Is ghrelin a signal of decreased fat-free mass in elderly subjects?

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Abstract

Objective: Aging is associated with appetite decline, weight loss, reduced fat-free mass (FFM), and increased fat mass (FM). Ghrelin and leptin are short- and long-term determinants of energy balance respectively, whose dysregulation could alter food intake. We evaluate the relationship of circulating ghrelin and leptin responses to standardized oral mixed nutrient load (SOMNL) with body composition, daily food intake, and insulin sensitivity in healthy elderly subjects (ES).

Design and methods: Twenty-six ES (12/14 M/F, 69 ± 4 years) and ten young healthy controls (LY) (5/5 M/F, 27 ± 3 years) were studied at the International Center for the Assessment of Nutritional Status (Milan, Italy) with air plethysmography, dual energy X-ray absorptiometry, indirect calorimetry, and dietary intake assessment. Basal and postprandial ghrelin, leptin, testosterone, glucose, insulin and C-peptide concentrations, and insulin resistance (homeostasis model assessment (HOMA-R)) and sensitivity (quantitative insulin-sensitivity check index (QUICKI)) were evaluated.

Results: Basal ghrelin levels were similar in ES and LY, whereas leptin was higher in ES than LY, in agreement with the higher amount of FM. Basal and percentage change in ghrelin were inversely related to FFM, appendicular skeletal muscle mass (SMM), and QUICKI, but not to FM. Basal and percentage change in leptin were directly related to FM and not to FFM indexes. Ghrelin basal concentration was negatively correlated with energy and protein intake and with QUICKI. Percentage change in Ghrelin after SOMNL correlated negatively with protein intake, but positively with resting energy expenditure and energy intake, and glucose, insulin, C-peptide basal concentrations, and HOMA-R.

Conclusion: In ES, basal and postprandial ghrelin increases with FFM, specifically SMM, reduction, whereas leptin increases with relative FM increases.

Introduction

It is well established that appetite and food intake decline with aging, resulting in weight loss and leading to protein-energy malnutrition and wasting. Moreover, aging is characterized by changes in body composition, including a decrease in bone and muscle mass and an increase in the amount of fat (1, 2). The age-related loss of skeletal muscle mass (SMM) is caused both by a reduction in anabolic factors such as growth hormone (GH), androgens and estrogens, physical activity and appetite, and an increase of catabolic factors such as inflammatory cytokines, like interleukin-1β, tumor necrosis factor-α, and interleukin-6, leading to the atrophy of muscle fibers, particularly of type-II (3). These processes, which worsen the nutritional status in the elderly, are associated with increased frailty, morbidity, and mortality (4). The mechanisms causing the physiologic decline of food intake are likely multifactorial and not completely understood. Although common exogenous causes like drug consumption, co-morbidity, feeding problems, and socioeconomic limitations significantly affect meal patterns, endogenous factors such as the dysregulation of the peripheral signals regulating appetite and energy homeostasis may play important roles in determining the anorexia of aging by reducing hunger and increasing satiety (5). Among the numerous gut peptides involved in the regulation of food intake, ghrelin, a hormone that may affect orexigenic pathways, has gained much interest (6, 7). Ghrelin plays a key role in the stimulation of the hypothalamic appetite centers (8, 9) and in the coordination of energy homeostasis (10, 11). In humans, plasma ghrelin concentration increases during fasting and decreases quickly after a meal (12), a pattern consistent with a role in meal initiation and short-term control of energy balance (13). A recent study demonstrated that ghrelin antagonizes leptin actions and promotes the production of orexigenic hypothalamic neuropeptides, such as neuropeptide Y (10).

Circulating ghrelin concentration has been shown to increase from early adulthood to middle age in humans (13) and subsequently to decrease in old age (14). The effect of the interaction between aging and food intake on ghrelin concentration was investigated by
Sturm et al. (15), who found that undernourished older women had higher plasma ghrelin concentration, but a similar suppression after voluntary food intake, compared to well-nourished women, suggests that the most important cause of the anorexia of aging could be a reduced basal hunger and appetite and not an early satiety that would involve an altered ghrelin suppression after food ingestion.

Aging is typically characterized by deterioration in glucose tolerance (16) that results from impaired insulin-stimulated glucose metabolism in skeletal muscle (17) for the increase of intramuscular fat content. The relationship between increased muscle triglyceride content and insulin resistance in muscle is the result of an age-associated reduction in mitochondrial oxidative and phosphorylation activity (18), probably due to the accumulation of mutations in control sites for mitochondrial DNA replication (19). Ghrelin changes after meal ingestion occur in the appositive direction and are inversely related to those of insulin (20–22).

It has also been demonstrated that plasma ghrelin levels change in a different manner according to the macronutrient ingested. Glucose ingestion consistently decreases ghrelin levels, a protein load has no effects, whereas, at least in women (23), lipid administration decreases ghrelin levels. On the contrary, a low fat diet fed ad libitum produces progressive weight loss and leptin level reduction without increase in ghrelin levels (24).

Although the relationship of leptin with body composition and habitual dietary intakes is well established (body fat mass (FM) regulates circulating levels of leptin and dietary carbohydrates, and fat regulates the biologically active leptin concentration) (25–27), for ghrelin, such a relationship, to the best of our knowledge, is still unclear.

The aim of the present study was, therefore, to evaluate whether ghrelin response to a standardized oral mixed nutrient load (SOMNL) is related to fat-free mass (FFM) in healthy elderly subjects (ES) in different nutritional status.

Materials and methods

Subjects

The characteristics of the subjects at the time of enrolment are shown in Table 1. Twenty-six healthy ES (14/12 females/male, 68.6 ± 4 years) were recruited: according to body mass index (BMI) classification, 16 were overweight or obese (BMI range, 25.5–33.6 kg/m²) and ten had normal weight (BMI range, 19.0–24.9 kg/m²). Moreover, ten young healthy normal weight subjects (LY) (BMI range, 19.0–24.9 kg/m²) were enrolled as control group. Exclusion criteria were: the presence of any disease causing significant impairment of the nutritional status (i.e. Crohn’s disease, neoplasia, end-stage renal failure, cirrhosis, congestive heart failure, and chronic infection), the presence of endocrine diseases (i.e. hyper-hypothyroidism and diabetes mellitus), the assumption of medications affecting endocrine function within the previous 2 months, the recent (< 1 month) occurrence of acute illness or injury, weight loss or gain (> 5 kg) in the last year, and treatment with special diets.

All procedures and meal preparation took place at the International Center for the Assessment of Nutritional Status (ICANS, Milan, Italy). The protocol was approved by the Institutional Ethical Committee and a consent form was signed by all subjects before enrolment.

![Table 1 Demographic, anthropometric and body composition variables of the study population. Mean values with s.d.](image-url)
Study design

After an overnight fast, each subject underwent the protocol shown in Fig. 1. The subjects were asked to abstain from caffeine, smoking, and alcohol from midnight before the study.

Nutritional status assessment – anthropometric measurements

Anthropometric measurements were collected by the same operator, according to conventional criteria and measuring procedures (28). Body weight (BW, in kilograms) and body height (BH, in centimeters) were measured to the nearest 0.1 kg and 0.5 cm respectively. BMI was calculated using the formula:

\[ \text{BMI (kg/m}^2) = \frac{\text{BW (kg)}}{\text{BH}^2 (\text{m}^2)} \]

Waist and hip circumferences were measured as proposed by Lohman et al. (28) and waist:hip ratio (WHR) was calculated to assess abdominal fat.

Body composition assessment

Air-displacement plethysmography (BOD POD; Body Composition System, Life Measurement Instruments, Concord, CA, USA) was used to measure body volume and thoracic lung volume according to the protocol proposed by McCrory et al. (29). It places low demands on subjects' performance and it is, therefore, the most convenient in the elderly (30). The BOD POD software (version 1.69) calculated whole body density (D) as BW divided by body volume and the percentage of FM using Siri's equation (31):

\[ \text{FM BOD POD (\%)} = (495/D) - 450 \]  

Fat-free mass (FFMBOD POD) was then calculated as the difference between BW and FM

Dual energy X-ray absorptiometry (DXA) was performed using a Lunar DPX-IQ (software version 4.6 d) whole body absorptiometer (Lunar Corporation, Madison, WI, USA). Subjects were made to lie supine with arms and legs at their sides during the 15-min scan; radiation exposure was <7 μSv. All the scans were performed by the same operator and daily quality assurance tests were performed according to the manufacturer's directions. Manufacturer's software version 4.6b was used for the analysis of bone mineral content (BMCDXA). Lean body mass (LBMBOD POD) was obtained by subtracting BMCDXA from FFM BOD POD. Appendicular SMMDXA was calculated by the sum of appendicular lean body mass by DXA (LBMDXA = LBMDXA arms + LBMDXA legs).

We calculated FM index (FMBOD POD/BH²) and FFM index (FFMBOD POD/BH²), because they are potentially useful in evaluating body composition parameters in aging by effectively eliminating differences in FM and FFM associated with height (1).

Resting energy expenditure (REE)

REE was estimated by indirect calorimetry using an open-circuit ventilated-hood system (Sensor Medics 29, Anaheim, CA, USA). All measurements were made in a thermoneutral environment (24–26 °C) with no external stimulation. Approximately, 30 min of respiratory gas exchange data were collected. The data collected during the first 5–10 min were discarded, as recommended by Isbell et al. (32). This allowed the subjects to acclimatize to the canopy and instrument noise. The average of the last 20 min of measurements was used to determine 24-h REE according to standard abbreviated Weir equation (33):

\[ \text{REE (kcal/day)} = (3.941 \text{ VO}_2 (\text{ml/min}) + 1.106 \text{ VCO}_2 (\text{ml/min}))1.44 \]  

Meal load

A SOMNL consisting of one toast (40 g of bread, 30 g of cheese, and 40 g of cooked ham), a glass of fruit juice (200 ml) and yoghurt (150 g) providing 478 kcal (19% protein, 35% lipids, and 46% carbohydrates) was administered at 0.900 h, while the subjects were postabsorptive.

Figure 1 Experimental protocol.
Biochemical parameters

A blood sample was obtained before (T0) and 60 min after (T1) the administration of the SOMNL for the determination of the following circulating compounds: ghrelin, leptin, testosterone, glucose, insulin, and C-peptide concentrations. HOMA-R (34) and QUICKI (35) were also evaluated. HOMA-R was calculated as ((fasting glucose (mg/dl) × fasting insulin (mU/l))/405 and QUICKI as 1/(log fasting insulin + log fasting glucose). Circulating ghrelin was measured by an RIA kit for total ghrelin (Phoenix Pharmaceuticals, Belmont, CA, USA); the lower detection limit was 0.15 ng/ml; the intra- and interassay coefficients of variation (CV) were <12%. Circulating leptin was measured using the RIA kit for human leptin (Linco Research, St Louis, MO, USA); the detection limit was 0.5 ng/ml; the intra- and the interassay CV values were 5.9 and 6.9% respectively. The levels of circulating testosterone, glucose, insulin, and C-peptide were measured in duplicate by commercial methods previously described (36).

Dietary intake data

The daily food intake was estimated by the Italian European Prospective Investigation of Cancer questionnaire, a validated semi-quantitative instrument (37) administered by trained dieticians to improve the accuracy. The food questionnaire and its nutritional evaluation are described in detail elsewhere (38). Briefly, it contains 248 questions concerning 188 different food items. The respondent indicates the number of times a given food item is consumed (per day, week, month or year) on the form, from which the absolute frequency of consumption of each item is assessed. The quantity of the food consumed is assessed from the respondent’s selection of an image of a food portion, or by selection of a predefined standard portion, when no image is available.

A computer program, Nutrition Analysis of Food Frequency Questionnaire (NAF), was developed ad hoc to convert questionnaire dietary data into frequencies of consumption and average daily quantities of foods, energy, and nutrients consumed. For the present analysis, NAF was linked to the Italian Food Composition Table for Epidemiological studies (39) for the energy and nutrients estimation. This method is validated and highly reliable: it is the most practical approach to examine the usual diet and was used in several trials to assess food intake in elderly (40, 41).

Statistical analysis

The results are expressed as mean ± s.d. Comparisons between groups were performed by unpaired Student’s t-test. Pearson correlation coefficient analysis was used to examine the relationship of the various anthropometric and body composition indexes, energy and macronutrient daily intake, and metabolic variables with ghrelin and leptin basal concentrations and their responses to preload, expressed as the percent change from premeal to postmeal levels (%Δghrelin, %Δleptin). Analyses were performed by means of Statistica for Windows software (Release 4.5B; StatSoft, Inc., Tulsa, OK, USA). Differences were considered statistically significant when P<0.05.

Results

Descriptive statistics

Demographic and nutritional variables of the population under investigation are shown in Table 1. ES showed greater BMI, waist circumference, WHR, and FM than the LY control subjects. Overweight/obese ES did not differ from normal weight elderly in FM% (37 ± 6 vs 33 ± 10), but for FFMBOD POD (52 ± 13 vs 42 ± 9 kg, P = 0.049) and SMMDXA (23 ± 6 vs 18 ± 4 kg, P = 0.035). Compared to LY, only lean elderly had a significant reduction of FFM (P<0.02). Data related to REE and preload indexes are reported in Table 2. REE normalized for BW was lower in elderly than in LY, but was not different when normalized to FFM. Since mean total REE was comparable between elderly and control, and mean total REE between normal weight elderly and LY differed only by 16% (1505 ± 109 vs 1306 ± 274 respectively.

| Table 2 | Resting energy expenditure variables, daily food intakes and preload indexes of the study population. |
|---|---|---|---|---|---|---|
| | Elderly (n=26) | Controls (n=10) | t-Test P |
| **REE variables** | | | | |
| REE (kcal/day) | Mean | s.d. | Mean | s.d. | 0.851 |
| REE/BW | 1528 | 357 | 1505 | 109 | 0.000 |
| REE/FFMBODPOD | 20.8 | 2.8 | 25.1 | 2.8 | 0.176 |
| **Preload indexes** | | | | |
| Preload/REE (%) | 32.4 | 5.0 | 29.9 | 3.9 | 0.727 |
| Preload/EI (%) | 33.9 | 7.6 | 33.0 | 2.5 | 0.053 |
| Preload/BW | 7.0 | 1.5 | 8.1 | 1.3 | 1.176 |

REE, resting energy expenditure; BW, body weight; FFM, fat free mass; BODPOD, air plethysmography; EI, energy intake.
-test, \( P = 0.062 \), all subjects received the same SOMNL energy content.

Basal ghrelin concentrations did not differ between elderly and LY, whereas leptin, glucose, insulin, C-peptide concentrations, and HOMA-R index were significantly higher and QUICKI was lower in elderly (Table 3). As expected, on the basis of similar FM, overweight/obese elderly had similar leptin concentration to normal weight elderly. On comparing the overweight/obese and lean elderly, we found that basal ghrelin and leptin concentrations did not differ, whereas glucose, insulin, C-peptide concentrations, and insulin sensitivity indexes were significantly higher in overweight elderly.

The administration of SOMNL, which was completed within 15 min by all subjects, induced a significant reduction in plasma ghrelin and leptin concentrations both in elderly and in LY (Table 3).

Simple regression analysis was performed in order to assess the relationship between serum ghrelin and leptin concentrations before and after SOMNL, and body composition in elderly. Basal leptin concentration was positively related to both FM indexes, but not to FFM and SMM in elderly (Fig. 2). In contrast, \( \% \Delta \)ghrelin concentration after SOMNL was unrelated to body composition. Basal ghrelin concentration was unrelated to both FM indexes, but showed an inverse relationship to FFM and SMM in elderly (Fig. 3). Similarly, \( \% \Delta \)ghrelin concentration after SOMNL was unrelated to FM, but it was inversely related to FFM and SMM. Sex hormones, especially testosterone, have been found to modulate ghrelin levels and testosterone is also associated with LBM; a multiple regression analysis adjusted for testosterone level was performed, but the relationship between ghrelin and FFM remained significant (\( P < 0.05 \)).

The suppressive action of meal on ghrelin was more potent in normal weight than in overweight ES (\( \% \Delta \)ghrelin, \( -23.3 \pm 10.3 \) vs \( -12.4 \pm 13.3 \) respectively, \( P = 0.046 \), whereas no difference was observed for \( \% \Delta \)leptin.

### Table 3

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Basal (pg/ml)</th>
<th>Post load (pg/ml)</th>
<th>Percentage of charges from basal</th>
<th>( % \Delta ) (P value)</th>
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<td>10.3</td>
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</table>

\( \text{Mean values with } \text{s.d. HOMA, } \text{glucose} \times \text{insulin} / 22.5; \text{QUICKI} = 1 / (\log \text{insulin} + \log \text{sglucose}). \)

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As expected, SOMNL elicited a significant increase in glucose, insulin, and C-peptide levels both in elderly and LY that were not proportionally different among groups, although the elderly reached significantly higher glucose, insulin, and c-peptide plateaus.

Pearson’s correlation coefficients among ghrelin and leptin concentration hormone responses, and other nutritional and metabolic parameters were calculated. Ghrelin basal values in elderly correlated negatively with BW ($r = -0.57, P < 0.05$), but not with BMI and WHR. Energy and protein absolute intakes and QUICKI negatively correlated with ghrelin basal concentration ($r = -0.61, -0.80, -0.71$ respectively, $P < 0.05$).

No significant correlation was found between leptin basal values and resting energy expenditure variables and daily food intake.

The $\%\Delta$ ghrelin after SOMNL was positively related to BW, BMI, and resting energy expenditure and energy intake ($r = 0.83, 0.71, 0.60$ respectively, $P < 0.05$) and was negatively related to protein intake ($r = -0.71, P < 0.05$). In addition, a positive correlation was detected between $\%\Delta$ ghrelin and glucose, insulin, and C-peptide levels.

Figure 2 Relationship between basal and postload leptin concentration and body composition variables.

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C-peptide basal concentrations, and HOMA-R ($r = 0.60, 0.77, 0.81, 0.80$ respectively, $P < 0.05$). %ΔLeptin after load did not correlate with the variables investigated.

**Discussion**

Previous studies have evaluated ghrelin and leptin concentrations in healthy ES (14,15); however, to the best of our knowledge, this is the first study on ES carefully evaluated with respect to body composition parameters.

Data for the estimation of body fat by plethysmographic method (BOD POD) have been reported to agree closely with the traditional gold standard, hydrodensitometry (underwater weighing), and accurately predict FM and FFM. In order to correlate better the FFM component (which also includes BMC and extracellular water) to the SMM, a second technique was employed, i.e. limbs body composition analysis by DXA. The stratification of ES according to BMI...
classification showed that normal weight ES differed from overweight ES only by the amount of FFM (significantly higher in overweight), since FM was similar in overweight/obese and in normal weights. The evaluation of nutritional status based on BMI could be misleading in the elderly: both sarcopenia and sarcopenic obesity can occur in individuals classified as normal weight or overweight by BMI. For these reasons, we directly measured body composition with different and sophisticated techniques and performed correlation analyses across the different BMI conditions.

Our data indicate that ghrelin concentrations in ES, both basal and after meal suppression, are sensitive to negative variations of FFM (and specifically to SMM), in a way similar to the well-established changes of leptin relative to increase of FM. In particular, concerning ghrelin, both techniques showed that its basal and postload concentrations in elderly were inversely related to FFM or LBM content after statistically adjusting for androgen levels.

In contrast to leptin, ghrelin was unrelated to both FM indexes. Fasting ghrelin was not significantly different between elderly and LY control subjects whereas leptin fasting concentrations were significantly more elevated in ES than in LY, in agreement with the difference of FM amount between groups.

Several previous studies failed to find an association between FFM and ghrelin (42, 43). Consistent with our observations, other investigators (44, 45) observed an association between plasma ghrelin levels and LBM. In particular, Moran et al. (44) found that in overweight/obese adult subjects, lean mass predicted fasting ghrelin levels and Nagaya et al. (45) showed that ghrelin administration increases LBM in humans. However, a relationship between ghrelin and LBM has not been previously reported in the elderly.

In agreement with our data, Sturm et al. (15), evaluating basal and postload ghrelin concentrations in well-nourished and undernourished ES and in well-nourished young subjects, found higher ghrelin values in undernourished ES, but no significant differences between well-nourished ES and young subjects at baseline.

Our data confirm the well-established increase in fasting serum glucose concentration and reduction in insulin sensitivity reported in elderly (46). The stratification of ES, according to BMI, in normal and overweight subjects showed that basal glucose, insulin, and C-peptide values were significantly higher (but within normal values) in overweight/obese subjects without differences among them in load responses. Ghrelin changes after meal ingestion occur in the opposite direction and are inversely related to those of insulin, showing an inverse relationship with insulin sensitivity. Given the strict observed relationship between ghrelin and FFM in the present study, as well as its strong association with markers of glucose metabolism, it could be speculated that insulin is an important modulator not only in the long-term regulation of body adiposity, but also body FFM. Lucidi et al. (47) demonstrated that hyperinsulinemia reduces ghrelin levels, suggesting that insulin modulates the meal-related changes in plasma ghrelin concentration in an indirect manner through changes in glucose disposal. In further studies, it would be interesting to evaluate glucose kinetics in order to support this hypothesis in the elderly. Nonetheless, the specific relationship of ghrelin to FFM and not to FM suggests that other factors beyond insulin sensitivity (which is exquisitely inversely related to body adiposity) are involved.

One aim of this study was to evaluate the relationship between basal and postload ghrelin and leptin concentrations and daily food intake. Our results show that basal ghrelin concentration is negatively related with both energy intake and protein intake, whereas ghrelin suppression after SOMNL is positively related to habitual energy intake, suggesting that in the ‘less eating’ ES, both signals to hunger and early satiety are stronger. This hypothesis is also supported by our finding that the percentage of preload on energy intake index (preload/EI%) was directly related to the amount of ghrelin suppression after load (data not shown; \( r^2 = 0.36, P < 0.05 \)). Although it may be tempting to speculate that the intense postload decline in serum ghrelin levels in the ‘less eating’ ES might be one of the causes of aging anorexia, the nature of our study does not allow us to conclusively establish such a causal relationship. This hypothesis may be tested in prospective investigations with a larger sample size.

In conclusion, the physiologic age-dependent decline in BW, caused by a reduction in energy intake (48–50), the so-called anorexia of aging, is thus associated with a disproportionate loss of lean tissue (sarcopenia) or another frequent type of nutritional impairment, called ‘sarcopenic obesity’, and defined as excess fat with loss of LBM (51–53). Within this context, the present data, taking advantage of an extensive evaluation of body composition and suggesting that increased ghrelin concentrations signal FFM depletion in older subjects, could also represent a starting point to envision novel therapeutical approaches for age-related sarcopenia.

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References
Ghrelin and fat-free mass


