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Bacterial Endotoxin Induces Biphasic Changes in Plasma Ghrelin in Healthy Humans

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Context: Ghrelin is a gut hormone with a highly preserved biological activity, which seems not to be restricted to the regulation of food intake, body composition, and growth. Continuous research is unraveling new properties of ghrelin, among others cardiovascular and antiinflammatory activities. Ghrelin is recently implicated in the host response to bacterial endotoxin in rodents and suggested as a possible therapeutic tool in sepsis.

Objective: This study aimed to investigate plasma ghrelin levels during human bacterial endotoxemia.

Design and Setting: We conducted a randomized, placebo-controlled, crossover clinical trial at a university medical center.

Study Participants: Participants included 10 healthy men.

Intervention: After an overnight fast, study subjects were randomized to 2 ng/kg *Escherichia coli* endotoxin [lipopolysaccharide (LPS)] or placebo and monitored for 6 h.

Main Outcome Measures: We measured ghrelin, GH, ACTH, cortisol, glucose, free fatty acids, TNF-α, IL-6, and IL-1 receptor antagonist.

Results: LPS administration induced a rapid ghrelin surge at 120 min (Δ ghrelin 100.2 $\pm$ 30.3 *vs.* 7.2 $\pm$ 26.4 pg/ml on the placebo day, $P = 0.042$). This ghrelin peak occurred 30 min after the TNF-α peak and corresponded with IL-6, GH, and ACTH peaks. Starting from 120 min and thereafter, ghrelin continuously decreased, reaching a nadir at 5 h after LPS administration (Δ ghrelin, -43.8 ± 28.4 compared with 70.3 $\pm$ 38.2 pg/ml on the control days, $P = 0.038$).

Conclusions: Ghrelin is one of the first hormones rapidly increasing in the human physiological response to bacterial endotoxic shock. Plasma ghrelin might be part of the complex immuno-neuroendocrine mechanisms activated by systemic infection and inflammation in humans. **(*J Clin Endocrinol Metab* 92: 3930–3934, 2007)**

SYSTEMIC INFECTION ACTIVATES the immune and neuroendocrine systems, which closely interact to coordinate the response to this homeostatic challenge (1, 2). The identification of possible drug targets needs a better understanding of the complex underlying pathophysiological mechanisms (3). The most widely used model for studying the innate immunity in humans is the administration of bacterial lipopolysaccharide (LPS), part of the Gram-negative bacterial cell wall (4). Similarly to septicemia, LPS increases proinflammatory cytokines and activates the hypothalamic-pituitary-adrenal (HPA) axis. It also induces anorexia, insulin resistance, and changes in plasma concentrations of leptin and other adipokines in humans (5–7). Studies performed in rodents have implicated in the host response to endotoxic shock another appetite-modulating hormone, ghrelin (8, 9).

Ghrelin is produced primarily in the endocrine cells of the oxyntic mucosa of the gastric fundus and was originally described as a potent endogenous GH-stimulating factor (10, 11). It induces short-term food intake and long-term adiposity by acting at the hypothalamus (12, 13). Ghrelin is highly conserved throughout evolution and is responsible for many central and peripheral endocrine and nonendocrine effects (14, 15). Several studies suggest a novel role of ghrelin, namely its antiinflammatory properties (16, 17). Ghrelin administration attenuates the response to endotoxin in rodents and has been suggested as a possible therapeutic tool in sepsis (8, 18, 19).

There are no data on the profile of circulating ghrelin levels in sepsis or similar models in humans. At the same time, it is known that nutrients modulate cytokine production and potency, and sepsis is accompanied by anorexia (20). Based on this, as well as on data from rodent studies, we hypothesized that plasma ghrelin might change in response to bacterial endotoxin in healthy human subjects.

Subjects and Methods

Subjects

The protocol was approved by the Institutional Review Board of the Medical University of Vienna, and informed consent was obtained from all subjects. Ten healthy human males (aged between 21 and 39 yr old) were included in the study. Participants had no concomitant disease and no febrile illness in the last month and were not smoking, abusing alcohol, or taking any medication (including nonprescription drugs). Before enrollment, they underwent a thorough physical examination and biochemical screening (full blood count; plasma glucose, electrolytes, cholesterol, and triglycerides; and renal, hepatic, and thyroid function). All tests were normal.

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Abbreviations: FFA, Free fatty acids; HOMA, homeostasis assessment model; HPA, hypothalamic-pituitary-adrenal; IL-1RA, IL-1 receptor antagonist; LPS, lipopolysaccharide.

Study protocol

The study was designed as a randomized, placebo-controlled, crossover trial. Subjects were examined on two occasions separated by a minimum of 3 wk and randomly assigned to receive LPS or placebo. They were studied at 0800 h after an overnight fast and no alcohol consumption in the preceding 12 h and fasted throughout the study. Two indwelling catheters were placed in the antecubital veins of the right and left forearm for infusions and blood sampling, respectively. An infusion of isotonic saline was started at time point −30 min, given at 500 ml/h for the first 30 min, 200 ml/h during 0–90 min, and then maintained at 100 ml/h during the remaining study period (90–360 min). At time point 0, subjects received iv either a bolus of placebo or LPS (20 IU/kg body weight, corresponding to 2 ng/kg; National Reference *Escherichia coli* endotoxin; U.S.P. Convention Inc., Rockville, MD). The subjects were studied during the 6 h after endotoxin administration. Heart rate and electrocardiogram were monitored online (Lohmeier M607; Siemens, Munich, Germany), and blood pressure was measured every 30 min. Body temperature was determined orally. All subjects tolerated LPS well. Approximately 8 h after LPS administration, they were discharged in good health.

Blood sampling and assays

Blood samples were obtained at time points 0, 60, 90, 120, 180, 240, 300, and 360 min in tubes containing EDTA, immediately cooled on ice, and then centrifuged for 10 min at 3000 rpm at 4 C.

All samples taken on both study days from an individual subject were analyzed in one assay and in duplicates. Plasma ghrelin was measured via a commercial RIA using ^{125}I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody against the C-terminal end of human ghrelin that does not differentiate between acylated and nonacylated ghrelin (Peninsula Laboratories, San Carlos, CA). The inter- and intraassay variations were 5 and 8%, respectively.

Plasma glucose was measured immediately on site by the glucose oxidase method (Beckman Instruments, Inc., Fullerton, CA). Insulin and C-peptide were determined using commercially available RIAs (Linco, St. Charles, MO). Plasma free fatty acid (FFA) concentrations were determined using a microfluorimetric method (Wako Chemicals Inc., Richmond, VA). GH was measured using an immunoradiometric assay (DiaSorin, Saluggia, Italy) and ACTH using a RIA (Peninsula).

TNF-α, IL-6, and IL-1 receptor antagonist (IL-1RA) were measured simultaneously from the same plasma sample using the Fluorokine MultiAnalyte Profiling Base Kit A (R&D Systems, Minneapolis, MN).

Statistics

Homeostasis assessment model (HOMA) index was calculated using HOMA calculator 2.2 (www.dtu.ox.ac.uk). Results are presented as absolute or Δ mean values ± SEM. Repeated-measures ANOVA was used to test the differences in response to LPS compared with placebo. When this test was positive, *post hoc* comparison was performed by means of a paired *t* test. $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the study subjects are presented in Table 1. On the placebo study days, there were no significant changes in T, heart rate, BP, cytokines, and HPA axis (Figs. 1, A and B; 2, A–C; and 3, C and D). As already shown in other studies, plasma ghrelin continuously increased by fasting (Fig. 3A) (21).

As expected, LPS administration produced a transient febrile illness. Pyrexia and the increase in heart rate became significant at time point 135 min ($P < 0.001$) and remained so until the end of the study (Fig. 1, A and B). TNF-α peaked at 90 min (210.7 ± 70 pg/ml *vs.* undetectable levels on the placebo days, $P = 0.008$) and remained significantly elevated at 2 h ($P = 0.001$) and at 3 h ($P < 0.001$) (Fig. 2A). IL-6 also increased significantly (194.3 ± 67.8 pg/ml, $P = 0.001$), as did IL-1RA, which reached maximal detectable levels at 2 h and remained so during the remaining study period ($P < 0.001$) (Fig. 2, B and C).

TABLE 1. Basal characteristics of the study subjects

	Mean ± SEM
Age (yr)	25.5 ± 5.8
Weight (kg)	75.6 ± 9.7
BMI (kg/m^2)	23.1 ± 1.7
Fasting glucose (mg/dl)	80.9 ± 5.8
HOMA-IR index	1.39 ± 0.36
Total cholesterol (mg/dl)	178.3 ± 38.6
HDL cholesterol (mg/dl)	60.1 ± 8.6
Triglycerides (mg/dl)	103.7 ± 33.9
TSH (μU/ml)	1.6 ± 0.6
CRP (mg/dl)	0.06 ± 0.03

Data are presented as mean ± SEM. Normal ranges are as follows: TSH, 0.44–3.77 μU/ml; CRP, <0.1 mg/dl. HOMA-IR, HOMA of insulin resistance.

Plasma ghrelin increased rapidly from 606.8 ± 183.9 to 707 ± 211.8 pg/ml within 2 h after LPS administration. The respective ghrelin values on the placebo days were 683.8 ± 197.68 pg/ml at time point 0 and 691 ± 170.3 pg/ml at 2 h. Because of the high spread of basal (time point 0) plasma ghrelin levels in our study group (ranging from 365-1050 pg/ml), we decided to consider the relative changes in plasma ghrelin for additional calculations; Δ ghrelin was 100.2 ± 30.3 pg/ml 2 h after endotoxin and only 7.2 ± 26.5 pg/ml 2 h after placebo ($P = 0.042$).

Afterward, plasma ghrelin continuously declined, reaching significantly decreased levels 5 and 6 h after LPS administration (at 5 h, Δ ghrelin −43.8 ± 28.4 pg/ml compared with 70.3 ± 38.2 pg/ml on the control days; at 6 h, Δ ghrelin −14.2 ± 22.2 pg/ml compared with 79 ± 38.1 pg/ml on the

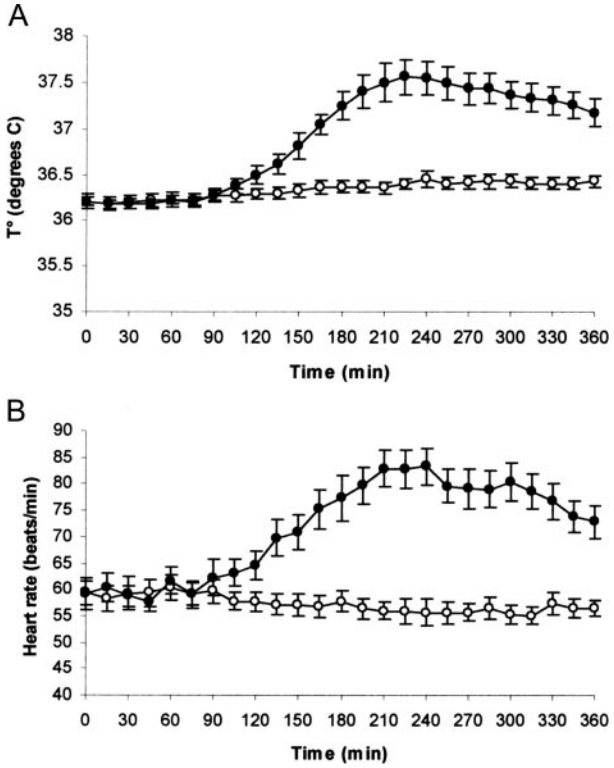

FIG. 1. Clinical response to endotoxin. LPS (given at time point 0) increased body temperature (A) and heart rate (B). Data are presented as mean ± SEM (●, LPS; ○, placebo).

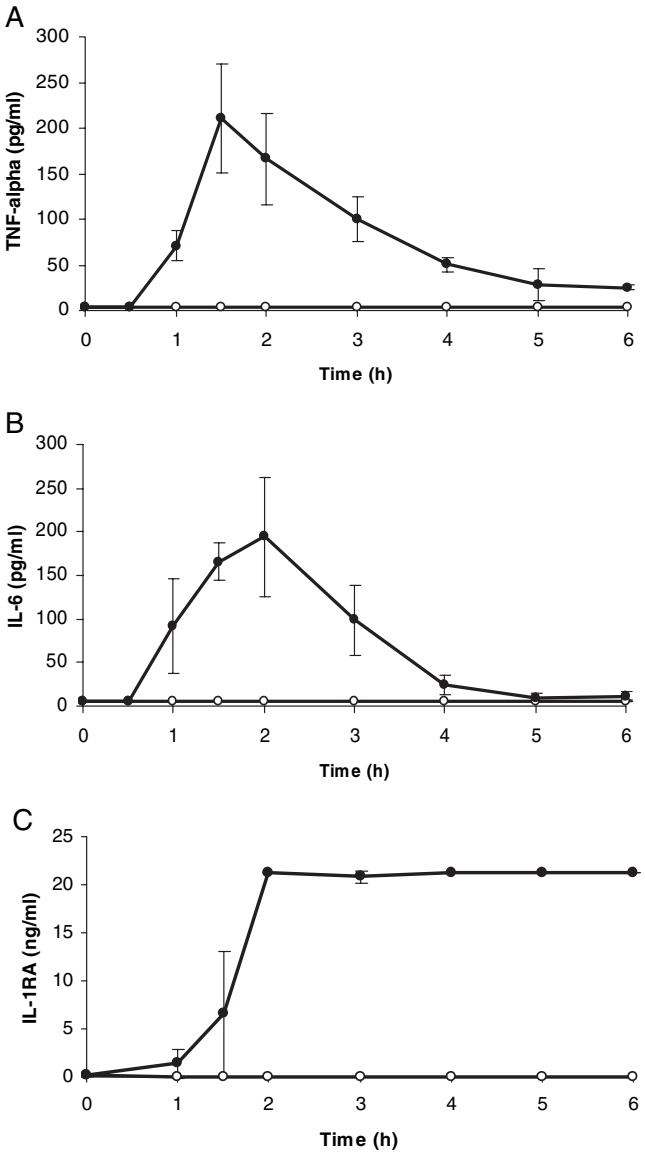

FIG. 2. Cytokine responses to endotoxin. After LPS infusion (time point 0), TNF-α increased starting from the 60th minute and reaching a peak at the 90th minute (A), IL-6 peaked at 2 h (B), and IL-1RA reached a high plateau at 2 h (C). Data are presented as mean ± SEM (●, LPS; ○, placebo).

placebo days; both $P < 0.05$) (Fig. 3A). The absolute ghrelin values 6 h after LPS were 560 ± 133.3 pg/ml on the LPS days *vs.* 762.8 ± 219.7 on the placebo days ($P = 0.037$).

Plasma GH surged at 2 h (18.98 ± 5.9 ng/ml compared with 1.4 ± 0.61 ng/ml on the placebo days, $P = 0.03$) (Fig. 3B). ACTH rose significantly at 2 h (71.5 ± 22.1 pg/ml *vs.* 18.95 ± 4.8 pg/ml in the placebo day, $P = 0.024$), remained significantly elevated at 3 h ($P = 0.017$), and decreased thereafter (Fig. 3C). Plasma cortisol increased slowly from the 60th minute, reached significantly elevated levels at the second hour ($P = 0.002$), and remained so until the fifth hour ($P = 0.017$). Maximal cortisol values were 19.07 ± 4.7 μg/dl (Fig. 3D).

LPS induced no significant changes in plasma glucose (Fig. 3E), insulin, and C-peptide levels (results not shown) but continuously increased FFA (Fig. 3F). The FFA increase became significant at 3 h after endotoxin and remained so until the end of the observation period ($P < 0.001$). The maximal FFA levels were observed at 5 h (Δ FFA = 681 ± 132 μmol/liter *vs.* 89.2 ± 114 μmol/liter on the placebo days, $P < 0.001$).

Discussion

The data presented in this study show that endotoxin-induced inflammation leads to biphasic changes in plasma ghrelin levels in humans. Ghrelin rose abruptly, reaching its maximum 2 h after LPS administration, being one of the first hormones responding to LPS. Surprisingly, ghrelin levels in the plasma decreased continuously thereafter until reaching their lowest values 5 h after LPS (Fig. 3A). The expected cytokine shock started with TNF-α, reaching its peak at 90 min (Fig. 2A). IL-6 peaked at 120 min (Fig. 2B), whereas IL-1RA reached its high plateau at 180 min (Fig. 2C). The impact of LPS on vital signs (thought to be cytokine mediated) became significant at time point 135 min (Fig. 1).

These rapid changes in a widely used model of infection/inflammation suggest a key role of ghrelin linking innate immunity with appetite control. The mechanisms through which endotoxin administration achieves such a rapid effect on plasma ghrelin levels in humans are not known. Studies in rats suggest the participation of IL-1 and prostaglandin pathways (9). At the same time, gastric cells contain the toll-like receptor 4 (22), so one could speculate on a direct effect of endotoxin on ghrelin release. Finally, we might also consider the mediation of vagal fibers, known to partially control ghrelin secretion (23, 24). We have measured only the response of total plasma ghrelin to endotoxin and cannot exclude possible different effects of LPS on acylated and nonacylated ghrelin.

The biphasic pattern of ghrelin changes and the reasons it rises and subsequently decreases in response to LPS are intriguing. Ghrelin is thought to be the signal telling the brain throughout evolution when it is time to eat (14). The host response to infection coordinates signals from the immune and neuroendocrine systems with the priority to combat the foreign pathogens (2). Because it is known that nutrients modulate cytokine production and potency, it is speculated that the provision of nutrients needed for the optimal function of the immune system cannot be left to chance and is therefore obtained from endogenous sources (20).

LPS modulates peripheral glucose utilization in a biphasic pattern, similar to that of ghrelin. Using the same endotoxin dose as in our protocol and performing a euglycemic hyperinsulinemic clamp in healthy humans, Agwunobi *et al.* (5) found that glucose utilization increases 120 min after LPS and decreases thereafter, and insulin resistance is established at 420 min. This analogy to the ghrelin plasma profile presented in this study (Fig. 3A) is not surprising, because ghrelin was shown to increase the glucose utilization rate of white and brown adipose tissue (25).

Intravenous administration of synthetic human ghrelin to humans elevates within 15 min plasma GH and ACTH (26). We observed that the LPS-induced significant increases in ghrelin, ACTH, and GH levels happen in parallel. These three parameters reach peak levels at 2 h, whereas TNF-α already at 90 min after endotoxin administration (Figs. 3, B and C, and 2A). Plasma GH falls continuously and parallel to ghrelin starting from 2 h after endotoxin, whereas ACTH

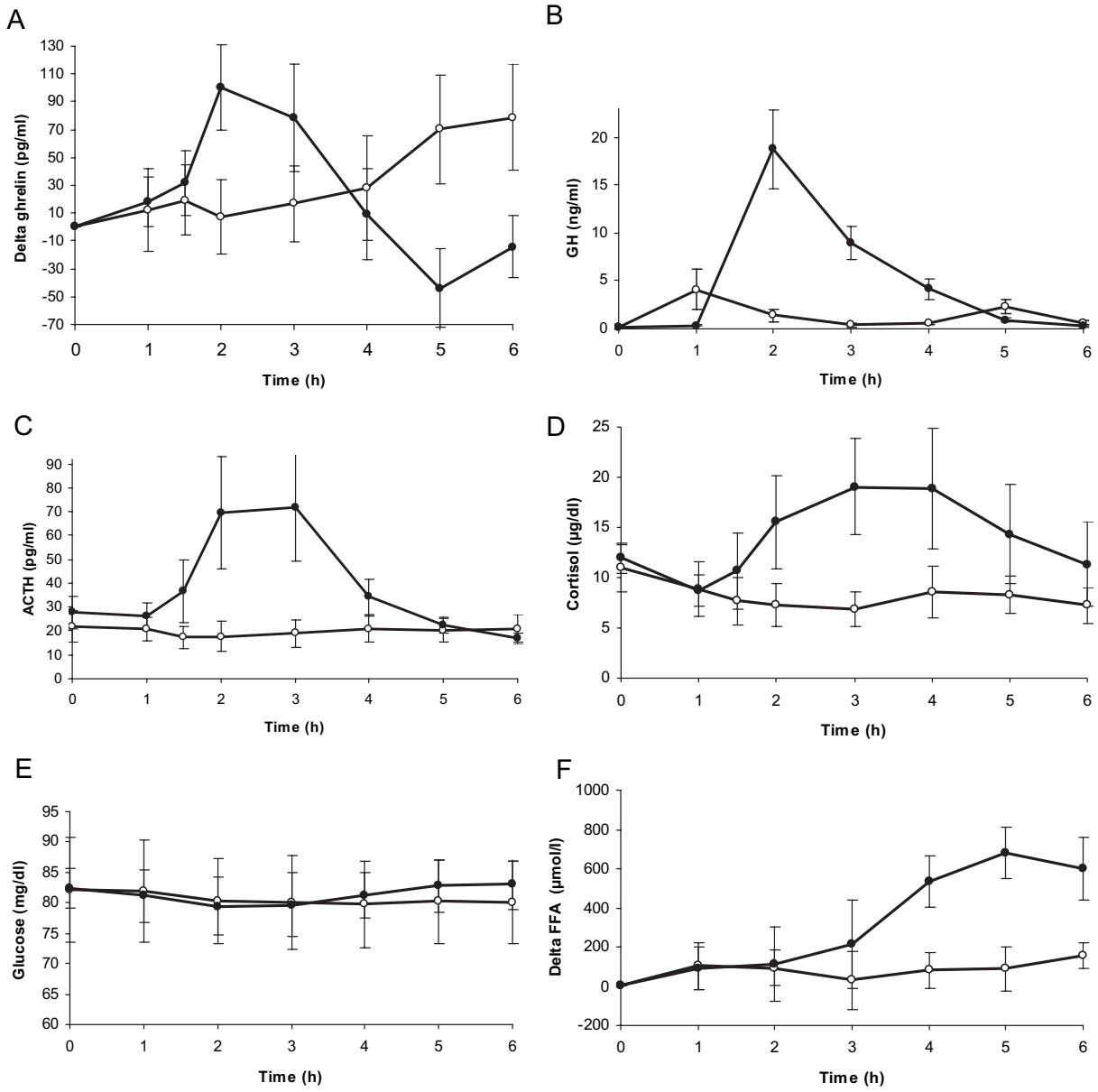

FIG. 3. Hormone and metabolic changes induced by endotoxin. LPS infusion (time point 0) induced within 2 h a rapid increase in plasma ghrelin followed by a second decline reaching a nadir at 5 h (A). LPS increased significantly GH (B), ACTH (C), cortisol (D), and FFA (F) but had no significant effects on plasma glucose (E). Data are presented as mean ± SEM (●, LPS; ○, placebo).

remains longer significantly elevated. Therefore, we hypothesize that ghrelin may in part mediate the effect of endotoxin on GH. Nevertheless, given the strong evidence on direct effects of cytokines on ACTH and GH release (1, 2, 27), ghrelin might not be the principle cause mediating ACTH and GH responses to endotoxin.

Recently, a few studies have revealed that ghrelin exerts also antiinflammatory properties. It inhibits proinflammatory pathways in human monocytes, T cells, and endothelial cells in primary culture (16, 19). Ghrelin down-regulates proinflammatory cytokine release in rats, and this effect is mediated by the vagus nerve (19, 28). By interacting with the opioid system, ghrelin inhibits inflammatory pain in rats (29).

There have been until now no data on a possible antiinflammatory role of ghrelin in systemic infection and inflammation in humans. Nevertheless, such properties might be partially expected given the fact that ghrelin activates the HPA axis and increases cortisol levels in normal subjects (26).

Not entirely in line with this hypothesis is the fact that ghrelin levels gradually decline after the second hour reaching a nadir 5 h after endotoxin. We suggest that the underlying cause is a multiple feedback regulation. The constant decrease in plasma ghrelin coincides with the increase in FFA and the persistently high IL-1RA levels. FFA administration is known to decrease circulating ghrelin (30). IL-1RA decreases the sensitivity to IL-1 and thereby might interrupt the IL-1-mediated changes in ghrelin (9). The reduction in ghrelin coincides also with constantly high levels of cortisol (Fig. 3, A and D), which ensure the antiinflammatory and anabolic effects in reply to infection. This might be another possible mechanism controlling circulating

ghrelin, because cortisol was found to inversely correlate with ghrelin during fasting (31).

To our knowledge, this is the first presentation on the participation of ghrelin in the immuno-neuroendocrine response to infection/inflammation in healthy humans. A few studies have addressed this question in rodents. Fasting plasma ghrelin was reduced 3 h after ip administration of low dose (0.1 mg/kg) LPS (9). Another study found that iv injection of 5 mg/g LPS increased ghrelin levels 24 h after administration. Repeated LPS (10 mg/kg iv) injections induced a rise in plasma ghrelin, too (8). Several trials using different protocols reveal a positive therapeutic effect of ghrelin on the endotoxic shock in rodents. Administration of ghrelin inhibited LPS-induced cytokine release (19), improved wasting symptoms, induced body weight gain (8), resulted in less pronounced hypotension, and reduced mortality in rodents (18). The authors suggest a possible therapeutic effect of ghrelin in sepsis. Nevertheless, some important questions need to be answered before testing this hypothesis in humans. The response of ghrelin −/− mice to endotoxin would answer the question whether ghrelin is a key regulator or just a by-player in the complicated immuno-neuroendocrine mechanisms. Ghrelin was found to be significantly elevated in 25 surgical patients with postoperative intraabdominal sepsis (32), but more studies and a better profiling of ghrelin fluctuations in acute infections are needed before testing its possible therapeutic effect in humans.

In summary, the data presented here reveal that ghrelin is an early response hormone in the cascade of events constituting the normal human response to bacterial endotoxin. We discuss the factors that might be involved in the biphasic regulation of plasma ghrelin by endotoxin as well as the possible role and eventual therapeutic application of ghrelin in sepsis.

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