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OSTEOPENIA AND ENDOTHELIN-1-MEDIATED VASCONSTRICTOR TONE IN POSTMENOPAUSAL WOMEN

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Abstract

Low bone mineral density is highly prevalent in postmenopausal women. Osteopenia in postmenopausal women is a predictor of adverse cardiovascular events. A potential mechanism contributing to the increased cardiovascular risk in postmenopausal women with osteopenia is endothelial vasomotor dysfunction. Endothelin (ET)-1 is a potent vasoconstrictor peptide that is associated with endothelial vasomotor dysfunction and increased cardiovascular risk. Currently, there is no information on osteopenia and ET-1 vasoconstrictor activity in postmenopausal women. We tested the hypothesis that ET-1 mediated vasoconstrictor activity is greater in postmenopausal women with osteopenia compared with those without. Forearm blood flow responses to intra-arterial infusion of BQ-123 (100 nmol/min for 60 min), a selective ET_A receptor antagonist, were determined in postmenopausal women: 10 with osteopenia (age: 56±1 yr, lumbar spine T-score between -1.5 and -2.5) and 12 without osteopenia (age: 60±2 yr, T-score > -1.5). In women with osteopenia, forearm blood flow increased approximately 25% (P<0.05) in response to BQ-123. However, in the women without osteopenia, resting forearm blood flow was not significantly changed. In conclusion, these results suggest that osteopenia is associated with greater ET-1-mediated vasoconstrictor tone. Increased ET-1 vasoconstrictor activity may contribute to the elevated cardiovascular risk in postmenopausal women with osteopenia.

Keywords

endothelial function; vasoconstriction; low bone mineral density; postmenopausal women; osteopenia

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Introduction

Osteopenia is a condition characterized by low bone mineral density that is highly prevalent among postmenopausal women [1]. It is estimated that approximately half of women in the United States aged 50 years and older have osteopenia [2]. Importantly, in addition to increased risk for bone fracture, postmenopausal women with low bone mineral density are at increased risk for developing cardiovascular diseases. Low bone mass is associated with increased incidence of stroke, coronary artery disease and cardiovascular disease-related mortality [3–5]. The increased cardiovascular disease risk observed in postmenopausal women with osteopenia may be due, in part, to endothelial vasomotor dysfunction. Indeed, impaired endothelial vasomotor function has been etiologically linked to the initiation and progression of atherosclerotic vascular disease [6,7]. The vascular endothelium plays a critical role in vasomotor regulation through the synthesis and release of opposing vasoactive substances, notably the vasodilator nitric oxide and the potent vasoconstrictor endothelin-1 (ET-1). Furthermore, it has been suggested that a disruption in the balance between the effects of these vasoactive substances, favoring ET-1-mediated vasoconstriction, underlies vasomotor dysregulation. While it has previously been demonstrated that low bone mineral density is associated with impaired nitric oxide-mediated endothelium-dependent vasodilation in postmenopausal women [8], it is currently unknown whether ET-1-mediated vasoconstrictor tone is elevated in this population. Accordingly, we tested the hypothesis that ET-1-mediated vasoconstrictor tone is greater in postmenopausal women with osteopenia compared with those without osteopenia.

Materials and Methods

Twenty-two sedentary, postmenopausal women were studied: 12 without osteopenia (lumbar spine T-score > -1.5) and 10 with osteopenia (lumbar spine T-score between -1.5 and -2.5). All subjects were non-smokers and free of overt cardiovascular or metabolic disease as assessed by medical history, physical examination, fasting blood chemistries and resting and maximal exercise electrocardiograms. None of the subjects were taking medications (including vitamins), or engaged in regular physical exercise for at least 1 year prior to the start of the study. Additionally, all subjects were at least 1 year postmenopausal and had never taken or had discontinued use of hormone replacement therapy at least 1 year prior to the start of the study. Before participation, the subjects provided written informed consent according to the guidelines of the University of Colorado at Boulder.

Bone Mineral Density and Body Composition

Lumbar spine (L2–L4) bone mineral density and body composition were assessed by dual energy X-ray absorptiometry (Lunar Corporation, Madison, WI). Bone mineral density results were categorized according to published guidelines [9]. Body mass was measured to the nearest 0.1 kg with a medical beam balance (Detecto, Webb City, MO). Body mass index was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines [10].

Metabolic Measurements

Fasting plasma lipid, lipoprotein, glucose and insulin concentrations were determined with standard techniques. Plasma concentrations of C-reactive protein were determined by high-sensitivity enzyme immunoassay (ALPCO Diagnostics, R&D Systems, Windham, NH) as previously described by our laboratory [11].

Intra-Arterial Infusion Protocol

All studies were performed between 7 am and 10 am in a temperature-controlled room following a twelve-hour overnight fast. A 5-cm, 20-gauge catheter was inserted in the brachial artery of the non-dominant arm under local anesthesia (1% lidocaine). Forearm blood flow was measured in both the experimental (non-dominant) and contralateral (dominant) forearm using strain-gauge venous occlusion plethysmography. Forearm blood flow was measured at baseline and in response to BQ-123 (Clinalfa, AG), a selective ET_A receptor antagonist. BQ-123 was infused at a rate of 100 nmol/min for 60 min. This dose of BQ-123 has been demonstrated to completely inhibit the vasoconstrictor response to ET-1 in the adult forearm [12,13]. Forearm blood flow was measured every ten minutes throughout the infusion of BQ-123.

Statistical Analysis

Differences in subject characteristics were determined by analysis of variance. Group differences in the forearm blood flow response to BQ-123 were determined by repeated measures analysis of variance. Relations between variables of interest were assessed by linear and regression analysis. Plasma C-reactive protein concentrations were log transformed to satisfy parametric testing assumptions. However, the absolute values for C-reactive protein are presented to facilitate clinical interpretation. All data are presented as mean $\pm$ standard error of the mean. Statistical significance was set *a priori* at $P < 0.05$.

Results

Selected subject characteristics are presented in Table 1. All subjects were normal weight, normotensive, normolipidemic and normoglycemic. By design, women with osteopenia had lower ($P < 0.05$) lumbar spine bone mineral density (1.01 ± 0.02 g/m²) compared with women without osteopenia (1.28 ± 0.03 g/m²). Baseline blood flow was not different between the women without and with osteopenia (Experimental arm: 3.9 ± 0.3 ml/100ml tissue/min vs 4.7 ± 0.4 ml/100ml tissue/min; Control arm: 2.9 ± 0.2 ml/100ml tissue/min vs 3.3 ± 0.3 ml/100ml tissue/min, respectively). The forearm blood flow responses to selective blockade with BQ-123 were markedly different ($P < 0.01$) between groups in the experimental arm (Figure 1). In the women without osteopenia, resting forearm blood flow was not altered significantly in response to BQ-123. In contrast, a significant vasodilator response to BQ-123 was observed in women with osteopenia. Forearm blood flow increased ~25% from baseline to selective ET_A receptor antagonism. In the overall study population, there was a strong inverse correlation between lumbar spine bone mineral density and vasodilator response to BQ-123 ($r = -0.44$; $P < 0.05$). The infusion protocol produced no changes in the forearm blood flow from baseline in the control arm in either group of women.

Discussion

The primary novel finding of the present study is that ET-1-mediated vasoconstrictor tone is greater in postmenopausal women with osteopenia compared with those without osteopenia. To our knowledge, this is the first study to determine whether postmenopausal women with osteopenia have elevated endothelin-1-mediated vasoconstrictor tone. Our findings represent a potentially important link between osteopenia and increased cardiovascular disease risk.

Loss of bone mineral density is associated with an increased risk of cardiovascular events, particularly in postmenopausal women [14]. The mechanisms responsible for this increased risk are not well understood. Traditional cardiovascular risk factors are not considered to contribute to the differences in risk as, by in large, postmenopausal women with osteopenia

present with an unremarkable cardiometabolic profile that is often similar to that of women without osteopenia. A potential mechanism underlying this increased risk accompanying loss of bone mineral density in postmenopausal women is endothelial vasomotor dysfunction. It has previously been shown that low bone mineral density is associated with impaired endothelium-dependent vasodilation [8]. The results of the present study compliment and extend these findings demonstrating that ET-1-mediated vasoconstrictor tone is higher in postmenopausal women with osteopenia. This may be a potential mechanism underlying endothelial vasodilator dysfunction in this population. Indeed, in response to BQ-123, women with osteopenia demonstrated a significant vasodilator response (~25%), whereas those with normal bone mineral density displayed no change from baseline. This indicates that osteopenia is associated with increased ET-1-mediated vasoconstrictor tone that is mediated via the ET_A receptor. Notably, increased ET-1 vasoconstrictor activity has been etiologically linked to coronary artery disease and chronic heart failure [15]. Therefore, increased ET-1 system activity may contribute to the increased risk of cardiovascular diseases observed in postmenopausal women with low bone mineral density.

The link between osteopenia and increased vasoconstrictor tone is currently unclear. However, it is possible that elevated systemic ET-1 is instrumental in both these pathophysiologic processes in post-menopausal women. It has recently been demonstrated that postmenopausal women with osteoporosis have higher circulating ET-1 than those without osteoporosis [16]. In addition, in postmenopausal women without osteoporosis there is a significant association between BMD and serum ET-1 levels [16]. A review by Lekontseva, et. al., has clearly demonstrated that estrogens have an inhibitory influence on ET-1 expression at multiple levels in vitro and in animal models [17]. In postmenopausal women there is evidence that estrogens decrease [18], increase [19] or do not change [20] ET-1 levels. It is likely these varied results are due to differences in hormone formulations and duration of use. The clinical variability in circulating peptide levels in postmenopausal women suggests that there are other modulating factors. Additional investigation will be necessary to completely elucidate the contributors.

A significant influence of ET-1 on bone metabolism has already been demonstrated [21]. Both bone and parathyroid cells contain ET receptors and synthesize and release ET-1 [22–26]. ET-1 can stimulate osteoblast proliferation and differentiation in vitro [27]. However, ET-1 can also stimulate osteoclast bone resorption by potentiating an interleukin-1 induced increase in interleukin-6 [25]. This combination of influences on bone cells may produce increased bone turnover which is believed to predispose postmenopausal women to the development of osteopenia and osteoporosis [28].

It is possible that other inflammatory cytokines play a role in this systemic process. C-reactive protein has been shown to promote bone resorption and osteoclastogenesis [29] and increased ET-1 production [30]. However, in the present study, we did not observe differences in circulating levels of C-reactive protein between the groups. Additionally, there was no significant correlation between C-reactive protein concentrations and vasomotor response to BQ-123. Thus, our results suggest that C-reactive protein does not underlie the increase in ET-1-mediated vasoconstrictor tone with osteopenia. However, it should be noted that, the lack of an association between one inflammatory marker and the vascular responses to ET-1 does not completely rule out inflammation as a potential mechanism mediating the relation between low bone mineral density and cardiovascular diseases. For example, other inflammatory makers, such as tumor necrosis factor- α , have also been implicated in postmenopausal bone loss [31]. Furthermore, tumor necrosis factor- α has been linked to increased ET-1 production and thus may influence ET-1 system activity with osteopenia [32].

Another potential mechanistic link between low bone mineral density and enhance ET-1-mediated vasoconstrictor tone may be oxidative stress. Indeed, recent evidence suggests that reactive oxidant species upregulates ET-1 synthesis in cultured endothelial and vascular smooth muscle cells [33,34]. Moreover, reactive oxidant species enhance osteoclastic activity and inhibit differentiation of bone cells [35]. Thus, although not measured in the present study, reactive oxidant species are increased with age in women [36,37] and may represent a causal link between low bone mineral density and ET-1 system activity.

There are a few experimental considerations that deserve mention. First, we used a cross-section study design, thus we cannot ignore the possibility that genetic and/or lifestyle factors may have influenced the results of our group comparisons. However, to minimize lifestyle behaviors, we studied postmenopausal women who were non-smokers, sedentary, not taking vitamins or medications, and similar in age and years since menopause. Second, our study focused exclusively on postmenopausal women. While the prevalence and incidence of osteopenia is highest in this population, low bone mineral density can also present in premenopausal women and men. These populations may exhibit a different endothelial phenotype. Third, venous occlusion plethysmography cannot distinguish between the relative contributions of changes blood flow within muscle or skin vascular beds. There is however, evidence for endothelial regulation of blood flow through the ET_A receptor in both muscle [38,39] and skin [40,41]. Therefore it is likely the blood flow responses noted are a combination of changes in the muscle and skin vascular beds. Finally, plasma ET-1 concentrations were not measured in the study population. Endothelial ET-1 is predominantly (>80%) released abluminally toward the vascular smooth muscle [42]. Therefore, circulating levels generally do not correlate with the vascular response but rather reflect variable spillover into, and clearance from, the bloodstream [43]. While it remains possible that circulating ET-1 contributes to bone metabolism, the findings of the current study more specifically link ET-1 mediated vasoconstrictor tone, an indicator of vascular risk, with osteopenia.

In conclusion, the results of the present study demonstrate that endothelin-1-mediated vasoconstrictor tone is elevated in postmenopausal women with osteopenia. It is possible that increased ET-1 activity plays a mechanistic role in the development of osteopenia and osteoporosis. Future studies are necessary to establish an etiologic link between the ET-1 system and bone density. Nevertheless, greater ET-1 vasoconstrictor activity may contribute to the increased cardiovascular disease risk observed in postmenopausal women with low bone mineral density.

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Figure 1.

Forearm blood flow responses to BQ-123 in postmenopausal women with and without osteopenia. The P value refers to the difference in FBF response to BQ-123 between groups. Values are mean $\pm$ SEM.

Table 1

Selected subject characteristics

Variable	No Osteopenia (n = 12)	Osteopenia (n = 10)
Age, yr	56±1	60±2
Years since menopause	9±2	11±2
BMD (g/m ²)	1.28±0.03	1.01±0.02 *
Body mass, kg	70.4±3.5	67.2±3.2
BMI, kg/m ²	26.3±1.4	26.2±1.1
Body fat, %	39.8±2.1	41.1±2.4
Waist circumference, cm	83.0±4.0	82.2±2.9
Waist-to-hip ratio	0.79±0.03	0.79±0.03
Systolic BP, mmHg	119±3	120±4
Diastolic BP, mmHg	76±2	74±3
Total cholesterol, mmol/L	5.2±0.2	5.3±0.2
LDL-cholesterol, mmol/L	3.1±0.2	3.2±0.2
HDL-cholesterol, mmol/L	1.7±0.1	1.6±0.1
Triglycerides, mmol/L	1.0±0.2	1.0±0.1
Glucose, mmol/L	5.1±0.2	5.0±0.1
Insulin, pmol/L	27.8±4.4	27.8±8.6
HOMA-IR	0.9±0.1	0.9±0.3
CRP, mg/dL	3.7±0.83	2.5±1.1

Values are mean±SEM. BMD: bone mineral density; BMI: body mass index; BP: blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HOMA-IR: homeostasis model of insulin resistance; CRP: C-reactive protein.

*
P < 0.05