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Fracture Risk in Perimenopausal Women Treated with Beta-Blockers

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Abstract. β2-adrenergic receptors have been identified on human osteoblastic and osteoclastic cells, raising the question of a sympathetic regulation of bone metabolism. We investigated effects of treatment with β-adrenergic receptor antagonists (β-blockers) on bone turnover, bone mineral density (BMD), and fracture risk. Within the Danish Osteoporosis Prevention Study (DOPS) a population based, comprehensive cohort study of 2016 perimenopausal women, associations between treatment with β-blockers and bone turnover and BMD were assessed in a cross-sectional design at the start of study. Moreover, in a nested case-control design, fracture risk during the subsequent 5 years was assessed in relation to treatment with β-blockers at baseline. Multiple regression- and logistic regression-analyses were performed. Treatment with β-blockers was associated with a threefold increased fracture risk (OR_{adj} 3.3; 95% CI: 1.1–9.4). Analyses on duration of treatment showed that women who had been treated for more than 8 years had a higher fracture risk (OR_{adj} 5.3; 95% CI: 1.1–26.3) than those treated for less than 8 years (OR_{adj} 2.4; 95% CI: 0.6–9.5). In addition, cross-sectional data showed 20% lower serum osteocalcin levels (an osteoblastic marker of bone formation) in women treated with β-blockers compared to untreated women ($P < 0.001$), whereas BMD at the lumbar spine and femoral neck did not differ between groups. β-blockers may decrease the activity of bone-forming cells and thereby increase fracture risk. However, confirmative studies and studies exploring mechanisms of action are needed.

Beta-adrenergic receptor antagonists (β-blockers) are extensively used in the treatment of arterial hypertension, ischemic heart diseases, certain cardiac arrhythmias, and migraine. β2-adrenergic receptors have been identified on human osteoblastic and osteoclastic cells [1, 2], raising the question of a sympathetic regulation of bone metabolism [3]. *In vitro* studies in different lines of bone cells as well as *in vivo* studies in experimental animals have demonstrated effects of β-adrenergic receptor agonists and antagonists on bone metabolism, but the results reported have been conflicting. In different studies, β-adrenergic stimulation and inhibition have been reported to cause anabolic as well as catabolic effects on bone [3–7].

Further evidences on a sympathetic regulation of bone metabolism have been provided in recent studies on effects of leptin on bone metabolism [2, 8, 9]. Leptin is an adipocyte-derived hormone that circulates to the hypothalamus, thereby providing a feedback-loop to the central nervous system (CNS) about the state of body fat. In addition, a relation between leptin levels and bone mass has been suggested. Leptin may regulate bone metabolism either through a direct effect on bone cells and/or through central actions via its binding to receptors in the CNS [8, 9]. Recently, leptin has been shown to inhibit bone formation through the sympathetic nervous system via osteoblastic β-adrenergic receptors [2].

In humans, there are only few and conflicting data on the effects of β-blockers on bone metabolism and fracture risk. In a previous study from our group on patients with hyperthyroidism, the β-adrenergic antagonist propranolol did not affect bone loss, as determined by measurement of bone mineral content and histomorphometric measures of bone turnover [10]. However, in a recent analysis from the Geelong Osteoporosis Study, Pasco et al. [11] found a reduced fracture risk and an increased BMD in users of β-blockers compared to controls.

In the setting of the Danish Osteoporosis Prevention Study (DOPS) [12], a population-based, comprehensive cohort study, we investigated associations between treatment with β-blockers and bone turnover, bone mineral density (BMD), and fracture risk.

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Subjects and Methods

Design

The present study was performed within DOPS, which is a 20-year prospective, open-label multi-center study (4 centers) on the effect of hormone replacement therapy (HRT) on BMD and fracture risk in 2016 perimenopausal women. DOPS is a population based, comprehensive cohort study [13] including a randomized arm (HRT or no treatment) and an arm based on self-selection (HRT or no treatment) in order to increase the external validity of the study. As first choice, participants received oral sequential therapy with Trisequens™ (estradiol/norethisterone), Novo Nordic, Denmark. If a change in the type of HRT was requested for reasons not requiring permanent discontinuation, a number of HRT alternatives were available, as previously detailed [14]. All drugs were delivered free of charge from the manufactures. The design is pragmatic, attempting to mimic the normal clinical situation. Study design and recruitment of participants have been detailed elsewhere [12]. The Central and Regional Ethical Committees and The Danish National Board of Health approved the study. Each participant gave informed consent and the study was conducted according to the Declaration of Helsinki II. The Good Clinical Practice (GCP) unit at Aarhus University monitored the study.

Participants

We recruited participants by direct mailing to a random subsample of Danish Caucasian females aged 45–58 years. Subjects were 3–24 months past their last menstrual bleeding or having experienced perimenopausal symptoms (including menstrual irregularities) in combination with elevated serum FSH levels. We excluded individuals with metabolic bone disease, osteoporosis defined as non-traumatic vertebral fractures, current estrogen use, ever treated with glucocorticoids for more than 6 months, current or past malignancy, chronic disease if newly diagnosed or out of control, and hospitalization due to alcohol or drug addiction.

Data Collection

All participants were clinically examined and were structurally interviewed at baseline in order to assess risk factors and prognostic factors for osteoporosis, including age, menopausal age, anthropometric variables, physical activity (hours per week spent on jogging, gymnastics, cycling, swimming, and standing/walking on the job), previous and present illnesses, gynecological history, and use of drugs, vitamin supplements, alcohol and tobacco [12]. We used a 7-day food record to assess current daily intakes of vitamins and minerals. A dietician using food models and photographs during a 15-min. validation interview evaluated serving sizes and cooking habits. The diet contents of energy, vitamins, and minerals were analyzed using a computerized database (''Dankost'', software version 1.3b), containing information on diet composition based on official Danish food tables [15]. After 5 years of follow-up, participants were asked whether they had sustained a fracture during the study period. If so, reported fractures were validated by review of hospital discharge records. In addition, X-rays of the spine were obtained as lateral projections of the spine covering Th4 to L5 at inclusion and after 5 years. The images were reviewed by trained radiologists. A fracture was defined as more than 20% reduction in the height of a vertebrae compared to the highest vertical distance of that vertebrae.

BMD Measurements. At baseline, we measured BMD at the lumbar spine (L2-L4) and femoral neck. We used carefully cross-calibrated QDR 1000/W and 2000/W densitometers (Hologic, Inc, Waltham, MA) in all four centers [12]. The *in vivo* precision errors (BMD) were 1.5% (spine) and 2.1% (femoral neck), respectively. We assessed the long-term stability of the equipment by daily scans of an anthropometric phantom in each center. Changes were $< 0.2\%$/year [12].

Biochemistry. We collected blood and urine specimens at baseline. Serum 25-hydroxyvitamin D was measured by a radioimmunoassay (RIA) using rachitic rat kidney cytosol as a binding protein [16]. The intra- and interassay coefficients of variation were 9.4% and 13.5%, respectively. Serum osteocalcin was measured by RIA using rabbit antiserum against bovine osteocalcin [17]. Intact, purified bovine osteocalcin verified by amino acid analysis and antisera against bovine osteocalcin was generously provided by J. Poser (Procter and Gamble Company, Cincinnati, OH). The sera showed full cross-reactivity between human and bovine osteocalcin. The intra- and interassay CVs were 5 and 10%, respectively. Total alkaline phosphatase activity in serum was measured spectrophotometrically and serum bone isoenzyme alkaline phosphatase activity (bone-ALP) was determined by lectin precipitation [18]. The intra-assay CV was 8% and the inter-assay CV was 25%. The renal excretion of hydroxyproline was collected on a gelatine-restricted diet (second void morning spot urine) and determined spectrophotometrically with p-dimethylaminobenzaldehyde as substrate, according to the manufacturer's direction (Organon Teknika, Boxtel, The Netherlands). The hydroxyproline excretion was expressed as a ratio relative to the renal creatinine excr etion i.e., hydroxyproline/creatinine-ratio (urinary OHP/creatinine ratio, μmol/mmol).

Assessments of Associations Between use of β-Blockers and Bone Mineral Density and Fracture Risk

Separate analyses were carried out in order to determine associations between use of β-blockers and BMD, bone turnover, and fracture risk:

Cross-sectional Studies on Bone Mineral Density and Bone Turnover. Cross-sectional analyses were carried out on data collected at baseline to assess associations between use of β-blockers and BMD of the lumbar spine and femoral neck. Also the relationship between use of β-blockers and bone turnover was assessed by measurements of biochemical markers of bone formation (osteocalcin and bone-ALP) and bone resorption (urinary hydroxyproline/creatinine-ratio).

Nested Case-control Study. During the 5-year follow-up period, 163 participants sustained a fracture (cases). For each fracture case, we selected 6 subjects from the population who had not sustained a fracture during the study period (controls). The controls were randomly selected except that they were matched to cases on the use of HRT during the study period (no HRT use, intermittent use, or continuous use).

Statistics

We assessed differences between groups using the chi-square test (Fisher's Exact test) for categorical variables and two-sample *t*-test or Mann-Whitney *U*-test for continuous variables, as appropriate, after testing for normal distributions. As several of included variables were not normally distributed, descriptive data are provided as a median value with the in-

Table 1. Characteristics of studied subjects at baseline. Median with interquartile range[1]

	Beta blocker group (n = 38)	Non-beta blocker group (n = 1978)	P value
Age (years)	50 (49–52)	50 (48–52)	0.60
Years postmenopausal	0.9 (0.2–1.6)	0.5 (0.2–1.5)	0.39
No. (%) with a previous fracture	8 (21%)	389 (20%)	0.83
Anthropometry			
Body weight (kg)	69.7 (60.6–76.1)	65.8 (59.8–73.9)	0.60
Diet and lifestyle			
Physical activity (hours/week)	18 (8–27)	19 (9–30)	0.40
Total energy intake (KJ/day × 10^3)	6.8 (5.8–7.7)	7.6 (6.5–8.9)	0.004
Dietary calcium intake (mg/day)	743 (543–884)	811 (628–1046)	< 0.05
Dietary vitamin D intake (μg/day)	2.0 (1.8–3.4)	2.2 (1.6–3.2)	0.81
No. (%) using vitamin D supplements	24 (63%)	1244 (63%)	0.98
Alcohol (g/day)	7.8 (3.0–15.8)	9.9 (3.1–19.0)	0.40
No. (%) current smokers	22 (58%)	809 (41%)	0.04
Biochemestry			
Serum creatinine (μmol/l)	75 (69–83)	73 (67–79)	0.19
Serum 25(OH)-vitamin D (nmol/l)	21 (16–28)	23 (16–31)	0.38
Serum bone-ALP (U/l)	62 (51–82)	65 (51–82)	0.74
Serum osteocalcin (ng/ml)	12 (10–16)	16 (13–21)	< 0.001
Urinary OHP/creatinine (μmol/mmol)	19 (15–26)	21 (16–26)	0.23
Bone mineral density			
Lumbar spine (g/cm^2)	1.003 (0.949–1.081)	1.023 (0.931–1.116)	0.46
Femoral neck (g/cm^2)	0.787 (0.710–0.829)	0.789 (0.715–0.868)	0.48
No. (%) current users of medicine			
Thiazide diuretics	10 (26%)	55 (3%)	< 0.001
Loop diuretics	4 (11%)	50 (3%)	< 0.01
Antipsychotic/anxiolytic/antidepressant	2 (5%)	75 (4%)	0.64

[1] Interquartile range: the distance between the third quartile (75th percentile) and the first quartile (25th percentile) values

Table 2. Type and dose of beta-blockers used by studied subjects

	Number of subjects	Median dose (mg)	Dose by number of subjects
Atenolol	14	50	50 mg/d: n = 7; 100 mg/d: n = 5; 200 mg/d: n = 1; unknown dose: n = 1
Metoprolol	10	100	25 mg/d; n = 1; 50 mg/d: n = 2; 100 mg/d: n = 6; 200 mg/d: n = 1
Propranolol	9	80	15 mg/d: n = 1, 40 mg/d: n=2; 60 mg/d: n = 1; 80 mg/d: n = 3; 120 mg/d: n = 1; 160 mg/d: n = 1
Bisoprolol	4	5	5 mg/d: n = 4
Sotalol	1	160	160 mg/d: n = 1

terquartile range (the distance between the first quartile (25th percentile) and the third quartile (75th percentile) values) as a measure of the spread of data.

Multiple regression analyses (all variables entered) were used to study associations between treatment with β-blockers and bone turnover and BMD in order to adjust for potential influence of other covariates on BMD. Assumptions for multiple regression analyses were tested using normal probability plots, and models were only accepted if residual plots were compatible with a normal distribution. In multiple regression analyses, treatment with medicine as well as current smoking and use of vitamin supplements were coded as "1", whereas no use of medicine, non-smoking status and no use of vitamin supplements were coded as "0".

Finally, logistic regression analyses (all variables entered) were used to study differences between subjects with a fracture and control subjects. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 8.0) for Windows.

Results

A total of 38 patients (2%) were treated with β-blockers at baseline. Characteristics of studied subjects are outlined in Table 1. Subjects in the β-blocker group had been treated with β-blockers for a median time period of 8 years. β-blocker-treated subjects had a lower dietary calcium intake and lower plasma osteocalcin levels than untreated subjects. In addition, smoking and use of thiazide and loop diuretics was more frequent in β-blocker-treated subjects than in the untreated group. β-blockers were prescribed for the treatment of hypertension (n = 30), migraine (n = 4), cardiac arrhythmia (n = 2), tremor (n = 1), and unknown (n = 1). Table 2 displays type and dose of β-blockers used.

Table 3. Nested case-control study. Baseline characteristics of studied subjects. Median with interquartile range[1]

	Characteristics of studied subjects, median (interquartile range, 25%–75%)		
	Cases (n = 163)	Controls (n = 978)	*P-value*
Age (years)	50 (48–52)	50 (48–52)	0.92
Years postmenopausal	0.7 (0.3–1.7)	0.6 (0.2–1.5)	0.13
No. (%) with a previous fracture	44 (27%)	189 (19%)	0.03
Anthropometry			
Weight (kg)	66.2 (60.0–73.2)	66.3 (60.0–74.4)	0.84
Diet and lifestyle			
Physical activity (hours/week)	20 (10–30)	20 (10–30)	0.91
Total energy intake (KJ/day × 10^3)	7.5 (6.3–8.7)	7.5 (6.5–9.8)	0.34
Dietary calcium intake (1000 mg/day)	0.79 (0.59–1.01)	0.81 (0.62–1.02)	0.49
Dietary vitamin D intake (μg/day)	2.3 (1.5–3.2)	2.2 (1.6–3.2)	0.79
No (%) using vitamin D supplements	104 (64%)	616 (63%)	0.86
Alcohol (no. of drinks per day)	0.7 (0.1–1.6)	0.8 (0.3–1.6)	0.84
No.(%) current smokers	67 (41%)	384 (39%)	0.72
Use of medicine			
No. (%) beta-blockers	6 (3.7%)	17 (1.7%)	0.10
No. (%) thiazide diuretics	1 (0.6%)	32 (3.3%)	0.06
No. (%) loop diuretics	3 (1.8%)	26 (2.7%)	0.54
No. (%) antipsychotic/anxiolytic/antidepressant	5 (3.1%)	25 (2.6%)	0.71
Bone mineral density (g/cm^2)			
Lumbar spine (L2–L4)	0.973 (0.870; 1.223)	1.033 (0.935; 1.260)	< 0.001
Femoral neck	0.744 (0.672; 0.938)	0.797 (0.724; 1.008)	0.001
Biochemestry			
Serum creatinine (μmol/l)	72 (66–80)	73 (68–79)	0.18
Serum 25(OH)-vitamin D (nmol/l)	22 (17–34)	24 (17–33)	0.76
Serum bone-ALP (U/l)	68 (55–84)	65 (51–83)	0.23
Serum osteocalcin (ng/ml)	17 (13–21)	16 (13–21)	0.19
Urinary OHP/creatinine (μmol/mmol)	22 (17–27)	20 (16–26)	0.20

[1] Interquartile range: the distance between the third quartile (75th percentile) and the first quartile (25th percentile) values

Effects of β-Blockers on Bone Turnover

Serum osteocalcin levels were lower in women treated with β-blockers than in untreated women ($P < 0.001$) (Table 1). Additionally, multiple regression analysis including all variables shown in Table 1, showed that treatment with β-blockers was an independent determinant of serum osteocalcin levels (dependent variable), with lower levels in β-blockers treated subjects than in untreated subjects (β coefficient –2.8, $P = 0.004$). However, multiple regressions analyses revealed no differences between users- and non-users of β-blockers on serum 25-hydroxyvitamin D levels ($P = 0.86$), serum bone-ALP levels ($P = 0.18$), or urinary OHP/creatinine ratio ($P = 0.60$).

Effects of β-Blockers on Bone Mineral Density

Baseline BMD at the lumbar spine and femoral neck did not differ between treated and untreated subjects (Table 1). Similarly, multiple regressions analyses revealed no associations between users- and non-users of β-blockers and BMD at the lumbar spine ($P = 0.09$) or femoral neck ($P = 0.75$).

Nested Case-Control Study

During the 5-year follow-up period, 140 study subjects sustained a fracture of the appendicular skeleton. In addition, X-ray of the spine revealed incident vertebral fractures in 26 subjects. Three subjects had both a vertebral fracture and a fracture of the appendicular skeleton. Thus, a total of 163 study subjects sustained a fracture during the 5-year follow-up period (cases). For each case, we randomly selected 6 control subjects from the same cohort, matched on whether they had received HRT during the study period.

Table 3 shows baseline characteristics of cases and controls. More cases than controls had sustained a fracture before the start of the study ($P = 0.03$) and compared to controls, cases had a lower baseline BMD at the lumbar spine ($P < 0.001$) and femoral neck ($P < 0.001$). Table 4 presents the crude and adjusted ORs from logistic regression analyses on associations between risk of fracture and treatment with β-blockers. In an unadjusted analysis, treatment with β-blockers was associated with non-significant increased fracture risk (crude odds ratio 2.2; 95% CI: 0.8 to 5.6). After controlling for the influence of variables shown in Table 3, the risk of fracture was statistically significantly in-

Table 4. Nested case-control study. Crude and multivariate odds ratio (OR) for fracture according to treatment with β-blockers

	Crude OR (95% CI)	Multivariate OR[1] (95% CI)
Treatment with beta-blockers	2.2 (0.8–5.6)	3.3 (1.1–9.4)*
Duration of beta-blocker treatment		
No treatment	1.00 (reference)	1.00 (reference)
Less than 8 years	1.8 (0.5–6.7)	2.4 (0.6–9.5)
More than 8 years	2.6 (0.7–10.3)	5.3 (1.1–26.3)*

*$p < 0.05$[1] Multivariate OR's are adjusted for the potential effects on fracture risk of all the covariates shown in Table 3

creased (adjusted odds ratio 3.3; 95% CI: 1.1 to 9.4). In addition, the increase in fracture risk was more pronounced in women who had been treated with β-blockers for more than 8 years than in women who reported treatment for less than 8 years (Table 4).

In order to study whether the underlying diseases necessitating β-blocker treatment influenced the association between fracture risk and treatment with β-blockers, we also studied associations between fracture risk and treatment with non-β-blocker cardiovascular drugs. In our cohort, 6 women were treated with calcium channel blockers and 25 with ACE inhibitors. As each of the two groups was too small to perform the analyses, they were combined ($n = 31$). Logistic regression analyses, similar to those detailed above, on associations between risk of fracture and treatment with one of the two drugs, did not reveal any significant relationship (crude OR = 0.3; 95% CI: 0.4 to 2.3). Nor did we find any significant association between risk of fracture and treatment with ACE inhibitors/calcium channel blockers after controlling for the influence of variables shown in Table 3 (adjusted OR = 0.5; 95% CI: 0.1 to 4.2).

To explore potential interactions among treatments with β-blockers, HRT, and fracture risk we restricted the analysis to those who had not received HRT during the 5-year follow-up. The analysis showed an increased fracture risk (adjusted odds ratio 5.0; 95% CI: 1.4 to 17.5). Similarly, restricting the analysis to those who had received HRT continuously during the study period, a trend towards an increased fracture risk was found (adjusted odds ratio 2.8; 95% CI: 0.2 to 47.3).

Discussion

In a sample of perimenopausal Danish women, we found treatment with β-blockers to be associated with a threefold increased fracture risk and an increase in fracture risk with increased duration of treatment. Moreover, the increase in fracture risk was lower in subjects who had received estrogen treatment during the study period than in non-estrogen-treated subjects, indicating that estrogen treatment may partly reverse the increased fracture risk associated with β-blocker treatment. In addition, treatment affected bone metabolism, as serum osteocalcin levels were lower in women treated with β-blockers than in untreated women. However, we were unable to demonstrate any significant effect of β blockers treatment on BMD at the lumbar spine or femoral neck.

Effect of β-Blockers on Bone Mass (BMD) and Fracture Risk

Conflicting results have been reported on the effect of β-blockers on bone mass and BMD [3]. In animal experimental studies, β-adrenergic blockade has been reported to cause a positive effect on bone mass. Minkowitz et al. [5] found increased trabecular bone formation in rats treated with propranolol for 12 weeks, and Takeda et al. [2] reported an increased bone formation rate and an increased number of osteoblasts, causing an increased bone volume, in mice treated with propranolol for 5 weeks. Thus, according to these studies in experimental animals, a reduced osteoblastic cell activity may be compensated for by an increased number of osteoblastic cells, causing a net positive effect on bone. However, adrenergic antagonists on different cell lines have demonstrated not only bone anabolic but also catabolic action [3]. In addition, conflicting results have been reported on the effects of adrenergic agonists. For example, in one study, adrenergic agonists were shown to exert anabolic effects on cancellous bone in ovariectomized rats [19], whereas in another study adrenergic agonists were found to stimulate bone resorption in neonatal mouse calvarias [1].

Only a few studies have been published on the effect of β-blockers on human bone. In a previous study from our group, propranolol treatment did not affect the accelerated bone loss in patients with hyperthyroidism [10]. Similarly, in the present study, we were unable to demonstrate any difference in BMD between β-blocker treated- and untreated subjects. Conversely, in a recent study from Australia, treatment with β-blockers was found to be associated with an increased BMD and a decreased fracture risk in postmenopausal women [11]. The reason for the discrepancy between our findings and those in the Australian study is not obvious. However, important differences exist in study design between the two studies. In the study from Australia, Pasco et al. [11] recruited women older than 50 years who sustained a fracture during a 2-year period. After the fracture episode (median 59 days post-fracture) study subjects were interviewed and BMD was measured. A total of 559 fracture cases were included. In

addition, 775 women who did not sustain a fracture were included in the study as controls. In contrast, our study subjects were recruited before the occurrence of a fracture. A clinical examination, including an interview and osteodensitometric measurements, was performed at a baseline visit. After the baseline visit, we followed our studied subjects for 5 years. In contrast to our design, selection bias may occur if studied subjects are sampled due to a fracture episode. Following fracture, mortality may increase and a fracture may be associated with co-morbidity, making it difficult for subjects with a fracture to participate in a subsequent scientific study [20, 21].

Most subjects treated with β-blockers receive the treatment due to cardiovascular diseases. Potentially, a fracture may have more severe consequences in patients with cardiovascular diseases than in otherwise healthy subjects. If so, the true number of fractures in subjects treated with β-blockers (i.e., subjects with cardiovascular diseases) may be underestimated if studied subjects are recruited after the fracture episode. Other differences between ours and the Australian study are the completeness of fracture assessment and the age of studied subjects. In our study, X-rays of the spine were obtained in all subjects at inclusion and at the 5-years follow-up visit, whereas in the Australian study, spine X-rays were only obtained in subjects in whom a vertebral fracture was suspected. Our study subjects were perimenopausal women with a median age of 50 years at baseline, whereas the women in the Australian study had a median age of 70 years. However, it is uncertain to what extent this may affect treatment with β-blockers on fracture risk. Further studies are needed to resolve this question.

In the Australian study, treatment with β-blockers was associated with an increased BMD at the hip and ultradistal forearm [11]. However, as osteodensitometric measurements were performed approximately 2 months after the fracture had occurred, certain limitations may apply to this finding, as BMD may be affected by the fracture itself [22–24]. If the cardiovascular diseases necessitating treatment with β-blockers affect the co-morbidity associated with a fracture, it may be difficult to make interpretations on associations between treatment with β-blockers and BMD, if osteodensitometric measurements are performed after the fracture occurs. Actually, in the Australian study, no significant effect of treatment with β-blockers on BMD was found in the group of women who had not sustained a fracture, i.e., in the control group (112 users compared to 663 non-users of β-blockers). This may indicate that interactions exist between treatment with β-blockers, fracture risk, and BMD levels measured after the fracture episode. Further studies are needed to resolve this question.

Our finding of an increased fracture risk in users of β-blockers despite absence of a difference in BMD could indicate that the fracture risk was not mediated by differences in BMD, but perhaps rather by an altered bone biomechanical competence not reflected in BMD (e.g. an altered microarchitecture) or by an increased risk of falls. Falls may be the results of a decrease in blood pressure, an increased presence of dizziness or cardiac rhythm disturbances (bradycardia and AV-block in particular). However, it is difficult to establish a link between falls and fractures in users of drugs because not all falls may be recorded and not all falls may result in a fracture. Thus, further studies are needed in animals as well as in humans to clarify effects of adrenergic antagonist and agonists on bone.

Our findings of decreased serum osteocalcin levels in the group of β-blocker-treated subjects compared to the untreated group may be explained by a direct effect of β-adrenergic antagonists on osteoblasts. β2-adrenergic receptors have been identified by gene expression analyses in primary mouse osteoblasts and in human SaOS-2 osteoblastic cells [2], and sympathectomy induced by guanethidine, which specifically destroys sympathetic adrenergic fibers has been shown to reduce the activity of osteoblasts [4]. Moreover, β-adrenergic agonists (isoproterenol and norepinephrine) and antagonists (propranolol) have been shown to affect the function of osteoblasts [2]. Normally, β2-adrenergic receptors are G-coupled receptors that signal through the cAMP pathway [25]. Accordingly, treatment of osteoblastic cells with β-adrenergic agonists increases, whereas β-adrenergic antagonists decreased cAMP production [2, 7].

The cAMP pathway is a major intercellular signal transduction mechanism that regulates osteoblastic function and metabolism, including regulation of osteocalcin expression [26]. Similar to β2-adrenergic receptors, the parathyroid hormone (PTH) receptor activates intracellular signal transduction through the cAMP pathways, and *in vitro* studies have shown that PTH increases osteocalcin synthesis through a cAMP-dependent mechanism of action [26, 27]. Moreover, in human studies, treatment with PTH and PTH-related peptide have been shown to increase plasma osteocalcin levels [28, 29], whereas decreased osteocalcin levels have been found in subjects with idiopathic hypoparathyroidism and following surgery for primary hyperparathyroidism [30–33]. Thus, the association in our study between treatment with β-blockers and low serum osteocalcin levels is most likely explained by a direct reduction in osteoblastic cell activity by β-blockers, i.e., a decreased cAMP production due to a pharmacological blockade of β2-adrenergic receptors.

Limitations to Study

Strengths of our study are the prospective design of fracture assessment, a long study duration, and the fact

that identification of fractures was not reliant on self-reports, but was validated against hospital records. In addition to fracture assessment, we measured BMD and levels of biochemical markers of bone turnover in order to assess not only fracture risk but also effects of β-blockade on bone metabolism. Moreover, we included a large number of potential confounders known to affect bone metabolism and fracture risk. Inclusion of these confounders turned out to be important as a statistical significant increase in fracture risk emerged after confounder adjustment. However, although we adjusted for several potential confounding factors, our results may still be influenced by potential confounding factors not included in the analyses. As most of our subjects were treated with β-blockers due to arterial hypertension, it may be speculated that hypertension per se may increase fracture risk. However, conflicting results have been reported on whether arterial hypertension influences BMD [34, 35], and hypertension has not been shown to increase risk of fracture [36, 37]. Moreover, our additional analyses on women treated with non-β-blocker cardiovascular drugs (ACE-inhibitors or calcium channel blockers) did not reveal associations between fracture risk and drug treatment/cardiovascular diseases. Therefore, it is unlikely that the underlying cardiovascular diseases cause the increased fracture risk observed by us.

Due to the relatively small number of subjects treated with β-blockers in our cohort, our ability to perform sub-analyses was limited. However, in a stratified analysis on duration of use of β-blockers, we found a biological plausible increased fracture risk with increased duration of treatment. Further studies should focus on whether a dose-effect relationship also exists. Moreover, certain characteristics that vary among different types of β-blockers may affect their ability to affect bone, e.g., differences in lipid solubility, relative affinity for β1 and β2 receptors.

Another study limitation, due to the relatively small number of subjects treated with β-blockers, is a low statistical power for detecting differences in BMD between treated and untreated subjects. For example, our statistical power to detect a 5% difference in BMD at the femoral neck between treated and untreated subjects was as low as 33% at a 5% significant level. Thus, our data do not exclude the fact that treatment with β-blockers may affect BMD. Further studies with larger samples are needed to resolve this question. Moreover, reanalyzing of previous randomized studies on treatment with β-blockers may also help.

Conclusions

In perimenopausal women, treatment with β-blockers was associated with a threefold increased risk of fracture. Moreover, duration of treatment was positively associated with risk of fracture. Women treated with β-blockers had lower osteocalcin levels than untreated women which may suggest decreased bone formation as the mechanism of action by which β-blockers increase risk of fracture. However, confirmative studies and studies exploring mechanisms of action are needed.

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