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ORIGINAL CONTRIBUTION

Fasting ghrelin is related to skeletal muscle mass in healthy adults

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Abstract *Background/Objectives* The determinants of plasma ghrelin concentrations including the effects of aging, gender, and body composition, are unclear. Appetite and energy intake decrease with advancing age, and there is a corresponding decline in total body lean tissue, and an increase in fat mass. *Methods* We measured fasting plasma ghrelin and insulin concentrations in 52 healthy subjects aged 22–82 years, and assessed body composition by dual energy X-ray absorptiometry. Energy intake was estimated from diet diaries. *Results* Fasting ghrelin concentrations were not significantly correlated with age and energy intake ($R = 0.07$, $P = 0.62$; and $R = -0.14$, $P = 0.34$ respectively) on univariate regression analysis, and ghrelin concentrations were higher in females than males (2886.8 ± 182.1 pg/ml vs 2082.5 ± 121.2 pg/ml; $P = 0.001$). Ghrelin was inversely related to body mass index ($R = -0.328$, $P = 0.018$), fat-free body mass ($R = -0.428$, $P = 0.002$), and total skeletal muscle mass ($R = -0.439$, $P = 0.001$), but not related to body fat mass ($R = 0.177$, $P = 0.208$). On multiple regression analysis, total skeletal muscle mass (corrected for height) was the only significant negative predictor ($P < 0.0001$) of fasting ghrelin concentrations. *Conclusions* In conclusion, in healthy adults, plasma ghrelin concentrations are not significantly influenced by age or energy intake per se, but relate to skeletal muscle mass.

Key words ghrelin – body composition – age – gender – skeletal muscle mass – food intake

Introduction

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, has a number of actions, including stimulation of hunger and energy intake [23]. Consumption of food suppresses ghrelin release, while plasma ghrelin concentrations increase in the fasted state [13, 34], characteristically reaching a peak just before the next meal [9, 34]. Ghrelin may thus play a role in meal initiation [9]. Possibly in response to chronic energy imbalance, circulating ghrelin concentrations are increased in undernourished states such as anorexia nervosa [25, 38] and decreased in obesity [48].

Determinants of postprandial ghrelin concentrations that have been identified include plasma glucose concentrations [7]. The role of insulin in determining postprandial ghrelin concentrations is unclear, with some studies demonstrating no significant effect [7, 41], and others showing a suppression in ghrelin with a rise in insulin concentrations, which may be

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either a direct effect of insulin or indirectly related through consumption of the meal [9, 32, 40, 45]. Glucagon administration also results in suppression of ghrelin [1, 45], but does not appear to play a role in determining fasting ghrelin concentrations [45].

Previous studies have produced conflicting data on the effect of healthy aging on circulating ghrelin concentrations [3, 9, 26, 28, 29, 37, 38, 42, 46]. In part this uncertainty appears to arise from the potential confounding effects of age-related body composition changes on ghrelin concentrations. In several of these studies age-related differences in ghrelin concentrations present on univariate analysis were no longer apparent when body composition measures were corrected for in multivariate analysis [29, 37]. Some studies have also demonstrated gender differences in fasting ghrelin concentrations, being higher in females than males [14, 28, 31].

On average body weight, as reflected in body mass index, increases until about age 60 years and declines somewhat thereafter [39], body fat stores increase throughout adult life, whereas lean tissue declines [4]. Increased body mass index has been associated consistently with reduced ghrelin concentrations [26, 28, 42, 48], but it is not clear if this association is due to increased fat tissue, increased lean tissue or both [5, 6, 17, 20, 31, 48]. Only limited analysis of the relationship between ghrelin concentrations and the components of lean tissue mass has been undertaken [5].

Healthy aging is associated with a decline in appetite and food intake which has been termed the 'anorexia of aging' [8]. In at-risk older people this predisposes to pathological under-nutrition, which is associated with increased morbidity and mortality [8]. The causes of the physiological anorexia of aging appear to be multiple, and are poorly understood. An understanding of these causes may help to develop ways of preventing and treating undernourished older people. One possible cause is age-related reductions in the orexigenic effects of ghrelin.

The aim of the present study was to determine the relationship of fasting plasma ghrelin concentrations to age, body composition (including the components of lean tissue) and energy intake in healthy adults across a wide age range. We hypothesized that circulating ghrelin concentrations would decline with age, independent of age-related changes in body composition and energy intake.

Subjects and methods

Subjects

Fifty-two healthy subjects (26 men, 26 women), aged 22–82 years (49.2 ± 2.4 years), with a mean body mass index (BMI) of 23.7 ± 0.3 kg/m^2, were recruited through advertisement. The BMI of the subjects over 65 years was not significantly different to those less than 65 (24.6 ± 0.5 vs 23.4 ± 0.4 kg/m^2, $P = 0.12$). Subjects did not have any gastrointestinal disease or symptoms, or significant respiratory, renal, or cardiac disease, take any medications known to affect gastrointestinal motility or appetite, smoke >10 cigarettes per day, or consume >20 g of alcohol per day. Pregnancy was excluded in women of reproductive age by a urine test at the time of the screening visit. Pre-menopausal women were studied between days 1 and 14 of their menstrual cycle. All subjects were asked to maintain their usual diet and physical activity prior to, and throughout, their participation in the study.

The study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital. Written, informed consent was obtained from each participant before their inclusion in the study.

Study design

At 8:30 a.m., after a 12 hour overnight fast, a venous blood sample was obtained in each subject for measurement of fasting ghrelin and insulin concentrations. The blood was placed in ice-chilled EDTA tubes containing 1,000 kallikrein inhibitory units aprotinin (Trasylol) per milliliter of blood. Plasma was separated by centrifugation (3,200 rpm for 15 min at 4°C) within one hour of collection and stored at −70°C until assayed. On a separate study day, body composition was quantified by dual energy X-ray absorptiometry (DEXA; Norland Densitometer XR36). Each subject was asked to maintain a three-day food diary as close as possible prior to the DEXA scan, including two consecutive weekdays and one weekend day, from which their total baseline energy intake was estimated [2].

Measurements

Food intake

The average energy intake was quantified from the 3-day food diary [2], using Foodworks 3.10 (Xyris Software, Highgate Hill, Australia).

Body composition

Dual-energy X-ray absorptiometry (DEXA; Norland densitometer XR36 Norland Medical Systems, Fort Atkinson, Wisconsin, USA) was used to measure total body fat mass (BFM) and fat-free mass (FFM; which includes bone mineral content). Appendicular lean

soft tissue (ALST) was calculated as the sum of the lean soft tissue mass in the upper and lower limbs, and includes skeletal muscle, skin, and connective tissue, minus the bone mineral content [22]. These measurements by DEXA have been found to correlate well with those obtained by direct measures from computed tomography (CT) and magnetic resonance imaging (MRI) [27, 50]. Total body skeletal muscle mass (total SM) was calculated using the following validated formula [22]: Total SM = (1.13 × ALST) − (0.02 × age) + (0.61 × sex) + 0.97, where sex = 0 for females, 1 for males). BFM, FFM, and total SM were corrected for height to give height-normalized indices (fat-free mass index FFMI, body fat mass index BFMI, and skeletal muscle mass index SMI), as suggested in a previous study [49].

Gastrointestinal hormones

Total plasma ghrelin (picograms per milliliter, pg/ml) was measured by radioimmunoassay with minor modifications to a previously published method [36]. The radiolabel was supplied by GE Healthcare, Biosciences, UK (Amersham IM347), incubation time was increased to 3 days and the second antibody was added after the incubation of antibody and label with standard. The minimum detectable level was 40 pg/ml, inter-assay coefficient of variation (CV) was 18% and intra-assay CV was 5%.

Serum insulin was measured using microparticle enzyme immunoassay on the Abbott AxSYM analyzer, and the CV for insulin at 9.5 U/l was 2.5%.

Statistical analyses

Relationships between ghrelin and age and other variables were determined using simple regression analyses. All variables showing a correlation with a P value of <0.15 were then entered into a stepwise multivariate regression model, with ghrelin concentration as the dependent variable. Age and energy intake were also included in the final regression model, regardless of P values, as these were pre-defined parameters of interest. The residuals for ghrelin concentration as the dependent variable were normally distributed. Male and female subgroups and older (≥65 years) and younger subgroups were compared separately using an independent samples t test. All statistical analyses were performed using SPSS for Windows Version 11.5 statistical software. Results are expressed as mean ± SEM and $P < 0.05$ was considered statistically significant.

Results

Subject details are shown in Table 1. The women were similar in age to the men, but weighed less, had lower BMIs, more fat tissue, less lean tissue, and lower bone mineral content in the arms and legs. Fasting ghrelin concentrations were higher in females than males ($P = 0.0006$), however males had a higher average energy intake than females ($P = 0.007$).

As shown in Fig. 1 there was no significant correlation between fasting ghrelin concentrations and age by simple regression analysis ($R = 0.07$, $P = 0.62$).

Table 1 Characteristics of the study group, with comparison between male and female and older and younger subgroups

	All subjects ($n = 52$)	Female ($n = 26$)	Male ($n = 26$)	P value for male versus female subgroups	P value for older (≥65 years; $n = 12$) vs. younger ($n = 40$) subgroups
Age (years)	49.2 ± 2.4	48.2 ± 3.4	50.1 ± 3.3	0.69	<0.0001
Body weight (kg)	69.4 ± 1.3	64.2 ± 1.3	74.7 ± 9.2	<0.0001	0.62
BMI (kg/m^2)	23.7 ± 0.3	23.0 ± 0.4	24.3 ± 0.4	0.04	0.12
Bone mineral content (BMC) of arms (kg)	0.41 ± 0.01	0.36 ± 0.01	0.46 ± 0.01	<0.0001	0.25
Bone mineral content (BMC) of legs (kg)	1.02 ± 0.02	0.94 ± 0.02	1.09 ± 0.03	<0.0001	0.09
Fat-free mass (FFM) (kg)	45.1 ± 1.5	36.6 ± 0.8	53.7 ± 1.5	<0.0001	0.47
Fat-free mass index (FFMI) (kg/m^2)	15.3 ± 0.4	13.1 ± 0.3	17.5 ± 0.4	<0.0001	0.85
Body fat mass (BFM) (kg)	22.8 ± 0.9	26.3 ± 1.3	19.4 ± 4.3	<0.0001	0.55
Body fat mass index (BFMI) (kg/m^2)	7.9 ± 0.3	9.4 ± 0.4	6.3 ± 0.3	<0.0001	0.17
Appendicular lean soft tissue (ALST) (kg)	19.4 ± 0.7	15.4 ± 2.2	23.4 ± 0.7	<0.0001	0.23
Total skeletal muscle mass (Total SM) (kg)	22.2 ± 0.8	17.4 ± 0.5	27.0 ± 0.8	<0.0001	0.15
Total skeletal muscle mass index (SMI) (kg/m^2)	7.5 ± 0.2	6.2 ± 0.2	8.8 ± 0.2	<0.0001	0.38
Energy intake/day (kJ)	8,593 ± 294 (2,053 ± 70 kcal)	7,820 ± 421 (1,868 ± 101 kcal)	9,365 ± 358 (2,237 ± 86 kcal)	0.007	0.73
Fasting plasma ghrelin (pg/ml)	2,485 ± 122.1	2,887 ± 182.1	2,083 ± 121.2	0.001	0.35
Fasting plasma insulin (mU/l)	5.6 ± 1.2	5.1 ± 1.5	6.1 ± 1.9	0.69	0.29

Values are means ± SEM
FFMI = FFM ÷ (height)^2; BFMI = BFM ÷ (height)^2; and SMI = total SM ÷ (height)^2

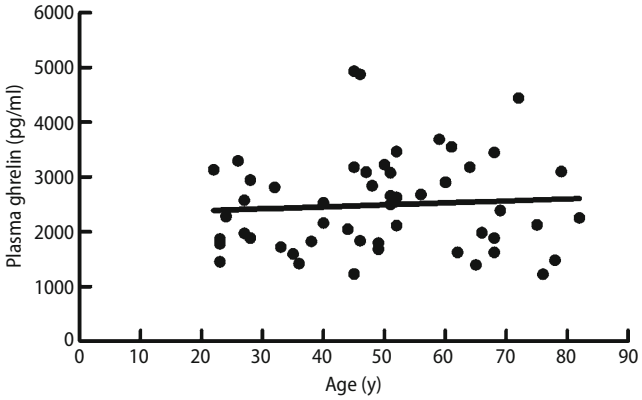

Fig. 1 Relationship between fasting plasma ghrelin concentrations and age for the entire group ($R = 0.07$, $P = 0.62$)

The results of univariate regression analyses between ghrelin and other variables are shown in Table 2. Ghrelin was inversely related to body mass index and a number of measures of lean mass and skeletal muscle mass. There was a trend toward bone mass of the arms and legs being a negative predictor of ghrelin concentrations, but these relationships did not achieve statistical significance. There was a weak negative correlation between energy intake and ghrelin concentrations, which did not achieve statistical significance. In a separate analysis, energy intake was not significantly related to BMI ($R = 0.13$, $P = 0.37$) or age ($R = 0.12$, $P = 0.42$), but was positively related to lean body mass components ALST ($R = 0.37$), FFMI ($R = 0.47$), SMI ($R = 0.41$), BMC of arms ($R = 0.37$), LM of arms ($R = 0.36$) and LM of legs ($R = 0.37$) (all $P \leq 0.01$), and negatively related to the body fat mass variable BFMI ($R = -0.43$, $P = 0.001$).

Table 2 Simple regression analyses of fasting plasma ghrelin concentrations with body composition and other variables (abbreviations as in Table 1)

Variable	*R*	*P*
Age (years)	0.070	0.621
Gender	0.461	0.001
BMI (kg/m^2)	−0.328	0.018
BMC of arms (kg)	−0.269	0.054
BMC of legs (kg)	−0.234	0.096
Fasting plasma insulin (mU/l)	0.097	0.492
Lean muscle mass (LM) of arms (kg)	−0.396	0.004
LM of legs (kg)	−0.429	0.001
FFM (kg)	−0.428	0.002
FFMI (kg/m^2)	−0.451	0.001
BFM (kg)	0.177	0.208
BFMI (kg/m^2)	0.223	0.113
ALST (kg)	−0.432	0.001
Total SM (kg)	−0.439	0.001
SMI (kg/m^2)	−0.475	<0.0001
Energy intake/day (kJ)	−0.135	0.340

Values are means ± SEM

Table 3 Stepwise multiple regression analysis of fasting plasma ghrelin with other covariates (abbreviations as in Table 1)

	Standardized coefficient (*B*)	*t*	*P*
SMI	−0.475	−3.82	<0.0001
Age	0.043	0.340	0.735
BMI	−0.113	−0.779	0.440
BMC of arms	0.058	0.356	0.724
BMC of legs	0.072	0.462	0.646
ALST	0.111	0.309	0.759
FFMI	0.046	0.104	0.917
BFMI	−0.143	−0.875	0.386
LM of arms	0.048	0.195	0.846
LM of legs	0.129	0.358	0.722
Gender	0.220	1.021	0.312
Energy intake/day	0.070	0.513	0.610

Values are means ± SEM

As many of the measured variables are correlated with each other, multiple regression analysis with plasma ghrelin as the dependent variable was performed, as shown in Table 3. The only significant independent predictor of ghrelin was total body skeletal muscle mass index, which was correlated negatively with fasting ghrelin concentrations ($P < 0.0001$), and accounted for 23% of the variance in ghrelin.

Discussion

The major observations in this study are that: (1) healthy aging does not appear to be associated with changes in fasting plasma ghrelin concentrations; (2) skeletal muscle mass is an independent negative predictor of fasting ghrelin concentrations; (3) plasma ghrelin and body mass index are inversely related, even in non-obese individuals; (4) while healthy women have higher fasting ghrelin concentrations than men, this is accounted for by differences in body composition; and (5) energy intake does not predict ghrelin concentration, which is a novel finding in this study.

Previous studies have reported that circulating ghrelin concentrations are higher [9, 37], lower [3, 29, 38] or no different [5, 12, 26, 28, 46] (as we found), between healthy older and young adults on univariate analysis. There is thus no consistent evidence that circulating ghrelin levels increase or decrease with age, even before taking other factors into account. The inconsistencies in previous studies may be attributable to differences in the subject groups studied, particularly in age and body weight range, and the confounding effects of age-related body composition changes. In both of the studies referred to above [29, 37] where an age-related difference was evident on univariate analysis and a multivariate analysis was also performed, there was no remaining effect of age

after body composition parameters were accounted for [29, 37]. In contrast, another study reported an increase with age which was only present on multivariate analysis [28]. Our finding of no relation between age and ghrelin concentrations on multivariate analysis, including body composition and food intake parameters, is therefore consistent with results of most previous studies. It appears that age per se has minimal, if any, influence on circulating ghrelin concentrations and that reduced circulating ghrelin concentrations are unlikely to contribute significantly to the anorexia of aging. We did not however study the 'old–old' (aged 85 years and over), and the study may have thus been underpowered to exclude a potential influence of age. A few recent studies have suggested an influence of age on postprandial serum ghrelin dynamics. Despite a comparable rise in postprandial serum total ghrelin concentrations within four hours of a mixed meal in healthy young and older individuals, hunger sensations were diminished during the postprandial period in the latter group [12]. Furthermore, older individuals do not demonstrate the pulsatile secretion of active (acylated) ghrelin seen in young individuals 2–4 h after ingestion of a mixed meal, which may influence subsequent meal initiation [11]. The effects of aging on sensitivity to the orexigenic effects of exogenous ghrelin have, to date, not been reported, and it is likely that chronic diseases and under-nutrition which are frequently found in older persons will impact on any observed abnormalities.

In our study, fasting ghrelin concentrations were 38% higher in the women than men, a difference that was highly significant. Some [14, 28, 31], but not all [3, 26, 37] studies have also found higher levels in women. The reason for these discrepant observations is unclear. Multivariate analysis have been performed in three of the four studies, including ours [28, 31], where ghrelin concentrations were higher in women. In two of the three ([31] plus our study), there was no gender difference after correcting for body composition differences by multivariate analysis, whereas in the other, ghrelin remained higher in women [28]. The interaction between body composition and ghrelin in our study appears to be due to the substantially lower skeletal muscle mass in women; skeletal muscle mass was a powerful, and statistically significant, negative predictor of ghrelin levels. Our study suggests that across a wide adult age range, ghrelin levels are higher in women than men, and that this difference is accounted for by body composition, rather than gender per se.

The relative effects of changes in energy intake and body composition on circulating ghrelin concentrations have not been defined. Fasting acutely elevates ghrelin levels, which are also increased chronically in under-nourished young [38] and older [46] adults. Weight loss, whether induced by diet or surgery (which reduces food intake), is associated with an increase in ghrelin levels [10, 18, 21, 51]. Conversely, eating acutely suppresses ghrelin levels [9]. The inverse relationship between energy intake and ghrelin appears to be mediated, at least in part, by the suppressive effects of insulin on ghrelin [3, 26]. In the stable state, energy intake, as assessed by diet diaries, is reported to be inversely related to circulating ghrelin concentrations [5, 29]. This relationship is not strong, however, perhaps due to limitations in measures of energy intake, and did not achieve statistical significance in our study.

It is not clear how much of the increase in ghrelin levels with weight loss (and vice versa for weight gain) is due to the accompanying changes in body composition, rather than to reduced energy intake per se. Most studies show an inverse relationship between body mass index and ghrelin concentrations [26, 28, 42, 48] and this was so in the present study even across a relatively narrow range of BMIs. Consistent with this, ghrelin concentrations are lower in obese than normal weight individuals [44]. On average energy intake is positively related to body weight [43]; extra energy is needed to maintain the greater weight. It is possible, therefore, that the inverse relationship between body weight and ghrelin levels is accounted for, at least in part, by differences in energy intake. However, in the present study, we found that daily energy intake did not significantly correlate with BMI. Furthermore, there was no significant relationship between energy intake and ghrelin concentrations on either univariate or multivariate analysis. This suggests that there is an association between ghrelin levels and body composition independent of energy intake.

The body composition component related to ghrelin in this study was skeletal muscle mass. Unlike some previous studies [6, 17, 20, 48] the amount of fat tissue was not apparently related to ghrelin, on either univariate or multivariate analysis. Our finding of an inverse association with skeletal muscle mass concurs with that of studies where lean tissue was found to be inversely related to ghrelin levels [5, 16, 31]. Bertoli et al. [5] also found no relation with fat mass and a significant inverse relationship between ghrelin levels and both fat free mass and skeletal muscle mass measured by DEXA in young and older adults. They found a significant negative correlation between energy intake and ghrelin concentrations, but did not undertake a multivariate analysis for independent associations. Our findings, therefore, confirm theirs of an inverse relation with skeletal muscle mass and extend them to indicate no apparent association with nutritional intake.

Both ghrelin and its receptor are found in skeletal muscle [19, 35] and short-term ghrelin administration increases lean body mass [33]. Furthermore, in vitro studies have shown that ghrelin stimulates differentiation of rodent muscle cells [15, 52]. The inverse relationship we have identified between plasma ghrelin concentrations and skeletal muscle mass further supports the existence of a connection between skeletal muscle and ghrelin secretory pathways, and suggests that skeletal muscle may exert a negative feedback effect on ghrelin secretion. If so, this may enable resistance and strengthening exercises in dieting, overweight, people to reduce the appetite-stimulating increase in ghrelin concentrations that accompanies weight loss, by increasing muscle mass. It is of interest that subjects in the Rancho Bernardo Study who exercised at least three times per week, and were therefore likely to have greater muscle mass, had lower levels of ghrelin than those who did not, even after adjustment for BMI [26]. We therefore postulate that ghrelin concentrations are determined by, and probably also a determinant of, total body skeletal muscle mass. Possible mechanisms may include an upregulation of growth hormone secretagogue receptors with an increase in skeletal muscle bulk, which subsequently has a negative feedback effect on ghrelin secretion.

While short-term resistance exercise apparently has no effect on ghrelin concentrations [47], the effect of such exercise in the longer term has not been defined. Interestingly, a comparison of different modalities of resistance exercise has shown that repetitive concentric, but not eccentric, muscle contractions results in a suppression of ghrelin concentrations during the recovery period of the exercise session [24]. It may be that frequent repetitions of resistance-type exercise over a longer duration of time, rather than an acute session of either high- or low-intensity exercise, have a suppressive influence on ghrelin concentrations.

Our study has some limitations. We examined plasma ghrelin concentrations only in the fasted state, but these are known to correlate closely to 24-h area under the curve or pooled ghrelin concentrations [37, 51], in both younger [9, 37] and older [51] individuals. Furthermore, we did not measure active ghrelin concentrations. Total (active and inactive) ghrelin has, however, been shown to correlate closely with active ghrelin concentrations in 20 lean and 20 obese subjects [30].

In conclusion, in this study of healthy adults across a sixty year age range, plasma ghrelin concentrations were not significantly influenced by age, were inversely correlated with BMI, and were higher in females than males, although not after correction of body composition differences. After accounting for other covariates, total body skeletal muscle, but not fat, mass was a significant negative predictor of ghrelin concentrations.

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