Neuroendocrine regulation of human bone metabolism
Vlug, A.G.

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Chapter 2

Bone resorption is increased in pheochromocytoma patients and normalizes following adrenalectomy.

Veldhuis-Vlug AG, El Mahdiui M, Endert E, Heijboer AC, Fliers E, Bisschop PH.

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ABSTRACT

Context: The sympathetic nervous system (SNS) controls bone turnover in rodents, but it is uncertain whether a similar role for the SNS exists in humans. Pheochromocytomas are catecholamine producing neuro-endocrine tumors. Since catecholamines are the neurotransmitters of the SNS, we hypothesized that pheochromocytoma patients have increased bone turnover.

Objective: Our objective was to compare bone turnover in pheochromocytoma patients and controls.

Design and setting: This retrospective case-control study was performed at the Endocrine Department of the Academic Medical Center of the University of Amsterdam (AMC/UvA) in the Netherlands from 2007 until 2011.

Patients: All patients were screened for pheochromocytoma. Cases (n=21) were identified by 24-hour urinary excretion of fractionated metanephrines above the institutional reference value and confirmed by histology following adrenalectomy. All patients screened and diagnosed as not having pheochromocytoma, served as controls (n=126).

Main outcome measure: The difference in bone turnover markers C-terminal cross-linking telopeptides of collagen type I (CTx) and procollagen type 1 N propeptide (P1NP) between cases and controls.

Results: CTx concentrations were higher in cases (343 ng/L (interquartile range (IQR) 295 ng/L)) than in controls (232 ng/L (IQR 168 ng/L)) (p=<0.001) and decreased following adrenalectomy (before 365 ng/L (IQR 450 ng/L), after 290 ng/L (IQR 241 ng/L), p=0.044). The effect remained after adjustment for possible confounders. P1NP concentrations did not differ.

Conclusions: This study shows that pheochromocytoma patients have increased bone resorption, which normalizes following adrenalectomy. This finding supports the concept of regulation of bone remodeling by the sympathetic nervous system in humans.
INTRODUCTION

Over the last decade, the sympathetic nervous system (SNS) has been identified as an important regulator of bone turnover in mice (1). Disruption of sympathetic signaling by deletion of the adrenergic beta-2 receptor leads to a high bone mass phenotype (2). In addition, pharmacological manipulation of the SNS by administration of beta-blockers or beta-agonists induces an increase and decrease, respectively, in bone mass (3-6).

Whether this ‘central control’ of bone metabolism also holds true for humans remains a question. Several retrospective case-control and cohort studies have investigated the risk of fracture during beta-blocker treatment and whereas some showed a reduction in fracture risk, others found no change. A meta-analysis did show an overall reduction in fracture risk (7). For beta-agonist treatment, three studies failed to demonstrate an effect on fracture risk in patients with chronic obstructive pulmonary disease (8-10). The observational nature of these studies, however, makes it difficult to draw any definite conclusions.

To study the effect of sympathetic stimulation on bone in humans, we investigated bone metabolism in pheochromocytoma patients. Pheochromocytomas are catecholamine producing neuro-endocrine tumors (11). Norepinephrine, a catecholamine, is the main neurotransmitter of the sympathetic nervous system. We hypothesized that pheochromocytoma patients would have an increase in bone turnover resulting from sympathetic overstimulation. Therefore, we conducted a case-control study comparing bone turnover markers in pheochromocytoma patients and controls.

MATERIALS AND METHODS

Study design and setting

This retrospective case-control study was performed at the Endocrine Department of the AMC/UvA in the Netherlands. All patients screened for pheochromocytoma in 2007 and 2008 were included in the study; the inclusion of case patients was extended to 2009, 2010 and 2011. The screening protocol for pheochromocytoma was carried out as reported before (12). The Institutional Review Board of the AMC approved this study.

Study population

Patients were biochemically screened for pheochromocytoma because of 1) symptoms and signs suggesting pheochromocytoma, 2) adrenal incidentaloma or 3) a genetic
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predisposition for pheochromocytoma (multiple endocrine neoplasia type 2 or succinate dehydrogenase complex subunit D mutation). Patients with a history of pheochromocytoma, pregnancy, alcohol or drug abuse or age under 18 years were excluded.

Screening consisted of measurement of 24 hour urinary excretion of fractionated metanephrines. Patients with measured concentrations above the institutional age adjusted reference value underwent imaging of the adrenal gland. The diagnosis of pheochromocytoma was confirmed by histology following adrenalectomy and these patients were defined as cases. All other patients were defined as controls. Of all patients age, sex, length, weight, smoking status and medication use were recorded.

Analytical procedures

C-terminal cross linking telopeptides of collagen type I (CTx) and procollagen type 1 N propeptide (P1NP) are parameters reflecting bone resorption and bone formation, respectively, and were the main outcome measures. CTx and P1NP were measured using immunoassays (Modular Analytics E 170, Roche Diagnostics Corporation, Indianapolis, IN, and Orion Diagnostica, Espoo, Finland, respectively), as described earlier (13).

To establish the diagnosis pheochromocytoma, urinary metanephrines and normetanephrines were measured. Urine samples were acidified to pH <2, boiled for 30 minutes and diluted in pasteurized plasma protein solution (Albuman, Sanquin, Amsterdam, The Netherlands). Thereafter urine samples were analyzed as plasma samples and the fractionated metanephrines were determined by automated online solid-phase extraction HPLC-tandem Mass spectrometry as described by de Jong et al (14).

All measurements were performed on serum samples collected in the morning after an overnight fast following the standardized protocol during the pheochromocytoma screening (12) and stored at -20°C until assayed. For some case patients measurements were repeated after adrenalectomy following the same protocol.

Statistical analysis

The statistical analysis was done using PASW Statistics for Windows, version 18.0 (SPSS Inc. Chicago, Illinois, USA). The mean and standard deviation (SD) or the median and interquartile ranges (IQR) are reported depending on the distribution. Differences between cases and controls were tested using the Mann-Whitney-U test and differences before and after adrenalectomy using the Wilcoxon signed rank test since the bone turnover markers follow a skewed distribution. Bone turnover markers were log-transformed to perform multiple linear regression analysis to test for confounding and effect modification by interaction. Assumptions underlying the linear regression model were met. All tests were two-sided and a p-value of <0.05 was considered significant.
RESULTS

Patients

During 2007 and 2008 180 patients were screened for pheochromocytoma. Of 36 patients the blood samples could not be retrieved. 10 patients were excluded because of a history of pheochromocytoma. 8 cases were identified and the remaining 126 patients were controls. During 2009-2011 13 additional cases were identified, therefore the total number of cases is 21. In all cases, the diagnosis was confirmed by pathological examination after surgery. Characteristics of the cases and controls are shown in table 1. Cases and controls were comparable except for the 24-hour urinary (nor) metanephrines secretion.

<table>
<thead>
<tr>
<th>TABLE 1 Patient Characteristics</th>
<th>Controls</th>
<th>Cases</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>127</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Sex % male</td>
<td>39</td>
<td>48</td>
<td>0.476</td>
</tr>
<tr>
<td>Age - years (SD)</td>
<td>59 (14)</td>
<td>55 (12)</td>
<td>0.266</td>
</tr>
<tr>
<td>BMI - kg/m² (SD)</td>
<td>27.3 (4.8)</td>
<td>24.2 (3.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Smoking %</td>
<td>35</td>
<td>38</td>
<td>0.729</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroid use %</td>
<td>2</td>
<td>0</td>
<td>0.475</td>
</tr>
<tr>
<td>Hormonal replacement therapy use %</td>
<td>6</td>
<td>5</td>
<td>0.882</td>
</tr>
<tr>
<td>Bisphosphonate use %</td>
<td>3</td>
<td>0</td>
<td>0.408</td>
</tr>
<tr>
<td>Thiazide diuretic use %</td>
<td>22</td>
<td>33</td>
<td>0.268</td>
</tr>
<tr>
<td>Beta-blocker use %</td>
<td>24</td>
<td>14</td>
<td>0.333</td>
</tr>
<tr>
<td>Normetanephrine excretion in 24-h urine - μmol (IQR)</td>
<td>1.63 (0.99)</td>
<td>5.74 (15.28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metanephrine excretion in 24-h urine - μmol (IQR)</td>
<td>0.45 (0.31)</td>
<td>2.66 (22.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Bone turnover markers and pheochromocytoma

CTx concentrations were higher in cases than in controls (343 ng/L (IQR 295 ng/L) versus 232 ng/L (IQR 168 ng/L) p=<0.001) (figure 1A). Multiple linear regression analysis confirmed the association between pheochromocytoma and CTx after adjustment for age, sex, BMI, smoking status and medication use (thiazide diuretics, glucocorticoids, hormonal replacement therapy, bisphosphonates and beta-blockers) (model: constant 2.378, pheochromocytoma B 0.203 ± SE 0.058, 95% CI 0.088-0.318, p=0.001). Of these possible confounders, only the use of beta-blockers (n=33) and bisphosphonates (n=3) were significant predictors for CTx. Beta-blocker users had a lower median CTx concentration (200 ng/L (IQR 164 ng/L)) than non-users (297 ng/L (IQR 202 ng/L)) (p=0.005).
P1NP concentrations did not differ between cases and controls (cases 45 μg/L (IQR 43 μg/L) versus controls 41 μg/L (IQR 24 μg/L) p=0.408) (figure 1B). Multiple linear regression analysis did not show any other significant predictors for P1NP.

Adrenalectomy

In 16 pheochromocytoma patients a blood sample following adrenalectomy was available. CTx concentrations decreased following adrenalectomy from 365 ng/L (IQR 450 ng/L) to 290 ng/L (IQR 241 ng/L) (p=0.044) (figure 1C). After adrenalectomy CTx concentrations were no longer different from controls. P1NP concentrations did not change following adrenalectomy (before adrenalectomy 41 μg/L (IQR 35 μg/L), after adrenalectomy 35 μg/L (IQR 20 μg/L), p=0.623) (figure 1D).

**FIGURE 1** Plasma CTx and P1NP in patients with pheochromocytoma and controls (A and B). Plasma CTx and P1NP in patients with pheochromocytoma before and after adrenalectomy (C and D).
DISCUSSION

This study shows that pheochromocytoma patients have increased bone resorption, which normalizes following adrenalectomy. This finding supports the concept of regulation of bone remodeling by the SNS in humans.

Previous experiments in mice have shown that activation or inhibition of the sympathetic nervous system influences bone metabolism and that this effect is mediated by the beta-2 adrenergic receptor on the osteoblast (2). Pharmacological stimulation of the beta-2 adrenergic receptor increases bone resorption and decreases bone formation leading to a low bone mass whereas inhibition of the receptor has the opposite effect (3-6).

In humans, the beta-2 adrenergic receptor is expressed by bone cells (3;15). Human studies on the role of the SNS in bone remodeling have focused primarily on the association between fracture risk and the use of beta-blockers. Most beta-blockers are selective for the beta-1 adrenergic receptor, which makes it difficult to interpret the conclusions from these studies. Some attempts have been made to study the effect of beta-agonists on bone remodeling in humans, but the results of these studies were all negative. These studies were carried out in patients with chronic obstructive pulmonary disease. The negative results are likely explained by the use of beta-2 agonists as inhalers which limits the systemic availability necessary to reach bone tissue, the use of corticosteroids as co-medication which may have overwhelmed the effect of beta-2 agonists on bone and finally the severity of the pulmonary disease which may have confounded the results (8-10).

To overcome these objections, we searched for a human model of disease in which the beta-2 adrenergic receptor is implicated. Pheochromocytomas produce excess catecholamines, capable of stimulating the beta-2 adrenergic receptor. Therefore we hypothesized that the catecholamine excess in pheochromocytoma patients could stimulate bone turnover in humans, as was confirmed in this study. Adrenalectomy, the surgical removal of the catecholamine producing tumor, relieves the catecholamine excess and this was indeed accompanied by a normalization of bone resorption. Therefore the present study endorses the hypothesis that the sympathetic nervous system exerts a control over bone metabolism in humans.

During physiological bone remodeling, bone resorption and bone formation are coupled, securing the balance in bone mass. Many pathological bone conditions, such as osteoporosis and osteopetrosis, are characterized by uncoupling of bone resorption and formation, leading to a loss or gain in bone mass (16). Uncoupling was also observed in the adrenergic beta-2 receptor knockout mice (2). In the present study we also observed uncoupling of bone formation and resorption, since we found an increase in CTx, the bone resorption marker, but no difference in P1NP, the bone formation marker. This
suggests that in pheochromocytoma patients, uncoupling of bone resorption and bone formation takes place, ultimately leading to a decrease in bone mass. Unfortunately, since this was a retrospective study, we were not able to assess the bone mass in these patients. For future studies, it would be interesting to assess bone mass changes by DEXA scanning in pheochromocytoma patients during disease and following recovery.

A potential limitation of this study is the use of a single marker for bone resorption (CTx) and formation (P1NP). Possibly our result could have been strengthened by addition of multiple makers. However, CTx and P1NP are recommended as the preferred reference markers for bone resorption and formation in clinical studies by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine since CTx and P1NP are the most specific for bone compared to the other markers and because of the wide availability of an automated assay which has been well characterized, enabling comparison among different studies (17).

We included 126 control patients, yet only 21 cases were collected, 16 of which had blood samples taken before and after adrenalectomy. This limitation reflects the rare nature of the disease. However, 21 cases yield enough statistical power to conclude that there is a difference in bone resorption in cases compared to controls.

Our data suggest that it is the catecholamine excess resulting in sympathetic overstimulation that causes the increase in bone resorption since we have accounted for many possible confounding factors such as age, sex, body mass index, smoking and medication use. However, we cannot exclude the possibility that there are other confounding factors in pheochromocytoma patients we have not accounted for. We have tried to minimize this possibility by including control patients from the same screening cohort as the pheochromocytoma patients instead of including healthy controls, making them as comparable as possible. Of course, future studies in other cohorts will be needed to confirm our results.

To summarize, this study supports the concept of sympathetic control of bone remodeling in humans by demonstrating an increased bone resorption in pheochromocytoma patients which normalizes following adrenalectomy.

ACKNOWLEDGEMENTS

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