Gender differences in plasma ghrelin and its relations to body composition and bone – an opposite-sex twin study

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Summary

Background Ghrelin, a peptide hormone that plays a role in the regulation of appetite and body adiposity, may also play a role in bone metabolism.

Objectives We used the opposite-sex twin model to study associations of plasma ghrelin levels with measures of bone mass and body composition, and determine how such associations were influenced by gender and age.

Patients and measurements We measured total plasma ghrelin by radioimmunoassay (RIA) and bone mass/body composition parameters by dual energy X-ray absorptiometry in 79 pairs of opposite sex twins (n = 158 subjects). To examine the effect of age, the study population was divided by median age into two groups: under 51.2 years (38 pairs) and over 51.2 years (41 pairs).

Results Women had higher plasma ghrelin levels than men (median 1063 vs. 869 ng/l, P < 0.01). Age was a significant predictor of plasma ghrelin levels after adjustment for gender, fat mass and body size. In the older age group, plasma ghrelin levels were inversely associated with fat mass measures in men and women, but there were gender differences in the nature of these associations. In women, plasma ghrelin correlated inversely with body mass index (BMI, r = −0.32), total fat mass (r = −0.30) and fat mass/lean mass ratio (r = −0.42), whereas in men associations with abdominal fat mass (r = −0.31) and fat distribution index (r = −0.33) were observed. Plasma ghrelin was associated with alcohol consumption in older men and women. In the obese subgroup (BMI > 30) no significant gender differences in plasma ghrelin were found. Plasma ghrelin levels were not significantly associated with bone mineral density (BMD) generally, except for hip BMD in younger women (r = −0.39).

Conclusion Plasma ghrelin levels are associated with age, gender, alcohol intake and fat mass measures but only weakly to bone mass measures.

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Introduction

Ghrelin is a recently described peptide hormone that is secreted by endocrine cells in the gastrointestinal tract. It has been shown that ghrelin stimulates appetite, promotes adipogenesis, decreases energy metabolism, and stimulates cortisol release, all of which potentially affect bone metabolism indirectly. In humans, plasma ghrelin levels increase during fasting and decrease following feeding. The ghrelin receptor, known as the growth hormone secretagogue receptor (GHS-R), is found in many organs, including the stomach, heart, lung, pancreas, intestine, kidney, testes and ovary, as well as in the hypothalamus and in adipose tissues. The wide distribution of this receptor indicates that ghrelin, which is produced in and secreted mainly from the stomach, may have a variety of regulatory functions both in the brain and peripheral tissues. A recent in vitro study of Maccarinelli et al. demonstrated an increase in osteoblast proliferation after ghrelin administration. Moreover, a study of Fukushima et al. has reported that ghrelin directly promotes bone formation and increases BMD in rats. The reports on relationship between plasma ghrelin levels and bone in humans are contradictory. While Misra et al. reported that ghrelin was an independent predictor of bone density at the lumbar spine and the hip in healthy adolescents girls, the studies of Weiss et al. in the large cohort of men and postmenopausal women of Rancho Bernardo, CA, USA and Oh et al. in middle-aged Korean men found no association between serum ghrelin levels and BMD. Numerous studies reported that plasma ghrelin levels are associated with body composition measures such as BMI and body fat mass. These findings, together with the evidence that growth hormone (GH) is well known to promote bone formation, raise the possibility that ghrelin may play a role in bone metabolism.

There have been only a few studies that reported gender differences in plasma ghrelin levels and the findings of these studies have been contradictory. Thus some authors did not find any sex differences in plasma ghrelin levels, whereas others reported higher basal plasma ghrelin levels in females. The effect of
age on plasma ghrelin levels also remains uncertain. In the present study, we examined the associations of plasma ghrelin levels with measures of bone, body composition and lifestyle factors and whether any such associations were influenced by gender or age.

Materials and methods

Subjects

Study subjects were healthy adult (over 18 years old) opposite-sex twin pair volunteers recruited as part of the Northern Sydney Twin Study at the Department of Rheumatology of the Royal North Shore Hospital, Australia. Subjects taking medications that could potentially affect results were not excluded, but such medications were recorded (see Results section). The hospital’s Human Research Ethics Committee approved the study. Some of these data have been reported elsewhere. After providing written informed consent, each twin was interviewed separately in accordance with a standard questionnaire to collect demographic, lifestyle and medical history data.

Body composition and bone mineral density measurements

Baseline characteristics included age, height (m), weight (kg), BMI (weight/height\(^2\)), and menopausal status for women. Lifestyle characteristics included smoking, alcohol intake and physical activity. Smoking was recorded as 0 – never smoked, 1 – current smoker and 2 – previous smoker. Alcohol consumption was categorized as 1 – never, social occasions only, 2 – up to 14 drinks per week and 3 – more than 14 drinks per week. Physical activity was recorded as the sum of work, home and leisure activities for the last 12 months, graded from 1 – inactive to 4 – heavy).

Whole body, lumbar spine (L1–L4), hip and distal forearm were scanned by fan-beam dual-energy X-ray absorptiometry (DEXA) using a QDR 4500 W (Hologic, Waltham, MA, USA). Bone mineral density (BMD) of the lumbar spine (LSBMD), total hip (HIPBMD) and forearm (FORBMD) were obtained from DEXA scans using standard protocols as previously described.

Total body BMD (TOT BMD), total fat mass (TOT FM), trunk fat mass (TRUNK FM), total lean mass (TOT LM) were obtained directly from whole body DEXA body composition analysis. The abdominal fat (ABF) region was defined by cursor manipulation extending between the top of the second and the bottom of the fourth lumbar vertebrae and laterally to the inner aspects of the rib cage. All scans were analysed by one operator. The intraobserver variation of ABM was assessed by the calculating intraclass correlation coefficient (ICC) and the coefficient of variation (CV) of 30 repeat scans. The ICC = 0·998 (P < 0·001) and CV = 1·5%. Leg fat mass (LEG FM) was calculated as the sum of both leg’s fat mass. Body fat distribution was assessed by trunk/leg fat mass ratio (TR FM/LEG FM). Total fat mass to total lean mass ratio (TOT FM/TOT LM) was also calculated from the DEXA scan.

Plasma ghrelin levels were measured by radioimmunoassay (RIA) using a double antibody/PEG technique (Linco Research Inc, St Charles, MO, USA). This assay has a sensitivity of 93 ng/l with an intra-assay precision of 10%. Samples were run in duplicates.

Statistical analysis

Mean values for the measured variables were compared between the male and female co-twins, using paired t-tests. The Mann–Whitney test was run to compare non-normally distributed data of plasma ghrelin levels. Uni- and multivariate regression analyses were performed to evaluate the strength of relationship between plasma ghrelin levels and body composition or bone mineral variables. Generalized estimating equation (GEE) models were used to take into account similarities between twin family members, when all study subjects were pooled for the analyses. A 95% confidence interval was used to describe the strength of association; P < 0·05 was considered significant. The data set was evaluated for outliers, defined as those lying outside 3 SD. All statistical analyses were performed using SPSS for Windows 11·5 software (SPSS, Chicago, IL, USA).

Results

The characteristics of the 79 opposite-sex twin pairs are given in Table 1. Their age ranged from 18 to 73 years. Among study subjects, two men (1·3%) had diabetes mellitus, 13 (8·2%; 8 men, 5 women) self-reported hypercholesterolaemia and were taking statins (LIPITOR, atorvastatin calcium, Pfizer; PRAVACHOL, pravastatin sodium, Bristol-Myers Squibb Pharmaceuticals, Princeton, NJ, USA; ZOCOR, simvastatin, Merck Sharp & Dohme, Hertford, UK). No patients were taking glitazones. Twenty-one participants were current smokers (13·3%; 13 men, 8 women), 21 subjects (13·3%; 19 men, 2 women) were taking more than 14 units of alcohol per week. Nineteen women were current and seven past HRT users. None of the studied individuals were taking other medications affecting bone metabolism. To examine the effect of age, twins were divided by median into two age groups: under 51·2 years (38 pairs) and over 51·2 years (41 pairs). The median age cut point was used rather than the menopause status of the female twin to simplify comparison between genders, although the results were unchanged using the latter. All females in the older age subgroup were postmenopausal.

In both age groups men were taller and heavier, had more lean and abdominal fat mass but less total fat mass than women. The ratio between trunk fat mass and leg fat mass was also higher in men of any age indicating that in males, fat mass was mostly deposited in the upper body.

Median plasma ghrelin levels were higher in women than in men (1062·8 vs. 869·1 ng/l, P < 0·01). When compared within the age subgroups, this difference was statistically significant in the younger age group (1056·1 vs. 859·8 ng/l, P < 0·01). Comparison of plasma ghrelin levels between genders of different age groups is presented in Fig. 1.

To examine the association between plasma ghrelin levels and bone density/body composition measures, nonparametric Spearman correlations were performed. These correlations are presented in Table 2.

Plasma ghrelin levels did not show any significant correlation with body composition measures in the younger age group in either gender. Nor was it correlated with any of the lifestyle factors. In the older age group, plasma ghrelin values were significantly correlated with various fat mass measures in men and women. There were gender differences in the nature of these associations. In women, plasma ghrelin concentrations were inversely associated with total FM
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Table 1. Characteristics of study subjects: comparison of gender and age subgroup

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Age under 51-2 (years)</th>
<th>Age over 51-2 (years)</th>
<th>Age subgroups comparison (P-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n = 79)</td>
<td>Women (n = 79)</td>
<td>Men (n = 38)</td>
<td>Women (n = 38)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.46 ± 13.24</td>
<td>37.48 ± 8.97</td>
<td>59.15 ± 5.85</td>
<td>0.474 0.335</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.07</td>
<td>1.63 ± 0.07 *</td>
<td>1.77 ± 0.07</td>
<td>1.64 ± 0.07 *</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.7 ± 14.2</td>
<td>70.6 ± 13.9 *</td>
<td>85.1 ± 15.6</td>
<td>68.7 ± 15.1 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 ± 3.8</td>
<td>26.5 ± 5.0</td>
<td>27.2 ± 4.3</td>
<td>25.6 ± 5.5</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>19.1 ± 8.3</td>
<td>25.8 ± 10.5 *</td>
<td>18.8 ± 9.6</td>
<td>22.8 ± 11.5</td>
</tr>
<tr>
<td>Abdominal fat mass (kg)</td>
<td>2.1 ± 1.10</td>
<td>1.8 ± 1.0 *</td>
<td>1.8 ± 1.1</td>
<td>1.4 ± 0.9 *</td>
</tr>
<tr>
<td>Trunk fat/leg fat</td>
<td>2.1 ± 0.8</td>
<td>1.2 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>1.0 ± 0.3 *</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>57.7 ± 7.1</td>
<td>38.8 ± 5.0 *</td>
<td>58.4 ± 7.3</td>
<td>40.1 ± 5.3 *</td>
</tr>
<tr>
<td>Fat mass/lean mass</td>
<td>0.33 ± 0.13</td>
<td>0.67 ± 0.24 *</td>
<td>0.32 ± 0.15</td>
<td>0.57 ± 0.24 *</td>
</tr>
<tr>
<td>Ghrelin (ng/l)</td>
<td>869.1</td>
<td>1062.8 *</td>
<td>859.8</td>
<td>1056.1 *</td>
</tr>
</tbody>
</table>

Categorical variables n (%)

Smoking
- Never: 39 (49.4%) 52 (65.8%) 23 (60.5%) 23 (60.5%) 16 (39%) 29 (70.7%)
- Past: 27 (34.2%) 19 (24.1%) 4 (10.5%) 11 (28.9%) 23 (56.1%) 8 (19.5%)
- Current: 13 (16.5%) 8 (10.1%) 11 (28.9%) 4 (10.5%) 2 (4.9%) 4 (9.8%)

Alcohol consumption
- Never, rarely: 20 (25.3%) 39 (49.4%) 9 (23.7%) 16 (42.1%) 11 (26.8%) 23 (56.1%)
- Up to 14 units per week: 40 (50.6%) 38 (48.1%) 23 (60.5%) 21 (55.3%) 17 (41.5%) 17 (41.5%)
- More than 14 units per week: 19 (24.1%) 2 (2.5%) 6 (15.8%) 1 (2.6%) 13 (31.7%) 1 (2.4%)

All data are presented as mean ± SD, except for ghrelin, where data shown as medians (25–75 percentile). P-values are age subgroups comparisons.

*Significantly different from men (P < 0.05).

For conversion of ghrelin from metric units to SI units: ng/l × 3.371 (pmol/l).

BMI, body mass index.

\( r = -0.299, P < 0.05 \) and FM/LM ratio \( r = -0.419, P < 0.01 \), whereas in men associations were seen with abdominal fat mass \( r = -0.307, P < 0.05 \) and fat distribution TR FM/LEG FM ratio \( r = -0.334, P < 0.05 \). The correlation between abdominal fat mass and ghrelin was weak and not statistically significant in women \( r = -0.280, P > 0.05 \), while the correlation with fat distribution index in women did not suggest any relationship \( r = -0.081, P > 0.05 \).

In older women, plasma ghrelin levels were also associated with BMI \( r = -0.352, P < 0.05 \).

Alcohol intake was correlated with plasma ghrelin levels in older men and women \( r = -0.490, P < 0.001 \) and \( r = -0.277, P < 0.05 \) for men and women, respectively. Smoking was only associated with ghrelin in older men \( r = -0.307, P < 0.05 \), while physical activity showed significant correlations in older women \( r = -0.392, P < 0.05 \).

Plasma ghrelin levels were not associated with BMD in men of any age. In women in the younger age group, plasma ghrelin levels showed an inverse relationship with BMD, which was statistically significant only for total hip \( r = -0.390, P < 0.01 \) even after adjustment for fat mass, body size and lifestyle factors (Table 3). In older women no such relationships were also present.

The inverse association between plasma ghrelin levels and fat mass measures lead us to investigate for differences in plasma ghrelin between obese and normal subjects. We subdivided the dataset into subgroups based on the World health Organization (WHO) obesity classification. Thirty-five women (44.3%) and 24 men (30.4%) had a normal BMI (18.51–24.99 kg/m²), 26 women (32.9%) and 38 men (41.1%) were considered overweight (BMI: 25.00–29.99 kg m²), and 18 women (22.8%) and 17 men (21.5%) were obese (BMI ≥ 30 kg m²).
The results are presented in Fig. 2. Obese women had lower geometric mean plasma ghrelin levels compared to those in the overweight or normal BMI range (973·9; 116·5 and 1219·2 ng/l, respectively). The difference was statistically significant only between obese and normal subgroups ($P < 0·01$). Geometric mean ghrelin levels were also lower in obese men compared to overweight and normal, but the difference was not statistically significant.

Women within the normal or overweight BMI range had significantly higher plasma ghrelin levels to those in men of the same BMI range (1219·2 vs. 1057·3 ng/l for normal and 1116·5 vs. 952·1 ng/l for overweight women and men, respectively). There were no gender differences in plasma ghrelin levels between the obese BMI subgroups though.

The results of multiple regression analyses with plasma ghrelin as the dependent variable are presented in Table 4. In Model 1, gender, age and BMI showed a statistically significant association with plasma ghrelin levels, with women having lower levels compared to men. The difference was statistically significant between men and women ($P < 0·05$) and between age groups ($P < 0·01$ and $P < 0·01$ for age under and over 51·2 years, respectively). BMI also showed a statistically significant association with plasma ghrelin levels, with lower levels in obese men compared to normal and overweight men ($P < 0·01$ and $P < 0·01$, respectively).

**Table 2.** Nonparametric correlations (Spearman) between ghrelin levels and body composition/bone mineral variables

<table>
<thead>
<tr>
<th></th>
<th>All ($n = 79$)</th>
<th>Age under 51·2 (years)</th>
<th>Age over 51·2 (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Age</td>
<td>0·129</td>
<td>0·068</td>
<td>0·190</td>
</tr>
<tr>
<td>Height (m)</td>
<td>−0·053</td>
<td>0·002</td>
<td>−0·154</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>−0·147</td>
<td>−0·117</td>
<td>−0·158</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>−0·168</td>
<td>−0·196</td>
<td>−0·063</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>−0·119</td>
<td>−0·163</td>
<td>−0·035</td>
</tr>
<tr>
<td>Abdominal fat mass (kg)</td>
<td>−0·118</td>
<td>−0·137</td>
<td>−0·097</td>
</tr>
<tr>
<td>Trunk fat/leg fat</td>
<td>−0·099</td>
<td>−0·111</td>
<td>−0·277</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>−0·085</td>
<td>−0·69</td>
<td>−0·191</td>
</tr>
<tr>
<td>Fat mass/lean mass</td>
<td>−0·099</td>
<td>−0·191</td>
<td>0·009</td>
</tr>
</tbody>
</table>

Categorical variables:

- Alcohol intake
- Smoking
- Physical activity
- Statins use
- HRT

Bone mineral density (gms/cm²):

- Whole body BMD
- Lumbar spine BMD
- Total hip BMD
- Total forearm BMD

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

HRT, hormone replacement therapy; BMD, bone mineral density.

**Table 3.** Multivariate regression analysis for hip bone mineral density (BMD) and ghrelin levels in women under 51·2 years of age

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized coefficients B</th>
<th>Standard error</th>
<th>Standardized coefficients Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>1·569</td>
<td>0·484</td>
<td>−0·003</td>
<td>0·003</td>
</tr>
<tr>
<td>Ghrelin log</td>
<td>−0·091</td>
<td>0·041</td>
<td>0·310</td>
<td>0·033</td>
</tr>
<tr>
<td>Abdominal fat mass</td>
<td>0·056</td>
<td>0·016</td>
<td>0·522</td>
<td>0·001</td>
</tr>
<tr>
<td>Height</td>
<td>−0·015</td>
<td>0·016</td>
<td>0·011</td>
<td>0·940</td>
</tr>
<tr>
<td>Smoking</td>
<td>−0·023</td>
<td>0·016</td>
<td>−0·216</td>
<td>0·156</td>
</tr>
</tbody>
</table>

**Fig. 2** Plasma ghrelin levels comparison between genders of different body mass index (BMI) groups.
ghrelin levels. Of the different measures of fat mass, abdominal fat mass showed the strongest association and hence was included in Model 2. This model demonstrates that abdominal fat mass and alcohol consumption were significant predictors of plasma ghrelin levels independent of gender, age or body size (height). However, the models explain only a small proportion of the variability in plasma ghrelin concentrations (7–11%). Physical activity, smoking, hormone replacement therapy or statins were not associated with plasma ghrelin and did not change the results when included in the regression models.

Comparison of plasma ghrelin levels of different categories of alcohol intake is presented in Fig. 3. There were no significant difference in plasma ghrelin levels between participants taking up to 14 units of alcohol per week (849·0 ng/l, \( n = 40 \) for men and 1141·0 ng/l, \( n = 38 \) for women) and those who drink on social occasions only or not at all (863·8 ng/l, \( n = 20 \) and 1012·1 ng/l, \( n = 39 \) men and women, respectively), regardless of gender. Women and men who had more than 14 drinks per week (1187·6 ng/l, \( n = 19 \) for men and 2312·3 ng/l, \( n = 2 \) for women) had significantly higher plasma ghrelin levels.

The data was also analysed using a classic twin method approach where intrapair differences were used as studied variables. Intra-pair differences were obtained by subtracting the variable of the female twin from the corresponding male twin. The results of multiple linear regression analysis between intrapair gender differences in ghrelin levels and differences in body composition measures are shown in Table 5. The intrapair differences in ghrelin levels were associated with those in abdominal fat mass and fat distribution, with the models explaining 14% of the variance (\( P < 0·05 \)), and total lean mass (15%, \( P < 0·05 \)), confirming the association between ghrelin and fat mass measures showed by correlation and regression analysis.

Table 4. Multiple regression analysis of covariates to plasma ghrelin concentration*

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Standard error</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>7·040</td>
<td>0·190</td>
<td>0·000</td>
</tr>
<tr>
<td>Gender</td>
<td>0·128</td>
<td>0·051</td>
<td>0·013</td>
</tr>
<tr>
<td>Age</td>
<td>0·004</td>
<td>0·002</td>
<td>0·050</td>
</tr>
<tr>
<td>BMI</td>
<td>−0·017</td>
<td>0·006</td>
<td>0·004</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>7·162</td>
<td>0·020</td>
<td>0·000</td>
</tr>
<tr>
<td>Abdominal fat mass</td>
<td>−0·068</td>
<td>0·020</td>
<td>0·001</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>0·519</td>
<td>0·014</td>
<td>0·000</td>
</tr>
<tr>
<td>Gender</td>
<td>0·140</td>
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<td>0·002</td>
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<tr>
<td>Age</td>
<td>0·005</td>
<td>0·002</td>
<td>0·036</td>
</tr>
<tr>
<td>Height**</td>
<td>−0·377</td>
<td>0·334</td>
<td>0·260</td>
</tr>
</tbody>
</table>

Plasma concentrations of ghrelin (ng/l) have been log transformed for regression analyses. Log ghrelin is dependent variable.

*GEE-models were used for taking into account similarities between twin family members.

**Height was used instead of body mass index (BMI) as body size parameters to eliminate interaction between body weight and fat mass.

Discussion

Since the discovery of ghrelin, relatively few studies have examined its plasma levels in normal healthy populations and these have generally been in small samples.\(^{17,18,21-23,27}\) The study has confirmed that there is a gender difference in plasma ghrelin levels. Plasma ghrelin was affected by age and alcohol intake and inversely associated with BMI and fat mass measures. The correlation between plasma ghrelin levels and fat mass distribution index measured by DEXA in older men is a novel finding, not previously reported. Bone density measures were not related to ghrelin in general, except for a weak association between hip BMD and plasma ghrelin levels in younger women.

Our observation that women had higher levels of plasma ghrelin levels than men is in agreement with some previous reports\(^{19,21}\) but contradict others where no gender differences were found.\(^{17,20,22,23,27,28}\) However, the majority of the previous studies had smaller numbers of participants, and men and women were not matched for age.

In accordance with other studies, we observed that measures of body composition, such as BMI or body fat mass, had inverse associations with plasma ghrelin.\(^{18-22,27,34}\) In our study these associations were significant only in the older age group. We also observed gender differences in ghrelin/fat mass associations, which have not been reported previously. In women plasma ghrelin levels had a stronger relationship with BMI, total fat mass and fat mass/lean mass ratio and in men with abdominal fat mass and fat distribution index. The correlation between abdominal fat mass and ghrelin was weak and not statistically significant in women, whereas the correlation with fat distribution index in women did not suggest any relationship. Associations with central obesity have been observed previously, when measured as waist circumference and waist-hip-ratio.\(^{18,19}\)
It has been reported that plasma ghrelin levels are lower in obese individuals than in normal controls, and decreased in morbidly obese compared with nonmorbidly obese patients. Our study confirms some of these observations. Based on the BMI classification, obese subjects of either gender had lower plasma ghrelin levels compared to those in overweight or normal BMI range. Women within the normal or overweight BMI range had significantly higher plasma ghrelin levels to those in men of the same BMI range. The difference in plasma ghrelin levels between men and women in the obese BMI subgroups, however, was not statistically significant, suggesting that there is a stronger relationship with fat measures than with gender in the obese population.

The effect of age on ghrelin levels also remains uncertain. In two smaller studies, Shiiya et al. and Greenman et al. did not find significant differences in plasma ghrelin concentrations by age or gender in normal subjects. On the other hand, Purnell et al. found that plasma ghrelin levels were positively correlated with age in a study of 60 subjects. We did not find statistically significant correlations between ghrelin and age when twins were subdivided into gender and age subgroups. When we pooled all the study subjects in the multiple regression analysis (n = 158), age was a significant predictor of plasma ghrelin levels after adjustment for gender, fat mass and body size. However, age only accounted for 15–20% of the variance in plasma ghrelin levels. Thus, statistical power might play a role in the previous findings where study subject numbers were relatively small.

A study in rats by Fukushima et al. has reported that ghrelin directly promotes bone formation, increases BMD in rats. In the study by Ki Won Oh et al., serum ghrelin levels were not correlated with BMI in middle-aged Korean men. In the recent study of Weiss et al., the association between BMD measurement and plasma ghrelin became nonsignificant after adjustment for BMI and age. In our study, plasma ghrelin levels were not significantly associated with bone mineral density (BMD) generally, except at one site (i.e. total hip) in younger women. Previous studies have shown that hip BMD has strong associations with body composition measures and fat mass in particular. The weight-bearing nature of the hip site might partly explain this relationship. Our previous study has shown that the body composition/bone measure relationships were stronger in younger women.

The inverse relationship between obesity and ghrelin reported earlier is confirmed by our results. Thus, the associations between plasma ghrelin levels and hip BMD in younger women were consistent with the previous reports. The results of our study confirm the inverse relationship between obesity and ghrelin which has been reported earlier. We also observed gender differences in ghrelin/fat mass associations, which have not been reported previously. This study also suggests that in the obese population the relationship between total plasma ghrelin levels is stronger with fat measures than with gender. Our study also showed that ghrelin levels were associated with alcohol intake. This is in agreement with the findings of the Langenberg et al. and Kraus et al. Kellokoski et al. have reported that oestrogen therapy increases plasma ghrelin in postmenopausal women. Accordingly we included hormone replacement therapy as a variable in the multiple regression analysis. No effect was seen in our analysis, but only 19 women were currently taking hormone therapy.

This study has certain strengths and limitations. The main strength of this study is that opposite-sex twins were used to make gender comparisons of all the variables studied. Most previous studies have recruited men and women at best from the same population and of a similar age distribution. The use of opposite-sex twin pairs has the advantage of controlling for some of the important determinants of bone mass and body size such as age, genetic influences (half of the genetic make-up in opposite-sex twin is common to both members of a twin pair) and some early childhood/adolescent influences on bone and body composition such as diet. The results of the present study are based on a relatively large population of healthy men and women. The wide range of age in our study population has allowed us to observe age and gender differences in plasma ghrelin levels and their relation to body composition and bone mineral measures. Nevertheless more opposite-sex twins may have allowed us to detect more subtle differences in the study variables. Although we had greater power than many previous studies, we have also made multiple comparisons. However, in general our comparisons between groups were supported by the multiple regression analysis.
Our study examined the hypothesis that ghrelin affects surrogate measures of fracture risk such as bone density. Our study demonstrated that in healthy subjects, plasma ghrelin levels were influenced by age, gender and body composition measures such as fat mass. However, we found no evidence that ghrelin plays a role in human bone metabolism, as assessed by bone densitometry. This study suggests that measurement of plasma ghrelin is unlikely to have clinical utility in assessing bone health; however, further studies of the relationship between ghrelin and other markers of bone metabolism, such as biochemical markers of bone formation and resorption would be of interest.

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References


