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ORIGINAL ARTICLE

The relationship between circulating adiponectin, leptin and vaspin with bone mineral density (BMD), arterial calcification and stiffness: a cross-sectional study in post-menopausal women

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Abstract
Objective To explore the relationship between circulating adiponectin, leptin and vaspin with bone mineral density (BMD), arterial stiffness and vascular calcification in post-menopausal women. We hypothesised that adipokines produced by adipose tissue may be mediators of bone and cardiovascular disease (CVD) and explain, in part, the observed association between osteoporosis and CVD.
Design We studied 386 ambulant community dwelling postmenopausal women aged (mean [SD] 61 [6.4] years). BMD at the lumbar spine, femoral neck (FN), and total hip (TH), body composition; fat mass (FM) and lean mass (LM) as well as abdominal aortic calcification (AAC) were determined by dual energy X-ray absorptiometry. Pulse wave velocity (PWV) and augmentation index, markers of arterial stiffness were measured. Fasting adiponectin, leptin and vaspin were quantified in serum.
Results A positive independent association was observed between vaspin and BMD at the FN ($p = 0.009$), TH ($p = 0.037$) in the whole study population adjusted for confounders including age, FM, LM and lifestyle variables. Using the same model, a negative association was seen between adiponectin and BMD at the FN in women with osteoporosis ($p = 0.043$). Serum adiponectin was significantly higher in women with fractures (20.8 [9.3] µg/ml compared to those without (18.5 [8.6] µg/ml, $p = 0.018$) and associated with a significant increased risk of fracture (HR 1.032, 95% CI 1.003–1.063, $p = 0.032$). Leptin was not associated with BMD or fracture risk after adjustment. Adiponectin was independently associated with AAC ($p = 0.007$) and significantly higher in women with AAC scores > 1; (19.2[9.2]) compared to those with no or low AAC scores (<1); 16.8 [8.0], $p = 0.018$). In adjusted analyses, PWV velocity was positively associated with circulating vaspin ($p = 0.039$) and AI was negatively associated with serum leptin ($p = 0.002$).
Conclusion Adiponectin, leptin, vaspin are related to markers of bone and vascular health and may contribute to the observed association between osteoporosis and CVD.

Keywords Adiponectin · Leptin · Vaspin · Bone density · Vascular calcification

Introduction

Cardiovascular disease (CVD) and osteoporosis are common age-related chronic disorders [1]. Both conditions are important public health problems with a high incidence in post-menopausal women. Several epidemiological studies have demonstrated that low bone mineral density (BMD), bone loss, and fractures are independently associated with significant increases in cardiovascular deaths in post-menopausal women [2]. Vascular calcification (VC) increases arterial stiffness and is associated with CVD-related events and mortality. VC is also highly linked with prevalent vertebral fractures [3].

Several biological pathways involving endocrine, autocrine/paracrine factors have been implicated in the pathogenesis of both VC and osteoporosis [4, 5]. It has also

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been postulated that the link between osteoporosis, fragility fractures and CVD may be via adipose tissue. Epidemiological studies show that fat mass is positively associated with BMD and may thus protect against osteoporosis in post-menopausal women, although recent evidence is emerging which indicates that obesity is associated with an increased risk of fracture [6–8]. Excess adiposity has also been shown to be related to increased risk of CVD, an effect which could be mediated, at least partly, through its effect on arterial compliance [9, 10]. The mechanisms linking adipose tissue to bone metabolism and CVD are not completely understood and are complex. Adipose tissue is metabolically active and produces a multitude of adipokines, which may have direct effects on the skeleton as well as on vascular structure and function [11, 12].

Several studies have shown significant associations between adiponectin and leptin with BMD, although evidence of a direct effect on bone metabolism or turnover in vivo remains unclear. In a meta-analysis, leptin was positively associated with BMD in post-menopausal women, although the associations were attenuated after adjusting for BMI [13]. A negative association between adiponectin and BMD has been reported which appears to be more pronounced in post-menopausal women, although the effect after adjustment for body fat mass is conflicting [13–15]. The effect of adiponectin on bone in in vitro or in animal models is positive and contrasts with epidemiological observations. Adiponectin stimulates bone formation and inhibits osteoclastogenesis [16–18] in experimental models. On the other hand, adiponectin knock out mice have increased bone mass [19]. The effect of vaspin on bone health is relatively understudied. In vitro studies show a positive effect on osteoblasts and inhibitory effects on osteoclasts [20, 21]. There have been no studies to date on the association between circulating vaspin and bone health in post-menopausal women.

Adipokines are also thought to play an important role in CVD by directly modulating endothelial and vascular smooth muscle cell (VSMC) activation and proliferation [22]. In the main, adiponectin is thought to have cardioprotective effects [23]. In clinical studies, the association between circulating concentrations of adiponectin with VC and CVD remains unclear and may be confounded by the presence of other CVD risk factors [24–27]. Leptin is involved in vascular inflammation and is thought to have a negative cardiovascular profile [19]. However, leptin has also been reported to be negatively associated with measures of vascular stiffness [27] which contrast with several studies [10, 28]. Vaspin has been shown to have anti-atherosclerotic and anti-inflammatory effect and is thought to be an insulin sensitiser [29]. However, there is no information as to whether vaspin is involved in the development of VC and arterial stiffness in non-diabetic, non-obese post-menopausal women.

Thus, the reported associations between adiponectin, leptin and vaspin with bone mass and CVD in post-menopausal women are unclear. Many studies have not looked at the relationship between these adipokines with measures of bone and cardiovascular health together in the same cohort. Our aim was to explore the relationship between adiponectin, leptin and vaspin with BMD, arterial stiffness and VC in a well-characterised cohort of community dwelling post-menopausal women. We hypothesise that these factors derived from adipose tissue may play a role in bone and cardiovascular health and, thus, explain the association between CVD and osteoporosis.

Materials and methods

Study population

This cross-sectional study consisted of 386 ambulant community dwelling postmenopausal women aged between 50 and 81 years (mean [SD] 61 [6.4] years). The study participants were recruited from the osteoporosis unit when they attended following referral for a DXA scan for assessment of their BMD and through community advertising for volunteers for the study. Ethical approval was obtained from the St Thomas' Hospital Research Ethics Committee and the study was carried out in accordance with the Helsinki's declaration. Written informed consent was obtained from each volunteer before entry into the study. At attendance, consecutive subjects were given a patient information sheet describing the study. Those who agreed to take part were entered in the study following informed consent. All study participants were asked to complete a questionnaire to capture their past, current medical and drug history, fracture history, lifestyle factors including smoking habits, alcohol intake. BMD was measured on all participants at the lumbar spine (LS), neck of femur (FN) and total hip (TH). Lean and fat mass were also determined by DXA. Blood pressure, pulse wave velocity (PWV) and augmentation index (AI), markers of arterial stiffness and cardiovascular risk, were measured. Fasting blood samples (overnight fast from 10.00 pm the previous day) were taken at the same visit and serum/plasma stored at −80 °C until analysis of the biomarkers. Exclusion criteria were history of metabolic bone diseases such as Paget's disease, chronic kidney disease-mineral bone disorder (CKD-MBD) or vitamin D deficiency, endocrine/biochemical abnormalities such as thyroid disorders, primary hyperparathyroidism and other secondary causes for osteoporosis. None of the participants were on current treatment with oestrogens, progestin, selective oestrogen receptor modulators, glucocorticoids,

teriparatide or lipid-lowering drugs. None of the participants had a history of diabetes mellitus or previous major cardiovascular events.

Assessment of bone mineral density (BMD), body composition and aortic abdominal calcification (AAC) by dual energy X-ray absorptiometry (DXA)

BMD was measured on all study participants. This was determined by DXA (Hologic QDR DXA Discovery scanner, Hologic, Inc. USA) at the lumbar spine (LS), femoral neck (FN) and total hip (TH) to assess BMD. The participants were divided into 3 groups based on their BMD in accordance with the WHO criteria for the diagnosis of osteoporosis and those with normal BMD, osteopenia or osteoporosis. The CV for BMD measurement using the spine phantom was 0.37%.

Vertebral fracture assessment (VFA) imaging of the thoracic and lumbar spine was done using DXA. This was used to assess abdominal aortic calcification (AAC) using a validated quantitative measure of AAC; the 24-point scoring system [30]. The trained reader was blinded to the participants' characteristics (with the exception of age) and the data of other investigations at the time each image were evaluated.

Body composition which included total lean mass (LM) and fat mass (FM) was also measured using DXA in accordance with the manufacturer's instructions. Percentage fat mass was derived from the proportion of total tissue mass made up of total fat mass. Fat mass distribution in the android and gynoid regions was derived as previously described [31] with android fat defined with the caudal limit at the top of the iliac crest and the cephalic limit at the base of the skull. This was set to 20% of the distance from the iliac crest to the base of the skull. The android fat mass includes both visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT is derived by subtracting SAT from the total android fat. Fat distribution in the gynoid region includes the hips and upper thighs, and overlaps both the leg and trunk regions. The upper demarcation used to derive gynoid fat is below the top of the iliac crest at a distance of 1.5 times the android height. The results were expressed as the android/gynoid ratio. The CVs for the measurement of total lean and fat mass are 1%. The VAT coefficient of variation is less than 10%.

Determination of pulse wave velocity (PWV) and Augmentation Index (AI)

Arterial stiffness is an important determinant of cardiovascular risk. Augmentation index (AI) and pulse wave velocity (PWV) are 2 non-invasive measures of arterial stiffness and established surrogate markers of early CVD. PWV is a marker of central arterial stiffness and is a predictor of cardiovascular and all-cause mortality [32]. AI is a measure of systemic arterial stiffness and is affected by macrovascular as well as microvascular function. AI is defined as the difference between the first and second peaks of the central arterial waveform, and is expressed as a percentage of the pulse pressure. Higher AI has been shown to be associated with adverse cardiovascular events [33]. AI and PWV were measured using the SphygmoCor system (AtCor Medical LTD, Australia). All measurements were done in triplicate by a single operator and the average value used.

Routine biochemical analyses

Routine biochemical tests including serum creatinine, albumin corrected calcium were measured by standard laboratory methods using Roche automated analysers (Roche diagnostics Limited, West Sussex RH15 9RY, UK). Estimated glomerular filtration rate (eGFR) was calculated using the MDRD formula. Blood glucose was measured on a sub-group of subjects ($n = 205$). Serum intact PTH was measured using Roche reagents on the Roche Elecsys 2010 analyser. Assay CVs were <5% at mean PTH concentrations of 41 and 105 ng/L.

Measurement of circulating adiponectin, leptin and vaspin

Serum adiponectin and Leptin were measured in serum by sandwich enzyme-linked immunosorbent assay (ELISA) technique (Duoset, R&D Systems Europe Ltd, Abingdon, UK) according to the manufacturers instructions. The assay detected all circulating molecular forms of adiponectin. Standard curves were constructed using concentrations of adiponectin, leptin and vaspin ranging from 0 to 8, 0 to 4, 0-4 mg/ml, respectively. All serum samples were diluted 1:5000 fold using reagent diluent prior to adiponectin analysis and 1:200 before serum leptin measurement. All results were multiplied by the respective dilution factor. Neat serum was used for vaspin measurement. The Intra- and inter-assay CV for serum adiponectin and leptin ranged from 7.9 to 8.9 and 1.7 to 4.1%, respectively. Intra-assay CV ranged from 0.4 to 3.2% and inter-assay CV was 11.5% for serum vaspin.

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 22.0. The characteristics of study participants are described as mean and standard deviation (SD) and were derived for all continuous variables. Non-parametric data were log-transformed to ensure normal distribution. Serum vaspin was log-transformed as it was not normally

distributed. Values are shown as median (5th–95th percentile). Bivariate analyses were used to explore the relationship between the adipokines with BMD, AAC and measures of arterial stiffness. Multivariable linear regression models were used to examine the associations between BMD at the LS, FN, TH as dependent variable and leptin, adiponectin and vaspin as independent variables after adjustment for age, years since menopause, body composition (fat mass, lean mass), VATm, android/gynoid ratio, fracture history, smoking habits, alcohol intake. The confounding factors were chosen based on their previously documented association with BMD and correlations following the bivariate analyses. Binary logistic regression was used to analyse the association between adiponectin, leptin or vaspin with fracture history (no/yes) following adjustment for the same covariates listed above. AAC, PWV and AI were analysed as dependent variables in a similar model as for BMD but other cardiovascular risk factors (serum lipids, blood pressure) which can impact on arterial calcification and compliance were also entered as covariates in the model. Group means were compared using the unpaired student's 't' test. A '*p*' value of <0.05 (95% confidence interval) was considered as statistically significant.

Results

Study population

Three hundred and eighty six post-menopausal women were studied. The study population demographics are summarised in Table 1. Eighty eight (22.8%) women had normal BMD, 215 (55.7%) had osteopenia (T score $<-1 > -2.5$) and 83 (21.5%) had osteoporosis (T score <-2.5) at either the LS, FN or TH. One-hundred and twenty-five women (32.4%) had previously sustained a fracture. Eighty three women (21.5%) had previously been or were currently on bisphosphonate for a duration of mean [SD] 5.3 [4.3] years. BMI was significantly higher in women with normal BMD; 26 [5.4] kg/m2 compared to those with osteopenia; 24 [3.9] kg/m2 and osteoporosis; 23 [3.7] kg/m2, $p < 0.001$. In the whole study population, PWV was mean [SD] 8.9 [1.75] m/s and AI 30 [7.1]%. There was a significant difference in PWV in women with osteoporosis; 9.2 [1.9] m/s compared to those with normal BMD; 8.6 [1.5] m/s, $p = 0.045$. This was no longer significant when adjusted for age. There was a significant negative association between PWV and BMD at the femoral neck ($p = 0.027$) following correction for confounders including age, body composition, serum lipids concentration, blood pressure. There was no significant difference in AI between the 3 groups. PWV was significantly higher in subjects who had previous fractures; 9.2 [2.1] m/s compared to those with no history of fractures; 8.7 [1.6] m/s, $p = 0.038$ and those on bisphosphonate; 9.4 [2.0] m/s compared to those not on bisphosphonate; 8.7 [1.6] m/s, $p = 0.008$. There was no significant difference in AI or AAC. There was a negative correlation between serum leptin and adiponectin ($r = -0.149$, $p = 0.003$) and a positive relationship between leptin and vaspin ($r = 0.132$, $p = 0.009$). In the whole study population, circulating total adiponectin was 19.3 [8.9] µg/ml, leptin was 24.3 [18.5] ng/ml and vaspin (median [5–95th percentile]) was 203 [39–1516] pg/ml. Adiponectin concentrations were significantly higher in the groups with osteopenia; 19.7 [8.8] µg/ml and osteoporosis; 20.2 [9.2] µg/ml compared to normal BMD; 17.5 [8.6] µg/ml, $p < 0.05$. In contrast, serum leptin was significantly lower in subjects with osteopenia; 22.8 [16.5] ng/ml and osteoporosis; 22 [20.3] ng/ml compared to those with normal BMD 29.6 [20.2] ng/ml, $p < 0.01$. There were no significant differences in serum vaspin

Table 1 Summary of study population characteristics

Parameters mean [SD]	Normal BMD $n = 88$	Osteopenia $n = 215$	Osteoporosis $n = 83$
Age (years)	60 (5.4)	61 (6.2)$^{\#}$	63 (7.23)**
BMI (kg/m^2)	26.0 (5.4)	24 (3.9)$^{\#\#}$	23 (3.7)**
Fat mass (kg)	28.0 (9.6)	23.9 (7.0)$^{\#\#}$	21.2 (6.8)**
Lean mass (kg)	43.5 (6.5)	39.8 (4.4)$^{\#\#}$	37.5 (3.9)**
Android/gynoid ratio	0.91(0.14)	0.86 (0.16)$^{\#}$	0.83 (0.17)**
Years since menopause	10.5 (6.74)	12 (7.19)$^{\#}$	13 (8.60)**
Hip total BMD (g/cm^2)	0.969 (0.07)	0.837 (0.08)$^{\#\#}$	0.731 (0.07)**
Femoral neck BMD (g/cm^2)	0.842 (0.07)	0.703 (0.07)$^{\#\#}$	0.61 (0.06)**
Spine total BMD (g/cm^2)	1.04 (0.07)	0.896 (0.09)$^{\#\#}$	0.758 (0.08)**
Pulse wave velocity (m/s)	8.6 (1.5)	8.9 (1.8)	9.17 (1.9)*
Augmentation index (%)	30 (7.2)	29.4 (6.6)	30.5 (8.0)

* $p < 0.05$, ** $p < 0.01$ normal BMD versus osteoporosis, $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ normal BMD versus osteopenia

between the groups. There was no significant difference in serum adiponectin and vaspin in the group of women on bisphosphonates compared to the treatment naïve subjects. Serum leptin was significantly lower in those on bisphosphonate (20.5 [14.5] ng/ml compared to women who were not taking bisphosphonate (25.4 [19.3] ng/ml, $p = 0.013$). The difference was no longer significant when adjusted for FM and LM. No significant differences in eGFR, serum lipid concentrations, PTH concentrations were observed between subjects with osteoporosis, osteopenia or normal BMD. Mean blood glucose in a sub-group of study participants ($n = 205$) was 4.9 [0.47] mmol/L. The unadjusted biochemical data are summarized in Table 2.

Relationship between adipokines, BMD and fractures

Bivariate analyses showed significant inverse correlations between serum adiponectin with BMD; LS ($r = -0.126$, $p = 0.016$), FN ($r = -0.138$, $p = 0.007$), and TH ($r = -0.19$, $p < 0.001$) and a positive correlation between leptin and BMD; LS ($r = 0.143$, $p = 0.006$), FN ($r = 0.183$, $p < 0.001$), and TH ($r = 0.202$, $p < 0.001$). When the association between serum adiponectin, leptin and vaspin was investigated in the whole study population using multivariable linear analysis, the following correction for age, body composition and confounders which included years since menopause (YSM), lifestyle variables including smoking habits, alcohol intake, bisphosphonate treatment, the relationship between adiponectin, leptin with BMD, seen in the bivariate analyses at all skeletal sites, was no longer significant. We observed a significant independent positive association between serum vaspin and BMD at the FN ($p = 0.009$) and TH ($p = 0.037$) Table 3. In the sub-group of women with osteoporosis, an independent negative association between adiponectin and BMD at the FN was observed ($p = 0.043$). Serum adiponectin was significantly higher in women with fractures (20.8 [9.3] µg/ml compared to those without (18.5 [8.6] µg/ml, $p = 0.018$). Following binary logistic regression, the risk of fracture was significantly associated with serum adiponectin only adjusted for age, years since menopause, body composition; fat mass, lean mass, android/gynoid ratio, VAT, and lifestyle variables; smoking habits, alcohol intake (HR 1.032, 95% CI 1.003–1.063, $p = 0.032$) Table 4. In sub-group analyses, using the same models, in participants who were not on bisphosphonates, we observed a significant association between serum vaspin and BMD at the FN ($p = 0.04$). The fracture risk was also positively associated with adiponectin with a similar size effect, although the results just failed to reach significance (HR 1.034, 95% CI 0.998–1.071, $p = 0.06$).

Table 2 Adiponectin, leptin and biochemical parameters of post-menopausal women with normal BMD, osteopenia and osteoporosis

Parameters mean [SD]	Normal $n = 88$	Osteopenia $n = 215$	Osteoporosis $n = 83$
Adiponectin (µg/ml)	17.5 (8.6)	19.7 (8.8)$^{\#}$	20.2 (9.2)*
Leptin (ng/ml)	29.6 (20.2)	22.8 (16.5)$^{\#\#}$	22 (20.3)**
Vaspin (pg/ml) median (5th–95th percentile)	161 (30–1570)	196 (44–1340)	257 (20–1760)
GFR (ml/min)	77.9 (13.3)	79.0 (15.1)	77.6 (15.2)
Albumin corrected calcium (mmol/L)	2.38 (0.07)	2.38 (0.10)	2.4 (0.08)
Parathyroid hormone (PTH) (ng/L)	35.8 (12.0)	37.7 (12.0)	39.1 (10.4)
Serum cholesterol (mmol/L)	5.9 (0.92)	6.1 (0.98)	6.0 (0.89)
Serum triglycerides (mmol/L)	1.1 (0.4)	1.0 (0.4)	1.1 (0.6)

* $p < 0.05$, ** $p < 0.01$ normal BMD versus osteoporosis, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ normal BMD versus osteopenia

Table 3 Association between adipokines and BMD at the femoral neck (g/cm^2) in the whole study population following adjustment for covariates including age, years since menopause, body composition (fat mass, lean mass), VATm, android/gynoid ratio, fracture history, smoking habits, alcohol intake, *$p < 0.05$

	Dependent variable: Femoral Neck (FN) (g/cm^2)		
	standardised coefficient Beta	unstandardized co-efficient B confidence interval (95%)	p value
Adiponectin (ug/ml)	0.000	−0.001 to 0.001	0.997
Log Vaspin (pg/ml)	0.127	0.007–0.048	0.009*
Leptin (ngml)	−0.064	−0.001 to 0.000	0.325

Table 4 Adipokines association with fractures (yes/no) in the whole study population following binary logistic regression adjusted for age, body composition (fat mass, lean mass), VATm, android/gynoid ratio, smoking habits, alcohol intake, *$p < 0.05$

	Fractures (no/yes)		
	Odds ratio	Confidence interval (95%)	*p* value
Adiponectin (ug/ml)	1.032	1.003–1.063	0.032*
Log vaspin (pg/ml)	0.810	0.500–1.315	0.391
Leptin (ng/ml)	1.006	0.989–1.023	0.495

Relationship between adipokines with AAC and arterial stiffness; PWV and AI

Adiponectin was positively correlated with AAC in bivariate analyses ($r = 0.19$, $p = 0.001$). This remained significant in multivariable analysis when adjusted for confounders including age, blood pressure, FM, LM, serum lipids (total cholesterol and triglycerides) Table 5A. Serum adiponectin was significantly higher in women with AAC scores > 1; (19.2 [9.2]) compared to those with no or low AAC scores (<1); 16.8 [8.0] ug/ml, $p = 0.018$) Fig. 1. There was no significant association between serum leptin and AAC in adjusted and unadjusted models. When adjusted for age, body composition, systolic blood pressure, serum lipids, AI was negatively associated with serum leptin ($p = 0.005$) Table 5B.

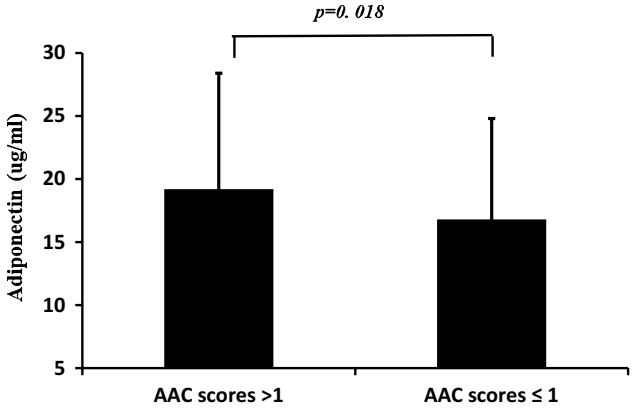

Fig. 1 Serum adiponectin concentrations in post-menopausal women with AAC scores >1 compared to those with no or low AAC score

In the same model, we observed any significant independent association between PWV and serum vaspin ($p = 0.039$) Table 5C. Sub-analyses, using the same model in the participants who were not on bisphosphonates, still showed significant associations between AAC and adiponectin ($p = 0.008$), PWV and serum vaspin ($p = 0.01$) and serum leptin with AI ($p = 0.003$). There was a significant correlation between PTH with PWV ($r = 0.12$, $p = 0.036$) and leptin ($r = 0.157$, $p = 0.002$) in bivariate analyses but the relationship failed to reach significance in the adjusted models.

Table 5 **A** Association between adipokines and AAC in the whole study population after adjustment for covariates including age, body composition (fat mass, lean mass), blood pressure, serum lipids. **B** Association between adipokines and measure of arterial stiffness; augmentation index (AI) in adjusted analyses, **$p < 0.01$. **C** Association between adipokines and pulse wave velocity (PWV) in adjusted analyses

A	Dependent variable: AAC		
	standardised co-efficient Beta	Unstandardized co-efficient B Confidence Interval (95%)	*p* value
Adiponectin (ug/ml)	0.17	0.013–0.085	0.007**
Leptin (ng/ml)	0.015	−0.021 to 0.025	0.86
Log vaspin (pg/ml)	0.021	−0.495 to 0.708	0.727
B	Dependent variable: AI		
	standardised co-efficient Beta	Unstandardized co-efficient B confidence interval (95%)	*p* value
Adiponectin (ug/ml)	0.026	−0.065 to 0.106	0.631
Leptin (ng/ml)	−0.231	−0.148 to −0.034	0.002**
Log vaspin (pg/ml)	0.039	−0.897 to 1.992	0.456
C	Dependent variable: PWV		
	standardised co-efficient Beta	unstandardized co-efficient B confidence interval (95%)	*p* value
Adiponectin (ug/ml)	−0.038	−0.028 to 0.013	0.47
Leptin (ng/ml)	−0.102	−0.025 to 0.003	0.114
Log vaspin (pg/ml)	0.104	0.017–0.684	0.039*

Discussion

To our knowledge, this is the first study to show a positive significant association between serum vaspin and BMD at the FN in a cohort of community dwelling post-menopausal women. Our findings also show a significant independent association between serum adiponectin, vaspin and BMD at the FN in the sub-group with osteoporosis. Adiponectin was associated with an increased risk of fractures. Adiponectin was positively associated with AAC and there was an inverse relationship between serum leptin and AI, a measure of arterial stiffness. Serum vaspin was positively associated with PWV.

Data from in vitro studies suggest that vaspin has a positive effect on bone density as it increases bone formation and reduces resorption [20, 21]. However, clinical data are lacking. Small studies in patients with inflammatory bowel disease and multiple sclerosis have failed to show any relationship with bone density [34, 35]. Thus, our finding of a positive effect of circulating vaspin on BMD at the hip site in post-menopausal women is interesting and novel. This positive association is biologically plausible as vaspin has been shown to modulate bone cell function [19, 20]. In addition, we speculate that vaspin may also influence BMD though its function as an insulin sensitiser [36] which could impact on insulin-like growth factor-1 (IGF-1). IGF-1 is thought to be down-regulated in the clinical setting of insulin resistance and the metabolic syndrome [37]. The effect of IGF-1 on peak bone mass in young adults and the maintenance of bone health in the elderly is well-documented [38]. We observed a significant independent association between femoral neck BMD and adiponectin in women with osteoporosis as previously reported [39]. Women with osteoporosis have been shown to have higher adiponectin concentrations compared to those without osteoporosis [30], in agreement with our own findings. The association between adiponectin and fracture risk has been previously reported in men but not in women [40, 41]. In the current study, we extend this finding to post-menopausal women and demonstrate that increased adiponectin is a risk factor for fractures, independently of other classical risk factors. The mechanism underlying the association with fracture risk and the contrasting biological and clinical effects of adiponectin on bone is unknown. One explanation is that the actions of adiponectin on bone may vary depending on whether its effects are mediated through autocrine/paracrine or endocrine pathways. Thus, we can speculate that locally derived adiponectin in the bone marrow environment has a positive effect on bone formation. However, the endocrine effect of adiponectin on bone may be negative as high circulating concentrations may suppress bone formation. Previous studies have shown that adiponectin levels are higher in peripheral compared to bone marrow plasma in post-menopausal women [42], although no data are available in post-menopausal women with osteoporosis. Further studies are needed to compare the local bone marrow concentrations and circulating concentrations in various populations. Circulating leptin was not associated with BMD, when adjusted for fat mass as previously shown [13]. Our data suggest that strategies that target and modulate adiponectin and vaspin production may be useful in the management of osteoporosis and fracture prevention.

Adiponectin, leptin and vaspin have been shown to play a role on cardiovascular pathophysiology. They regulate pathways linked with atherosclerosis, VC and stiffness. We showed that higher adiponectin concentrations were associated with increased AAC scores. Our findings contrast to previous studies where lower levels of adiponectin have been linked with coronary artery calcium [24]. The differences in the observed relationship may be related, at least in part, to differences in the extent of vascular calcification or prevalent CVD. Our population did not have any evidence of clinical CVD and, thus, it can be postulated that in this clinical context, increases in adiponectin may be a protective mechanism in an attempt to reduce oxidative stress and inhibit progression of vascular calcification as previously found in a study of young adults aged 33–45 years [43]. This may also explain why some studies have found higher adiponectin levels in people with a history of CVD [26]. Similar mechanism may also explain the positive association between serum vaspin and PWV in our population. Some but not all studies have shown positive associations between serum vaspin and coronary atherosclerosis and stenosis [44–46]. Analogous to the relationship between adiponectin and AAC, we can hypothesise that vaspin secretion may have a compensatory effect on the metabolic abnormalities which lead to decreased vessel compliance. It is not known whether vaspin is produced by endothelial cells but we can speculate that the effect on PWV may be related to its endocrine effect.

Increased leptin concentration is thought to predispose to ischaemic heart disease and induce vascular dysfunction through its pro-inflammatory effect [22]. In the current study, we observed a significant negative-independent association between leptin and AI (a measure of systemic arterial stiffness). An inverse relationship between serum leptin and arterial stiffness has been reported in a younger community-based population with no evidence of CVD [27]. These observations are in agreement with ours and extend the findings to an older female population. Our data thus suggest a protective role of leptin on arterial stiffness. One explanation is that leptin may exert differential effects on large central compared to smaller peripheral arteries. Another explanation is increased leptin production that

may have a positive effect on vascular remodelling. Leptin has been shown to exert vasodilatory effects and enhance endothelial cell proliferation [47, 48]. However, although we have shown a beneficial effect of leptin on arterial stiffness, determined by the measurement of AI, in post-menopausal women, we cannot infer causality and further studies are required to assess the underlying mechanisms. The clinical implications of our findings remain to be established but our data suggest that these adipokines could become new targets in the management or monitoring of CVD risk.

The study has several limitations. We were unable to evaluate the causal relationship between adiponectin, leptin and vaspin with bone and cardiovascular health due to the cross-sectional design. The observed associations may be influenced by confounders, although the study population is well characterised and we were able to adjust for several variables in our analyses. We did not assess vitamin D status which has been implicated in vascular function [49]. Finally, our findings may not be generalisable to other populations.

In summary, we have shown that circulating vaspin is positively associated with BMD at the femoral neck and hip in post-menopausal women. Adiponectin is associated with increased fracture risk. All 3 adipokines were associated with markers of vascular health. Further longitudinal studies are required to examine whether these adipokines have a direct effect on the pathogenesis of osteoporosis and CVD.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Ethical approval was obtained from the St Thomas' Hospital Research Ethics Committee and the study was carried out in accordance with the Helsinki's declaration.

Informed consent Written informed consent was obtained from each volunteer before entry into the study.

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