Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects

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The discovery of ghrelin as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R) was a long awaited scientific breakthrough (18) considering that synthetic ligands had been known for decades and that the growth hormone (GH) secretagogue receptor (GHS-R) was identified in 1996 (16). The GHS-R is present not only in the hypothalamus and the pituitary gland but also in several peripheral tissues (15, 28), suggesting pleiotropic effects of ghrelin.

Ghrelin is an acylated 28-amino acid peptide produced primarily in the gastric mucosa and the hypothalamic arcuate nucleus (18) but also in other tissues (15). As expected, administration of ghrelin is associated with pronounced stimulation of GH secretion. Additional endocrine effects of ghrelin include stimulation of adrenocorticotropic hormone (ACTH) and prolactin secretion (3, 33). There is accumulating evidence to support the hypothesis that ghrelin plays an important role in the regulation of food intake (8, 10, 26, 27, 32, 38), energy metabolism (2, 5, 36), and gastric motility (23), and, when administered to humans, ghrelin increases left ventricular function and improves vasodilation (12, 24, 25, 35). Moreover, in rodents, ghrelin administration induces adiposity (36), whereas absence of ghrelin or the GHS-R prevents the development of obesity (37, 40).

Circulating ghrelin levels increase before feeding followed by suppressed postprandial levels (8). In patients with anorexia nervosa, a compensatory increase in ghrelin levels are reported (34), but hyperphagia secondary to Prader-Willi syndrome seems to be driven by increased ghrelin secretion (7).

In the literature, ghrelin has so far been administered to ~300 healthy subjects and patients. Ghrelin bolus doses between 1 and 10 μg/kg body wt, administered subcutaneously (12), and ≤10 μg/kg body wt, administered intravenously (24), have resulted in systemic ghrelin increments from 2- to 61-fold. When ghrelin is infused at a rate of 5 pmol·kg body wt$^{-1}$·min$^{-1}$ (corresponding to 16.9 ng·kg body wt$^{-1}$·min$^{-1}$), ghrelin levels are usually reported to reach steady state after 60–90 min (9, 23, 27, 38).

To our knowledge only two articles have reported pharmacokinetic data (1, 24), and both describe first-order elimination in a one-compartment model with a half-life between 9 and 31 min.

Ghrelin levels are low in obesity (11, 19, 22). It remains to be studied whether this decrease is caused by increased ghrelin degradation or decreased secretion and if any biochemical variables predict ghrelin turnover. In this regard it is noteworthy that acylated ghrelin binds to high-density lipoproteins (HDL) in vitro (4).

In view of the potential therapeutic applications of ghrelin it is relevant to evaluate in more detail the pharmacokinetics and pharmacodynamics of exogenous ghrelin. In the present double-blind crossover study, 17 healthy young men received a 3-h constant intravenous infusion of ghrelin and placebo. This enabled calculation of mean steady-state levels, exponential coefficients, half-lives, rate of elimination, mean residence time (MRT), and distribution volume ($V_{SS}$) of ghrelin. The pharmacodynamic measurements included the secretion of GH, prolactin, ACTH, insulin levels, and glucose homeostasis, as well as satiety and hunger.

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MATERIALS AND METHODS

Study subjects. Seventeen healthy men aged 23.1 ± 0.4 yr with body mass indexes (BMIs) of 23.0 ± 0.3 kg/m² volunteered in this study. None of the study participants was smoking or abusing alcohol or taking any medication. All had a normal physical examination. Fasting blood glucose, triglyceride, and cholesterol levels and hematological, renal, and hepatic functions assessed by biochemical screening were normal in all participants. Baseline characteristics are provided in Table 1.

The study was conducted in accordance with the Helsinki Declaration, and all subjects gave their oral and written informed consent to participate in the trial. The study protocol was approved by the local Ethics Committee of Aarhus County, the Danish Medicines Agency, and the Good Clinical Practice (GCP) Unit of Aarhus University Hospital. According to the International Committee of Medical Journal Editors, the protocol was registered (Clinicaltrials.gov; ID NCT00116025) before the onset of enrolment.

Preparation of synthetic ghrelin. Synthetic human acylated ghrelin (NeoMPS, Strasbourg, France) was dissolved in isotonic saline and sterilized by double passage through a 0.8/0.2 μm pore size filter (Super Acrodics; Gelman Sciences, Ann Arbor, MI) by the local hospital pharmacy.

Protocol. Subjects were studied at 0800 in a quiet, thermoneutral indoor environment following a minimum of 9 h of fasting. The subjects fasted during the trial. Two intravenous cannulae were inserted in the antecubital region, one for infusion and one for blood sampling. All subjects were examined on two occasions (ghrelin or placebo) in random order, separated by a minimum of 3 wk. In a double commercial monoclonal immunofluorometric assay (DELFA; PerkinElmer, Wallac, Turku, Finland) was used to measure serum growth hormone (s-GH), cortisol (s-cortisol), and insulin (s-insulin). Plasma glucose (p-glucose) levels were measured in duplicate on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Levels of serum free fatty acids (s-FFA) were determined using a commercial kit (Wako Chemicals, Neuss, Germany). Plasma ACTH (DELFIA; PerkinElmer, Wallac, Turku, Finland) was used to measure serum ACTH levels (s-ACTH).

Pharmacokinetic parameters. The postinfusion serum ghrelin concentration time data from each subject were analyzed by nonlinear least-squares regression analysis using a monoexponential and a biexponential decay model. Pharmacokinetic analyses were done by means of the computer program GraphPad Prism v.4.00 for Windows (GraphPad Software, San Diego, CA). The preferred model was chosen on the basis of visual inspection of the concentration time data and the extra sum-of-squares F-test. To correct the s-ghrelin values for naturally occurring ghrelin, the mean of the s-ghrelin measurements during placebo treatment were subtracted from each serum value.

A biexponential equation

\[ C = A \cdot e^{-\lambda_1 \cdot T} + B \cdot e^{-\lambda_2 \cdot T}, \]

representing a two-compartment open model with first-order elimination gave the best fit.

As ghrelin infusion was given over a 180-min period, the intercepts A' and B' were corrected according to the following equations:

\[ A' = \frac{A \cdot \lambda_1}{1 - e^{-\lambda_1 \cdot T}}, \]

and

\[ B' = \frac{B \cdot \lambda_2}{1 - e^{-\lambda_2 \cdot T}}, \]

where \( T \) is the infusion period (20).

The pharmacokinetic variables were then calculated using the corrected intercepts A and B and slopes \( \lambda_1 \) and \( \lambda_2 \).

The distribution half-life \( t_{1/2}(\lambda_1) \) and the elimination (terminal) half-life \( t_{1/2}(\lambda_2) \) were calculated as

\[ t_{1/2}(\lambda_1) = \frac{\ln(2)}{\lambda_1}, \]

and

\[ t_{1/2}(\lambda_2) = \frac{\ln(2)}{\lambda_2}, \]

respectively. The area under the serum concentration vs. time curve, AUC, was calculated as


Table 1. Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 17)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.1±0.4</td>
<td>21–26</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.84±0.01</td>
<td>1.70–1.96</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.6±1.5</td>
<td>68.8–88.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.0±0.3</td>
<td>20.7–25.6</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>4.9±0.1</td>
<td>4.2–6.0</td>
</tr>
<tr>
<td>Fasting serum ghrelin, μg/l</td>
<td>0.74±0.06</td>
<td>0.41–1.23</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>3.98±0.16</td>
<td>2.90–5.70</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.58±0.09</td>
<td>1.1–2.7</td>
</tr>
<tr>
<td>Plasma albumin, μmol/l</td>
<td>676.0±5.6</td>
<td>624–713</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index.

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AUC_\text{c} = \frac{A}{\lambda_1} + \frac{B}{\lambda_2}

The area under the first moment curve (AUMC) was calculated as:

AUMC = \frac{A}{\lambda_1} + \frac{B}{\lambda_2}

Total clearance (Cl), the volume of distribution of the central compartment (V_c), the volume of distribution during the terminal phase [V(\lambda_2)], and the volume of distribution at steady state (V_{ss}) were calculated as follows:

Cl = \frac{\text{dose}}{\text{AUC}_c}

V_c = \frac{\text{dose}}{A + B^2}

V(\lambda_2) = \frac{\text{dose}}{\text{AUC}_c \cdot \lambda_2}

V_{ss} = \frac{\text{dose} \cdot \text{AUMC}}{\text{AUC}_c^2}

MRT was calculated as

MRT = \frac{\text{AUMC}}{\text{AUC}_c}

Micro constants k_{12}, k_{21}, and k_{01} were calculated from

k_{12} = \frac{A \cdot \lambda_2 + B \cdot \lambda_1}{A + B}

k_{01} = \frac{\lambda_1 \cdot \lambda_2}{k_{12}}

and

k_{21} = \lambda_1 + \lambda_2 - k_{12} - k_{01}.

Simulation of the serum concentration during the infusion was done according to the following equation (31):

C = f_1 \cdot \frac{R_0 \cdot (1 - e^{-\lambda_1 t})}{Cl} + f_2 \cdot \frac{R_0 \cdot (1 - e^{-\lambda_2 t})}{Cl}.

where R_0 is the rate of infusion and f_1 and f_2 denote the fraction of elimination associated with the first and last exponential term, respectively:

f_1 = \frac{A/\lambda_1}{\text{AUC}_c}

and

f_2 = \frac{B/\lambda_2}{\text{AUC}_c}.

Serum concentration at steady state (C_{SS}) was calculated as

C_{SS} = \frac{R_0}{Cl}.

Statistical analyses. Results are expressed as means ± SE. Systemic levels of FFA, glucose, insulin, GH, prolactin, ACTH, and cortisol were analyzed by two-way analysis of variance (ANOVA). The interaction between time and treatment (“time × treatment”) was considered the term of interest. The Bonferroni correction was used to account for multiple comparisons. Data were examined by Student’s two-tailed paired t-test when appropriate. Appetite scores were analyzed by comparing the changes in VAS by a paired t-test. In backward regressions analyses, BMI and total and HDL cholesterol levels were included as independent variables, and AUC, half-lives, k_{01}, or Cl were included as dependent variables, respectively. A P value <0.05 was considered significant. Statistical analysis was performed using SPSS version 13.0 for Windows.

RESULTS

Ghrelin pharmacokinetics. The mean total ghrelin serum concentrations (s-ghrelin) vs. time profile are shown in Fig. 1. According to comparison of two nested nonlinear models (1- and 2-phase exponential decay) by the extra sum-of-squares F-test, the pharmacokinetics of infused ghrelin was best fitted to the latter two-compartment model (F = 256.9, P < 0.0001).

Summary pharmacokinetic parameters are presented in Table 2. Linear regression analysis revealed a significant increase in s-ghrelin levels after 100 min of ghrelin infusion (r = 0.94, P = 0.02). During this period we attained s-ghrelin C_{max} (4.41 ± 0.29 μg/l) above baseline levels. The observed s-ghrelin C_{max} level was not different from the level predicted by the model (expected C_{180} = 4.35 ± 0.28 μg/l, P = 0.45), but we did not reach a true steady-state level because the measured C_{max} was significantly smaller than the predicted C_{SS} (5.00 ± 0.35 μg/l, P = 0.003). The initial disposition rate constant, λ_1, was 0.033 ± 0.003/min, and the terminal rate constant, λ_2, was 0.007 ± 0.003/min. After termination of the ghrelin infusion, s-ghrelin was eliminated with Cl = 3.68 ± 0.30 ml·kg^{-1}·min^{-1}. The initial and terminal s-ghrelin half-lives, t_{1/2}(λ_1) and t_{1/2}(λ_2), were 24.2 ± 2.5 and 146.0 ± 35.6 min, respectively. The fraction of ghrelin eliminated from the terminal phase (f_2) was 33 ± 5%. MRT was 92.7 ± 16.3 min and V_{SS} was 0.35 ± 0.06 l/kg.

Hormones and metabolites. Circulating levels of FFA, GH, glucose, prolactin, insulin, ACTH, and cortisol are depicted in Fig. 2. FFA increased after 40 min of ghrelin infusion (0.57 ± 0.06 vs. 0.37 ± 0.06 mmol/l, P = 0.04) to levels almost twice by 10.220.33.5 on June 28, 2017 http://ajpendo.physiology.org/ Downloaded from as high as those after placebo at t = 200 min (0.97 ± 0.08 vs. 0.56 ± 0.07 mmol/l, P < 0.001) and were normalized by termination of the postinfusion period (t = 360 min). As

![Fig. 1. Levels of total serum ghrelin above baseline vs. time. Data are means ± SE. Ghrelin was infused from t = 0 to 180 min. The curve from 180 to 360 min is the best-fit curve from nonlinear regression analysis, resulting in a 2-compartment model. The curve from 0 to 180 min is the infusion curve derived from the best-fit postinfusion pharmacokinetic parameters.](http://ajpendo.physiology.org/)](http://ajpendo.physiology.org/)}
expected, serum GH increased rapidly in response to ghrelin infusion and reached the peak levels after 60 min of infusion (36.8 ± 4.7 vs. 1.5 ± 0.6 ng/ml, P < 0.001). Eighty minutes after termination of ghrelin infusion and throughout the study we observed a rebound decrease of GH levels. Plasma glucose increased −0.3 ± 0.1 mmol/l after 120 min of ghrelin infusion (P < 0.01) and returned to baseline levels shortly after termination of ghrelin infusion. Serum prolactin increased after 60 min (9.8 ± 0.6 vs. 4.3 ± 0.2 μg/l, P < 0.001) and remained elevated throughout the infusion period. Serum ACTH more than doubled after 60 min of ghrelin infusion compared with placebo (25.8 ± 3.5 vs. 11.5 ± 1.4 ng/ml, P = 0.002), followed by a decline toward baseline after 180 min. Serum cortisol increased after 60 min of ghrelin infusion (336.3 ± 28.9 vs. 212.9 ± 20.6 nmol/l, P < 0.01), and the elevation persisted throughout the infusion period. Cortisol levels returned to baseline 80 min after termination of the ghrelin infusion, whereafter a further decrease was recorded at t = 360 min (150.1 ± 20.3 vs. 198.6 ± 15.0 nmol/l, P = 0.02). Serum insulin levels remained constant during ghrelin infusion despite elevation in p-glucose levels. At t = 180 min, insulin levels increased (24.1 ± 2.4 vs. 17.3 ± 2.1 pmol/l, P = 0.004), and the elevation was sustained throughout the postinfusion period. Overall, ANOVA for repeated measurements uncovered a highly significant increase in insulin levels.

**Insulin sensitivity.** Insulin sensitivity as determined by the revised QUICKI method (30) decreased after 40 min of ghrelin infusion and outlasted both the infusion period and the postinfusion interval [P (interaction of ghrelin) < 0.001; Fig. 2G].

**Correlations.** MRT correlated positively with both BMI (r = 0.51, P < 0.001; Fig. 3A) and HDL cholesterol (r = 0.75, P < 0.001; Fig. 3B). The correlation between MRT and BMI was to a large extent carried by the two observations with the highest MRTs. If those observations were removed, the relationship was still significant (r = 0.53, P = 0.04). However, backward regressions analyses revealed no correlation between total cholesterol levels, HDL cholesterol levels, and BMI as independent variables and AUC, half-lives, k01, or Cl as dependent variables, respectively.

**Appetite.** The VAS scores at baseline were comparable between the two study days. The VAS scores increased during both placebo and ghrelin infusion and only reached significantly higher levels in the response to ghrelin with regard to “lack of satiety” (P = 0.03; Fig. 4).

**DISCUSSION**

The present study demonstrates that the distribution kinetics of constantly infused ghrelin follows a two-compartment model with a biexponential decay curve after termination of the infusion. Of note, we recorded significant positive correlations between the mean residence time of total ghrelin levels and both BMI and HDL.

Endogenous ghrelin exhibits a distinct ultradian rhythm; in fact, frequent blood sampling has revealed up to 22 pulses/24 h (39). The rapid and dynamic ghrelin fluctuations suggest that the half-life of ghrelin is short. Indeed, Nagaya et al. (24) reported that total ghrelin levels disappeared from plasma with a half-life of 10 min after a bolus injection. The pharmacokinetics of ghrelin were further analyzed by Akamizu et al. (1), who reported half-lives of both total (t1/2 27 to 31 min) and acylated plasma ghrelin (t1/2 9 to 13 min) in a one-compartment model following two different doses of ghrelin bolus injections and blood samples taken every 15 min. These previously reported half-lives of total ghrelin are in line with the initial half-life (t1/2 24.2 min) in our present study.

We compared a mono- and a biexponential description of the decay curve of total ghrelin levels after termination of a 180-min infusion period. By statistical means we found a significantly better description using a biexponential equation. This contrasts former pharmacokinetic ghrelin studies favoring the monoeponential model (1, 24). One explanation for this apparent discrepancy can be attributed to the frequency of blood sampling, since the concentration-time curve appears monophasic when the fraction of a substance eliminated by the last exponential term (f2) is relatively large (in this study 33%) and blood is drawn less frequently. To circumvent this problem, we collected blood samples every 5 min for the first hour after termination of the ghrelin infusion to detect the two distinct slopes of the decay curve.

Based on the estimated pharmacokinetic parameters from the biexponential decay curve, we simulated the expected concentration-time curve during the infusion period. The concentration-time curve even tended to overestimate the experimentally obtained ghrelin levels, indicating that simplifying distribution kinetics of ghrelin to a one-compartment model is inadequate. However, by means of the estimated pharmacokinetic parameters and the two-compartment model, the currently presented mathematical model adequately explains our experimental observations. Peak ghrelin concentration observed was very similar to the mathematically predicted level, but a steady-state level was not reached. Our observations contrast previous ghrelin infusion studies reporting steady state within 60–90 min (9, 23, 27, 38). However, none of the previous reports provides the pharmacokinetic approach, making it difficult to draw direct comparisons with our results.

**Table 2. Pharmacokinetic parameters of infused ghrelin**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>s-Ghrelin</th>
</tr>
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<tbody>
<tr>
<td>A0, μg/l</td>
<td>3.32 ± 0.32</td>
</tr>
<tr>
<td>A, μg/l</td>
<td>18.38 ± 1.73</td>
</tr>
<tr>
<td>λ1, min⁻¹</td>
<td>0.033 ± 0.003</td>
</tr>
<tr>
<td>B0, μg/l</td>
<td>1.35 ± 0.14</td>
</tr>
<tr>
<td>B, μg/l</td>
<td>2.42 ± 0.30</td>
</tr>
<tr>
<td>λ2, min⁻¹</td>
<td>0.007 ± 0.003</td>
</tr>
<tr>
<td>AUC (trap) 0–360, μg·min⁻¹</td>
<td>804.6 ± 50.6</td>
</tr>
<tr>
<td>AUC, μg·min⁻¹</td>
<td>900.1 ± 61.9</td>
</tr>
<tr>
<td>Cl, ml·kg⁻¹·min⁻¹</td>
<td>3.68 ± 0.30</td>
</tr>
<tr>
<td>Cmax, μg/l</td>
<td>5.00 ± 0.35</td>
</tr>
<tr>
<td>Measured Cmax, μg/l</td>
<td>4.41 ± 0.29</td>
</tr>
<tr>
<td>Expected Cl180, μg/l</td>
<td>4.35 ± 0.28</td>
</tr>
<tr>
<td>MRT, min</td>
<td>92.7 ± 16.3</td>
</tr>
<tr>
<td>k01, min⁻¹</td>
<td>0.023 ± 0.001</td>
</tr>
<tr>
<td>k12, min⁻¹</td>
<td>0.011 ± 0.001</td>
</tr>
<tr>
<td>k21, min⁻¹</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td>Vss, l/kg</td>
<td>0.35 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. AUC, area under the serum concentration vs. time curve; Cl, total clearance; Cmax, concentration at steady state; Cmax, maximal concentration; MRT, mean residence time.
We measured total ghrelin levels only. Whether acylated ghrelin degrades to desacylated ghrelin remains to be convincingly demonstrated. If so, desacylated ghrelin levels should increase when the fatty acid side chain of acylated ghrelin is cleaved off during breakdown. With respect to this, divergent observations exist; one in vitro study revealed no increase in desacylated ghrelin levels along the degradation of acylated ghrelin (17), whereas the study by Akamizu et al. (1) indicated that acylated ghrelin accounted for only ~50% of the increase in total ghrelin levels after administration of acylated ghrelin. Gauna et al. (14) detected higher total serum ghrelin levels after administration of acylated ghrelin than after an equal dose of desacylated ghrelin. They suggested that the apparent increase in desacylated ghrelin following administration of acylated ghrelin was not solely explained by degradation of acylated to desacylated ghrelin.

Fig. 2. The hormonal and metabolite responses to ghrelin and placebo infusion. All measured variables revealed a significant interaction of time and treatment [P (time × treatment interaction) < 0.001; A–H]. Asterisks in A–H refer to post hoc comparison between treatment groups (ghrelin vs. placebo) at different time points. A: serum levels of free fatty acids (FFA); *P < 0.05. B: serum growth hormone (GH); *P < 0.05. C: plasma glucose; *P < 0.05. D: serum prolactin; *P < 0.01. E: serum insulin; *P < 0.01. F: serum ACTH; *comparison at t = 60 min, P = 0.002. G: insulin sensitivity as determined as revised QUICKI; *P < 0.05. H: serum cortisol; *P < 0.05.
but rather stemmed from endogenous release of desacylated ghrelin.

In the present study, we revealed a positive correlation between BMI and the MRT of ghrelin. Thus, the counterregulatory decline in ghrelin levels that presumably serves to compensate for a positive energy expenditure in obese individuals (11, 19, 22) is probably caused by a decrease in ghrelin secretion rather than an increase in ghrelin degradation. This is also in accord with the observation that a constant ghrelin infusion increases circulating ghrelin levels more in obese than in lean subjects (10), and it is compatible with previous studies (11, 19, 22) reporting that obese individuals have a reduced postprandial decrease in ghrelin levels compared with lean subjects.

We also revealed a positive correlation between MRT and HDL cholesterol levels. Although correlation does not imply causality, it has previously been shown that the majority of circulating acylated ghrelin is bound to larger molecules (29), and the HDL fraction has been demonstrated to bind acylated ghrelin in vitro (4). HDL cholesterol levels could thus be an independent biological determinant of ghrelin bioavailability in humans.

After 180 min of sustained supraphysiological ghrelin levels we detected a significant decrease in the sensation of “satiety” but no effects on either hunger, the desire to consume a meal, or an individual expected amount of food to be consumed if an ad libitum meal was served. This contrasts the more pronounced orexigenic effects of ghrelin reported previously (10, 27, 32, 38). Temporal and concentration differences between the earlier reports and the present results may help to explain these discrepancies. Wren et al. (38) studied the orexigenic and appetite effects of more physiological increments (~2-fold elevations from baseline), significantly less than the 6.5-fold increase we obtained. High ghrelin levels may entail compensatory mechanisms, such as internalization of the GHS-R from the cell surface, to desensitize the cell responsiveness. The internalization is maximal after ~20 min and the receptor level rises to basal levels after 360 min following removal of its agonist (6). The acute orexigenic effects of ghrelin administration were demonstrated by Druce et al. (10) and Schmid et al. (32). They observed significant orexigenic effects after 45 and 60 min following initiation of ghrelin administration, respectively. Moreover, 90 min of ghrelin infusion caused increased energy intake and meal appreciation in cancer patients suffering cachexia (27).

As expected, we observed significant elevations in circulating levels of GH, ACTH, cortisol, prolactin, glucose, and FFA, in accord with most previous reports (1, 3, 9, 10, 12, 14, 18, 21, 24, 25, 27, 32, 33, 38). We also recorded a small but significant increase in plasma glucose levels, but in contrast to one previous study (5) this was associated with an increase rather than a decrease in insulin levels. This translated into a decrease in insulin sensitivity following ghrelin infusion, as estimated by the revised QUICKI model (30). The decrease was evident after only 40 min and lasted throughout the study period. This effect may partly be attributable to the increase in GH levels. Interestingly, the reduced insulin sensitivity remained after
normalization of both GH and glucose levels, suggesting that the effect could be caused by the ghrelin infusion per se. The present results are in accord with earlier observations (14) reporting ghrelin-induced insulin resistance. In that study, concomitant GH administration surprisingly ameliorated the impairment of metabolic control induced by ghrelin. This apparent discrepancy may be attributable to the differences in ghrelin doses and thus differences in GH responses and intrinsic effects of ghrelin. At first glance, the increase in cortisol levels contrasts the previous established inverse correlation between ghrelin and cortisol observed during fasting (13). However, the ACTH concentration-time curve reveals a transient increase in ACTH levels after 60 min of ghrelin infusion only, whereas ACTH levels return to baseline levels. The present ghrelin infusion period is not sufficient to disclose any effect of ghrelin on cortisol levels after normalization of ACTH levels, but the decrease in cortisol levels at t = 360 min indicates that the long-term effect of ghrelin may actually be to inhibit cortisol levels.

An important limitation applies to the present results. We measured total ghrelin levels only, and as such we report the resulting levels of both acylated and desacylated ghrelin probably along with other molecules possessing ghrelin-like immunoreactivity.

In conclusion, the kinetics of infused ghrelin follow a two-compartment model, and the MRT of infused ghrelin correlates positively with both HDL cholesterol and BMI. Supraphysiological ghrelin levels decrease insulin sensitivity. It remains to be convincingly demonstrated whether the reduced insulin sensitivity is caused by ghrelin per se or by the concomitant increase in GH levels. The present data are useful when tailoring ghrelin infusion rates in clinical experiments.

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