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Serum leptin, adiponectin and ghrelin concentrations in post-menopausal women: Is there an association with bone mineral density?

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ABSTRACT

Objective: Adipokines and ghrelin exert well-documented effects on energy expenditure and glucose metabolism. Experimental data also support a role in bone metabolism, although data from clinical studies are conflicting. The purpose of this cross-sectional study was to investigate the association of serum concentrations of leptin, adiponectin and ghrelin with bone mineral density (BMD) in post-menopausal women.
Methods: BMD in lumbar spine and femoral neck, and circulating leptin, adiponectin and ghrelin concentrations were measured in 110 healthy post-menopausal women. Patients with secondary causes of osteoporosis were excluded.
Results: Osteoporosis was diagnosed in 30 (27%) women and osteopenia in 54 (49%). Serum leptin concentrations were positively correlated with both lumbar spine ($r = 0.343$, $p < 0.01$) and femoral neck BMD ($r = 0.370$, $p < 0.01$). Adiponectin concentrations were negatively associated with BMD at both sites ($r = -0.321$, $p < 0.01$ and $r = -0.448$, $p < 0.01$ respectively). No significant correlation between ghrelin concentrations and BMD was found. Osteoporotic women had lower body weight, body mass index (BMI) and leptin concentrations, but higher adiponectin concentrations compared with non-osteoporotic women. In multivariate stepwise regression analysis, the association of adiponectin concentrations with BMD remained significant only for femoral neck, after adjustment for body weight or BMI.
Conclusions: An inverse association between adiponectin and femoral neck BMD was found in post-menopausal women, independently of body weight. The positive association between leptin and BMD was dependent on body weight, whereas no effect of ghrelin on BMD was evident.

1. Introduction

Obesity and osteoporosis are important public health problems with increasing prevalence in the developed world. Obesity was initially considered protective for the skeleton, as a positive correlation between body weight and bone mineral density (BMD) at all skeletal sites was confirmed in many studies, in both sexes [1]. However the association between body weight and fracture risk is more complex [2]. Several theories have been proposed in order to explain the correlation of body weight with BMD: increased mechanical loading of the skeleton in obese individuals, increased insulin concentrations due to insulin resistance, increased estrogen aromatization in adipose tissue, reduced levels of sex hormone-binding globulin (SHBG) and common origin of osteoblasts and adipocytes [1].

Due to the discovery of adipokines, adipose tissue is not considered just a fat depot but a complex endocrine organ contributing to the regulation of many metabolic processes. The first to be discovered and best studied adipokine is leptin, followed shortly by adiponectin. Leptin acts at the hypothalamic level, as a satiety signal, controlling appetite and energy expenditure, according to the levels of energy stored as body fat. It is elevated in obese patients and insulin resistance states. Hyperleptinemia is also associated

Abbreviations: BMD, bone mineral density; BMI, body mass index; CV, co-efficient of variation; DXA, Dual-energy X-ray Absorptiometry; ELISA, enzyme-linked immunosorbent assay; IRMA, immunoradiometric; IQR, interquartile range; RANK, receptor activator of nuclear factor k; RIA, radioimmunoassay; SHBG, sex hormone-binding globulin; WHR, waist-to-hip ratio.

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with systemic inflammation and atherosclerotic disease [3]. On the other hand, adiponectin concentrations are reduced in obesity states. Adiponectin exerts insulin sensitizing, anti-inflammatory and anti-atherosclerotic properties [4]. Ghrelin, although it does not belong to adipokines, it is of special interest since it is considered the endogenous leptin antagonist, exerting orexigenic properties. It was named for its GH-releasing activity (Growth Hormone RElease Inducing), but there is evidence of pleiotropic effects including increased appetite, increased gastrointestinal motility, regulation of glucose metabolism and anti-inflammatory action [5–7]. Except for their role in adiposity and insulin resistance, a role of these peptides in bone metabolism has also been proposed, although current data are not conclusive.

Although the "BMD – body weight" and "body weight – adipokine" associations have been extensively explored, a direct link between BMD and adipokines is far less evaluated. The aim of this study was to investigate whether circulating levels of leptin, adiponectin and ghrelin are possible mediators of the relationship between body weight and BMD in post-menopausal women.

2. Methods

2.1. Patients

One hundred and ten post-menopausal women, aged 46 – 80 y, that had been referred to the Department of Endocrinology, 424 General Military Hospital, Thessaloniki, Greece for bone status evaluation, were included in the study. Menopause was defined by absence of menses for at least 12 months. Osteoporosis and osteopenia were defined according to the World Health Organization (WHO) criteria [8]. All participants were in apparently good health. Exclusion criteria were: (a) use of anti-osteoporotic agents at any time in the past; (b) any other treatment that could influence BMD (i.e. corticosteroids, thyroid medication, diuretics, β-blockers, calcium, antiepileptic drugs, anti-vitamin K agents); (c) surgical menopause; (d) secondary causes of osteoporosis (i.e. endocrine disorders, malignant disease, connective tissue disorders, malabsorption syndromes, chronic liver disease, chronic obstructive pulmonary disease, chronic renal disease, organ transplantation, immobility, rheumatoid arthritis, gastrectomy); (e) history of fracture; and (f) nicotine or excess alcohol consumption (>3 units/d) [9]. All participants had normal serum glucose, calcium, and parathyroid hormone concentrations, as well as, liver and renal function tests. The reason for applying such a large set of exclusion criteria was the avoidance of confounding factors, which is of particular importance in cross sectional studies.

2.2. Methods

With the subjects wearing light clothes and no shoes, height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight per square of height in meters (kg/m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest and hip circumference at the level of the trochanter. Waist-to-hip ratio (WHR) was also calculated. BMD at the lumbar spine (L2-L4) and femoral neck was determined in each subject by Dual-energy X-ray Absorptiometry (DXA) (Hologic Discovery A QDR Series). Daily quality control was performed with lumbar spine phantom (Hologic DXA Quality Control Phantom serial no 25647, area 54.3 cm^2, bone mineral content: 50.6 g and BMD: 0.93 g/cm^2). All BMD measurements were performed by the same operator using the same DXA equipment. According to the department's internal quality control, the precision for the specific technologist was 1.2% for lumbar BMD and 1.6% for neck BMD. These correspond to Least Significant Change values of 3.3% and 4.4%, respectively.

2.3. Hormone measurements

Blood samples were obtained from all participants after an overnight fasting. Samples were allowed to clot and then centrifuged, separated and stored at −30 °C, until they were analyzed. Leptin concentrations were measured using a commercially available radioimmunoassay (RIA) kit (Leptin-Ria-CT, KIPMR44, DIA Source Immunoassays S.A., Belgium). The intra-assay co-efficient of variation (CV) was 4.4% and 4.8% at a mean concentration of 19.67 and 6.95 ng/ml, respectively. The analytical sensitivity of the assay was 0.1 ng/ml. Total ghrelin concentrations were also assessed by RIA (Ghrelin Human RIA Kit RK-031-30, Phoenix Pharmaceuticals Inc., Belmont CA). The lowest detection limit of the method was 0.1–0.6 ng/ml and the intra-assay CV was 5%. Serum adiponectin concentrations were assessed by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Adiponectin ELISA KAPME09, DIA Source, Belgium). The analytical sensitivity of the assay was <0.6 ng/ml and the intra-assay CV < 5%. Circulating concentrations of insulin and estradiol were determined by using a commercial immunoradiometric (IRMA) and RIA Kit, respectively (Insulin IRMA kit IM3210, Beckman Coulter, with analytical sensitivity of 0.5 μIU/ml, intra-assay CV 4.3%; RIA Estradiol A21854, Beckman Coulter, analytical sensitivity <6 pg/ml, intra-assay CV 12.1%).

2.4. Statistics

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 20.0 (SPSS Inc, Ill, USA). A Shapiro-Wilks test for normality was used to assess data distribution. Summary descriptive statistics are expressed as mean ± standard deviation (SD) for normally distributed variables and as median and interquartile range (IQR, 25th and 75th percentiles) for variables that did not follow the normal distribution. Linear relations among variables were tested using Spearman rank correlation coefficients. Statistical significance was defined as <0.05. Subjects' division into osteoporotic and non-osteoporotic group was made "a posteriori". Student's T-test for normally distributed and Mann Whitney *U* test for not normally distributed variables were used to compare differences between subgroups. A multi-variate stepwise regression analysis with BMD at lumbar spine and femoral neck as dependent variables and body weight, BMI, leptin, adiponectin and insulin concentrations as independent variables was performed. Two models were created, due to collinearity between body weight and BMI.

The main study outcome was to prove an association between serum adipokines (leptin, adiponectin) and both lumbar and femoral neck BMD. Given a type I error (α) of 0.05, a type II error (β) of 0.20 and an effect size (|r|) of 0.3, a sample size of 84 women was calculated. The study recruited eventually 110 women in total.

3. Results

Anthropometric, biochemical and BMD data are presented in Table 1. According to the WHO criteria [8], 30 women (27%) were osteoporotic, 54 (49%) were osteopenic and 26 (24%) had normal BMD.

Table 2 illustrates the correlation coefficients between lumbar spine and femoral neck BMD, anthropometric parameters and serum leptin, adiponectin and ghrelin concentrations. Body weight, BMI, waist and hip circumference, waist to hip ratio, serum leptin and serum insulin concentrations were positively correlated with BMD at both sites. A negative correlation was found between serum adiponectin concentrations and both lumbar and femoral