The circulating concentration and ratio of total and high molecular weight adiponectin in post-menopausal women with and without osteoporosis and its association with body mass index and biochemical markers of bone metabolism

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Received 10 March 2009; received in revised form 24 May 2009; accepted 1 June 2009
Available online 10 June 2009

Abstract

Objectives: There is increasing evidence suggesting that adiponectin plays a role in the regulation of bone metabolism.

Design and methods: This was a cross-sectional study of 34 post-menopausal women with and 37 without osteoporosis. All subjects had body mass index (BMI), bone mineral density (BMD), total-, high molecular weight (HMW)-adiponectin and their ratio, osteoprotegerin (OPG), a marker of bone resorption (βCTX) and formation (P1NP) measured.

Results: We observed a positive correlation between BMI and BMD (r=0.44, p<0.001). When normalised for BMI, total-, HMW-adiponectin concentrations and HMW/total-adiponectin ratio were significantly lower in obese compared to lean subjects but there was no difference between those with or without osteoporosis. There were significant negative correlations between HMW/total-adiponectin ratio and BMI (r=−0.27, p=0.030) and with OPG (r=−0.44, p<0.001).

Conclusions: Our data suggests that there is no significant difference in the circulating concentration of fasting early morning total- or HMW-adiponectin in post-menopausal women with or without osteoporosis. The correlation between HMW/total-adiponectin ratio and OPG may indicate that adiponectin could influence bone metabolism by altering osteoblast production of OPG thereby affecting osteoclasts mediated bone resorption.

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Keywords: Adiponectin; Osteoporosis; Body mass index; Bone mineral density; Osteoprotegerin; Bone biomarkers

Introduction

Adiponectin is an adipocytokine that is highly expressed in human adipose tissue circulating in serum at concentrations of more than 10 mg/L [1,2]. It has been shown to have anti-diabetic [3], anti-atherogenic [4] and anti-inflammatory properties [5]. It is well known that adiponectin is decreased in adults with obesity and type-2 diabetes mellitus [6,7].

Several reports suggest that adiponectin was negatively correlated with bone mineral density (BMD) [8–12] but others have demonstrated no significant correlation [13–16]. One recent study by Kanazawa et al. [8] measured total and high molecular weight (HMW)-adiponectin in Japanese men and post-menopausal women with type 2 diabetes. They found that serum total-adiponectin was negatively correlated with total, lumbar spine and femoral neck BMD whereas HMW-adiponectin was negatively correlated with lumbar spine BMD alone in diabetic men. They also showed that total-adiponectin was positively correlated with serum osteocalcin and the presence of vertebral fractures. Conversely, in diabetic post-menopausal women there was no association between adiponectin and BMD at all sites studied or with the presence of vertebral fractures. However, total-adiponectin was again positively correlated with serum osteocalcin. This study [8] highlighted two important points: first, there appears to be specificity in the biological activity of circulating adiponectin isoforms on bone cells with total-adiponectin appearing to be

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doi:10.1016/j.clinbiochem.2009.06.003
more potent; second, gender and diabetic status appears to have a modifying role in the effect of adiponectin. The authors concluded that the association between serum adiponectin and BMD suggests that adiponectin may be a surrogate marker for BMD in assessing vertebral fracture in diabetic males.

It has been shown that the skeleton plays a pivotal role in the endocrine regulation of energy metabolism. Lee et al. demonstrated that mice lacking osteoblast-derived osteocalcin displayed decreased β-cell proliferation, glucose intolerance, and insulin resistance [17]. In co-culture experiments, osteocalcin from osteoblasts regulated adipocyte expression and secretion of adiponectin. This was the first study to demonstrate that the endocrine dialogue between bone and adipocytes involved adiponectin. It is well known that obesity protects mammals from osteoporosis with studies showing that leptin, also an adipocyte-derived hormone, regulates bone remodelling by acting on osteoblasts via neural pathways [18]. Thus, whether adiponectin expression and secretion in vivo protects against osteoporosis or contributes to its pathogenesis remains an untested hypothesis.

Adiponectin and its receptors (AdipoR1 and AdipoR2) are expressed in human osteoblasts [19,20] and osteoblast-like cell lines [21]. Adiponectin mRNA expression and adiponectin secretion increases during the differentiation of human osteoblasts in culture [22]. Two single nucleotide polymorphisms (SNPs), T45G and G276T, in exon 2 of the adiponectin gene have been shown to be associated with lumbar spine BMD suggesting a possible role for adiponectin in bone remodelling [21].

Adiponectin does not circulate as a monomer but rather, the main circulating forms in human plasma are a 180 KDa low molecular weight (LMW) hexamer and a ∼360 KDa high molecular weight (HMW) multimer [23,24]. There is also sexual dimorphism with females having higher levels of the HMW form [24]. One report has suggested that the ratio of HMW to total-adiponectin, not the total-adiponectin level per se, was responsible for the favourable metabolic effects such as increased insulin sensitivity [23]. Administration of HMW-adiponectin to mice also lowered glucose concentration in a dose-dependent manner [23]. Thus, it appears that the HMW form is the active form and that the ratio, by an unknown mechanism, may be an important determinant of the metabolic effects. At present it remains unclear which form of circulating adiponectin may have a role in bone metabolism although as stated above, one particular study [8] suggests that total-adiponectin may have a more potent effect on the bone.

Since adiponectin may play a role in the pathogenesis of osteoporosis, we examined the changes in the concentrations of plasma total- and HMW-adiponectin in post-menopausal women with and without osteoporosis. Importantly, because it is known that circulating plasma adiponectin concentration is affected by the Body Mass Index (BMI) and diabetes [6,7], we recruited both lean and obese post-menopausal women without diabetes. We investigated the relationship between total-, HMW-adiponectin, HMW/total-adiponectin ratio, osteoprotegerin (OPG), a marker of bone resorption (type 1 collagen C-telopeptide β-crosslaps, βCTX) and formation (type 1 procollagen amino-terminal pro-peptide, P1NP).

Materials and methods

Subjects

The local Research Ethics Committee approved the study and all volunteers gave informed written consent. All procedures in this study were conducted in accordance with the guidelines of The Declaration of Helsinki. We recruited 34 post-menopausal women with and 37 without osteoporosis from our Metabolic Bone Clinic. Subjects underwent a bone scan of the os calcis using the Peripheral Instantaneous X-ray Imager (PIXI) (GELunar Inc, Madison, WI) as part of an osteoporosis screening program. The subjects were categorized as having osteoporosis based on the T Scores, where > −0.5 was classified as normal and < −1.7 classified as osteoporosis [25]. Following an overnight fast of 12 h, blood samples were taken for electrolytes, calcium, phosphate, liver and renal biochemical profiles, β-CTX, P1NP, OPG, total- and HMW-adiponectin. Postmenopausal status was confirmed by elevated plasma FSH and LH concentrations and the absence of menstruation for at least 12 months. Information regarding alcohol consumption and smoking was obtained from all subjects. Information regarding fractures was obtained from the case notes. Subjects were excluded if they were on medications known to affect the skeleton such as corticosteroids; if they were on calcium and vitamin D supplements; whether they had ever been exposed to bisphosphonate therapy or if they were receiving hormone replacement therapy or had received it within the last year before the start of the study. None of the subjects were known to have diabetes mellitus. In this study, lean was defined as a BMI of 20−25 kg/m² whereas obese and overweight was defined as a BMI> 25 kg/m² because there is evidence that the latter are both associated with increased mortality in midlife [26].

Biochemical assays

Electrolytes, calcium, phosphate, liver and renal biochemical profiles were analysed on the Roche Modular System (Roche Diagnostic, Lewes, UK) using routine methods. Plasma adiponectin was measured by a high sensitivity direct enzyme-linked immunosorbent assay (ELISA) (BioVendor Laboratory Medicine, Inc., Heidelberg, Germany). This assay has been validated and used previously [27] and the kit insert reports an intra-assay coefficient of variation (CV) of 4.1% at 8.3 mg/L and 4.7% at 24.0 mg/L. The inter-assay CV was 4.0% at 7.57 mg/L and 7.4% at 19.3 mg/L. OPG was measured by a direct ELISA (Immunoendocrine Systems Ltd, Boldon, UK) with an intra- and inter-assay CV of <10% across the working range 0.1−30.0 pmol/L. β-CTX was measured using an electrochemiluminescence immunoassay (ECLIA) (ELECSYS, Roche Diagnostics, Lewes, UK) with an intra- and inter-assay CV of <5% across the working range 0.01−6 μg/L. P1NP was measured using an ECLIA on the Roche ELECSYS with an intra- and inter-assay CV of <5% across the working range of 5−1200 μg/L.

We also measured total and HMW-adiponectin using in-house developed assays as previously reported [28] with inter- and intra-assay CVs of <10% across the working range 0.5−30.0 mg/L.

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Table 1

| Patient characteristics, demographic details and biochemical markers of bone metabolism for post-menopausal women with and without osteoporosis. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Without osteoporosis | With osteoporosis | p value |
|                  | Lean (LWO) (n=14) | Obese (OWO) (n=23) | Lean (LO) (n=17) | Obese (OO) (n=17) |
| Age (range), yrs | 67.4 (45–84) | 68.0 (60–83) | 69.8 (60–80) | 69.4 (47–84) | 0.796 |
| Body mass index, kg/m² | 23.0 (1.4)a,b | 30.6 (3.3)c,d | 21.4 (2.4)c,e | 28.1 (2.3)b,d | <0.001 |
| T score (os calcis) | 0.51 (0.80)c | 0.87 (0.93)c,d | -2.5 (0.49)c,e | -2.2 (0.49)c,e | <0.001 |
| Current smoker (%) | 1 (7) | 1 (4) | 4 (24) | 6 (35) | 1.000 |
| Alcohol, units/week | 2.16 (2.5) | 1.09 (1.5) | 3.3 (5.8) | 6 (35) | 0.413 |
| Fracture in previous year (%) | 1 (7) | 4 (17) | 3.3 (5.8) | 3.9 (9.5) | 0.413 |
| Osteoprotegerin, mg/L | 0.43 (0.18) | 0.37 (0.18) | 0.53 (0.5) | 0.27 (0.1) | 0.062 |
| P1NP, μg/L | 28.4 (11.9) | 31.6 (22.7) | 37.4 (32.7) | 31.9 (15.7) | 0.728 |

Data are shown as mean (standard deviation) unless otherwise stated.

HMW — high molecular weight; β-CTX — Type 1 collagen C-telopeptide β-crosslaps; P1NP — Type 1 procollagen amino-terminal pro-peptide. Statistical significance denoted as follows: aLWO vs OWO; bLO vs OO; cLWO vs LO; dLWO vs OO; eOWO vs LO; fOWO vs OO.

Briefly, samples were treated with acid to allow the formation of dimers. For the measurement of HMW-adiponectin, the samples were treated with proteinase K [29] followed by acid treatment to stop proteinase K action. Both assays used microtitation wells coated with monoclonal adiponectin antibodies, biotinylated secondary antibodies and streptavidin conjugated horse radish peroxidise [29]. The colour formation with TMB substrate was measured spectrophotometrically at 450 nm and the absorbance was proportional to the concentration of total- and HMW-adiponectin respectively. These assays correlate well with other commercially available assays for concentration of total- and HMW-adiponectin respectively. The BioVendor and in-house total-adiponectin assays showed good correlation (r=0.97, p<0.001 in all cases; Fig. 1) The HMW/adiponectin ratio were also higher in lean subjects compared to obese subjects with or without osteoporosis (p<0.001; Fig. 2). Overall, there were significant differences in the concentration of total- and HMW-adiponectin between lean and obese subjects but no difference between subjects with or without osteoporosis.

Adiponectin, BMI and BMD

BMD was significantly and positively associated with BMI (r=0.44, p<0.001; Table 2). We did not observe any significant correlation between log total-adiponectin using the two assays (Table 2). The association of log HMW-adiponectin with BMI tended towards statistical significance (r=−0.23, p=0.057). However, there was a significant correlation between HMW/total-adiponectin ratio and BMI (r=−0.27, p<0.030). We found no significant correlations between total- or HMW-adiponectin and BMD (Table 2).
adiponectin ratio and OPG ($r = -0.44$, $p < 0.001$). There was also no association between total-, HMW-adiponectin and the HMW/total-adiponectin ratio with either the marker of bone formation – P1NP or the marker of bone resorption – $\beta$-CTX. We observed an association between P1NP and $\beta$-CTX ($r = 0.78$, $p < 0.0001$).

**Discussion**

In this study, we found significant differences in circulating total-HMW-adiponectin concentrations and HMW/total-adiponectin ratio between lean and obese post-menopausal women although there was no difference between those with or without osteoporosis. We also observed a significant and negative association between HMW/total-adiponectin ratio and OPG. To the best of our knowledge, at the time of investigation this was the first study evaluating the association between adiponectin isoforms and BMD, BMI and biochemical markers of bone metabolism.

In agreement with previous studies we found a significant and positive correlation between BMD and BMI [6,30]. Our findings are consistent with other reports that showed in non-diabetic women, serum adiponectin had no significant correlation with BMD [13–15]. In particular, similar to the Kanazawa et al. study [8] we found that in this cohort of non-diabetic post-menopausal women there was no association between total- or HMW-adiponectin and BMD. In addition, we found no difference between total- and HMW-adiponectin in post-
menopausal women with or without osteoporosis. We observed a strong association between P1NP and β-CTX \((r=0.777, p<0.001)\) suggesting intact coupling of bone remodelling but we found no correlation between a marker of bone resorption or formation and circulating total- or HMW-adiponectin. We also observed no correlation between total- and HMW-adiponectin with BMI although interestingly, we found that the HMW/total-adiponectin ratio to be significantly correlated with BMI. Our data suggest that there is no significant difference in the circulating concentration of fasting early morning total- or HMW-adiponectin in post-menopausal women with or without osteoporosis; however there is significant difference between lean and obese post-menopausal women.

We observed a significant and negative association between HMW/total-adiponectin ratio and OPG, which is a decoy receptor for the receptor activator of nuclear factor-κB ligand (RANKL) [31]. This is consistent with previous in vitro studies that showed adiponectin inhibited the production of OPG [20]. These observations suggest that adiponectin could influence bone metabolism by altering osteoblast production of OPG thereby affecting osteoclast mediated bone resorption. It has been shown that adiponectin exerts direct effects on the osteoblast via adipo R1 and/or adipo R2 receptors [19,20]. Adiponectin receptors have also been shown to be expressed on osteoclasts [32] and therefore adiponectin may directly or indirectly activate bone resorption. Further work is required to elucidate the exact mechanism(s) involved and the roles for the adiponectin isoforms in osteoclastic activity.

There are potential limitations of our study. There may be gender differences or a mediating role for menopausal status not shown here. Due to the cross-sectional nature of our study, no inferences of causality may be drawn. However, given the relative homogeneity of the subjects in the respective groups we were able to confidently identify potentially important variables.

### Conclusions

Our data suggest that there is no significant difference in the circulating concentration of fasting early morning total- or HMW-adiponectin in post-menopausal women with or without osteoporosis; however, the correlation between HMW/total-adiponectin ratio and OPG may indicate that adiponectin could influence bone metabolism by altering osteoblast production of OPG thereby affecting osteoclast mediated bone resorption.

### Disclaimers

All authors contributed to the design of the study, the analysis and interpretation of the data and in the final writing of the manuscript. R Sodi is the guarantor of the validity and originality of the data. We do not have any conflicts of interest to declare.

### References


