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Rapid Communication

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

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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 beta-2 agonist on bone turnover in healthy postmenopausal women in a randomized controlled trial. We hypothesized that systemic administration of a selective beta-2 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 were 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). Inter-assay 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 (IQRs) 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].