Original Article

High Blood Pressure, Bone-Mineral Loss and Insulin Resistance in Women

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Increasing evidence indicates that high blood pressure is associated with abnormalities in calcium metabolism. Sustained calcium loss may lead to increased bone-mineral loss in subjects with elevated blood pressure. Furthermore, recent findings indicate a possible linkage between abnormal calcium metabolism and insulin resistance. In the present study, we investigated the relationship(s) among bone-mineral density (BMD), blood pressure, calcium-related and bone metabolic parameters (plasma intact parathyroid hormone (I-PTH), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], osteocalcin, and urinary deoxypyridinoline), and insulin resistance, as assessed by a conventional homeostasis model (HOMA-R). We compared non-diabetic women with essential hypertension (WHT, n=34) with age-, body mass index- and menopause (yes or no)-matched normotensive, non-diabetic women (WNT, n=34). The BMD for WHT was significantly lower than that for WNT (0.596±0.019 *vs.* 0.666±0.024 g/cm^2, $p<0.05$). The BMD was correlated inversely with systolic blood pressure in all subjects examined ($r=-0.385$, $p<0.05$). The 24-h urinary calcium/sodium excretion ratio (Ux-Ca/Na) was significantly greater in WHT compared with WNT ($p<0.01$). In addition, a negative relationship was apparent between Ux-Ca/Na and BMD ($r=-0.58$, $p<0.05$). The plasma levels of PTH and 1,25(OH)$_2$D, and HOMA-R were significantly higher in WHT compared with WNT ($p<0.01$, $p<0.05$, and $p<0.05$, respectively), whereas the serum ionized calcium was lower in WHT compared with WNT ($p<0.05$). There were no significant differences in serum total calcium, inorganic phosphorus, osteocalcin, or urinary deoxypyridinoline between the two groups. These results indicate that high blood pressure is associated with abnormalities in calcium metabolism and insulin resistance in WHT. (*Hypertens Res* 2005; 28: 565–570)

***Key Words*: bone-mineral density, hypertension, insulin resistance, calcium metabolism**

Introduction

Recent studies have documented clinical, experimental and epidemiologically significant abnormalities in calcium metabolism in hypertension (*1–8*). Furthermore, intake of calcium has been shown to correlate inversely with blood pressure in clinical and experimental studies (*9*), although some contradictory results have also been published (*10*). Calcium supplementation has also been suggested to decrease blood pressure in human hypertension, and certain types of hypertensive patients, including those with hypertensive disorders of pregnancy and salt-sensitive individuals, may be more susceptible to the blood pressure-lowering action (*11*).

Parathyroid hormone (PTH), together with vitamin D and calcitonin, is the principle regulator of ionized calcium in extracellular fluid. Decreased circulating levels of calcium result in increased PTH, which has been implicated in the occurrence and/or development of hypertension (*12*), especially in women.

On the other hand, DeFronzo *et al*. (*13*, *14*) reported that insulin might act on renal distal tubules to enhance calcium excretion in humans, promote cellular uptake of phosphorus, and enhance tubular reabsorption of phosphorus at the proxi-

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Table 1. Clinical Characteristics

	WNT (n=34)	WHT (n=34)	p value
Age (years)	53.6±1.7	53.2±1.7	NS
Postmenopause (%)	58.8	58.8	NS
Body mass index (kg/m^2)	23.9±0.4	24.1±0.3	NS
Systolic BP (mmHg)	122.2±2.0	166.5±2.7	<0.001
Diastolic BP (mmHg)	69.7±1.4	93.2±2.0	<0.001
FPG (mmol/l)	4.1±0.1	4.5±0.1	<0.05
IRI (μU/ml)	4.9±0.1	8.9±0.2	<0.05
HOMA-R	0.90±0.15	1.68±0.20	<0.05
Total cholesterol (mmol/l)	5.15±0.14	5.23±0.07	NS
Triglycerides (mmol/l)	0.89±0.11	0.92±0.10	NS
HDL-cholesterol (mmol/l)	1.68±0.09	1.56±0.10	NS
Creatinine (μmol/l)	62.07±6.03	64.93±8.87	NS
Smoking (%)	26.4	39.4	NS
Drinking (%)	26.4	23.5	NS
Duration of hypertension (years)		18.1±1.5	

Values are expressed as mean±SEM. WNT, women with normotension; WHT, women with essential hypertension; NS, not significant; BP, blood pressure; FPG, fasting plasma glucose; HDL, high-density lipoprotein; IRI, immunoreactive insulin; HOMA-R, homeostasis model assessment of insulin resistance.

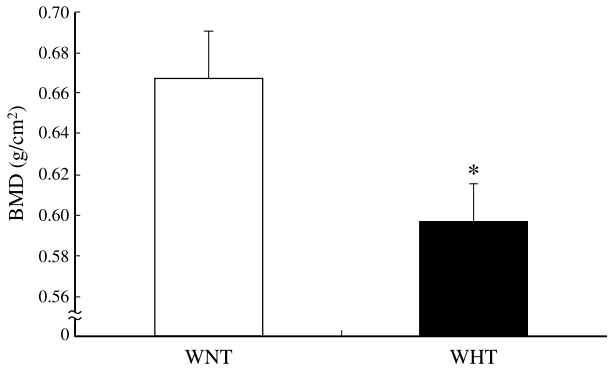

Fig. 1. *Bone-mineral density (BMD) in the lumbar spine of the central portion of a lateral scout view of the lumbar 2 to 4 vertebrae in women with normotension (WNT,* n=*34) and women with essential hypertension (WHT,* n=*34).* *p<*0.05 WNT* vs. *WHT.*

mal tubules in dogs.

Shimamoto *et al*. (*15*) reported that euglycemic hyperinsulinemia increased serum free calcium, decreased PTH, and induced calciuresis in non-obese normotensive subjects. Ohno *et al*. (*16*) also demonstrated that euglycemic hyperinsulinemia decreased intact PTH and increased fractional excretion of calcium in young, lean, normotensive male subjects. These basic science and clinical studies suggest that there is a link between insulin sensitivity or hyperinsulinemia and whole body calcium homeostasis.

Beginning at menopause, the rate of bone loss in women accelerates for approximately 7 years. The rate of spinal compression fractures is about eight times higher in women than in men, and the rate of wrist and hip fracture from all causes is about twice as high. Finally, the apparent gender-related differences may reflect the possible influence of sex hormones on calcium-regulating hormones (*12*).

Although type 1 diabetes mellitus has been associated with decreased bone-mineral density (BMD) (*17*), there have been conflicting reports about BMD in type 2 diabetes mellitus; some authors have reported elevated BMD, some have reported decreased BMD, and others have reported that BMD did not change (*18*).

In the present study, we investigated the relationship(s) among blood pressure, BMD, bone and calcium-related humoral factors, and insulin sensitivity in non-diabetic women with essential hypertension.

Methods

Subjects

Thirty-four non-diabetic women with essential hypertension (WHT; mean±SEM age, 53.2±1.7 years; range, 34–68 years) and 34 age-, body mass index- and menopause (yes or no)-matched non-diabetic women with normotension (WNT; mean±SEM age, 53.6±1.7 years; range, 35–68 years) were recruited.

Hypertension was defined as a systolic blood pressure (SBP) ≥140 mmHg and/or a diastolic blood pressure (DBP) ≥90 mmHg (*19*), recorded in a sitting position on at least three different occasions.

All measurements were performed at the outpatient clinic of Fukushima Rosai Hospital. Patients with secondary hypertension were excluded on the basis of a complete history, physical examination, radiological and ultrasound examinations, urinalysis, serum creatinine, potassium and sodium concentrations, renin activity, aldosterone, cortisol and catecholamine (adrenaline, noradrenaline, and dopamine) levels in the plasma and measurements of the 24-h urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids. Patients with a history of cardiovascular and/or cerebrovascular diseases, hyperlipidemia, liver dysfunction and renal diseases were also excluded from the study. Among the 34 WHT, 14 were untreated and 20 were receiving antihypertensive drugs. All antihypertensive drugs were discontinued at least 2 weeks before the study. The diagnosis of diabetes mellitus was based on a 75-g oral glucose tolerance test (*20*). Individuals with diabetes mellitus, which was defined as a fasting level of 7.0 mmol/l or greater and/or a plasma glucose level at 2 h after

Table 2. Calcium-Related and Bone Metabolic Parameters

	WNT (n=34)	WHT (n=34)	p value
Total calcium (mmol/l)	2.29±0.24	2.32±0.25	NS
Albumin (g/dl)	4.22±0.36	4.29±0.38	NS
Intact PTH (ng/l)	38.9±1.2	44.9±1.7	<0.01
1,25$(OH)_2$D (pmol/l)	90.2±4.3	96.7±4.6	<0.05
Ionized calcium (mmol/l)	1.28±0.06	1.23±0.03	<0.05
Inorganic phosphorous (mmol/l)	1.27±0.01	1.25±0.01	NS
Osteocalcin (ng/ml)	6.9±0.5	7.7±0.6	NS
Urinary DPD (nmol/mmol Cr)	6.5±0.6	7.0±0.6	NS
Urinary calcium (mmol/24 h)	3.47±0.21	4.52±0.22	<0.01
Urinary sodium (mmol/24 h)	146±9	143±11	NS
100 × Urinary Ca/Na ratio	2.38±0.17	3.17±0.22	<0.01

Values are expressed as mean±SEM; WNT, women with normotension; WHT, women with essential hypertension; NS, not significant; PTH, parathyroid hormone; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; DPD, deoxypyridinoline.

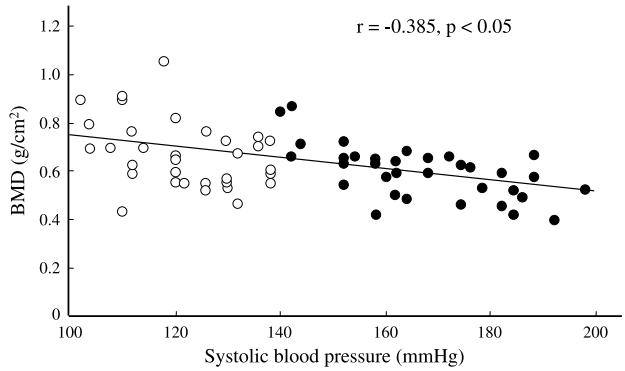

Fig. 2. *Correlation between bone-mineral density (BMD) in the lumbar spine of the central portion of a lateral scout view of the lumbar 2 to 4 vertebrae and systolic blood pressure in the combined group of women with normotension (WNT) and women with essential hypertension (WHT). Open (*n = *34) and closed (*n = *34) circles indicate WNT and WHT, respectively.*

glucose administration of 11.1 mmol/l or greater, were excluded from the study.

The study was approved by the Ethics Committee of Fukushima Rosai Hospital. All patients provided written informed consent to participate in the study.

Study Design

On hospitalization, both WHT and WNT were kept on a constant diet (NaCl: 7 g/day; K: 100 mEq/day; Ca: 600 mg/day; calories: 30 kcal/kg of standardized body weight) for 7 days. Compliance with the diet was confirmed by measurements of the 24-h urine electrolytes that were excreted on the fifth day. Determinations of biomedical valuables, as described below, and BMD were made in the morning on the seventh day.

Measurements

The BMD was evaluated at the lumbar spine (using the central portion of a lateral scout view of the lumbar 2 to 4 vertebrae) by dual energy X-ray absorptiometry using a Hologic QDR 2000 (Hologic, Bedford, USA). The *Z*-score is the number of SDs by which a given measurement differs from the mean for a sex-, age-, and race-matched reference population. The *T*-score is the number of SDs by which a given measurement differs from the mean for a normal young adult reference population. Biochemical measurements including serum electrolytes and lipids were performed using an autoanalyzer (model 7250; Hitachi Co., Ltd., Tokyo, Japan). Serum ionized calcium was determined anaerobically using an ion-sensitive electrode (NOVA Biomedical Co., Ltd., Waltham, USA). The serum levels of intact PTH, 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) and osteocalcin were measured by chemiluminescent immunoassay (Nichols Institute Diagnostic, San Clemente, USA), radioimmuno-assay (Immunodiagnostic System Co., Ltd., Boldon, UK) and immunoradiometric assay (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan), respectively. Urinary deoxypyridinoline was determined by an enzyme immunoassay (Quidel Corporation, San Diego, USA). Fasting plasma glucose and immunoreactive insulin (IRI) were measured by the glucose dehydrogenase and enzyme-linked immunosorbent methods, respectively. The homeostasis model assessment of insulin resistance (HOMA-R) values were calculated as described previously (*21*) according to the formula: HOMA-R = [fasting IRI (μU/l) × fasting plasma glucose (mmol/l)]/22.5.

Statistical Analysis

Values are expressed as the mean±SEM, unless otherwise noted. Statistical comparisons were performed using unpaired two-tailed Student's *t*-test, and Pearson's correlation method was used to assess the relationships between the variables. All statistical analyses were performed with StatView for Macintosh version 5.0 Software (Abacus Concepts, Inc., Berkeley, USA). A null hypothesis was rejected when $p<0.05$.

Results

Baseline Characteristics

Table 1 shows the clinical characteristics of the subjects. The

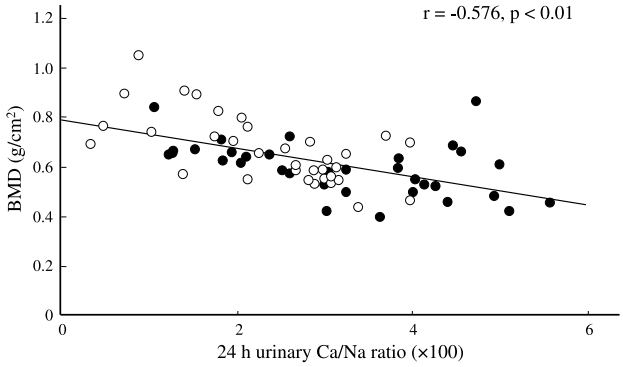

Fig. 3. *Correlation between bone-mineral density (BMD) in the lumbar spine of the central portion of a lateral scout view of the lumbar 2 to 4 vertebrae and 24-h urinary Ca/Na ratio in the combined group of women with normotension (WNT) and women with essential hypertension (WHT). Open (*n = *34) and closed (*n = *34) circles indicate WNT and WHT, respectively.*

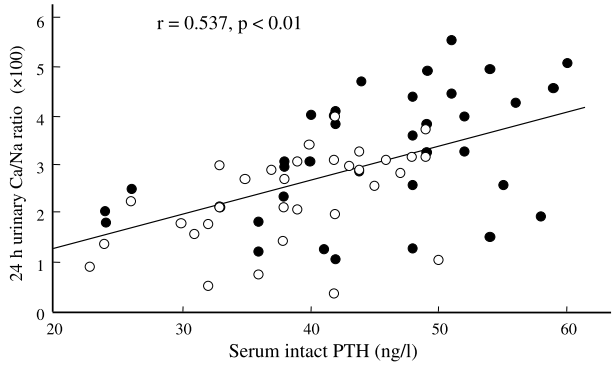

Fig. 4. *Correlation between serum intact parathyroid hormone (PTH) and 24-h urinary Ca/Na ratio in the combined group of women with normotension (WNT) and women with essential hypertension (WHT). Open (*n = *34) and closed (*n = *34) circles indicate WNT and WHT, respectively.*

fasting plasma glucose, IRI, and HOMA-R were all significantly greater in WHT compared with WNT. There were no significant differences in age, BMI, serum levels of lipid components and creatinine, or alcohol and/or cigarette habits between the two groups.

BMD

As shown in Fig. 1, the BMD was significantly lower in WHT compared with WNT (0.595±0.019 g/cm^2 *vs.* 0.666±0.024 g/cm^2, $p<0.05$). The *T*-score and *Z*-score were both significantly lower in WHT compared with WNT ($p<0.05$, each).

Parameters of Calcium and Bone Metabolism

The calcium and bone metabolic parameters are summarized in Table 2. The intact PTH, 1,25(OH)$_2$D and HOMA-R were significantly higher in WHT compared with WNT, whereas serum ionized calcium was lower in WHT compared with WNT. No significant differences were observed for serum total calcium, inorganic phosphorus, osteocalcin, or urinary excretion of deoxypyridinoline between the two groups. However, the 24-h urinary Ca/Na ratio was greater in WHT compared with WNT ($p<0.05$).

Relationship among Blood Pressure, BMD, HOMA-R and Bone Metabolic Parameters

As shown in Fig. 2, the BMD correlated inversely with SBP ($r=-0.385$, $p<0.05$) but not with DBP ($r=0.186$) in all subjects. The 24-h urinary Ca/Na ratio also correlated inversely with BMD ($r=-0.576$, $p<0.01$, Fig. 3). No significant relationships were found between BMD and HOMA-R, serum total calcium, ionized calcium, inorganic phosphorus, osteocalcin, or urinary excretion of deoxypyridinoline (data not shown). A positive correlation was observed between intact PTH and 24-h urinary Ca/Na ratio in all subjects ($r=0.537$, $p<0.01$, Fig. 4). Serum 1,25(OH)$_2$D did not correlate with the 24-h urinary Ca/Na ratio in any of the subjects ($r=0.216$, not shown).

Discussion

We have documented the existence of decreased bone mineral loss in WHT. The results of the present study confirm a previous report in essential hypertension (*22*). In the present study, WHT exhibited significantly higher levels of intact PTH, 1,25(OH)$_2$D, HOMA-R, and urinary Ca/Na ratio, and decreased serum ionized calcium. We found no differences in total serum calcium, inorganic phosphorous and urinary deoxypyridinoline between the two groups.

Several previous studies conducted under uncontrolled dietary conditions have also found that urinary calcium excretion is elevated in patients with essential hypertension (*1–3*). However, other studies have either not demonstrated elevated calcium excretion in hypertensive subjects (*23*) or have attributed the higher level of calcium excretion to differences in sodium excretion (*24*). In our study WHT exhibited higher calcium excretion than WNT when they consumed controlled diets. This finding suggests that failure to control dietary intake may obscure the detection of relative hypercalciuria, which is one of the distinguishing characteristics of hypertension. For example, a genetic abnormality of the renal calcium-sensing receptor (*25*) may be present in some patients with primary hypertension.

The present findings corroborate several previous reports that demonstrated that the serum ionized calcium concentration is lower in hypertensive persons than in normotensive controls (*26*), whereas serum albumin tended to increase in WHT compared with WNT, although this difference was not

statistically significant. Thus, these findings raise the possibility of the existence of an abnormality in protein binding of extracellular calcium in WHT.

We found that serum intact PTH concentrations were higher in WHT than in WNT. Hypertensive patients have previously been reported to exhibit elevated PTH levels (*1–4*). However, other studies have not demonstrated such elevations in hypertensive subjects (*7*). Acute administration of PTH results in decreased blood pressure (*27*) and relaxation of vascular tissue (*28*), suggesting that PTH is a direct vasodilator. By contrast, long-term attenuation of PTH by parathyroidectomy prevents the normal age-related increases in blood pressure in hypertensive and normotensive animals (*29*). Furthermore, primary hyperparathyroidism has been associated with a greater degree of prevention of hypertension (*30*). The exact reason for this discrepancy is currently unclear. The observed depression in serum ionized calcium may be the proximate stimulus for increased hormone synthesis and release by the parathyroid in hypertensive patients. Alternatively, the difference in findings may be related to a difference between the cohorts.

We also demonstrated that the serum $1,25(OH)_2D$ was elevated in WHT compared with WNT. Specific receptors for $1,25(OH)_2D$ have been found on cardiac muscle (*31*), vascular smooth muscle (*32*) and endothelial cells (*33*). It has been shown that $1,25(OH)_2D$ stimulates calcium uptake (*34*), calcium-ATPase activity (*35*) and proliferation of vascular smooth muscle cells (*36*). When administered to humans, $1,25(OH)_2D$ caused elevation of the blood pressure (*37*), and augmented the pressor response to norepinephrine in spontaneously hypertensive rats (SHR) (*38*). These findings, taken together with our present results, are in agreement with the concept that increases in $1,25(OH)_2D$ may play an important role in the development and/or maintenance of hypertension in humans.

We found no differences in urinary deoxypyridinoline, which is a marker of bone resorption, between the two groups. However, we found that serum intact PTH concentrations were higher in WHT compared with WNT. The discrepancy between normal urinary deoxypyridinoline excretion and slightly elevated PTH concentrations (within the normal range) might be, at least in part, dependent on the accuracy of the assay employed. However, it is also possible that osteal tissue might be less sensitive to PTH in WHT. Further studies will be necessary to clarify this intriguing issue.

In the present study, the HOMA-R value was higher in WHT compared with WNT. Abnormal calcium transport has been associated with essential hypertension (*39*). DeFronzo *et al*. (*13*, *14*) reported that insulin might act on renal distal tubules to enhance calcium excretion in humans, promote cellular uptake of phosphate and enhance tubular reabsorption of phosphate at the proximal and distal tubules in dogs. Shimamoto *et al*. (*15*) reported that euglycemic hyperinsulinemia increased serum free calcium, decreased PTH, and induced calciuresis in non-obese normotensive subjects. It has been also demonstrated that oral glucose loading suppresses PTH levels in parallel with an increase of the urinary calcium/creatinine ratio (*40*).

Insulin decreases intracellular calcium in cultured vascular smooth muscle cells. The effects of insulin on ionized calcium and intracellular calcium remain controversial (*15*, *40*), and a question has been raised as to whether the response to insulin might vary according to insulin resistant status or experimental conditions. In the present study, since 1) the dietary intake of calcium and sodium was kept constant throughout the study, and 2) the urinary excretion of sodium was not different between WHT and WNT, it seems less likely that insulin increased the urinary Ca/Na ratio *via* its direct action on the renal tubules to enhance sodium reabsorption. Draznin (*41*) has hypothesized that abnormal intracellular calcium homeostasis is attributed to insulin resistance that is associated with essential hypertension. It has been demonstrated that increasing intracellular calcium in isolated adipocytes results in significant inhibition of insulin-stimulated glucose transport and oxidation (*41*). Therefore, increased intracellular calcium may contribute to insulin resistance by inhibiting the dephosphorylation of insulin-sensitive glucose transporters and thereby decreasing its activity. Although insulin resistance and disturbed calcium metabolism are closely linked *in vivo*, only a few studies have attempted to investigate the relationship between insulin sensitivity/hyperinsulinemia and whole body calcium homeostasis (*14*, *40*).

In conclusion, the present study suggests that BMD provides an index of whole calcium balance, and implies that elevated blood pressure is inversely correlated with reduced BMD in WHT, thus indicating that high blood pressure is associated with abnormalities in calcium metabolism and insulin resistance in WHT.

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