

Bone Resorption Is Increased in Pheochromocytoma Patients and Normalizes following Adrenalectomy

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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 neuroendocrine tumors. Because 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 in The Netherlands from 2007 until 2011.

Patients: All patients were screened for pheochromocytoma. Cases ($n = 21$) were identified by 24-h urinary excretion of fractionated metanephrines above the institutional reference value and confirmed by histology after 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 was the main outcome measure.

Results: CTx concentrations were higher in cases [343 ng/liter; interquartile range (IQR), 295 ng/liter] than in controls (232 ng/liter; IQR, 168 ng/liter; $P < 0.001$) and decreased after adrenalectomy [before, 365 ng/liter (IQR, 450 ng/liter); after, 290 ng/liter (IQR, 241 ng/liter); $P = 0.044$]. 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 after adrenalectomy. This finding supports the concept of regulation of bone remodeling by the SNS in humans. (*J Clin Endocrinol Metab* 97: E2093–E2097, 2012)

In 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 β -2 receptor leads to a high

bone mass phenotype (2). In addition, pharmacological manipulation of the SNS by administration of β -blockers or β -agonists induces an increase and a decrease, respectively, in bone mass (3–6).

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Abbreviations: CTx, C-terminal cross-linking telopeptides of collagen type I; IQR, interquartile range; P1NP, procollagen type 1 N propeptide; SNS, sympathetic nervous system.

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 β -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 β -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 neuroendocrine tumors (11). Norepinephrine, a catecholamine, is the main neurotransmitter of the SNS. 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.

Patients and Methods

Study design and setting

This retrospective case-control study was performed at the Endocrine Department of the Academic Medical Center (AMC) of the University of Amsterdam 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 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 yr were excluded.

Screening consisted of measurement of 24-h 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 after adrenalectomy, and these patients were defined as cases. All other patients were defined as controls. Age, sex, length, weight, smoking status, and medication use of all patients were recorded.

Analytical procedures

C-terminal cross linking telopeptides of collagen type I (CTx) and procollagen type 1 N propeptide (P1NP) are pa-

rameters reflecting bone resorption and bone formation, respectively, and they 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 of pheochromocytoma, urinary metanephrines and normetanephrines were measured. Urine samples were acidified to pH <2, boiled for 30 min, 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, IL). The mean and SD or the median and interquartile range (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 because 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 <0.05 was considered significant.

Results

Patients

During 2007 and 2008, 180 patients were screened for pheochromocytoma. The blood samples of 36 patients could not be retrieved. Ten patients were excluded because of a history of pheochromocytoma. Eight cases were identified, and the remaining 126 patients were controls. From 2009 to 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-h urinary (nor) metanephrines secretion.

Bone turnover markers and pheochromocytoma

CTx concentrations were higher in cases than in controls [343 ng/liter (IQR, 295 ng/liter) *vs.* 232 ng/liter (IQR, 168 ng/liter); *P* < 0.001] (Fig. 1A). Multiple linear regression analysis confirmed the association between pheochromocytoma and CTx after adjustment for age, sex,

TABLE 1. Patient characteristics

	Controls	Cases	P value
n	127	21	
Sex (% male)	39	48	0.476
Age, mean (sd) (yr)	59 (14)	55 (12)	0.266
Body mass index, mean (sd) (kg/m ²)	27.3 (4.8)	24.2 (3.6)	0.007
Smoking (%)	35	38	0.729
Medication use (%)			
Corticosteroid	2	0	0.475
Hormonal replacement therapy	6	5	0.882
Bisphosphonate	3	0	0.408
Thiazide diuretic	22	33	0.268
β-blocker	24	14	0.333
Normetanephrine excretion in 24-h urine, median (IQR) (μmol)	1.63 (0.99)	5.74 (15.28)	<0.001
Metanephrine excretion in 24-h urine, median (IQR) (μmol)	0.45 (0.31)	2.66 (22.5)	<0.001

body mass index, smoking status, and medication use (thiazide diuretics, glucocorticoids, hormonal replacement therapy, bisphosphonates, and β-blockers) (model: constant 2.378; pheochromocytoma B 0.203 ± 0.058 SE, 95% confidence interval 0.088–0.318; P = 0.001). Of these possible confounders, only the use of β-blockers (n = 33) and bisphosphonates (n = 3) were significant predictors for CTx. β-Blocker users had a lower median CTx concentration [200 ng/liter (IQR, 164 ng/liter)] than nonusers [297 ng/liter (IQR, 202 ng/liter); P = 0.005].

P1NP concentrations did not differ between cases and controls [cases, 45 μg/liter (IQR, 43 μg/liter) vs. controls, 41 μg/liter (IQR, 24 μg/liter); P = 0.408] (Fig. 1B). Mul-

tiple linear regression analysis did not show any other significant predictors for P1NP.

Adrenalectomy

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

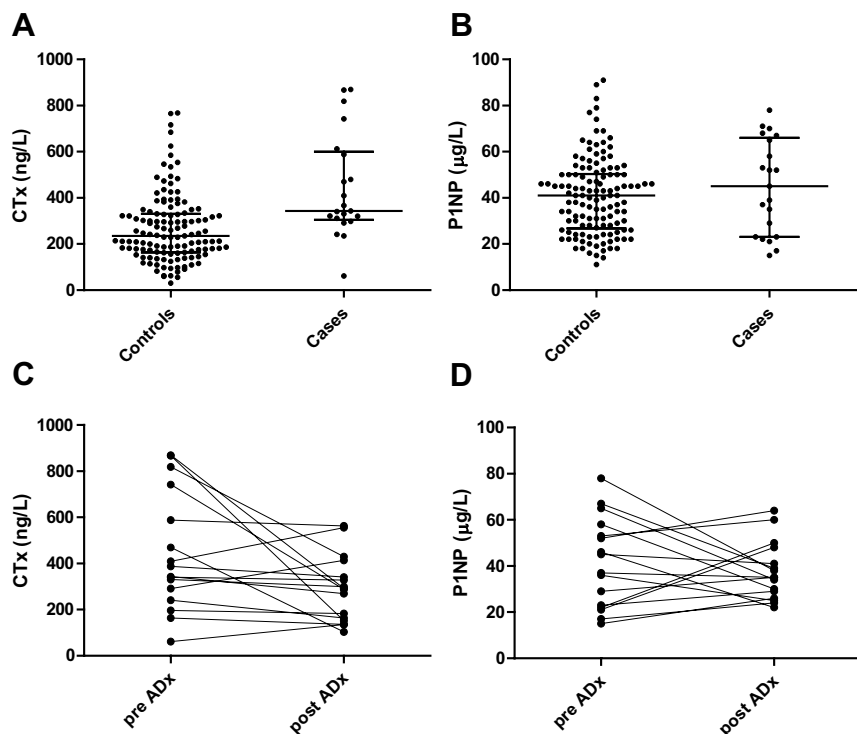


FIG. 1. A and B, Plasma CTx and P1NP in patients with pheochromocytoma and controls. C and D, Plasma CTx and P1NP in patients with pheochromocytoma before and after adrenalectomy.

Discussion

This study shows that pheochromocytoma patients have increased bone resorption, which normalizes after 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 SNS influences bone metabolism and that this effect is mediated by the β-2 adrenergic receptor on the osteoblast (2). Pharmacological stimulation of the β-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 β-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 β -blockers. Most β -blockers are selective for the β -1 adrenergic receptor, which makes it difficult to interpret the conclusions from these studies. Some attempts have been made to study the effect of β -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 β -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 β -2 agonists on bone; and finally, the severity of the pulmonary disease that may have confounded the results (8–10).

To overcome these objections, we searched for a human model of disease in which the β -2 adrenergic receptor is implicated. Pheochromocytomas produce excess catecholamines capable of stimulating the β -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 SNS 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 β -2 receptor knockout mice (2). In the present study, we also observed uncoupling of bone formation and resorption because 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, because 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 dual-energy x-ray absorptiometry scanning in pheochromocytoma patients during disease and after 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 the addition of multiple markers. 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 because CTx and P1NP are the most specific for bone compared with the other markers and because of the wide availability of an automated assay that 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 with controls.

Our data suggest that it is the catecholamine excess resulting in sympathetic overstimulation that causes the increase in bone resorption because 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 that 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 that normalizes after adrenalectomy.

Acknowledgments

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