Changes in gut hormone and glucose concentrations in relation to hunger and fullness1–3

Sofie G Lemmens, Eveline A Martens, Arnold D Kester, and Margriet S Westerterp-Plantenga

ABSTRACT
Background: The search for biomarkers of appetite is very active. Objectives: The aims were to compare dynamics of hunger and fullness ratings on a visual analog scale (VAS) with dynamics of glucagon-like peptide 1, peptide tyrosine-tyrosine, ghrelin, glucose, and insulin concentrations throughout different meal patterns—and thus different timings of nutrient delivery to the gut—by using a statistical approach that focuses on within-subject relations of these observations and to investigate whether appetite ratings are synchronized with or lag behind or in front of changes in hormone and glucose concentrations.

Design: Subjects (n = 38) with a mean (±SD) age of 24 ± 6 y and BMI (in kg/m²) of 25.1 ± 3.1 came to the university twice for consumption of a 4-course lunch in 0.5 or 2 h (randomized crossover design). Per subject regression slopes and R² values of VAS scores on hormone and glucose concentrations were calculated. We tested whether the means of the slopes were different from zero. Regarding possible lags in the relations, the analyses were repeated with VAS scores related to hormone and glucose concentrations of the relevant previous and following measurement periods.

Results: VAS scores and hormone and glucose concentrations changed synchronously (P < 0.005, R² = 0.4–0.7). Changes in ghrelin concentrations lagged behind (10–30 min) changes in hunger scores (P < 0.005, R² = 0.7) and insulin concentrations (P < 0.005, R² = 0.6), which suggests a role for insulin as a possible negative regulator of ghrelin. No major differences in slopes and R² values were found between the meal patterns.

Conclusions: This method may be useful for understanding possible differences in relations between VAS scores and hormone and glucose concentrations between subjects or conditions. Yet, the reported explained variation of 40% to 70% seems to be too small to use hormone and glucose concentrations as appropriate biomarkers for appetite, at least at the individual level and probably at the group level. This study started in 2007, which means that it was not registered as a clinical trial. Am J Clin Nutr 2011;94:717–25.

INTRODUCTION
The regulation of energy intake and appetite is a complex process involving, besides environmental and behavioral factors, physiologic factors such as the dynamics of gastrointestinal hormones and the possibly related feelings of hunger and fullness (1–3). The search for physiologic biomarkers of appetite is currently very active. Relevant potential biomarkers related to appetite (ie, hunger and fullness) may be the anorexigenic peptides GLP-14 and PYY, the orexigenic gut peptide ghrelin, and glucose and insulin concentrations (4, 5). GLP-1 and PYY are released from the endocrine L cells of the ileum and the colon and appear to reduce appetite (4, 6, 7). Ghrelin is a peptide secreted primarily by the stomach and appears to increase appetite (8, 9). Glucose is hypothesized to play a role in meal initiation, because feeding is usually preceded by a decrease in blood glucose concentrations (10–13). Glucose triggers insulin secretion by the β cells of the pancreatic islets (10, 11). Similar to blood glucose, insulin has been hypothesized to be involved in appetite regulation (14, 15).

Measured feelings of appetite expressed as ratings on VAS have been shown to be highly reproducible and therefore reliable (16). However, a possible association between VAS appetite ratings and physiologic measures remains the subject of debate. Several studies showed no relation between appetite ratings and endogenous GLP-1, PYY, and ghrelin concentrations (17–19), whereas others found significant correlations (P < 0.05, R² < 0.3) at a few time points or for the AUC (6, 20–22). The latter articles suggest a relation between appetite ratings and gastrointestinal hormone concentrations, although correlation coefficients are mostly too low to presume that the gastrointestinal hormones may serve as a reliable biomarker. Regarding glucose and insulin concentrations, a meta-analysis by Flint et al (5) showed that insulin concentrations were inversely correlated with feelings of hunger (P < 0.02, R² < 0.1), whereas glucose concentrations were not correlated with feelings of hunger or satiety. Literature has indicated that it is not clear yet whether blood glucose and insulin concentrations can act as biomarkers of appetite because the relation is confounded by many metabolic processes (5, 11).

In the studies cited above (5, 6, 17–22), correlation analyses were based on the calculated AUC or on the measured values per time point, not taking into account the factor time. The objective of our study was to compare the dynamics of VAS hunger and fullness ratings with hormone and insulin concentrations.
fullness ratings with the dynamics of GLP-1, PYY, ghrelin, glucose, and insulin concentrations using a statistical approach that includes the factor time by concentrating on the within-subject relations of these observations. We hypothesized that including the factor time might strengthen the possible relation between VAS appetite scores and hormone and glucose concentrations. Moreover, we investigated whether the changes in VAS scores are synchronized with, or lag behind or in front of, the changes in hormone and glucose concentrations. The study design consisted of consumption of a 4-course lunch spread over 2 h (staggered) and consumption of the same 4-course lunch in 0.5 h (nonstaggered). This design gave us the ability to measure and compare postprandial appetite and hormone and glucose dynamics throughout different meal patterns and thereby different timings of nutrient delivery to the gut.

SUBJECTS AND METHODS

Subjects

Thirty-eight healthy white subjects (18 men and 20 women) with a mean (±SD) age of 24 ± 6 y (range: 18–49 y) with a BMI (in kg/m²) of 25.1 ± 3.1 (range: 19.5–30.1) participated in this study. Subjects were recruited by advertisements in local newspapers and on notice boards at the university. Subjects underwent an initial screening that included measurement of body weight and height and completed questionnaires related to health, use of medication, smoking behavior, alcohol consumption, physical activity, and eating behavior (Dutch translation of the Three-Factor Eating Questionnaire; 23). All subjects gave written informed consent. The study was approved by the Medical Ethical Committee of the Maastricht University and was in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Study design

The study was conducted in a randomized crossover design. All subjects came to the university twice, on 2 separate days ≥1 wk apart, in a fasted state for either condition: 4-course meal consumption in 0.5 h without within-meal pauses (nonstaggered meal condition) compared with 4-course meal consumption in 2 h with 3 within-meal pauses of 20 to 25 min (staggered meal condition). The order of the 2 conditions was randomized across the subjects to prevent any order effects.

In the morning (0800), all subjects consumed a standardized breakfast drink at home (“Goede Morgen!”; Campina) The amount of the breakfast drink subjects had to consume corresponded to 10% of their individual DERs. For each subject the DER was calculated by multiplying the basal metabolic rate by the appropriate physical activity factor (1.5–1.8, derived from the screening questionnaire; 26). The basal metabolic rate (kcal/d) was calculated according to the equation of Harris-Benedict (27).

The lunch consisted of a 4-course meal, ie, a salad (iceberg lettuce, mozzarella, tomato, croutons, and dressing) with a slice of white bread as a starter, macaroni Bolognese as the first part of the main course, vegetable lasagna as the second part of the main course, and raspberry pudding as a dessert. The energy density of the total 4-course meal was 5.0 kJ/g and consisted of 14% of energy as protein, 54% of energy as carbohydrate, and 32% of energy as fat. The amount of the 4-course meal subjects had to consume corresponded to 40% of their DER (5.0 ± 0.1 MJ; starter: 8%; first part main course: 12%; second part main course: 12%; and dessert: 8% of the DER).

During the staggered meal condition, the starter and the first part of the main course were consumed between 1200 and 1300, whereas the second part of the main course and the dessert were consumed between 1300 and 1400. The amount of energy (20% of the DER) and the energy density (5.0 kJ/g) of the foods consumed between 1200 and 1300 and of the foods consumed between 1300 and 1400 were equal.

During the nonstaggered meal condition, subjects had 0.5 h to consume the 4-course meal. During the staggered meal condition the courses were offered at 1200, 1235, 1310, and 1340 (time points: 0, 35, 70, and 100 min), and the subjects had 10 min to consume each course. All subjects were instructed to consume the entire amount of food presented. Each participant was given 14.3 mL/kg body wt water to consume ad libitum throughout the test days (28).

Test meals

An overview of the nutritional information of the foods consumed on the test days is provided in Table 1. Beforehand, during screening, subjects had rated the food items for subjective liking (VAS) to check whether all food items were acceptable. All food items were scored as >60 mm on a 100-mm VAS.

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Table 1

<table>
<thead>
<tr>
<th>Energy</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast drink</td>
<td>300</td>
<td>70.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Course 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceberg lettuce</td>
<td>42</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>998</td>
<td>18.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Tomato</td>
<td>58</td>
<td>0.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Croutons</td>
<td>2370</td>
<td>7.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Dressing</td>
<td>950</td>
<td>0.6</td>
<td>9.6</td>
</tr>
<tr>
<td>White bread</td>
<td>1144</td>
<td>9.7</td>
<td>51.3</td>
</tr>
<tr>
<td>Course 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni Bolognese</td>
<td>396</td>
<td>3.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Course 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable lasagna</td>
<td>501</td>
<td>4.8</td>
<td>12.8</td>
</tr>
<tr>
<td>Course 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raspberry pudding</td>
<td>520</td>
<td>3.2</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Appetite profile

VAS (100 mm) were used to assess the appetite profile. The scales were anchored with “not at all” at one end and “extremely”
at the other end and combined with questions on feelings of hunger and fullness. The VAS were completed 5 times throughout the test day with the nonstaggered meal condition (at −10, 35, 65, 95, and 125 min) and 7 times throughout the test day with the staggered meal condition (at −10, 20, 50, 65, 85, 95, and 110 min).

**Blood sampling**

Venous blood samples were collected into EDTA-containing tubes, 5 times during the test day with the nonstaggered meal condition (at −5, 30, 60, 90, and 120 min) and 9 times during the test day with the staggered meal condition (at −5, 15, 30, 45, 60, 75, 90, 105, and 120 min). Blood samples were drawn to determine concentrations of plasma GLP-1, PYY, ghrelin, glucose, and insulin.

For the GLP-1 analysis, blood was collected into EDTA-containing tubes to which dipeptidyl peptidase IV inhibitor (10 μL/mL blood) was added. For the PYY analysis, blood was collected into EDTA-containing tubes in which dipeptidyl peptidase IV inhibitor (10 μL/mL blood) and aprotinin (500 KIU/mL blood) was added. After collection, blood samples were centrifuged for 10 min at 4°C at 3000 rpm. For ghrelin analysis, phenylmethylsulfonyl fluoride, dissolved in methanol, and hydrochloric acid were added to the plasma. Plasma samples were immediately frozen in liquid nitrogen and stored at −80°C until analyzed.

Plasma concentrations of PYY3–36 and active ghrelin were measured by radioimmunoassay (Linco Research Inc) and those of active GLP-1 by ELISA (EGLP-35K; Linco Research Inc). Plasma insulin concentrations were measured by means of radioimmunoassay to the manufacturer’s instructions (Human insulin-specific RIA kit; Millipore).

**Statistics**

Data were analyzed by using StatView 5.0 (SAS Institute Inc). To assess the strength of the within-subject relation between changes in VAS scores for hunger and fullness and changes in hormone and glucose concentrations, we calculated, separately for each subject, regression slopes and $R^2$ values for the regression of VAS scores on hormone and glucose concentrations for the corresponding measuring moments (fullness vs GLP-1, fullness vs PYY, hunger vs ghrelin, fullness vs glucose, and fullness vs insulin). To investigate whether the changes in VAS scores were synchronized with, or lag behind or in front of, the changes in hormone and glucose concentrations, first it was determined whether the peak or nadir values were synchronized to execute comparisons in the physiologically relevant directions. For instance, increases in GLP-1 and PYY concentrations after a meal coincide with the increases in fullness. Both responses reach a peak and then change direction. It should be avoided to compare a still increasing line with an already decreasing line, thus creating artificial inverse relations. Hence, the analysis was repeated with the VAS scores vs the hormone and glucose concentrations of the physiologically relevant previous (time shift “−1”) and of the following measuring moment (time shift “+1”). The means, 95% CIs for the means, and interquartile ranges (quartiles 1–3) of the observed slopes and $R^2$ values are provided (Table 2). Student’s one-sample $t$ tests were used to test whether the means of the regression slopes were different from zero.

For the staggered meal condition, the analysis was repeated to assess the strength of the within-subject relations between the cumulative energy intake and the changes in hormone and glucose concentrations. Paired Student’s $t$ tests were used to test

<table>
<thead>
<tr>
<th>Time shift</th>
<th>Slope</th>
<th>95% CI</th>
<th>IQR</th>
<th>$R^2$</th>
<th>95% CI</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fullness vs GLP-1 staggered</td>
<td>0</td>
<td>$12.6 \pm 1.6^{a,b}$</td>
<td>9.5, 15.7</td>
<td>$6.3, 15.2$</td>
<td>$0.5 \pm 0.05$</td>
<td>0.4, 0.6</td>
</tr>
<tr>
<td>Fullness vs GLP-1 nonstaggered</td>
<td>0</td>
<td>$9.8 \pm 1.2^{a}$</td>
<td>7.5, 12.1</td>
<td>$5.3, 12.2$</td>
<td>$0.6 \pm 0.04$</td>
<td>0.5, 0.7</td>
</tr>
<tr>
<td>Fullness vs PYY staggered</td>
<td>0</td>
<td>$0.9 \pm 0.2^{a}$</td>
<td>0.5, 1.3</td>
<td>$0.6, 1.4$</td>
<td>$0.5 \pm 0.05$</td>
<td>0.4, 0.6</td>
</tr>
<tr>
<td>Fullness vs PYY nonstaggered</td>
<td>0</td>
<td>$1.2 \pm 0.1^{a}$</td>
<td>0.9, 1.4</td>
<td>$0.6, 1.6$</td>
<td>$0.6 \pm 0.04$</td>
<td>0.5, 0.7</td>
</tr>
<tr>
<td>Hunger vs ghrelin staggered</td>
<td>0</td>
<td>$0.9 \pm 0.1^{a}$</td>
<td>0.7, 1.1</td>
<td>$0.5, 1.2$</td>
<td>$0.5 \pm 0.04$</td>
<td>0.4, 0.5</td>
</tr>
<tr>
<td>Hunger vs ghrelin nonstaggered</td>
<td>0</td>
<td>$0.9 \pm 0.1^{a}$</td>
<td>0.6, 1.1</td>
<td>$0.4, 1.4$</td>
<td>$0.4 \pm 0.04$</td>
<td>0.3, 0.5</td>
</tr>
<tr>
<td>Hunger vs ghrelin staggered</td>
<td>+1</td>
<td>$1.2 \pm 0.1^{a,b}$</td>
<td>1.0, 1.4</td>
<td>$0.7, 1.4$</td>
<td>$0.7 \pm 0.04^a$</td>
<td>0.6, 0.8</td>
</tr>
<tr>
<td>Hunger vs ghrelin nonstaggered</td>
<td>+1</td>
<td>$2.2 \pm 0.3^{a,b}$</td>
<td>1.7, 2.8</td>
<td>$1.2, 3.0$</td>
<td>$0.7 \pm 0.05^a$</td>
<td>0.6, 0.8</td>
</tr>
<tr>
<td>Fullness vs glucose staggered</td>
<td>0</td>
<td>$6.1 \pm 3.4^{a}$</td>
<td>$−0.9, 13.1$</td>
<td>$−3.6, 20.0$</td>
<td>$0.3 \pm 0.04$</td>
<td>0.2, 0.3</td>
</tr>
<tr>
<td>Fullness vs glucose nonstaggered</td>
<td>0</td>
<td>$15.6 \pm 2.0^{a}$</td>
<td>11.6, 19.6</td>
<td>$7.3, 22.7$</td>
<td>$0.4 \pm 0.1$</td>
<td>0.3, 0.5</td>
</tr>
<tr>
<td>Fullness vs insulin staggered</td>
<td>0</td>
<td>$0.6 \pm 0.1^{a}$</td>
<td>0.4, 0.7</td>
<td>$0.2, 0.7$</td>
<td>$0.5 \pm 0.1$</td>
<td>0.4, 0.6</td>
</tr>
<tr>
<td>Fullness vs insulin nonstaggered</td>
<td>0</td>
<td>$0.5 \pm 0.04^{a}$</td>
<td>0.4, 0.6</td>
<td>$0.3, 0.7$</td>
<td>$0.7 \pm 0.03$</td>
<td>0.6, 0.7</td>
</tr>
<tr>
<td>Ghrelin vs insulin staggered</td>
<td>0</td>
<td>$−0.5 \pm 0.1^{a,b}$</td>
<td>$−0.6, −0.3$</td>
<td>$−0.6, −0.1$</td>
<td>$0.5 \pm 0.04^a$</td>
<td>0.4, 0.6</td>
</tr>
<tr>
<td>Ghrelin vs insulin nonstaggered</td>
<td>0</td>
<td>$−0.1 \pm 0.04^{a}$</td>
<td>$−0.2, −0.1$</td>
<td>$0.2, −0.03$</td>
<td>$0.3 \pm 0.04$</td>
<td>0.2, 0.3</td>
</tr>
<tr>
<td>Ghrelin vs insulin staggered</td>
<td>−1</td>
<td>$−0.5 \pm 0.1^{a,b}$</td>
<td>$−0.7, −0.3$</td>
<td>$−0.6, −0.2$</td>
<td>$0.6 \pm 0.04^a$</td>
<td>0.5, 0.6</td>
</tr>
<tr>
<td>Ghrelin vs insulin nonstaggered</td>
<td>−1</td>
<td>$−0.2 \pm 0.05^{a}$</td>
<td>$−0.3, −0.1$</td>
<td>$−0.3, −0.1$</td>
<td>$0.6 \pm 0.05^a$</td>
<td>0.5, 0.7</td>
</tr>
</tbody>
</table>

1 $n = 38$. GLP-1, glucagon-like peptide 1; PYY, peptide YY. *Mean of the regression slopes significantly different from zero, $P < 0.005$ (Student’s one-sample $t$ test). $^a$Significantly different from time shift “0”, $P < 0.005$ (paired Student’s $t$ test). $^b$Significantly different from nonstaggered, $P < 0.005$ (paired Student’s $t$ test).

2 Time shift “+1”: analysis of visual analog scale fullness scores vs ghrelin concentrations of the following measuring moment; Time shift “−1”: analysis of ghrelin concentrations vs insulin concentrations of the previous measuring moment.

3 Quartiles 1–3.

4 Mean ± SEM (all such values).
whether the observed slopes and $R^2$ values differed between the synchronized state (time shift “0”) and the relevant time shifts (“+1” and/or “−1”) and between the staggered and non-staggered meal conditions. All tests were 2-sided, and differences were considered significant at $P < 0.005$. Values are expressed as means ± SEMs unless stated otherwise.

RESULTS

Comparison of the staggered and nonstaggered meal conditions showed that there were no major physiologically relevant differences for the regression slopes and $R^2$ values between the conditions (Table 2).

Fullness scores and GLP-1 concentrations over time

In the staggered and nonstaggered meal conditions, both VAS fullness scores and GLP-1 concentrations changed in parallel over time, reaching a peak value at the same time point (Figure 1, A and B). Because the responses peak at the same time point, only the synchronous comparison is physiologically relevant. In the staggered and nonstaggered meal conditions, VAS fullness scores changed synchronously with GLP-1 concentrations (Table 2; Figure 1, A and B).

Fullness scores and PYY concentrations over time

In the staggered and nonstaggered meal conditions, both VAS fullness scores and PYY concentrations changed in parallel over time, reaching a peak value at the same time point (Figure 2, A and B). Because the responses peak at the same time point, only the synchronous comparison is physiologically relevant. In the staggered and nonstaggered meal conditions, VAS fullness scores changed synchronously with PYY concentrations (Table 2; Figure 2, A and B).

Hunger scores and ghrelin concentrations over time

In the staggered and nonstaggered meal conditions, both VAS hunger scores and ghrelin concentrations changed in parallel over time; however, they did not reach a peak/nadir value at the same time point (Figure 3, A and C). After applying a time shift “+1”, the peak/nadir values were synchronized, which resulted in a stronger relation (time shift “0” vs time shift “+1” $P < 0.005$; Table 2; Figure 3, B and D). This time shift indicated that the ghrelin concentrations lagged behind the VAS hunger scores by ∼10–25 min.

Fullness scores and glucose concentrations over time

In the staggered meal condition, the peak values of the response in VAS fullness scores and glucose concentrations were not synchronized because of an early peak in glucose (Figure 4A), pointing to glucose utilization (29). Therefore, the relation between VAS fullness scores and glucose concentrations before and after the glucose peak was assessed separately. From time point −5 min until time point 60 min (glucose), the synchronized relation between VAS fullness scores and glucose concentrations was positive (regression slope: 19.6 ± 4.8; $R^2 = 0.6 ± 0.06; P < 0.001$), whereas from time point 60 min until time point 105 min (glucose) the synchronized relation became negative (regression slope: −14.2 ± 3.3; $R^2 = 0.4 ± 0.05; P < 0.001$). This implies that first VAS fullness scores increase related to increased glucose concentrations and that later VAS fullness scores increase related to glucose utilization, which is an energy-generating process.

In the nonstaggered meal condition, both VAS fullness scores and glucose concentrations changed in parallel over time, reaching a peak value at the same time point (Figure 4B). Because the responses peak at the same time point, only the synchronous comparison is physiologically relevant. In the nonstaggered meal condition, VAS fullness scores changed synchronously with glucose concentrations (Table 2, Figure 4B).

Fullness scores and insulin concentrations over time

In the staggered and nonstaggered meal conditions, both VAS fullness scores and insulin concentrations changed in parallel over time, reaching a peak value at the same time point (Figure 5, A and B). Since the responses peak at the same time point, only the synchronous comparison is physiologically relevant. In the staggered and nonstaggered meal conditions, VAS fullness scores changed synchronously with insulin concentrations (Table 2, Figure 5B).
scores changed synchronously with insulin concentrations (Table 2; Figure 5, A and B).

Ghrelin and insulin concentrations over time

Regarding the ghrelin response, we showed that meal consumption induced a delayed ghrelin suppressive response by 10–25 min. Several studies showed that insulin is required for postprandial ghrelin suppression (30–32). Moreover, Solomon et al (28) documented that there is a delay (~20 min) between responses of insulin and ghrelin, insulin leading ghrelin. Therefore, we analyzed the strength of the within-subject relation between ghrelin and insulin concentrations using the same statistics as those used for the analyses of the strength of the within-subject relation between VAS appetite scores and hormone and glucose concentrations.

In the staggered and nonstaggered meal conditions, both ghrelin and insulin concentrations changed in parallel, although inversely, over time; however, they did not reach a peak and nadir value at the same time point (Figure 6, A and C). By applying a time shift (~1”), the peak and nadir values were synchronized, which resulted in a stronger inverse relation ($P < 0.001$, Table 2; Figure 6, B and D). This time shift indicates that the ghrelin concentrations lagged behind the insulin concentrations by ~15–30 min. The synchronized relation between ghrelin and insulin concentrations was stronger in the staggered than in the nonstaggered meal condition ($P < 0.001$, Table 2).

Cumulative energy intake and hormone and glucose concentrations over time

In the staggered meal condition, analyses of the within-subject relations between the cumulative energy intake over the 4 courses (1.0 ± 0.03, 2.5 ± 0.07, 4.0 ± 0.1, and 5.0 ± 0.1 MJ) and the changes in hormone and glucose concentrations (time points 30, 60, 90, and 120 min) showed significant correlations ($P < 0.005$) with $R^2$ values of 0.3 to 0.8 (GLP-1: $R^2 = 0.8 ± 0.03$, $P < 0.001$; PYY: $R^2 = 0.7 ± 0.04$, $P < 0.001$; ghrelin: $R^2 = 0.8 ± 0.04$, $P < 0.001$; and insulin: $R^2 = 0.6 ± 0.05$, $P < 0.001$).

FIGURE 2. Mean (±SEM) plasma peptide peptide tyrosine-tyrosine (PYY) concentrations and visual analog scale (VAS) fullness scores ($n = 38$; synchronized) in the staggered (A) and nonstaggered (B) meal conditions.

DISCUSSION

The objective of our study was to compare the dynamics of VAS fullness scores and GLP-1, PYY, glucose, and insulin concentrations and of VAS hunger scores and ghrelin concentrations by using a statistical approach that includes the factor time. Moreover, we investigated whether the VAS scores are synchronized with, or lag behind or in front of, hormone and glucose concentrations. The study design consisted of 2 different meal patterns (staggered and nonstaggered) and thereby different timings of nutrient delivery to the gut.

Analyses of regression slopes and $R^2$ values showed that VAS appetite scores and hormone and glucose concentrations changed synchronously and that the mean explained variation was ~70% for fullness vs insulin, ~60% for fullness vs GLP-1 and PYY, ~50% for hunger vs ghrelin, and ~40% for fullness vs glucose. The question remains whether this explained variation of 40% to 70% is sufficient to presume that the hormone and glucose concentrations may serve as a reliable biomarker for appetite at the individual level. A biomarker is in general a substance that can provide reliable early indicators of a biological state (33). For a biomarker of appetite to be useful, it must meet certain criteria: the measurement of the biomarker must be feasible, measurable without invasive procedures, and reproducible under similar conditions; moreover, the biomarker must clearly relate to appetite physiology and be sensitive to changes in appetite (33). Taking the CIs and interquartile ranges into account, we suggest that the explained variation of 40% to 70% may be insufficient. However, the hormone and glucose dynamics, in relation to feelings of appetite may be useful to determine differences between experimental conditions or between different subject groups; yet, this still needs to be executed for each experiment one undertakes as well, to be able to judge whether in such a case certain changes in hormone concentrations may explain certain changes in appetite related feelings, up to a certain degree.

Overall, it appeared that VAS fullness scores changed synchronously with GLP-1, PYY, glucose, and insulin concentrations. In contrast, the changes in ghrelin concentrations lagged behind the changes in VAS hunger scores, with a delay of 10 min in the staggered meal condition and of 25 min in the nonstaggered
meal condition. This agrees with the study of Frecka and Mattes (34), which reported that the association between ghrelin and hunger was strongest when ghrelin lagged behind hunger by 30 min. This pattern is inconsistent with ghrelin causing the hunger rise, and Frecka and Mattes suggest that ghrelin concentrations rise in anticipation of eating rather than eliciting feeding.

**FIGURE 3.** Mean (±SEM) plasma ghrelin concentrations and visual analog scale (VAS) hunger scores (n = 38) in the staggered [synchronized (A) and time shift “+1” (B)] and nonstaggered [synchronized (C) and time shift “+1” (D)] meal conditions.

**FIGURE 4.** Mean (±SEM) plasma glucose concentrations and visual analog scale (VAS) fullness scores (n = 38; synchronized) in the staggered (A) and nonstaggered (B) meal conditions.
Meal consumption induced a delayed ghrelin suppressive response of 10 to 25 min. Prandial ghrelin suppression does not require luminal nutrient exposure in the stomach or duodenum—the principle sites of ghrelin production (35, 36). Instead, signals mediating this response originate further downstream in the intestine and from postabsorptive events (37). Several studies

FIGURE 5. Mean (±SEM) plasma insulin concentrations and visual analog scale (VAS) fullness scores (n = 38; synchronized) in the staggered (A) and nonstaggered (B) meal conditions.

FIGURE 6. Mean (±SEM) plasma insulin and ghrelin concentrations (n = 38) in the staggered [synchronized (A) and time shift “−1” (B)] and nonstaggered [synchronized (C) and time shift “−1” (D)] meal conditions.
showed that insulin may be required for postprandial ghrelin suppression (30–32, 34). Frecka and Mattes (34) showed that peak insulin concentrations preceded nadir ghrelin concentrations. Moreover, Solomon et al (28) reported that there is a delay of ~20 min between responses of insulin and ghrelin, insulin leading ghrelin. Our study confirmed the findings by Solomon et al (28): changes in ghrelin concentrations lagged behind the changes in insulin concentrations by ~15–30 min in the staggered meal condition and by ~30 min in the non-staggered meal condition. This may suggest a role for insulin as a possible negative regulator of ghrelin (38).

In the staggered meal condition, the analysis of the within-subject relation between the changes in glucose concentrations and VAS fullness ratings showed no significant synchronized relation. This may be explained by the phenomenon that, after consumption of the second course (time point: 60 min), glucose concentrations decreased because of glucose utilization (oxidation and storage) (29), whereas VAS fullness ratings and energy intake still increased. Therefore, the relation was analyzed in 2 parts. The phenomenon that the first part showed a stronger relation than the second part may have been due to its immediate contribution to glucose homeostasis (29, 39).

We were aware that, in the staggered meal condition, nutrient delivery was not evenly distributed among each eating occasion, which was taken into account to interpret the fluctuations in the hormone and glucose concentrations within that pattern. The analyses showed strong correlations between the cumulative energy intake over the 4 courses and the changes in hormone concentrations. These relatively high correlations indicate that the hormone releases are directly nutrient related.

Comparison of the staggered and nonstaggered meal conditions showed that there were no major physiologically relevant differences regarding the measured regression slopes and $R^2$ values. Thus, meal pattern had no major effect on the relation between VAS scores and hormone and glucose concentrations. With respect to the effect of meal pattern on hormone and appetite dynamics, we showed previously that the changes in appetite profile scores and in hormonal release were less pronounced in the staggered than in the nonstaggered meal condition (40).

In conclusion, the explained variation in the development of hunger and fullness scores over time by the development of GLP-1, PYY, ghrelin, glucose, and insulin concentrations over time was 40–70%. We suggest that this value was too small to use the changes in hormone and glucose concentrations as biomarkers for feelings of appetite at the individual level and probably at the group level. However, this approach appears to be a useful tool for characterizing the processes of hunger and fullness between changes. Changes in GLP-1, PYY, glucose, and insulin concentrations ran parallel to the changes in VAS fullness scores. Changes in ghrelin concentrations lagged behind the changes in VAS hunger scores and insulin concentrations, which probably suggests a role for insulin as a possible negative regulator of ghrelin.

We thank our subjects for their participation in this study. The authors’ responsibilities were as follows—MSW-P and SGL: designed the study; SGL and EAM: carried out the study and collected the data; SGL: analyzed the data and wrote the largest part of the manuscript; EAM and MSW-P: reviewed the manuscript; MSW-P: supervised the conduction of the study and the data collection; and ADK: supervised the data analysis. No conflicts of interest were reported.

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