Ghrelin plasma concentrations increase during fasting and fall rapidly after nutrient ingestion. We hypothesized that insulin or glucose could regulate ghrelin secretion by a feedback mechanism. In this randomized, double-blind, placebo-controlled crossover study, three different study days were carried out in nine healthy volunteers (age 26 ± 6 years). On each day, stepwise increasing systemic glucose concentrations of 5.0, 8.3, and 11.1 mmol/l were attained by intravenous infusion of glucose, representing fasting and postprandial conditions. Ghrelin plasma concentration was studied during concomitant exogenous hyperinsulinemia, inhibition of endogenous insulin production by somatostatin infusion, and placebo time control, respectively. Elevated glucose concentrations increased circulating insulin to 612 ± 85 pmol/l (P < 0.01), but they did not affect ghrelin concentrations. Prolonged hyperinsulinemia by exogenous infusion in circulating insulin of 1,602 ± 261 pmol/l (P < 0.01) and suppressed plasma ghrelin to 49.6% of baseline (P < 0.01). During administration of somatostatin, insulin concentration remained constant, but an even greater decrease in ghrelin to 39.5% of baseline was noted (P < 0.01). Hyperglycemia does not decrease ghrelin, and a reduction in ghrelin is only seen at supraphysiological insulin concentrations. In contrast, systemic ghrelin concentrations are decreased by somatostatin. The meal-related suppression of ghrelin appears not directly regulated by glucose or insulin. Diabetes 52:16–20, 2003

The 28–amino acid peptide ghrelin has been identified as the endogenous ligand of the pituitary growth hormone (GH) secretagogue receptor (1). Ghrelin is produced by a distinct endocrine cell type primarily in the gastric fundus (2,3) and increases GH levels in both animals (4) and humans (5–7). In addition, ghrelin exerts potent orexigenic and adipogenic effects (8,9), which are thought to be mediated by action on hypothalamic neurons (10–12). Ghrelin is assumed to be involved in a feedback loop regulating food consumption and meal initiation. However, access of peripherally secreted ghrelin to regions in the brain is limited by specific and selective transport mechanisms through the blood-brain barrier (13).

There is experimental evidence that food intake and body weight increase rapidly in rodents treated with ghrelin (4,10), and ghrelin levels rise in fasting animals and humans (10,11,14). A postprandial fall of ghrelin plasma levels has been demonstrated in both animals (15) and humans (16,17). Plasma ghrelin is elevated in cachectic patients with chronic heart failure (18), bulimia (19), and anorexia nervosa, where weight gain substantially reduces ghrelin concentrations (20). Conversely, ghrelin concentrations are suppressed in obese subjects (21) and increased by weight loss (22). However, food intake fails to decrease ghrelin levels in obese patients (23). Mutations in the human preproghrelin/ghrelin gene may be associated with obesity (24,25), but the influence of genetic polymorphisms on weight regulation is under debate (26).

The mechanisms controlling ghrelin secretion during fasting and postprandial suppression are unknown. Ghrelin levels were found to be reciprocal to those of glucose and insulin (14). Glucose or insulin might therefore regulate ghrelin release. To test this hypothesis, plasma ghrelin and GH concentrations were quantified during a glucose clamp at normal and high blood glucose concentrations, representing fasting and postprandial states. This was studied on three separate study days, under conditions of concomitant hyperinsulinemia on the first day, during inhibition of endogenous insulin production by somatostatin on the second day, and serving as placebo control on the third study day.

**RESEARCH DESIGN AND METHODS**

The study was approved by the ethics committee of the University of Vienna and complies with the principles outlined in the Declaration of Helsinki, including current revisions and the Good Clinical Practice guidelines. Written informed consent was obtained from all subjects before enrolment.

**Subjects.** Nine healthy male volunteers were included in this study. The subjects were between the ages of 19 and 36 years (mean ± SD, 26 ± 6 years) and had a BMI between the 15th and 85th percentile (27). All subjects were nonsmokers and drug free. In a complete health examination (including physical examination, electrocardiography, laboratory screening, and an oral glucose tolerance test) within 14 days before the first study day, no clinically relevant abnormalities were detected. Subjects were studied after overnight fasting, and experiments were started between 0800 and 0900. Studies were conducted in a quiet room with an ambient temperature of 22°C.
Experimental protocol. The study used a double-blind, placebo-controlled three-way crossover design. On three different study days separated by a washout period of at least 2 days, all subjects received insulin infusions of somatostatin or placebo, respectively. This was accompanied by a coinfusion of glucose to achieve stepwise increased venous glucose concentrations of 5.0, 8.3, and 11.1 mmol/l for a period of 90 min each on all three study days.

Intravenous cannulas were inserted into opposite arms for blood sampling and drug infusions, respectively. Baseline blood samples were drawn, and infusions were started and maintained for 270 min: insulin mixed with heparinized blood (to avoid adsorption to the syringe and to connecting tubing) (28) at 1.0 mU·kg⁻¹·min⁻¹ (Huminsulin; Lilly, Fegersheim, France), somatostatin at 61.1 pmol·kg⁻¹·min⁻¹ (UCB Pharma, Vienna, Austria), or placebo (0.9% saline). Blood glucose concentrations were determined every 5 min throughout the study (glucose analyzer; Beckman, Fullerton, CA), and glucose infusion rates (10% glucose; Mayerhofer Pharmazeutika, Linz, Austria) were adjusted accordingly.

Laboratory parameters. Blood samples for quantification of ghrelin were drawn every 30 min. Ghrelin was analyzed using a commercially available kit (Peninsula Laboratories, San Carlos, CA). Venous insulin, human GH, and IGF-1 concentrations were determined at baseline and at the end of the three different glucose level periods. Laboratory analyses were carried out according to standard procedures at the Department of Medical and Chemical Laboratory Diagnostics, Allgemeines Krankenhaus, Vienna.

Systemic hemodynamics. Blood pressure was measured by an automated oscillometric device on the upper arm at 15-min intervals during the study periods, and the pulse rate was monitored continuously by a finger pulse-oxymetric device (CMS patient monitor; Hewlett Packard, Palo Alto, CA) (29).

Statistical analysis. The individual area under the ghrelin concentration versus time curve (AUCg) was calculated for each 90-min interval of different glucose concentrations using Kinetica software (release 3.0; InnaPhase, Philadelphia, PA). Because of the skewed distribution of the parameters under study, nonparametric tests were used. Between-group comparisons were performed using the Mann-Whitney U test. Within groups, effects were tested by Friedman’s ANOVA and the Wilcoxon’s signed-rank test, respectively. All calculations were performed using a Statistica software package (release 5.1; StatSoft, Tulsa, OK). P ≤ 0.05 was considered significant. Values are expressed as the means ± SE unless indicated otherwise.

RESULTS
The drugs under study were well tolerated, and no side effects were reported. No differences in blood pressure or pulse rate were observed at baseline between the three study days, and the drugs under study had no effect on systemic hemodynamics (data not shown). Fasting glucose concentrations were comparable at baseline and in the normal range. The scheduled systemic glucose concentrations of 5.0, 8.3, and 11.1 mmol/l were achieved on all trial days (Figs. 1–3). However, the dose of exogenous glucose required was much higher during coinfusion of insulin than during placebo or somatostatin. During hyperinsulinemia, a mean of 2,269 ± 168 ml of a 10% glucose solution was infused over 270 min, whereas 753 ± 69 ml were administered during placebo and 382 ± 32 ml during somatostatin, respectively (P < 0.001 between groups). Baseline insulin or ghrelin concentrations were not different between the study days (Figs. 1–3).

Effect of hyperglycemia. During placebo, hyperglycemia caused an increase of endogenous insulin, from 69 ± 13 pmol/l to a maximum of 612 ± 85 pmol/l (P < 0.01) at the end of the study (Fig. 1). Increased glucose concentrations did not affect ghrelin concentrations, which were 193 ± 29 pmol/l at baseline and 220 ± 49 pmol/l at the end of the study (P = NS). The AUCg was 16.74 ± 2.18, 18.03 ± 2.81, and 17.27 ± 2.24 nmol·min⁻¹·l⁻¹ at glucose concentrations of 5.0, 8.3, and 11.1 mmol/l, respectively (P = NS). GH concentrations decreased slightly during hyperglycemia (P = NS). This was paralleled by a significant decrease in IGF-1 concentrations (P < 0.05) (Table 1).

Effect of exogenous hyperinsulinemia. Exogenous in-
DISCUSSION
This randomized controlled study suggests that systemic ghrelin concentrations are not directly regulated by changes in circulating glucose or insulin at physiological concentrations, but rather by somatostatin. Ghrelin concentrations were not affected by glucose concentrations of up to 11.1 mmol/l and an increase of insulin to 620 pmol/l.

Our findings also confirm results of a recent study in which intravenous glucose coadministered with subcutaneous insulin failed to suppress ghrelin concentrations (30). In contrast, other studies have reported a rapid, but transient, decrease in plasma ghrelin after a high-dose glucose bolus (21) or after food intake (17). This reciprocal pattern between glucose and ghrelin concentrations was not reproducible in our controlled study. Although it is possible that changes in ghrelin within periods shorter than 30 min could have been missed in our experiments, it is unlikely that increased glucose results in ghrelin suppression in healthy subjects when elevated to supraphysiological concentrations.

A reduction in ghrelin was seen during pharmacological hyperinsulinemia, at an insulin concentration of \(\sim 1,600\) pmol/l, with concomitant hyperglycemia. However, hyperinsulinemia at concentrations typically seen in insulin-resistant subjects did not affect plasma ghrelin in our experiments. This is compatible with findings in patients with type 2 diabetes, where ghrelin concentrations were in the normal range in lean subjects and only decreased in obese patients (21). In contrast, a recent uncontrolled study reports a decrease in ghrelin concentrations during euglycemia at insulin concentrations of \(\sim 600\) pmol/l (31). Of note, there is substantial variability of circulating ghrelin concentrations, and changes over time can be easily overinterpreted. On the basis of our data using similar insulin concentrations during euglycemia and hyperglycemia, it seems that insulin substantially decreased plasma ghrelin only at pharmacological concentrations. Glucose in combination with supraphysiological insulin concentrations might cross the blood-brain barrier more easily and influence central regulation of gastric ghrelin release. On the other hand, upregulation of ghrelin expression (11) and an increase of plasma ghrelin (32) have also been reported in animals after the administration of insulin. This was not confirmed in the healthy subjects under study, indicating that insulin is not responsible for the reduction in ghrelin concentrations after food intake, nor is it responsible for the observed increase after insulin administration.

Somatostatin suppressed the hyperglycemia-induced increase in endogenous insulin and reduced ghrelin concentrations significantly. This effect on ghrelin was apparent during euglycemia, and it was more pronounced during hyperglycemia. The placebo control experiment suggests
that this is due to a time-dependent effect rather than to hyperglycemia. The decrease in ghrelin concentrations may reflect a direct inhibitory action of somatostatin on ghrelin-releasing peripheral cells and may indicate a continuous constitutive release of ghrelin under normal conditions. It is well known that somatostatin inhibits hormone release from neuroendocrine cells (33) and that the gastrointestinal mucosa expresses somatostatin receptors (34). Thus, there could also be an interaction between somatostatin and the ghrelin-producing cells, but so far there is no direct evidence to support this hypothesis. Furthermore, the functional results obtained do not elucidate which cells are involved in the endocrine responses. The tissue distribution of ghrelin is widespread (35), and the physiological significance of this distribution is still unclear.

Apart from glucose or insulin, several other regulatory mechanisms appear to be involved in the regulation of plasma ghrelin after ingestion. The autonomic nervous system may play a role because vagotomy increases plasma ghrelin levels, and the vagal tone is low in fasting animals (32). Stimulation of gastric mechanoreceptors is probably of minor clinical importance, because ghrelin plasma concentrations were only reduced by ingestion of nutrients and not by the same volume of water in animals (8) and in humans (21). However, gastric bypass surgery abrogates the normal meal-related fluctuations and diurnal rhythm and decreases ghrelin concentrations (17). The potential roles of amino acids or lipids have been less investigated, but they may alter ghrelin release directly by luminal contact (36) or by increased plasma concentrations. This possibility needs to be addressed in future studies.

Hyperglycemia is known to inhibit GH secretion in normal subjects (37). This was also seen in our study, in which a small decrease in GH and IGF-1 was observed during hyperglycemia. This effect was more pronounced during insulin or somatostatin coinfusion, suggesting the involvement of different mechanisms. Because IGF-1 concentrations were also decreased at glucose concentrations at which no changes in GH could be detected, a direct action of glucose on IGF-1 production seems likely.

In conclusion, ghrelin plasma concentrations were significantly decreased by somatostatin. In contrast, no direct involvement of glucose or insulin at physiological concentrations in the regulation of ghrelin plasma concentrations in healthy humans was demonstrable.

ACKNOWLEDGMENTS

We are grateful for the assistance and administrative work of Carola Fuchs, RN.

REFERENCES


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**TABLE 1**

Human GH and IGF-1 plasma concentrations at baseline and during various venous glucose concentrations with coinfusion of placebo, insulin, or somatostatin

<table>
<thead>
<tr>
<th>Human GH (µg/l)</th>
<th>Placebo</th>
<th>Insulin</th>
<th>Somatostatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline glucose</td>
<td>0.17 ± 0.06</td>
<td>0.25 ± 0.19</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>5.0 mmol/l glucose</td>
<td>0.17 ± 0.05</td>
<td>0.36 ± 0.18</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>8.3 mmol/l glucose</td>
<td>0.09 ± 0.03</td>
<td>0.18 ± 0.12</td>
<td>0.02 ± 0.01*</td>
</tr>
<tr>
<td>11.1 mmol/l glucose</td>
<td>0.12 ± 0.05</td>
<td>0.03 ± 0.01*</td>
<td>0.02 ± 0.01*</td>
</tr>
</tbody>
</table>

TABLE 1

<table>
<thead>
<tr>
<th>Human GH (µg/l)</th>
<th>Placebo</th>
<th>Insulin</th>
<th>Somatostatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline glucose</td>
<td>37.3 ± 3.1</td>
<td>42.4 ± 6.0</td>
<td>37.2 ± 3.8</td>
</tr>
<tr>
<td>5.0 mmol/l glucose</td>
<td>36.5 ± 3.1</td>
<td>39.4 ± 6.0*</td>
<td>33.8 ± 3.7*</td>
</tr>
<tr>
<td>8.3 mmol/l glucose</td>
<td>35.6 ± 3.3</td>
<td>37.7 ± 6.3*</td>
<td>33.0 ± 3.8†</td>
</tr>
<tr>
<td>11.1 mmol/l glucose</td>
<td>34.6 ± 3.4*</td>
<td>36.5 ± 5.9†</td>
<td>31.4 ± 3.8†</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 9). *P < 0.05 vs. baseline; †P < 0.01 vs. baseline.