Effect of age and frailty on ghrelin and cholecystokinin responses to a meal test1–3

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ABSTRACT

Background: Ghrelin and cholecystokinin (CCK) are among the peripheral signals that regulate hunger and satiety.

Objective: The objective was to assess whether ghrelin and CCK responses to a standard nutritional load are related to age and frailty.

Design: Ghrelin, CCK, insulin, glucose, and 4-h visual analog hunger scale curves after a standard nutritional load test (380 kcal) were described and compared between 3 groups: old (>75 y) and frail persons (group A), old (>75 y) but nonfrail persons (group B), and young (25–65 y) adults (group C).

Results: Frail persons showed no postprandial ghrelin suppression, and old subjects, frail and nonfrail, showed no significant postprandial ghrelin recovery compared with young adults. Frailty was also associated with lower fasting ghrelin concentrations. No differences in fasting CCK were observed between young and old persons; however, postprandial CCK concentrations were enhanced in young persons, whereas no frailty effect on the CCK curve was observed in the old subjects. No correlations between mean ghrelin and hunger values over time were found, but strong negative correlations were shown between CCK and hunger (group A: \( r_s = -0.88, P = 0.009 \); group B: \( r_s = -0.86, P = 0.014 \); group C: \( r_s = -0.71, P = 0.071 \)) and insulin and hunger (group A: \( r_s = -0.901, P = 0.006 \); group B: \( r_s = -0.964, P < 0.001 \); group C: \( r_s = -0.929, P = 0.003 \)).

Conclusions: Advanced age determines a poorer ghrelin postprandial recuperation phase, a reduced CCK postprandial response, and an exaggerated postprandial insulin release. A loss of ghrelin prandial rhythm is present in old frail persons. The impaired response of these hunger regulatory hormones with age might contribute to the mechanisms of anorexia associated with aging. Am J Clin Nutr 2009;89:1410–7.

INTRODUCTION

Ghrelin is an orexigenic hormone secreted mainly in the stomach, which stimulates appetite and food intake and plays an important role in regulating the energy homeostasis of the organism (1, 2). Causes of anorexia of aging are not well known, but decreases in ghrelin concentration with age have been suggested as one of the possible reasons for reduced appetite in the elderly (3). However, only a slight decrease in fasting concentrations of this hormone in persons aged >70 y has been described, and its physiologic relevance remains unclear (4). It seems that the regulation of energy homeostasis through ghrelin is preserved until advanced ages, because different studies have shown greater ghrelin concentrations in thin and malnourished older persons than in well-nourished ones (5, 6). This dynamic mechanism of regulation responds to several stimuli related to energy stocks and nutritional status [eg, weight or body mass index (BMI)], ingestion, and other unknown factors through complex feedback processes. For this reason, it is difficult to interpret a single determination of fasting ghrelin. It would be interesting to determine how ghrelin concentrations respond to a standard stimulus, such as food intake, in a dynamic manner; thus, an inadequate response could indicate an alteration in this regulatory mechanism.

Cholecystokinin (CCK) is another peripheral signal that regulates appetite. This intestinal hormone is released by the proximal small intestine in response to the delivery of nutrients from the antrum, particularly of lipids and proteins (7). CCK reduces gastric emptying and also stimulates postprandial satiety at the hypothalamic level, so it can be considered as an orexigenic hormone. Some studies have shown higher concentrations of CCK in the elderly than in young persons (8, 9) and higher concentrations in malnourished than in well-nourished old persons (5). This is why CCK has been suggested among the major causes of anorexia of aging and is thought to play an important role in the development of malnutrition at advanced ages.

On the other hand, frailty has been described as a syndrome of decreased resilience and reserves, in which a mutually exacerbating cycle of functional decline across multiple systems results in negative energy balance, sarcopenia, and diminished strength and tolerance for exertion (10, 11). Independently of age, frailty might be related to an alteration in the ghrelin-based energy balance regulatory mechanism and with CCK response to in-
The aim of this study was to assess whether ghrelin and CCK responses to a standard nutritional load were related to age and frailty.

SUBJECTS AND METHODS

Study design

Three groups of persons were studied: old (>75 y) and frail persons (group A), old (>75 y) but nonfrail persons (group B), and nonobese (index of corporal mass <30) young (25–65 y) adults (group C). All subjects underwent a standard nutritional load test, and ghrelin, CCK, insulin, glucose, and 4-h hunger curves were compared.

Sample selection and recruitment

Persons aged >75 y were recruited from a convalescent home and a long-term care center if they fulfilled all selection criteria, and the young adults were healthy volunteers with no present or habitual treatment recruited among hospital workers. Exclusion criteria were dementia with a Global Deterioration Scale score >4 (moderate-to-severe), total or partial gastrectomy, active neoplasm, or severe dysphagia. Recruitment was done from May to June 2007. The study protocol was approved by the local ethical committee, and all persons signed an informed consent form before inclusion in the study.

Frailty definition

Old persons were considered “frail” if they fulfilled ≥3 of the following 5 criteria (10, 11): 1) weight loss [nonintentional weight loss >4 kg or 5% of weight or weight loss >10% of weight at age 60 y or a BMI (in kg/m²) <19], 2) self-reported exhaustion [low usual energy (≤3 on a visual analog scale; VAS)] or unusually tired or weak in the previous month), 3) low muscle strength (<20th percentile with the Jamar hand-held dynamometer: <7 kg in women and <14 kg in men), 4) walking slowness (>7 s when walking 4.5 m if ≤159 cm tall or ≥6 s when walking 4.5 m if >159 cm tall), and 5) poor physical activity (no outdoor life or <0.5 h of outdoor walking daily).

Standard meal test

After fasting overnight for ≥10 h, all subjects were administered a 380-kcal standard breakfast between 0800 and 0900. Two energetically and nutritionally equivalent options were offered, and the subjects chose the one they preferred: 1) 200 mL semiskim milk, 50 g white bread, 15 g vegetal margarine, and 15 g boiled ham, or 2) 100 g liquid yogurt, 30 g toast, 10 g olive oil, and 30 g boiled ham. This standard test meal provided approximately 14 g protein, 21 g lipids, and 32 g carbohydrate. A nurse registered the percentage of the meal ingested; if <70%, the test was considered invalid. Just before ingestion and 0.5, 1, 1.5, 2, 3, and 4 h after ingestion, a blood sample was collected to determine serum ghrelin, CCK, glucose, and insulin concentrations. Eating was not allowed during the 4-h follow-up period after the standard breakfast.

Biochemical determinations

All biochemical measurements were made by using validated commercialized kits. Total plasma ghrelin concentrations were measured with a human radioimmunoassay kit (Linco Research Inc, St Charles, MO). The detection limit was 93 pg/mL, with intra- and interassay CVs of 11.1% and 14.7%, respectively. Plasma CCK-8 (CCK 26–33) concentrations were measured with a validated commercial human radioimmunoassay kit (Euro-Diagnostica, Malmö, Sweden). The antiserum of this method was previously shown to have very low cross-reactivity toward gastrin-17 and sulfated gastrin (12). In addition, it binds all biological active forms of CCK, which share an identical sequence of the C-terminal octapeptide, with equimolar potency. The assay system shows a very high sensitivity, with a detection of 0.1 pmol/L and intra- and interassay CVs of 7.2% and 13.7%, respectively. Plasma insulin concentrations were measured by chemiluminescence (Immulite 2000 DPC; Siemens, Madrid, Spain) with the use of sheep and mouse anti-insulin antibodies. Serum glucose was measured with a commercially available test kit (Gluco-quant enzymatic Hexokinase; Roche Diagnostics GmbH, Mannheim, Germany) on a Hitachi 917 automatic analyzer (Hitachi Instrument Division, Ibaraki-ken, Japan), with intraassay CVs ranging from 0.7% to 0.9%, depending on the concentration.

Main outcome measures

Serum concentrations at the 7 time points previously defined were determined to calculate ghrelin, CCK, insulin, and glucose curves over a 4-h follow-up period. The following variables were defined to characterize these curves in each subject: fasting concentrations, ranges (maximum – minimum concentration), and the areas under the curve (AUCs) in the first, second, third, and fourth hours after eating. Incremental areas under or over the baseline value were calculated and the term AUC was used to refer to both values, which were delineated as negative AUC or positive AUC, respectively.

Other study variables

Sociodemographic variables and main comorbidities were registered. Hunger was also measured at all of these time points by means of a VAS (VAS-hunger), with scores ranging from 0 (no hunger at all) to 10 (maximum hunger). Nutritional status was assessed with the Mini Nutritional Assessment, and functional capacity with the Barthel score. Weight and height were measured, and BMI was calculated. A bioelectrical impedance analysis was performed in all persons to assess body composition and to measure basal metabolism. Hand grip of the nondominant hand was also measured by means of a hand-held dynamometer (JAMAR model). Creatinine, albumin, lipid (total, HDL, and LDL cholesterol and triglycerides), and C-reactive protein concentrations were also measured.

Statistical analysis

A descriptive analysis of the main characteristics of the study sample was initially performed by using means and SDs for continuous variables and percentages for categorical variables. For each of the study groups, a description of the specified ghrelin, CCK, and insulin curve was done. To assess the effect of
age on these variables, groups A and B were added to form an “old group” and compared with group C, or the “young group.” To assess the effect of frailty, group A was compared with group B. Incremental AUC with respect to the hour 0 value for the first, second, third, and fourth hours were calculated for ghrelin, CCK, insulin, glucose, and VAS-hunger. The chi-square test or Fisher’s exact test were used to compare proportions between groups. Kolmogorov-Smirnov (Lilliefors correction) and Shapiro-Wilk tests were used to test normality and, because most variables were not normally distributed, nonparametric tests were used. The Mann-Whitney U test or the Kruskal-Wallis test were used to compare medians between 2 groups or 3 groups, respectively, and to compare variables at different time points in the same groups in which Wilcoxon’s test (2 samples) or Friedman’s test (k samples) was used. However, basal ghrelin in the overall sample satisfied parametric assumptions, and a multiple linear regression was used to adjust the effect of age (young compared with old) or frailty for sex, age, and BMI when fasting ghrelin was considered the dependent variable. To compare ghrelin, CCK, insulin, glucose, and VAS-hunger curves between study groups, a repeated-measures multivariate analysis of variance was used. Although it is a parametric test, repeated-measures analysis of variance is robust to violations of normality and homogeneity of covariance assumptions. To assess the relations between ghrelin, CCK, insulin, and glucose with VAS-hunger, correlations estimated. To assess the increment effect of CCK, the increase in CCK from hour 0 to hour 0.5 was correlated with the increase in insulin concentration from hour 0 to hour 0.5. Spearman correlation coefficients (r_s) were used in all correlation analyses. Statistical significance was considered at a P value <0.05. Statistical analysis was performed with SPSS software (version 11.5; Statistical Product and Service Solutions, Ibérica SL, Madrid, Spain).

RESULTS

Forty-two subjects were recruited into the study: 15 in group A, 10 in group B, and 17 in group C. The main characteristics of the 3 study groups are presented and compared in Table 1, which shows poorer nutritional status, less muscle mass, and worse functional capacity in old frail persons than in nonfrail old subjects. No differences between frail and nonfrail old subjects were observed for the main comorbidities, except depression.

Ghrelin response

Ghrelin curves and AUCs are presented in Figure 1. In young persons, ghrelin concentrations showed a significant reduction during the first postprandial 1.5 h (950.4 pg/mL in hour 0 compared with 848.5 pg/mL in hour 1.5; P = 0.028) and then an important and significant recovery until the fourth hour of follow-up (848.5 pg/mL in hour 1.5 compared with 1192.0 pg/mL in hour 4; P < 0.001). The AUC showed an inhibitory response phase during the first 2 h and a positive recuperation phase after this period. Nonfrail persons showed a significant inhibitory response phase (1074 pg/mL in hour 0 compared with 917 pg/mL in hour 1.5; P = 0.017) but not a significant recuperation phase (917 pg/mL in hour 1.5 compared with 948.9 pg/mL in hour 4; P = 0.445), whereas frail persons showed no inhibitory response phase (734.6 pg/mL in hour 0 compared with 697.6 pg/mL in

### Table 1
Comparison of main characteristics of study groups

<table>
<thead>
<tr>
<th>Group A: old, frail (n = 15)</th>
<th>Group B: old, nonfrail (n = 10)</th>
<th>P (old, frail compared with old, nonfrail)</th>
<th>Group C: young adults (n = 17)</th>
<th>P^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>83.0 ± 7.3</td>
<td>80.01 ± 8.4</td>
<td>0.405</td>
<td>39.7 ± 9.8</td>
</tr>
<tr>
<td>Female sex [n (%)]</td>
<td>10 (66.7)</td>
<td>4 (40.0)</td>
<td>0.241</td>
<td>10 (58.8)</td>
</tr>
<tr>
<td>Weight loss/low weight [n (%)]</td>
<td>3 (21.4)</td>
<td>1 (10.0)</td>
<td>0.615</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Fatigue [n (%)]</td>
<td>13 (86.7)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Hand grip &lt; first quintile [n (%)]</td>
<td>3 (20.0)</td>
<td>1 (10.0)</td>
<td>0.626</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Slow walking speed [n (%)]</td>
<td>15 (100.0)</td>
<td>4 (40.0)</td>
<td>0.001</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Poor physical activity [n (%)]</td>
<td>14 (93.3)</td>
<td>2 (20.0)</td>
<td>&lt;0.001</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>28.7 ± 6.6</td>
<td>26.7 ± 3.0</td>
<td>0.482</td>
<td>25.2 ± 3.3</td>
</tr>
<tr>
<td>Hand grip (kg)</td>
<td>14.2 ± 8.5</td>
<td>15.7 ± 7.3</td>
<td>0.470</td>
<td>33.0 ± 13.6</td>
</tr>
<tr>
<td>Mini Nutritional Assessment [n (%)]</td>
<td>0.041</td>
<td>0.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnourished</td>
<td>3 (21.4)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>8 (57.1)</td>
<td>3 (30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-nourished</td>
<td>3 (21.4)</td>
<td>7 (70.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barthel score</td>
<td>57.0 ± 19.7</td>
<td>90.5 ± 17.4</td>
<td>0.001</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>Fatty mass (%)</td>
<td>36.1 ± 9.4</td>
<td>35.4 ± 5.6</td>
<td>0.815</td>
<td>26.2 ± 5.6</td>
</tr>
<tr>
<td>Muscular mass (%)</td>
<td>32.0 ± 6.6</td>
<td>37.8 ± 5.9</td>
<td>0.042</td>
<td>49.4 ± 5.0</td>
</tr>
<tr>
<td>Basal metabolism (kcal)^2</td>
<td>1245.2 ± 132.5</td>
<td>1351.5 ± 145.0</td>
<td>0.133</td>
<td>1565.4 ± 182.7</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.976 ± 0.40</td>
<td>1.1 ± 0.4</td>
<td>0.402</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.82 ± 0.59</td>
<td>4.3 ± 0.3</td>
<td>0.038</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>163.3 ± 38.4</td>
<td>179.5 ± 43.4</td>
<td>0.292</td>
<td>195.6 ± 32.3</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>0.99 ± 0.88</td>
<td>0.9 ± 1.4</td>
<td>0.129</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

^1 Fisher’s exact test was used to compare proportions between groups, and the Mann-Whitney U test or the Kruskal-Wallis test was used to compare means between 2 groups or 3 groups, respectively.

^2 Mean ± SD (all such values).

^3 Basal metabolism was estimated by bioelectrical impedance.
hour 2; $P = 0.394$) but a small recuperation phase ($697.6 \text{ pg/mL in hour 2 compared with 798.4 pg/mL in hour 4}; P = 0.027$) (Figure 1). No significant differences were observed between young and old individuals for fasting ghrelin concentrations, the overall ghrelin curve, and AUCs in the first and second postprandial hours. However, young subjects showed higher ghrelin concentrations and higher AUCs in the third and fourth hours than did old persons, which indicated a higher ghrelin release in the recuperation phase. Frail old persons had lower fasting ghrelin concentrations and lower ghrelin ranges than did nonfrail old persons, but no statistically significant differences were observed in the overall ghrelin curves in a comparison of these 2 groups. When the effect of frailty over fasting ghrelin was adjusted by age, sex, and BMI in old subjects, a $b$ coefficient of $433.0 (P = 0.027)$ was shown. Similarly, after adjustment for the effect of age (young compared with old) by sex and BMI, no significant effect of young compared with old was observed for fasting ghrelin, but significant effects were observed for ghrelin ranges, with a $b$ coefficient of $136.2 (P = 0.043)$. In all 3 study groups, basal ghrelin concentrations strongly correlated with all other ghrelin measures (from hour 0.5 to hour 4), which indicated that persons with high basal concentrations also had high ghrelin concentrations throughout the curve.

**CCK response**

CCK curves and AUCs are presented in Figure 2. In young adults, CCK showed a significant 3-fold increase in the first 0.5 h after ingestion, and these values remained high for the following 3 h and thereafter decreased sharply. In old persons, CCK showed a 2-fold increase 0.5 h after food intake and then showed a progressive decrease. Fasting CCK concentrations were similar between all 3 groups, but CCK concentrations increased more sharply in young than in old adults 0.5 h after ingestion ($1.10 \text{ pmol/L increase in the old group compared with a 2.17 pmol/L increase in the young group}; P = 0.007$). Significant differences were observed in the overall CCK curve between young and old persons ($P = 0.002$). The CCK concentration was significantly higher in the young group during the first 3 h of follow-up, but differences disappeared again 4 h after ingestion. No relevant differences were observed in the CCK curves between frail and nonfrail old persons. Mean ($\pm$SD) increases in CCK concentrations during the first 0.5 h after ingestion were $1.12 \pm 1.06 \text{ pmol/L in old frail subjects, 1.08 \pm 1.22 pmol/L in old nonfrail subjects, and 2.17 \pm 1.23 pmol/L in young adults} (P = 0.025$).

**Insulin and glucose responses**

The curves and AUCs for insulin and glucose during the 4-h test follow-up are shown in Figure 3. Significant differences between study groups were observed in the overall insulin and glucose curves. In young adults, the insulin concentration increased 3-fold 1 h postprandially and then decreased progressively until normalization 3 h after the meal. Frail persons showed a 5-fold increase and nonfrail old persons showed
a 9-fold increase in insulin concentrations 1 h after ingestion. Differences in insulin responses between young and old adults were highly significant. The mean (±SD) increases in insulin concentrations during the first 0.5 h after ingestion were 36.1 ± 23.2 mU/L in group A, 51.3 ± 25.9 mU/L in group B, and 24.2 ± 17.9 mU/L in group C (P = 0.024). The increases in CCK from hour 0 to hour 0.5 was not correlated with the increase in insulin from hour 0 to hour 0.5 in any group. In young adults, glucose concentrations varied less over time, but in the old groups it increased by nearly 60% 1 h after ingestion. Highly significant differences in glucose curves were observed between young and old persons during the first 2 h. On the contrary, differences in glucose curves between frail and nonfrail persons were observed after the first 2 h of ingestion, when the frail subjects had higher serum glucose concentrations. Old persons had higher insulin responses to ingestion and higher glucose concentrations than did young adults, which indicated a state of insulin resistance.

Hunger scores and related factors

Hunger curves and AUCs are presented in Figure 4. Hunger after a an overnight fast (hour 0) was higher in young than in old persons (VAS-hunger: 7.3 compared with 3.1; P < 0.001). The hunger score decreased 0.5 h after the ingestion of 380 kcal and then gradually recovered. The young persons also experienced

FIGURE 2. Mean (±SEM) cholecystokinin (CCK) responses during the 4 h after a 380-kcal breakfast in the study groups. “&” indicates repeated-measures multivariate ANOVA. AUC, area under the curve. *Significant difference between the young and old (frail + nonfrail) groups, P < 0.01 (Mann-Whitney U test); no significant differences were observed between the frail and nonfrail old persons (Mann-Whitney U test). n = 17 for the young group, 15 for the frail old group, and 10 for the nonfrail old group.

FIGURE 3. Mean (±SEM) glucose and insulin responses during the 4 h after a 380-kcal breakfast in the study groups. “&” indicates repeated-measures multivariate ANOVA. AUC, area under the curve. *Significant difference between the young and old (frail + nonfrail) groups, P < 0.01 (Mann-Whitney U test); no significant differences were observed between the frail and nonfrail old persons (Mann-Whitney U test). n = 17 for the young group, 15 for the frail old group, and 10 for the nonfrail old group.
an early and greater recovery in hunger than did the old persons: VAS scores of 2.2 compared with 1.1 (\( P = 0.026 \)) 1 h after ingestion and of 3.8 compared with 1.8 (\( P = 0.020 \)) 3 h after ingestion, respectively. Overall VAS-hunger curves between groups were nearly statistically significant (\( P = 0.068 \)). No relevant differences in hunger were observed between frail and nonfrail old persons. AUCs of VAS-hunger were significantly different between young and old adults, but no differences were observed between frail and nonfrail old persons. In a cross-sectional analysis at hour 0, fasting ghrelin concentrations were not related to VAS-hunger in any group and were correlated with BMI only in the young group (\( r = 0.44, P = 0.076 \)). Similarly, fasting CCK, insulin, and glucose were not correlated with VAS-hunger at hour 0 in any group. No correlations between mean ghrelin and hunger values between the hour 0 and hour 4 time points were observed, but strong negative correlations between CCK and hunger were observed in all groups (group A: \( r_s = -0.88, P = 0.009 \); group B: \( r_s = -0.86, P = 0.014 \); group C: \( r_s = -0.71, P = 0.071 \), insulin and hunger (group A: \( r_s = -0.901, P = 0.006 \); group B: \( r_s = -0.964, P < 0.001 \); group C: \( r_s = -0.929, P = 0.003 \), and glucose and hunger (group A: \( r_s = -0.847, P = 0.016 \); group B: \( r_s = -0.786, P < 0.036 \); group C: \( r_s = -0.679, P = 0.094 \)).

**DISCUSSION**

The main findings of the present study were that 1) frail old persons experienced no postprandial suppression of ghrelin, which indicated a loss of postprandial ghrelin rhythm; 2) nonfrail old persons experienced early postprandial ghrelin suppression without recovery at 4 h; 3) frail persons had a lower fasting ghrelin concentration than did nonfrail persons; 4) young individuals experienced ghrelin suppression 90 min postprandially and had a remarkable recovery thereafter until 4 h, in contrast with the old persons, who had no recovery; 5) no differences in fasting CCK concentrations were observed between young and old persons; 6) old persons experienced a lower postprandial CCK increase than did the young persons; and 7) young persons had higher fasting hunger scores. These results provide new evidence concerning the response of peripheral signals that control hunger and may contribute to the understanding of the mechanisms of anorexia of aging. It is known that the central regulation of appetite and satiety is influenced by several peripheral signals that stimulate \( \geq 2 \) main hypothalamic neural populations—those of the neuropeptide Y (NPY) and agouti-related peptide (AgRP) system, which stimulates hunger and inhibits satiety, and those of the proopiomelanocotin (POMC) network, which has the opposite effect (13). Ghrelin, produced mainly in the stomach, stimulates NPY neurons and is the only known orexigenic peripheral signal. All other peripheral short-term (CCK and peptide YY) and long-term (leptin and insulin) signals inhibit hunger and produce satiety. It has been postulated that increases in CCK concentrations and decreases in ghrelin concentrations with age may be major causes of anorexia of aging (14). The results of the present study do not fully agree with these hypotheses.

We found that age and frailty can affect the postprandial ghrelin response. In a similar study, Di Francesco et al (15) found no differences in fasting or postprandial ghrelin concentrations between the young and old groups. In contrast, we found strong late postprandial ghrelin recovery in the young group. This discrepancy might have been due to the younger and healthy study sample and the use of a higher caloric test meal (800 kcal) in the study by Di Francesco et al. Sturm et al (5) showed no differences between well-nourished old and young women in the 90-min ghrelin curve after a preload of 143 g vanilla ice cream. These results do not disagree with those of the present study, because differences in ghrelin curves are shown for 90 min after the meal, when ghrelin initiates its recovery. Similar postprandial ghrelin curves were shown in young volunteers by Blom et al (12), who showed a reduction of \( \approx 20\% \) during the first 2 h after ingestion, after which a recovery ensued. In a recent study, in which the acylated (active) ghrelin response to a meal was compared between young and elderly subjects Di Francesco et al (16) found similar and parallel postprandial acylated ghrelin profiles than those found in our study, i.e., a flat curve in elderly subjects and a significant recovery phase in the young group. Although acylated ghrelin is the only ghrelin form with orexigenic and metabolic effects, we do not think that the measurement of total ghrelin affected our conclusions. Acylated ghrelin is unstable and is a fraction of total ghrelin, and the active assays require the intact octanoyl group on the third amino acid and the total assays can read the truncated form without octanoyl modification. Des-acyl ghrelin may represent either
a preform of acyl-modified ghrelin or the product of its deacylation. Furthermore, the similarities between total and acylated ghrelin responses in these studies might suggest that the proportion of acylated ghrelin remains constant across the 4-h follow-up study period within groups. Ghrelin is secreted by the empty stomach and its blood concentrations are maximal just before a meal and decrease just after stomach filling. Old persons secreted similar ghrelin concentrations on an empty stomach to young persons. However, some studies have shown that old persons have delayed gastric emptying (17), which may explain the delayed ghrelin recovery observed in the present study and may contribute to the development of anorexia (18). Delayed gastric emptying may also produce prolonged satiety and may play an initial role in the anorexia of aging (9). Moreover, ghrelin also stimulates gastric motility, closing a feedback gastric regulatory mechanism. On the other hand, a frailty effect has been observed in relation to fasting ghrelin and ghrelin inhibition after ingestion. Loss of ghrelin production in frail old persons may also explain the unbalanced energy homeostasis they frequently suffer, with weight loss and sarcopenia. The results of the present study also showed no correlation between ghrelin concentrations and VAS-hunger at different time points, which suggests, as did the study by Sturm et al (5), that ghrelin does not play a major role in regulating short-term hunger or satiety.

Human plasma CCK occurs in multiple molecular forms; the major forms are CCK-58, -33, -22, and -8. The predominant CCK form in human plasma, both in the fasting state and postprandially, is CCK-33—a form that is accurately measured by the radioimmunoassay we used. No differences in fasting CCK were observed between young and old persons, but the CCK response was faster and greater in the young. No frailty effect on the CCK curve was observed. A close relation between CCK and hunger levels at different time points was observed in all 3 study groups, which indicated the short-term satiating effect of this hormone. These results agree with the idea that CCK determines satiety sensation after meals, but contrasts with the higher CCK concentrations in the elderly reported by other authors (5, 9, 19). CCK is mainly produced by the duodenum and jejunum in response to the delivery of nutrients from the antrum (7), and its plasma concentration increases 6-fold 15 min after a meal and lasts ~2 h (20). The main physiologic actions of CCK are postprandial gallbladder contraction and exocrine pancreatic secretion, but it also inhibits gastric motility. Bile concentrations in the duodenum regulate CCK liberation; therefore, high bile concentrations inhibit CCK release (21), and gallbladder emptying capacity seems to be one of the main determinants of postprandial CCK concentrations (22). A study by Di Francesco et al (9) reported that elevated postprandial CCK concentrations might sustain satiety and inhibit hunger in a group of elderly subjects with impaired gallbladder contraction. However, this might not be the case of older subjects with normal gallbladder contraction. Impaired gallbladder emptying (caused by gallstone disease, cholecystectomy, or blockade of bile salts in the duodenum) also increases postprandial CCK responses in young subjects (20–22). In addition, another study using CCK-1 receptor antagonists found that endogenous CCK delays gastric emptying (23). Taken together, the results on CCK release found in the present study and in the other studies (5, 9, 19, 22, 23) suggest that gallbladder and gastric emptying should be carefully monitored in studies assessing the postprandial profiles of these regulatory peptides and their effects on hunger and satiety in elderly patients. Slow gastric emptying and slow delivery of nutrients to the duodenum in the elderly may explain the low postprandial CCK liberation observed in this age group. Per such a hypothesis, abnormalities in gastrointestinal motility would be the origin of anorexia of aging. Although more studies are needed to explore this hypothesis in more detail, it has been recognized that aging is associated with slower gastric emptying of solids and liquids and that these changes may contribute to the development of anorexia, dyspepsia, and early satiety (18). The mechanisms that determine delayed gastric emptying in the elderly are not known, but some studies in animals have observed a significant reduction in neurons in the myenteric plexus with age (24). Finally, CCK has also been considered an incretin candidate, enhancing insulin release, although this insulinotropic effect of CCK is also controversial (20, 23). Some studies found that CCK reduced the increase in circulating glucose after meal ingestion and enhanced the increase in circulating insulin in both healthy persons and in persons with noninsulin dependent diabetes mellitus, which suggests an antidiabetogenic action of CCK (25–27). Data from the present study do not support this effect because young persons with a higher CCK response have a lower insulin response and vice versa in the elderly, and no correlation was observed between the increase in CCK and the increase in insulin in any study group.

Hyperglycemia may be another factor that contributes to the satiating sensation and anorexia in elderly persons (28). An inverse correlation between gastric emptying and plasma glucose concentrations has been described, with delayed emptying in situations of hyperglycemia and rapid emptying in hypoglycemia. Insulin is considered a satiety hormone (14). Insulin may have an indirect anorexigenic effect by enhancing the leptin signal in the hypothalamus (29) and by inhibiting ghrelin gastric expression and central sensitivity (30). The present study showed a strong negative correlation between insulin and hunger that supports this idea. Increased circulating concentrations of insulin associated with aging could be a cause of anorexia in old persons. However, the satiating effect of insulin is also controversial because evidence is limited. A satiating effect has been shown with the administration of high doses of insulin to animals (31), but euglycemic insulin infusions have not shown any effect on appetite and food intake in humans (32). The increased insulinemia in the elderly is due to insulin resistance related to changes in body composition, ie, an increase in fatty mass or adiposity (33). More studies are also needed to discover the role of insulin and changes in body composition in the genesis of anorexia of aging.

Anorexia is very common among elderly persons and has been related to a higher risk of malnutrition, lower muscular strength, and poorer functional capacity (34); therefore, the mechanism that regulates appetite and satiety in the elderly should be studied further. The present study showed some differences in ghrelin, CCK, and insulin responses to a standard test meal between old and young persons. Advanced age is associated with a poorer ghrelin postprandial recovery phase, a poorer CCK postprandial response, and an exaggerated postprandial insulin release. Moreover, a loss of prandial rhythm was observed in frail old persons, which probably aggravates the mechanism of hunger generation.
We thank all persons who collaborated in the study, especially Maria Roca (nutritionist), Georgina Miró (nurse), Laura Domínguez (laboratory), Cristina Mas (secretary), and the volunteers who participated in the study. The authors’ responsibilities were as follows—MS-P: involved in the study design, development of the protocol, coordination of the field work, analysis of the data, and writing of the manuscript; EP: involved in the data collection, the statistical analysis, and review of the manuscript; and PC and MP-D: provided significant advice in the study design and in the writing of the manuscript. All authors approved the final form of this manuscript. None of the authors declared a conflict of interest in relation to the present work.

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