Possible entrainment of ghrelin to habitual meal patterns in humans

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Frecka JM, Mattes RD. Possible entrainment of ghrelin to habitual meal patterns in humans. Am J Physiol Gastrointest Liver Physiol 294: G699–G707, 2008. First published January 10, 2008; doi:10.1152/ajpgi.00448.2007.—Ghrelin is reportedly a meal-initiation signal based on observations that concentrations increase before meals coincident with rising hunger. However, evidence that ghrelin peaks vary with feeding schedules suggests that it rises in anticipation of an expected meal, rather than eliciting feeding. To explore the entrainment of ghrelin profiles, this study investigated the association between varying habitual meal patterns and plasma ghrelin concentrations. Lean and obese adults following either a short intermeal interval (SII) pattern, with 2.5–3.5 h between their habitual breakfast and lunch times, or a long intermeal interval (LII) pattern, with 5.5–6.5 h between these eating occasions, participated. Food intake and appetite were recorded for 2 baseline days. On the subsequent test day, blood samples were collected over 8 h while participants ate a breakfast and lunch matched to their customary meals and pattern. Appetite ratings were obtained and ghrelin, insulin, glucose, and leptin concentrations were measured. Peak ghrelin concentrations differed significantly by group and occurred prior to each group’s respective lunch time. Ghrelin concentrations directly correlated with subjective hunger. This association was stronger when hunger preceded ghrelin, a pattern inconsistent with ghrelin causing the hunger rise. Ghrelin concentrations were inversely correlated with insulin, and peak insulin concentrations preceded nadir ghrelin concentrations postprandially. Ghrelin concentrations perimprandially, and over the entire test session, did not differ by meal group, likely because of similar intakes between groups. These data demonstrate that the timing of ghrelin peaks is related to habitual meal patterns and may rise in anticipation of eating rather than eliciting feeding.

Hormones secreted by adipose tissue reportedly signal chronic adiposity whereas peptides secreted from the gastrointestinal tract and pancreas provide more acute signals that may influence eating behavior (44). Multiple peptides may mediate meal size and frequency (41), but only ghrelin is posited to stimulate hunger and initiate meal ingestion. Support for this role is multifold. Intracerebroventricular and intraperitoneal ghrelin injections increase food intake and body weight in rodents (29, 43). Additionally, infusion of anti-ghrelin antibodies into the rat brain suppresses fasting-induced feeding. However, food intake and responses to starvation do not differ between ghrelin-null mice and wild-type littermates (37), suggesting that the relationship between ghrelin and intake is not necessarily causal or absolute. In humans, ghrelin increases preprandially (8, 14, 35) followed by a postprandial decline (4), and infusions have increased energy intake by 28% (15, 42). Additionally, slightly superphysiological ghrelin infusions increase subjective appetite ratings (15, 42), and a rising preprandial ghrelin concentration is closely associated with increased hunger (7). Most studies report increased hunger with ghrelin administration, but this is not the case for all individuals (2) and ghrelin is not necessary for eliciting the sensation (12).

Alternatively, ghrelin may only serve as a meal initiation signal. It is released following cephalic stimulation (1); the sites for its synthesis are proximal (10, 22, 24); and it stimulates gastric acid secretion (11, 26), gastrointestinal motility (13, 38), and pancreatic exocrine secretions (32), all of which increase in anticipation of eating. Indeed, stimulating these physiological responses may be ghrelin’s primary feeding-related function. Furthermore, its orexigenic actions are rapid and transient, characteristic of a signal acutely influencing prandial behavior (28, 43). However, studies have failed to find a relationship between preprandial ghrelin concentrations (3, 4, 7) or the rate of increase (4) and either the time of a spontaneous meal request or energy intake at the subsequent meal.

If hormone profiles reflect the anticipation of eating, it is likely that an individual’s habitual eating habits will entrain the release of appetite hormones at customary eating times. Whether ghrelin secretion is entrained to habitual meal patterns has not been well established. In vitro, ghrelin induces a phase advance in suprachiasmatic nucleus slices, suggestive of an influence on endogenous circadian rhythms (45). A transient ghrelin surge occurs before each meal in sheep entrained to two or four meals per day, whereas there is no significant change in plasma ghrelin in sheep fed ad libitum (36). A similar study in rats noted a significant increase in plasma ghrelin levels over the 2 h prior to meal time in meal-trained rats, but no increase in ad libitum-fed rats (14). The lack of a preprandial ghrelin increase in ad libitum-fed rats was also observed after both groups of rats were food deprived for an equivalent amount of time, suggesting that it was the habituated meal pattern, rather than energy deficit, that was responsible for the ghrelin increase. The limited human data indicate individuals who habitually consume three meals per day exhibit increased ghrelin concentrations around habitual mealtimes followed by a nadir ~2 h later (29). This observation suggests that changes in energy status are not required to alter ghrelin profiles and lends additional support for an anticipatory influence on ghrelin secretion.

It has been difficult to discern whether ghrelin entrainment to meal times exists in humans because much of the current literature investigating ghrelin profiles places participants on set eating schedules that often differ from their normal patterns. One way to explore whether ghrelin profiles are entrained to habitual diet is to study ghrelin patterns in individuals that follow very regular but discrepant eating schedules and to monitor ghrelin concentrations when they follow their
habitual patterns. The present research used this approach. If ghrelin concentrations peak differentially in individuals who vary in customary meal times, support would be provided for an anticipatory role of ghrelin secretion. To better characterize the mechanisms and consequences of this response, this study also investigated the associations between ghrelin, insulin, leptin, and glucose under normal eating conditions. Response patterns in lean and obese individuals, who have been reported to differ in their ghrelin profiles (16, 18), as well as male and female subjects were explored.

MATERIALS AND METHODS

Participant recruitment and screening. Participants were recruited by public advertisement. Eligibility criteria included age 18 to 50 yr, healthy, not taking medication other than contraceptives, body mass index (BMI) between 18.5 and 24.9 kg/m² or >30 kg/m², weight stable (less than ±5 kg change over the last 3 mo), and no endocrine or eating disorders. All participants habitually consumed breakfast and lunch without any snacks in between. An eating pattern was considered habitual if the participant followed the pattern at least 4 days/wk for a minimum of 2 mo. A short intermeal interval (SII) group was defined as having 2.5–3.5 h between their habitual breakfast and lunch times. A long intermeal interval (LII) group was defined as having 5.5–6.5 h between their habitual breakfast and lunch times. All participants provided signed informed consent prior to study initiation. This study protocol was approved by the University Institutional Review Board.

General protocol. The study consisted of 2 baseline days and one test visit. During the 2 baseline days, participants followed their habitual eating pattern and kept their intake similar. They tracked appetitive ratings throughout the days and recorded daily food intake. Several finger-stick blood glucose measurements were taken to verify participants adhered to their habitual intake patterns.

For the test visit, participants reported to the laboratory after an overnight (12-h) fast. Based on baseline diet records, the mean time of last meal ingestion of the SII and LII groups was 1,833 ± 38 and 1,928 ± 60 h, respectively, so a fast of this duration was not atypical. Upon arrival, blood glucose concentration was measured by finger stick to confirm that participants were in a fasted state. Next, participants were placed in a semisupine position and a catheter was inserted in a vein in the antecubital space of the arm. Blood was withdrawn by Vacutainer and, between draws, the line was flushed with sterile physiological saline. Following catheter insertion, participants rested for 15 min. An 8-ml baseline blood sample was then drawn and the participants completed appetitive questionnaires on a palm pilot. Next, a breakfast, matched to the participant’s habitual intake patterns (7) by 30-min increments over 90 min with recessed (lead condition) by 30-min increments over 90 min with 17.6%. Plasma leptin was also assessed in duplicate using a commercial RIA kit (Linco Research, St. Charles, MO) kit with a lower and upper detection limit of 90 and 6,000 pg/ml, respectively. The intra-assay coefficient of variation (CV) was 8.7% and the interassay CV was 17.6%. Plasma leptin was also assessed in duplicate by using a commercial RIA kit (Linco Research, St. Charles, MO). For this assay the lower detection limit is 0.5 ng/ml and the upper limit is 100 ng/ml and the inter- and intra-assay CVs were 9.0 and 19.8%, respectively. Serum insulin concentration was measured by an electrochemiluminescence immunoassay method using the Elecsys 2010 Immunoassay System (Roche Diagnostic Systems, Indianapolis, IN). Serum glucose was assessed by enzymatic colorimetry on the Cobas Integra 400 Analyzer (Roche Diagnostic Systems).

Statistical analysis. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), version 14.0. The criterion for statistical significance was \( P < 0.05 \), two-tailed. All data are expressed as means ± SE. Because there was considerable interindividual variability in hormone concentrations, all concentrations were calculated as both change from baseline and area under the curve (AUC) by the trapezoidal rule. Because of nonnormality, hormone concentrations were analyzed by the Mann-Whitney test. Appetitive measurements and energy intake were analyzed by paired \( t \)-tests. In investigation of changes around the lunch test meal, to account for the range in intermeal intervals within each meal group (2.5–3.5 h for the SII group and 5.5–6.5 h for the LII group), time points were standardized to lunch time (as specified below). Otherwise, time points were matched across the groups. Hormone profiles throughout the day were analyzed by repeated-measures analysis of variance. Associations between ghrelin and insulin and ghrelin and hunger were investigated by time series analyses. Insulin concentrations and hunger ratings were both advanced (lag condition) and recessed (lead condition) by 30-min increments over 90 min with respect to ghrelin concentrations. Associations were assessed by Pearson correlation coefficients or Spearman’s Rho in the case of nonnormality.

RESULTS

Participant characteristics. Thirty participants completed the protocol; however, nine either were not fully compliant with baseline intake patterns that deviated from their habitual patterns (7) or had physiological responses falling outside

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normal ranges (2) and so were excluded from analyses. Participant characteristics are summarized in Table 1. The SII group consisted of 12 (7 lean, 5 obese) individuals with a mean age of 27.5 ± 7.6 yr and the LII group was comprised of 9 (4 lean, 5 obese) individuals with a mean age of 31.6 ± 7.8 yr. There were no significant differences between the meal groups in age, BMI, baseline intake, or hormone concentrations (Tables 1 and 2). There were also no differences between included and excluded participants on any baseline characteristics or food intake at both the baseline days and test day.

Plasma ghrelin. There were no significant differences between lean and obese or male and female participants for baseline, daily mean, or total AUC ghrelin concentrations (Table 1). Additionally, there were no time×BMI or time×group×BMI interactions. As a result, all data were pooled within each meal group. Baseline plasma ghrelin (Table 1), daily means (SII 393.5 ± 28.4 pg/ml; LII 336.2 ± 17.6 pg/ml), and total AUC concentrations (SII 182485 ± 13,438 pg·ml⁻¹·min⁻¹; LII 155,851 ± 7,915 pg·ml⁻¹·min⁻¹) did not differ between the SII or LII groups. However, the ghrelin profiles did vary over the day.

Following breakfast, all participants experienced a reduction of ghrelin concentration with no statistically significant difference in postprandial nadir between the meal groups (postbreakfast nadir plasma ghrelin, Table 3). Ghrelin concentrations (calculated as change from baseline) were also similar among the two meal groups through 90 min after breakfast. Although the magnitude of ghrelin suppression did not reach statistical significance for the SII group, the change from baseline for the magnitude of ghrelin suppression did not reach statistical significance for the SII group, the change from baseline for

Serum insulin. Neither BMI nor meal groups differed in their baseline or mean insulin concentrations. However, total AUC insulin concentrations were significantly greater in the LII group (U = 19, P = 0.011). The temporal insulin profile followed a reciprocal pattern to ghrelin (Fig. 2). The concentrations of the hormones throughout the day were negatively correlated (r = -0.313, P < 0.001). The association between insulin and ghrelin was strongest when no time series adjustments were applied. However, the associations were stronger when insulin was lagged, as opposed to led, in relation to ghrelin, indicating a stronger relationship when insulin concentrations preceded ghrelin. These findings did not differ by group. Insulin levels significantly increased following consumption of both breakfast and lunch, whereas during the intermeal interval they decreased toward baseline. The peak insulin concentration following breakfast and lunch (postbreakfast/lunch peak plasma insulin, Table 3), as well as the time to reach each peak (breakfast/lunch to peak insulin, Table 3), did not differ between the two meal groups. However, in accordance with the results of the time series analysis, the insulin peaks after both meals slightly preceded ghrelin nadir concentrations (Table 3).

Consistent with ghrelin, the time from peak to nadir insulin concentrations between breakfast and lunch was greater for the LII group than the SII group (U = 0, P < 0.001) (postbreakfast peak to prelunch nadir insulin, Table 3). However, the time from the prelunch nadir to lunch did not differ between the two groups (adipar insulin to lunch, Table 3). There was also a significant time×group interaction [F(1,13) = 7.048, P < 0.001] and time×group×BMI interaction [F(1,13) = 9.186, P < 0.001]. Insulin concentrations were greater for the SII group for 15 (U = 20, P = 0.016) through 105 min after their lunch compared with the corresponding time points for the LII group. Following the LII group’s lunch, insulin concentrations over the final 90 min of the testing session were greater for the LII group than the SII group (U = 6,545, P = 0.011).

Serum glucose. Following each meal, serum glucose concentrations increased for all participants and then declined to

### Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
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<th>M</th>
<th>F</th>
<th>Age</th>
<th>BMI</th>
<th>Baseline Ghrelin, pg/ml</th>
<th>Baseline Leptin, ng/ml</th>
<th>Baseline Insulin, μU/ml</th>
<th>Baseline Glucose, mg/dl</th>
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<tbody>
<tr>
<td>SII</td>
<td></td>
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<td></td>
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<td></td>
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<td>Lean</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>24.3±1.6</td>
<td>23.3±0.6</td>
<td>395.5±50.3</td>
<td>3.2±0.7</td>
<td>7.7±1.0</td>
<td>77.9±1.6</td>
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<tr>
<td>Obese</td>
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<td>3</td>
<td>2</td>
<td>32.0±4.3</td>
<td>32.2±0.7</td>
<td>398.9±22.4</td>
<td>7.4±1.2</td>
<td>13.1±2.8</td>
<td>81.8±2.3</td>
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<tr>
<td>LII</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Lean</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>28.3±1.3</td>
<td>22.8±0.6</td>
<td>303.8±33.0</td>
<td>1.8±0.5</td>
<td>5.5±1.8</td>
<td>78.5±4.3</td>
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<tr>
<td>Obese</td>
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<td>4</td>
<td>1</td>
<td>34.2±4.0</td>
<td>33.5±1.1</td>
<td>358.4±27.8</td>
<td>5.0±2.2</td>
<td>13.1±2.3*</td>
<td>83.8±2.3</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. BMI, body mass index; SII, short intermeal interval; LII, long intermeal interval. *P < 0.05 compared with lean LII.
baseline levels (Fig. 3). Neither the magnitude of the peak glucose concentrations nor the time to reach the peaks differed between the meal groups. Similarly, nadir glucose concentrations between the meals were comparable between the groups. The time from the intermeal peak to nadir glucose concentrations was greater for the LII group, 181.1 ± 28.6 min, than the SII group, 51.5 ± 14.9 min (U = 20, P = 0.015). There was also a main effect of time [F(1.13) = 9.10, P < 0.001] as well as a time×group interaction [F(1,13) = 5.87, P = 0.001] for glucose concentrations throughout the day. This was attributable to a higher glucose concentration for the SII group 45 min (U = 21, P = 0.019) after their lunch compared to the corresponding time point for the LII group. This trend continued for an additional 120 min.

**Plasma leptin.** Baseline (r = 0.56, P = 0.009), daily mean (r = 0.51, P = 0.017), and total AUC (r = 0.50, P = 0.021) leptin concentrations all positively correlated with percent fat mass. Baseline leptin concentration was significantly higher in the obese than the lean group (U = 27, P = 0.049). Although leptin concentrations throughout the day appeared to be consistently and systematically higher in the SII than LII group, this did not reach statistical significance. There were no differences in baseline plasma leptin, daily means, or total AUC concentrations between the SII or LII groups (Table 1). Additionally, there was no change over time nor a time×group interaction for leptin concentrations. No meal group differences in leptin profiles were observed. Leptin concentrations throughout the day were positively correlated with both insulin (r = 0.16, P = 0.002) and glucose (r = 0.11, P = 0.042). The length of testing did not allow for analysis of a diurnal rhythm for leptin.

**Appetitive ratings.** Mean appetite ratings during the baseline days were similar between both meal and BMI groups. However, desire to eat something fatty [F(1,20) = 6.10, P = 0.023] and something salty [F(1,20) = 4.54, P = 0.046] were greater for the SII than the LII group, as was their preoccupation with food [F(1,20) = 4.87, P = 0.040].

There were no significant differences between the meal groups in their daily mean hunger, fullness, desire to eat, desire to eat something fatty, desire to eat something sweet, desire to eat something salty, prospective consumption, preoccupation with food, or thirst during the test day. The BMI groups also showed similar appetitive ratings, excluding hunger, which was greater for the lean participants than obese [F(1,20) = 4.68, P = 0.043]. Across the test day, all appetitive measures revealed significant time×meal group interactions with differences noted during the time prior to the LII group’s lunch. Additionally, the peak hunger rating was greater for the LII

| Table 3. Ghrelin and insulin parameters |

<table>
<thead>
<tr>
<th>Meal Group</th>
<th>SII</th>
<th>LII</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postbreakfast nadir plasma ghrelin, pg/ml*</td>
<td>-67.5 ± 18.8</td>
<td>-74.6 ± 16.8</td>
<td>0.57</td>
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<tr>
<td>Breakfast to nadir ghrelin, min</td>
<td>40.0 ± 7.7</td>
<td>56.7 ± 15.4</td>
<td>0.44</td>
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<tr>
<td>Pre-lunch peak plasma ghrelin, pg/ml*</td>
<td>73.3 ± 22.4</td>
<td>81.5 ± 23.1</td>
<td>0.78</td>
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<tr>
<td>Peak ghrelin to lunch, min</td>
<td>21.3 ± 10.2</td>
<td>27.2 ± 8.3</td>
<td>0.25</td>
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<tr>
<td>Post-lunch nadir plasma ghrelin, min</td>
<td>-93.7 ± 19.6</td>
<td>-34.8 ± 15.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Postbreakfast nadir to pre-lunch peak ghrelin, min</td>
<td>105.0 ± 10.3</td>
<td>247.8 ± 23.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Slope postbreakfast nadir to pre-lunch peak ghrelin, pg·ml⁻¹·min⁻¹</td>
<td>1.3 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>0.09</td>
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<tr>
<td>Lunch to nadir ghrelin, min</td>
<td>85.0 ± 21.1</td>
<td>58.3 ± 13.8</td>
<td>0.47</td>
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<tr>
<td>Postbreakfast peak plasma insulin, µU/ml*</td>
<td>7.6 ± 2.9</td>
<td>-3.8 ± 0.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Breakfast to peak insulin, min</td>
<td>26.3 ± 5.3</td>
<td>31.7 ± 6.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Pre-lunch nadir plasma insulin, µU/ml*</td>
<td>54.5 ± 8.6</td>
<td>89.2 ± 23.9</td>
<td>&lt;0.01</td>
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<tr>
<td>Nadir insulin to lunch, min</td>
<td>12.5 ± 5.2</td>
<td>17.2 ± 7.7</td>
<td>0.59</td>
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<td>Post-lunch peak plasma insulin, µU/ml*</td>
<td>66.4 ± 10.0</td>
<td>58.7 ± 17.7</td>
<td>0.32</td>
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<tr>
<td>Postbreakfast peak to pre-lunch nadir insulin, min</td>
<td>127.5 ± 9.1</td>
<td>282.8 ± 7.7</td>
<td>&lt;0.01</td>
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<tr>
<td>Lunch to peak insulin, min</td>
<td>23.8 ± 2.2</td>
<td>43.3 ± 8.8</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. *Data calculated as change from baseline.
group than the SII group (Fig. 4). However, nadir fullness ratings did not differ between the groups. The time between nadir to peak hunger ratings during the breakfast to lunch interval were significantly greater for the LII group. Likewise, the time between peak and nadir fullness ratings was greater for the LII group. Thus it appears that the participants' appetite ratings also reflected their habitual eating patterns. Ghrelin concentrations throughout the day were positively correlated with hunger ($r_{[H11005]} = 0.25, P_{[H11021]} < 0.001$) and negatively correlated with fullness ($r_{[H11005]} = 0.16, P_{[H11005]} < 0.003$). Furthermore, the association between ghrelin and hunger was strongest when hunger was lagged by 30 min in relation to ghrelin (Fig. 5). Additionally, all lag conditions resulted in stronger associations than their corresponding lead condition, indicating a stronger relationship when hunger preceded ghrelin, and similar trends were observed for both meal groups. After both breakfast and lunch, nadir hunger ratings occurred ~30 min prior to nadir ghrelin concentrations. Hunger ratings, therefore, also began increasing prior to changes in ghrelin concentration. The slopes over this time period confirmed this because ghrelin and hunger slopes for the 90 min after meal consumption were significantly different [$F(1,21) = -3.76, P < 0.001$] and only the hunger slope was positive [$F(1,21) = 3.38, P = 0.003$].

Food intake. Intake during the test day was well matched to the participants’ habitual intake because there were no significant differences in the gram weight, kilocalories, or macronutrient composition at either breakfast or lunch between the baseline days and test day (Table 2). Furthermore, the intakes of both meal and BMI groups were very similar with no significant differences at breakfast or lunch during the test day as well as overall intake during the baseline days.

There were no associations between intake and ghrelin concentrations for the SII group. LII breakfast intake was negatively correlated with ghrelin AUC from breakfast to lunch ($r = -0.72, P = 0.03$), and lunch intake was correlated with ghrelin AUC concentrations from lunch through the end of the testing period ($r = -0.72, P = 0.03$). In the SII group, caloric intake at breakfast was positively correlated with insulin ($r = 0.64, P = 0.026$) and glucose ($r = 0.70, P = 0.011$) AUC concentrations from breakfast to lunch. Caloric intake at lunch, however, was only correlated with glucose AUC for the 15 min following lunch ($r = 0.58, P = 0.048$). LII breakfast

Fig. 1. Ghrelin profiles (calculated as change from baseline or normalized using a logarithmic function) standardized to lunch time by meal group (SII, short intermeal interval, LII, long intermeal interval). Arrows designate the lunch times for each group. *Peak concentration is significantly greater than nadir, $P < 0.05$. †Time from nadir to peak concentration is significantly greater in LII than SII, $P < 0.05$.

Fig. 2. Temporal profiles of ghrelin (●) and insulin (○) concentrations (calculated as change from baseline) by meal group across the testing period. Arrows designate breakfast; bars designate the range of lunch times within each group.

Fig. 3. Temporal profiles of glucose (calculated as change from baseline) by meal group across the testing period. The arrow designates breakfast; bars designate the range of lunch times within each group.
intake was positively correlated with glucose AUC concentrations for the 60 min following consumption of the meal (r = 0.72, P = 0.03). Lunch intake in the LII group was correlated with insulin AUC concentrations from lunch through the end of the testing period (r = 0.83, P = 0.005). It was also correlated with glucose AUC concentrations for 30 (r = 0.90, P = 0.001) through 90 min (r = 0.72, P = 0.03) after lunch consumption. Total caloric intake during the test session was significantly correlated with mean insulin (r = 0.75, P = 0.007) and glucose (r = 0.82, P = 0.007) concentrations in the LII group.

**DISCUSSION**

There is considerable debate over whether ghrelin initiates meals or increases in anticipation of eating. Several studies have addressed this by investigating the relationship between ghrelin and food intake while isolating participants from time and food-related cues (4, 7, 29). Mixed findings have been reported. This may be attributed to the numerous factors, such as an individuals’ routine schedule, that guide the timing of many meals. This study measured ghrelin secretion profiles in people adhering to their individual habitual short or longer intermeal patterns. The findings suggest ghrelin secretion may be entrained to customary consumption patterns and the anticipation of an expected meal likely contributes to the preprandial ghrelin increase. Plasma ghrelin concentrations throughout the day were correlated with subjective hunger and fullness ratings, although preprandially, hunger ratings rose sooner than ghrelin concentrations, providing evidence that ghrelin may not directly produce a hunger signal. Plasma ghrelin concentrations also negatively correlated with insulin concentrations, and this association was stronger when postprandial insulin changes preceded ghrelin changes than the reverse, consistent with evidence that ghrelin may be regulated by insulin. We found no evidence to support a similar influence of leptin.

**Ghrelin entrainment.** The principal finding was that the time from preprandial nadir to peak ghrelin concentration differed between LII and SII groups. This reflected the differences in customary eating schedules of the two groups because both peaks occurred prior to their respective habitual lunch times. This suggests an entrainment effect on ghrelin. Therefore, the preprandial ghrelin rise may occur more as a conditioned response. This observation is supported by a study in sheep revealing that different feeding regimens alter ghrelin profiles (36). In Suffolk rams fed either ad libitum or entrained to a 2...
or 4 meal per day schedule, plasma ghrelin levels increased prior to each eating occasion in the meal-fed sheep whereas levels remained relatively stable in the ad libitum-fed animals. Similarly, when a restricted feeding schedule was imposed on rats, only the meal-fed rats experienced a ghrelin peak prior to their expected meal time whereas their ad libitum-fed counterparts did not (14). There is limited human literature to support ghrelin entrainment. One study concluding ghrelin secretion was largely under cephalic control found that when individuals were fasted for 24 h, plasma ghrelin increased around customary meal times (29). This observation suggests that changes of energy status are not necessary for the preprandial peaks and intermeal nadirs in ghrelin concentration. Our data confirm this finding in humans since statistically significant differences in ghrelin profiles were noted between the SII and LII groups despite similar energy intakes. We also noted that both insulin and glucose nadir concentrations following breakfast occurred at a later time in the LII group. Furthermore, peak insulin concentrations preceded nadir ghrelin concentrations. Thus it is possible that ghrelin entrainment in response to customary eating schedules occurs secondarily to alterations in insulin or glucose profiles.

The periprandial ghrelin changes in this study are consistent with other reported profiles (7, 8, 33) and did not vary by habitual eating schedule. Neither the rise from baseline to peak ghrelin concentrations nor absolute peak concentrations differed between the meal groups. Therefore, it is possible that a threshold ghrelin change precedes eating (either causally or in anticipation). Previous reports indicate the increase in plasma ghrelin following the postprandial nadir does not predict the timing of a spontaneous meal request (4). However, that study did not adhere to the participants’ customary eating pattern and used preloads varying in energy density. Other work suggests the intermeal interval is largely determined by customary eating patterns and the size of the previous meal (5). Additional studies that consider the influence of habitual eating patterns on ghrelin secretion may help to discern whether a threshold ghrelin change does precede an eating occasion and, if so, the magnitude of this change that is required.

**Ghrelin and appetite.** Several studies have reported a positive association between hunger and ghrelin (8, 42). The present data confirm this observation in lean and obese individuals. Although hunger and ghrelin followed a similar pattern, the LII group’s hunger ratings reached a greater magnitude than did the SII group, despite similar peak ghrelin concentrations. Thus the relationship between ghrelin and hunger is not straightforward and may be influenced by customary consumption patterns. The results of the present time-series analysis revealed that ghrelin and hunger correlated most strongly when hunger changes preceded ghrelin. The slopes of the recovery from nadir of ghrelin and hunger also confirmed that hunger increases prior to changes in ghrelin concentration. This is not consistent with the hypothesis that rising ghrelin generates a hunger signal. Instead, ghrelin and hunger may be under separate controls and the primary role of ghrelin may lie more in preparing the gut for processing the incoming load than as a meal-initiating signal.

**Ghrelin and energy intake.** Despite increased hunger in the LII group prior to lunch, there were no differences in the energy intake or macronutrient content of the meal between groups. The similarity in energy intakes between the groups may explain the similarity of their periprandial ghrelin concentrations. Nutrient ingestion may be responsible for suppressing ghrelin concentrations as similar energy intakes at both meals resulted in similar declines of postprandial ghrelin concentration between the meal groups. Energy intake was correlated with postprandial ghrelin concentrations in the LII group. Although a significant relationship did not exist in the SII group, the greater variation in ghrelin concentrations in that group likely accounts for this discrepancy. In rodents, ghrelin concentrations are decreased by the presence of nutrients, but not water, in the stomach (39). In another human trial, ghrelin reduction following feeding was proportional to the energy intake of the preceding meal (4). However, it is also possible that nutrient ingestion is not directly responsible for this decline. When gastric emptying is prevented with intragastric nutrient infusion, the decline is abolished (40). Signals driving the postprandial decline in ghrelin are likely not derived solely from the sites of ghrelin synthesis, the stomach or duodenum, which would be expected if nutrient exposure was directly suppressing ghrelin. Jejunal nutrient infusions decrease ghrelin concentrations to an equivalent extent as either gastric or duodenal infusions (30).

The results of this study raise additional questions about the role of ghrelin in meal initiation. One short-term study noted ghrelin and hunger increased prior to voluntary meal requests in the absence of time and food related cues (7). However, all participants in that study habitually consumed three meals per day and the present findings, coupled with others (29, 35, 36), indicate this may just reflect residual training effects. After a 4-day fast, ghrelin concentrations decrease (23), possibly reflecting an attenuation of the meal-related anticipatory rise. Ghrelin concentrations increase with prolonged undernutrition and starvation in humans (9, 34), but this may be attributable to associated changes of body fat stores and endocrine function more than feeding cycles (17, 19, 25, 28).

**Additional hormone interactions.** Consistent with several other reports, insulin and ghrelin profiles were negatively correlated throughout the testing session (3, 7). Furthermore, the postprandial peak insulin concentration preceded nadir

Fig. 5. Time series correlations between ghrelin and hunger. Subjective hunger ratings were adjusted (lagged or led) whereas ghrelin concentrations were held constant, $P < 0.05$. 
ghrelin concentrations at both meals and the association between ghrelin and insulin concentrations was stronger when insulin concentrations preceded those of ghrelin by 30, 60, or 90 min rather than followed it. This suggests that a change in insulin concentration may regulate postprandial ghrelin concentrations. Conversely, reduced insulin does not appear to be solely responsible for the rise of ghrelin concentrations. The preprandial insulin concentrations were lower for the LI group yet peak ghrelin concentrations did not differ between the meal groups.

Our results suggest that plasma leptin concentrations do not change acutely in response to meals and are in agreement with several other reports (20, 21). Additionally, the finding that total AUC leptin concentrations, as well as the absence of changes periprandially, did not differ between the two meal groups does not support a role for leptin in regulating plasma leptin concentrations. Conversely, reduced insulin does not appear to be responsible for the change acutely in response to meals and are in agreement with the absence of change acutely in response to meals and are in agreement with the absence of change acutely in response to meals for the two groups.

Summary. The present study indicates that ghrelin secretion is entrained to habitual eating patterns. Furthermore, it is not solely dependent on energy intake since the ghrelin secretion patterns differed between meal groups despite similar energy intakes. These findings are not consistent with previous reports that increasing ghrelin concentrations preprandially generate a hunger signal. If ghrelin secretion is not directly responsible for increasing hunger and meal initiation, there are several other explanations for the observed prandial ghrelin changes. Preprandial ghrelin increases may help to prepare the gastrointestinal tract for ingesta and to optimize digestion, absorption, and the utilization of nutrients (14). In the absence of these preparatory changes digestion and absorption are impaired (46). Additionally, ghrelin changes may occur secondarily to the influence of other hormones. Findings from this study in conjunction with other published data suggest insulin may play a role in ghrelin regulation. Finally, although the results of this study suggest ghrelin entrainment does occur, it is also possible that rather than ghrelin becoming entrained to habitual meal patterns, the ghrelin profiles of the participants in the two mea groups promoted the different dietary patterns. Requesting individuals to eat at times they customarily do not eat and assessing how their appetite and ghrelin profiles respond may be controlled in studies aimed at elucidating the controls of human feeding.

GRANTS
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REFERENCES


