Leptin Signaling and Hyperparathyroidism: Clinical and Genetic Associations

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BACKGROUND: The role of leptin in mediating calcium-related metabolic processes is not well understood.

STUDY DESIGN: We enrolled patients with hyperparathyroidism undergoing parathyroidectomy in a prospective study to assess postoperative changes to serum leptin and parathyroid hormone levels and to determine the presence of *LEPR* (leptin receptor) polymorphisms. Patients undergoing hemithyroidectomy under identical surgical conditions were enrolled as controls. Wilcoxon signed-rank test was used to analyze changes in leptin. Pearson correlations and Bland-Altman methods were used to examine the between-subject and within-subject correlations in changes in leptin and parathyroid hormone levels. Five single-nucleotide polymorphisms in the *LEPR* gene were genotyped, and linear regression analysis was performed for each polymorphism.

RESULTS: Among the 71 patients included in the clinical study, after-surgery leptin levels decreased significantly in the parathyroid adenoma ($p < 0.001$) and parathyroid hyperplasia subgroups ($p = 0.002$) and increased in the control group ($p = 0.007$). On multivariate analysis, parathyroid disease subtype, baseline leptin levels, age, body mass index, and calcium at diagnosis was associated with changes in leptin. Among the 132 patients included in the genotyping analysis, under a recessive model of inheritance, single-nucleotide polymorphism rs1137101 had a significant association with the largest parathyroid gland and total mass of parathyroid tissue removed ($p = 0.045$ and $p = 0.040$, respectively). When analyzing obese patients only, rs1137100 and rs1137101 were significantly associated with total parathyroid size ($p = 0.0343$ and $p = 0.0259$, respectively).

CONCLUSIONS: Our results suggest a role for the parathyroid gland in regulating leptin production. Genetic contributions from the leptin pathway might predispose to hyperparathyroidism. (J Am Coll Surg 2014;218:1239−1250. © 2014 by the American College of Surgeons)

Hyperparathyroidism is a common endocrine condition[1,2] that is treated surgically before hypercalcemic sequelae, such as nephrolithiasis, pancreatitis, peptic ulcer disease, and pathologic bone fractures, develop.[3] The exact mechanism underlying the development of hypersecreting parathyroid adenomas, constituting nearly 90% of hyperparathyroidism cases, remains poorly understood.[4] Despite this, several clinical associations have been observed among patients with hyperparathyroidism. Patients with primary hyperparathyroidism have significantly higher serum leptin levels than healthy subjects.[5] Those with comorbid obesity have even greater serum leptin levels than in the setting of either disease alone.[5] In women, leptin and parathyroid hormone (PTH) levels are positively correlated.[6] Patients with chronic kidney disease and in whom secondary hyperparathyroidism develops have abnormally elevated leptin levels due to poor renal clearance.[7-9] Parathyroid hormone levels correlate positively with body mass index (BMI) and fat mass consistently across studies.[10,11] There is also an increase in the prevalence of obesity and higher BMI values among patients with hyperparathyroidism,[12] suggesting

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Abbreviations and Acronyms
BMD = bone mass density
BMI = body mass index
LEP = leptin gene
LEPR = leptin receptor gene
PTH = parathyroid hormone
SNP = single-nucleotide polymorphism

that there exists a cause and effect relationship mediated by the leptin hormone.[13,14]

Multiple studies suggest a role for leptin in the regulation of pulsatile endocrine systems, especially in calcium-related metabolic processes. It appears that the diurnal rhythms of leptin and PTH secretion are intricately tied, as both have nearly identical mid-morning nadirs and nocturnal peaks that are independent of an endogenous circadian clock.[15-17] Leptin has been shown to affect bone mass in a dual manner; local administration to the hypothalamus results in indirect suppression of osteoblasts via independent sympathetic and serotonin-dependent systems, and systemic administration leads to PTH-like estrogenic bone formation.[18-22] In addition, leptin levels are higher in obese and premenopausal women[23] and correlates with increased bone mineral density (BMD) in the early postmenopausal state.[24]

Leptin, a 16.7-kDa protein of the obese gene (*LEP*), is predominantly synthesized in adipose cells at levels that positively correlate with BMI and modulates feeding behavior and energy expenditure through the leptin receptors (*LEPR*) in the hypothalamus.[25-27] Our laboratory has recently confirmed the presence of leptin production in the parathyroid glands (D Hoang and colleagues, unpublished data). Leptin is known to have anti-apoptotic activity,[28] have pro-angiogenic activity,[29] increase stem cell pools,[30] and act on cyclin D,[31] a key factor in the development of parathyroid neoplasia.[32] These studies suggest a mechanistic link between leptin levels and parathyroid neoplasia.

However, the relationship between leptin, PTH, and the parathyroid gland remains overall, poorly understood.[5-9,33-35] We therefore sought to determine the effect of parathyroidectomy on serum leptin and PTH levels, as well as to better characterize the genetic basis of the leptin signaling pathway in relation to hyperparathyroidism by evaluating the *LEPR* gene single-nucleotide polymorphisms (SNPs).

METHODS

Patients

From July 2010 to June 2011, one hundred and thirty-two patients were enrolled in a prospective study of leptin and hyperparathyroidism (Fig. 1). The Institutional Review Board of Yale University School of Medicine approved this study and all patients gave informed consent. Patients were enrolled based on their diagnosis of hyperparathyroidism (including primary, secondary, or tertiary hyperparathyroidism) and their candidacy for surgical intervention. Pathologists at our academic institution determined the pathologic diagnoses of parathyroid adenoma and hyperplasia. As controls, we recruited patients with a diagnosis of a unilateral thyroid nodule, but not hyperparathyroidism undergoing hemithyroidectomy under a similar surgical approach by the same surgeons, with identical incisions, anesthesia considerations, and lengths of procedures.

Available patient demographic and clinical information were recorded, including patient age, sex, BMI, BMD, medical history, radiographic imaging, clinical and intraoperative laboratory values, and surgical pathology reports.

Blood collection, clinical leptin hormone, and parathyroid hormone analyses

Blood samples were collected from study subjects at 4 time points during their routine clinical care. Time point 1 was 1 to 2 months before surgery as a preoperative baseline, time point 2 was 1 hour before surgery as a perioperative baseline, time point 3 was 1 hour after surgery to assess perioperative changes, and time point 4 was 1 month after surgery to assess long-term serum changes. A volume of 10 mL blood was collected by peripheral venipuncture at each of the described time points in blood-collection tubes, immediately centrifuged, and then serum was separated and stored at −80°C. Leptin serum levels were subsequently evaluated in duplicate according to the manufacturer's protocol using the Sensitive Human Leptin Radioimmunoassay kit (LINCO Research, cat. #SHL-81K). Parathyroid hormone measurements were made at the same time points as leptin as part of routine patient clinical care.

Statistical analysis of leptin and parathyroid hormone levels

Due to the non-normality of leptin and PTH values, the Wilcoxon signed-rank test was used to compare hormone levels measured at the different time points. An analysis of covariance model was used to compare the perioperative changes in leptin between control and parathyroid disease subgroups (adenoma and hyperplasia) and adjust for pre-surgery levels. Bivariate and multivariate analyses were performed to examine the effect of clinical variables on the perioperative changes.