Neuroendocrine regulation of human bone metabolism

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Chapter 4

The effects of beta-2 adrenergic agonist and antagonist on human bone metabolism: a randomized controlled trial.

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ABSTRACT

Purpose: Genetic knockout or pharmacological inhibition of the beta-2 adrenergic receptor (B2AR) increased bone mass, whereas stimulation decreased bone mass in rodents. In humans, observational studies support sympathetic nervous system regulation of bone metabolism, but intervention studies are lacking. We aimed to determine the effects of a selective beta-2 adrenergic agonist and non-selective antagonist on human bone metabolism.

Methods: 32 healthy postmenopausal women were included in a randomized controlled trial conducted in the Academic Medical Center Amsterdam. Participants were randomized to receive treatment with 17-β estradiol 2 mg/day; 17-β estradiol 2 mg/day and terbutaline 5 mg/day (selective B2AR agonist); propranolol 80 mg/day (non-selective B-AR antagonist); or no treatment during 12 weeks. Main outcome measure was the change in serum concentrations of procollagen type I N propeptide (P1NP) and C-terminal crosslinking telopeptides of collagen type I (CTx) as markers of bone formation and resorption after 12 weeks compared between the treatment groups. Data were analyzed with mixed model analysis.

Results: 17-β estradiol decreased bone turnover compared to control (P1NP p<0.001, CTx p=0.003), but terbutaline combined with 17-β estradiol failed to increase bone turnover compared to 17-β estradiol alone (P1NP p=0.135, CTx p=0.406). Propranolol did not affect bone turnover compared to control (P1NP p=0.709, CTx p=0.981).

Conclusion: Selective beta-2 adrenergic agonists and non-selective beta-antagonists do not affect human bone turnover although we cannot exclude small changes below the detection limit of this study.
INTRODUCTION

The sympathetic nervous system (SNS) is an important regulator of bone metabolism in rodents [1]. Osteoblast-specific beta-2 adrenergic receptor (B2AR) knockout mice display increased bone formation and decreased bone resorption, resulting in a high bone mass phenotype [2]. Likewise, pharmacological inhibition and stimulation of beta-adrenergic receptors increased and decreased bone mass, respectively [3, 4].

It is still unknown whether human bone metabolism is under sympathetic control. Sixteen retrospective cohort and case-control studies, summarized in a recent meta-analysis [5], have investigated the association between beta-blocker use and fracture risk. Half of the studies reported a significant reduction in fracture risk; the other half reported no reduction or even an increase. Study populations were highly heterogeneous and ranged from 200 to almost 400,000 subjects, with different sex and age distributions. The studies included populations using a range of beta-blocker preparations varying in dose and duration. Underlying diseases and co-medications also varied between the studies. Nevertheless the meta-analysis indicated that the use of beta-blockers was associated with a small but significant reduction in fracture risk. In contrast, only three studies have investigated the effect of beta-agonists on bone metabolism [6-8]. A major limitation of all three studies was the administration by inhalation precluding significant systemic exposure. In addition, the study populations consisted of patients with chronic obstructive pulmonary disease, who frequently used some form of glucocorticoids, which could easily have overwhelmed any effect of beta-agonists on bone. Therefore there is an urgent need for prospective studies investigating the effects of beta-blockers and beta-agonists on bone metabolism.

To date, only one prospective pharmacological intervention study on beta-adrenergic receptor modulation and bone metabolism has been reported [9]. The authors of this study concluded that the non-selective beta-blocker propranolol did not affect bone metabolism although serum osteocalcin concentration decreased significantly. The effect of selective beta-2 adrenergic receptor modulation has not been studied before. Therefore the aim of our study was to determine the effect of a selective beta2-agonist on bone turnover in healthy postmenopausal women in a randomized controlled trial. We hypothesized that systemic administration of a selective beta2-agonist would increase bone turnover, parallel to the rodent studies. Since bone turnover is already increased in postmenopausal women [10], we determined the effect in women during estradiol substitution. In addition we studied the effect of a non-selective beta-antagonist, comparable to the previously reported study.
MATERIALS AND METHODS

Study Design and Setting
This multi-arm parallel randomized controlled trial was performed at the Endocrine Department of the Academic Medical Center of the University of Amsterdam (AMC/UvA) in The Netherlands from May 2010 until September 2012. Subjects were randomly allocated to treatment using a computer-generated (nQuery Advisor version 7.0, Statistical Solutions, Cork, Ireland) block randomization list with a block size of 4. The investigators were blinded to treatment allocation, but after randomization the investigators and subjects were not blinded to treatment. Laboratory personnel analyzing the samples was blinded to treatment. The study was carried out in accordance with the principles of the Declaration of Helsinki and the Institutional Review Board of the AMC/UvA approved the protocol. The trial was registered in the Netherlands Trial Register (TC 2874) before start of the study.

Subjects
32 healthy postmenopausal women who had their last menstrual cycle 12 to 60 months before inclusion were recruited from the general population via advertisements in local newspapers. Exclusion criteria were conditions or use of medication influencing bone metabolism and contraindications to treatment with estrogen, adrenergic beta-agonists and adrenergic beta-antagonists. All subjects provided written informed consent before study inclusion.

Intervention
Subjects (n=8 per group) were randomized to receive treatment with 1] 17-β estradiol 2 mg daily (Zumenon, Abbott Products BV, Weesp, Netherlands), 2] 17-β estradiol 2 mg daily and terbutaline 5 mg daily (Bricanyl, AstraZeneca UK Ltd., Luton, UK), 3] propranolol slow release 80 mg daily (Propranolol retard, Pharmachemie BV, Haarlem, Netherlands) or 4] no treatment during 12 weeks.

Measurements
At baseline, the investigators took a complete history, measured weight and height, performed dual energy x-ray absorptiometry (DXA) scanning (Hologic Discovery, Bedford, MA, USA; APEX system software version 3.3) and electrocardiography and drew venous blood samples after an overnight fast to determine serum concentrations of calcium, albumin, phosphate, parathyroid hormone, 25(OH) vitamin D, bone turnover markers, creatinine, and urea. After 4, 8 and 12 weeks subjects filled out questionnaires
assessing study medication compliance and side-effects and provided venous blood samples after an overnight fast to determine the concentrations of bone turnover markers.

**Main outcome**

Changes in serum concentrations of the bone resorption marker C-terminal crosslinking telopeptides of collagen type I (CTx) and the bone formation marker procollagen type I N propeptide (P1NP) (together bone turnover markers) after 12 weeks were the main outcome measures. CTx and P1NP are recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine as international reference markers for bone resorption and formation. In addition, we determined changes after 12 weeks in serum concentrations of osteocalcin, the bone formation marker commonly used in clinical practice and reported in the previous intervention study [9].

**Analytical procedures**

CTx, P1NP and osteocalcin were measured using immunoassays (Modular Analytics E 170, Roche Diagnostics Corporation, Indianapolis, IN, Orion Diagnostica, Espoo, Finland, and BioSource, Nivelles, Belgium respectively). The assay was performed at the end of the study period in a single batch. Serum concentrations of calcium, albumin, creatinine, urea and phosphate were measured on a Roche Modular autoanalyzer Cobas 8000 using standard colorimetric techniques. Serum concentrations of parathyroid hormone and 25(OH) vitamin D were measured using an automated immunoassay (Roche Diagnostics Corporation, Indianapolis, IN and Diasorin, Stillwater, MN, USA respectively). Interassay coefficients of variation (CV) were as follows: CTx 3%, P1NP 8%, osteocalcin 8%, calcium 1.0%, albumin 1.6%, creatinine 1.2%, urea 1.9%, phosphate 1.4%, parathyroid hormone 2.3% and 25(OH) vitamin D 7.2%. All serum samples were collected in the morning between 7:00 and 9:00 h after an overnight fast and stored at -20°C until analysis.

**Statistical Analysis**

The statistical analysis was carried out with SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL, USA) and R statistical software for Windows (version 2.15, R Core Team. R: a language and environment for statistical computing 2013, package: nonlinear mixed effects (nlme). The mean and standard deviation (SD) or the median and interquartile ranges (IQR) are reported depending on the distribution. All statistical tests were two-sided and a P-value of 0.05 was considered significant. To assess the effect of the intervention including all timepoints, we performed a linear mixed model analysis with treatment and visit as categorical fixed effects, a random intercept to correct for variance in baseline concentrations and correction for heteroscedasticity and
repeated measurements (AR1). The assumptions of the model were met. To compare intervention groups after 12 weeks of the intervention period (post hoc) and correct for multiple testing, we used Tukey tests.

**Power and sample size calculation**

No specific methods exist to calculate power and sample size for linear mixed models. The best approximation is to employ an ANOVA model. It should be noted that the absence of the repeated measurements in the ANOVA model results in underestimation of the power. Using a one-way ANOVA with a two-sided significance level of 0.05 and assuming a standard deviation of 25%, a sample size of 8 per group will have 80% power to detect a difference in means of at least 30%, which is the smallest change considered clinically relevant [11].

**RESULTS**

**Subjects**

Recruitment yielded 89 responses of which 29 subjects did not meet the inclusion criteria, 18 declined to participate and 4 could not be reached by telephone. 38 subjects were randomized of which 2 did not complete the study due to side-effects of treatment (one subject experienced headache from beta-blocker treatment and one had vaginal discharge from 17-β estradiol treatment) and 4 participants did not receive the allocated intervention or did not comply with the assessments (1 subject in the control group and 1 subject in the 17-β estradiol group withdrew for personal reasons, 1 in the 17-β estradiol group refused medication and 1 in the 17-β estradiol combined with beta-agonist group was excluded due to corticosteroid use which became known after randomization). Table I shows the baseline characteristics of the participants. Compliance with study medication was 99% and there were no differences between treatment groups. No serious adverse events were recorded during the intervention.

**Bone turnover markers**

As expected from the randomization, the groups did not differ at baseline. P1NP, CTx and osteocalcin followed normal distributions. Mixed model analysis showed an overall significant effect of treatment and time for P1NP (p=<0.001 for both) and CTx (p=0.003 and p=0.015 respectively). There was no effect of treatment or time on osteocalcin (p=0.277 and p=0.437, respectively).

Post hoc tests showed that 17-β estradiol decreased P1NP and CTx (p<0.001 and p=0.003 respectively) as compared to the control group. Combined terbutaline and 17-β estradiol decreased P1NP significantly (p=0.039) but not CTx (p=0.242) as
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compared to the control group, however there was no difference in P1NP and CTx as compared to the 17-β estradiol group (P1NP p=0.135 and CTx p=0.406). There was also no difference in P1NP and CTx between the propranolol and the control group (P1NP p=0.709 and CTx p=0.981) (Figure I).

**TABLE 1** Baseline characteristics subjects

<table>
<thead>
<tr>
<th></th>
<th>17-B estradiol</th>
<th>17-B estradiol and terbutaline</th>
<th>Propranolol</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age</td>
<td>52 (2.9)</td>
<td>52 (2.7)</td>
<td>53 (3.4)</td>
<td>53 (2.9)</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>49 (3.0)</td>
<td>50 (3.8)</td>
<td>50 (3.0)</td>
<td>50 (1.6)</td>
</tr>
<tr>
<td>Body Mass Index [kg/m2]</td>
<td>26 (3.8)</td>
<td>23 (3.2)</td>
<td>25 (2.5)</td>
<td>25 (2.7)</td>
</tr>
<tr>
<td>Smoking [n]</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DXA T-score femoral neck</td>
<td>-0.6 (0.5)</td>
<td>-0.7 (0.8)</td>
<td>-0.7 (1.2)</td>
<td>-0.8 (0.9)</td>
</tr>
<tr>
<td>DXA T-score L1-L4</td>
<td>-0.7 (0.7)</td>
<td>-0.3 (1.5)</td>
<td>-0.5 (0.9)</td>
<td>-0.7 (1.1)</td>
</tr>
<tr>
<td>Serum concentrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium [2.2-2.6 mol/L]</td>
<td>2.33 (0.06)</td>
<td>2.34 (0.07)</td>
<td>2.35 (0.09)</td>
<td>2.34 (0.07)</td>
</tr>
<tr>
<td>25(OH) Vit D [75-250 nmol/L]</td>
<td>40 (26)</td>
<td>47 (25)</td>
<td>55 (20)</td>
<td>65 (36)</td>
</tr>
<tr>
<td>PTH [0.6-6.7 pmol/L]</td>
<td>4.8 (1.3)</td>
<td>5.3 (1.9)</td>
<td>5.8 (0.8)</td>
<td>4.6 (1.0)</td>
</tr>
<tr>
<td>Osteocalcin [0.4-4.0 nmol/L]</td>
<td>1.50 (1.40)</td>
<td>2.70 (0.78)</td>
<td>1.89 (0.86)</td>
<td>1.68 (1.46)</td>
</tr>
<tr>
<td>CTx [&lt;1008 ng/L]</td>
<td>293.6 (153.5)</td>
<td>351.1 (140.6)</td>
<td>388.6 (122.6)</td>
<td>302.6 (136.8)</td>
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<tr>
<td>P1NP [19-96 ug/L]</td>
<td>45 (9)</td>
<td>56 (14)</td>
<td>55 (11)</td>
<td>52 (13)</td>
</tr>
</tbody>
</table>

All measurements are expressed as mean (standard deviation). Reference ranges and units are provided in square brackets. No significant differences between groups were observed.
Figure 1 P1NP, CTx and osteocalcin concentrations at baseline and after 12 weeks per treatment group.
DISCUSSION

This randomized controlled trial shows that 12 weeks of treatment with terbutaline, a selective beta-2 agonist, or propranolol, a non-selective beta-antagonist, does not affect bone turnover in postmenopausal, healthy women as measured by changes in serum bone turnover markers CTx, P1NP and osteocalcin although we cannot exclude small changes below the detection threshold of this study. Therefore we could not confirm the effect of pharmacological beta-adrenergic stimulation or inhibition on human bone remodeling as has been observed in rodents.

Part of our results are supported by a previously published randomized controlled trial in postmenopausal women showing that 12 weeks of propranolol treatment (160 mg daily) compared to placebo, had no effect on P1NP and alkaline phosphatase activity, markers of bone formation [9]. Urine free deoxypyridinoline and serum CTx, markers of bone resorption, both decreased approximately 10% in the propranolol group, which was assumed to be caused by a change in glomerular filtration rate during the study period and was not considered a relevant effect although serum osteocalcin concentration decreased significantly by twenty percent. The conclusion from this study, that propranolol did not affect bone turnover, is in accordance with our result.

One year after the publication of the aforementioned trial in humans, two studies investigated dose effects of propranolol on bone turnover in rats and showed that low doses of propranolol (0.1 and 5 mg/kg) increased bone mineral density whereas higher doses (up to 20 mg/kg) did not [3, 12]. Therefore we lowered the dose to the lowest dose of propranolol available in a slow-release preparation, 80 mg of propranolol, which is approximately 1.2 mg/kg and this should have had an effect on bone metabolism.

Two recent studies supported SNS regulation of bone metabolism in humans. The first study showed that bone resorption was increased in phaeochromocytoma patients and normalized after adrenalectomy [13]. Phaeochromocytomas are tumors of the adrenal gland producing catecholamines, the natural ligands of adrenergic receptors and important neurotransmitters of the SNS. Although in this study the source of the neurotransmitters was the adrenal gland and not the SNS, the study suggests that stimulation of human bone turnover by the SNS is possible. A study on patients with malignant phaeochromocytoma and bone metastases showed a high prevalence of skeletal-related events [14]. The second study observed an inverse association of sympathetic activity, measured with microneurography, with trabecular microstructure and bone strength in women [15], highlighting the adverse effect of increased sympathetic activity on bone structure. In addition, sympathetic and parasympathetic tone, measured with sympathetic skin responses and heart rate variability, were increased and decreased respectively in women with osteoporosis compared to healthy women [16] and a rapid
resting heart rate was associated with an increased risk of osteoporotic fracture in elderly women [17].

Considering these observations, a regulatory influence of the SNS on bone metabolism seems to be present in humans. However, our study shows that pharmacological manipulation of the beta-2 adrenergic receptor does not influence bone turnover. A possible explanation for this discrepancy could be the contribution of other adrenergic receptors to bone turnover. Although expression of the beta-1 adrenergic receptor on bone cells is supposed to be low or absent, beta-1 adrenergic receptor knockout and beta-1/beta-2 double knockout mice have reduced bone mass unlike beta-2 receptor knockout mice [18, 19]. In addition, alpha-2a and alpha-2c adrenergic receptor female knockout mice had an increased bone mass despite elevated catecholamine levels [20]. We included early postmenopausal women in our study since they are vulnerable to bone loss because of estrogen depletion. Bone turnover in postmenopausal women is characterized by an increase in bone resorption and bone formation, resulting in a net loss of bone [10]. Since we hypothesized that terbutaline would also increase bone turnover, we combined the administration of terbutaline with 17-β estradiol to augment the chance of detecting an effect of terbutaline. As a control group, we added a group treated with 17-β estradiol alone. Subjects received 5 mg terbutaline daily (70 ug/kg), which is the recommended dose for systemic treatment of broncho-obstructive disease in humans. In rodents, administration of beta-2 agonists reduced bone mass, but it should be noted that the doses of beta-2 agonists used in rodent studies exceeded the therapeutic range in humans by 50 to 1000-fold [4].

The intervention period in this study was 12 weeks. The remodeling cycle of bone takes approximately 3 months [21]. During this period we observed no effect, nor a trend towards an effect, in the propranolol and control groups. In the estrogen and combined estrogen/terbutaline groups, CTx concentrations reached a plateau already after 4 weeks of treatment, with no trend towards an additive effect of terbutaline at 12 weeks. P1NP concentrations did not reach a plateau, but there was again no trend towards an additive effect of terbutaline after 12 weeks, suggesting that a 12 week study duration is adequate to detect clinically relevant changes in bone turnover.

In our power calculation before start of the study, we estimated the standard deviations of our outcome measures P1NP and CTx to determine the group size. For P1NP the assumptions were met to detect a change of 30% at the end of the study. However, at the end of the study period, the standard deviation of CTx was larger than expected. Therefore the minimal change in CTx concentration we could detect with 80% power was 55%. This change is still close to the lowest threshold for clinical relevance of 30% and a conservative approach compared to the changes in bone turnover markers observed.
with therapies such as bisphosphonates, teriparatide, denosumab and corticosteroids which easily exceed changes of 100%.

In conclusion, the SNS is an important regulator of bone metabolism in rodents; however, its role in humans remains speculative. Retrospective observational studies have shown a possible influence of the SNS, but prospective intervention studies are difficult to perform due to the functional heterogeneity of the SNS and the low accessibility of bone tissue. The present prospective randomized intervention trial does not show an effect of selective beta-2 adrenergic agonist or non-selective beta-adrenergic antagonist on bone metabolism in healthy postmenopausal women. Therefore, this does not seem a feasible approach to influence bone metabolism in humans.

ACKNOWLEDGEMENTS

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