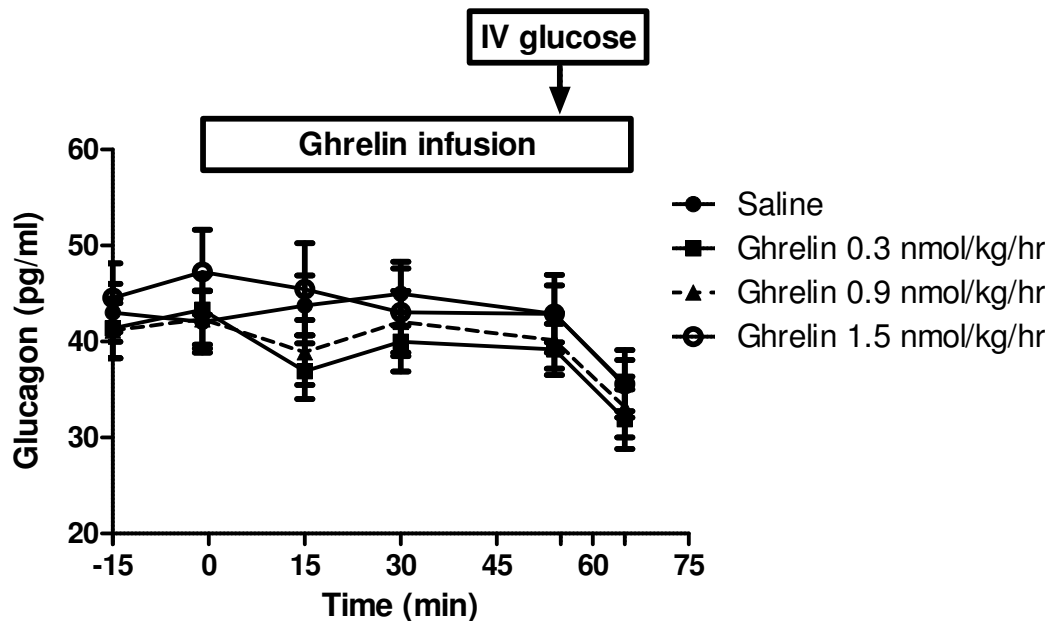
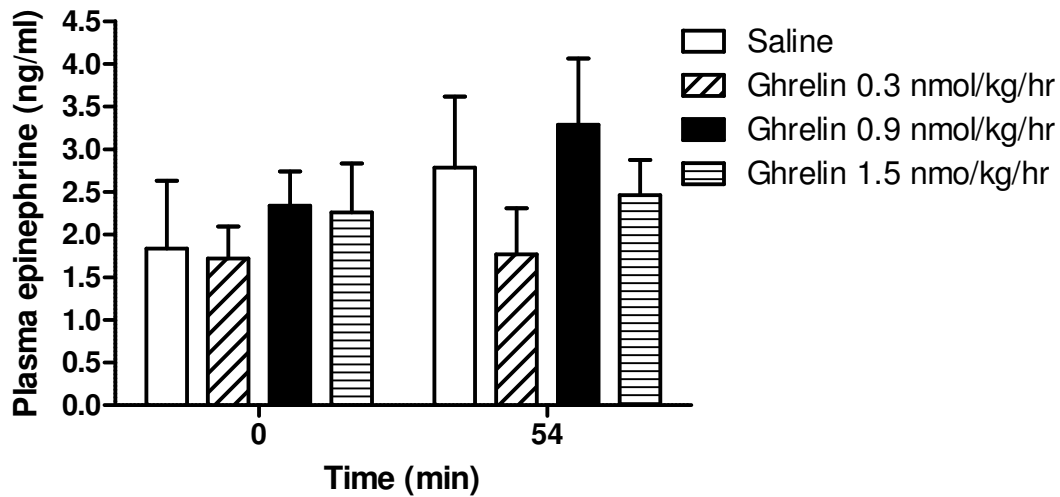


Figure 2: Acyl ghrelin had no effect on plasma catecholamine levels. Epinephrine and norepinephrine were measured as described in the Methods section. Plasma levels of both catecholamines were low and were unchanged throughout the study.

A.



B.

