Circulating Sclerostin Levels Are Decreased in Patients with Endogenous Hypercortisolism and Increase after Treatment

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Context: Increased bone fragility is a frequent complication of hypercortisolism due predominantly to suppression of bone formation. Sclerostin is an osteocyte-produced negative regulator of bone formation, which is up-regulated by glucocorticoids in mice.

Objective: Our objective was to assess the effect of endogenous hypercortisolism on circulating sclerostin and bone turnover in humans.

Design: We measured sclerostin, β-C-terminal telopeptide, amino-terminal propeptide of type 1 procollagen, and fibroblast growth factor 23 in blood samples of 21 patients with endogenous hypercortisolism and 21 age- and gender-matched controls. In 12 patients, measurements were repeated at various time intervals after successful surgical treatment (transsphenoidal surgery or adrenalectomy).

Results: Plasma sclerostin levels were significantly decreased in patients compared with controls (112 ± 49 *vs.* 207 ± 48 pg/ml, $P < 0.001$). In the 12 patients who were evaluated after surgical treatment, sclerostin levels increased from 121.4 ± 46.5 to 175.8 ± 78.5 pg/ml ($P = 0.003$). These changes in plasma sclerostin levels were accompanied by significant increases in levels of fibroblast growth factor 23 (from 44.2 ± 12.2 to 84.0 ± 58.8 pg/ml, $P = 0.017$) and of the bone turnover markers amino-terminal propeptide of type 1 procollagen (from 31.7 ±18.2 to 94.2 ± 92.2 ng/ml, $P = 0.037$) and β-C-terminal telopeptide (from 134.2 ± 44 to 409.2 ± 285 pg/ml, $P = 0.005$).

Conclusions: Contrary to the findings in mice, circulating sclerostin is decreased in patients with chronic endogenous hypercortisolism and increases after treatment. These findings suggest that in humans, chronic exposure to glucocorticoids affects the number or function of osteocytes rather than the production of sclerostin. **(*J Clin Endocrinol Metab* 97: E1953–E1957, 2012)**

Glucocorticoid excess increases bone loss and fragility leading to glucocorticoid-induced osteoporosis (1, 2). The action of glucocorticoids on bone is complex and involves local and systemic factors that adversely affect bone mass and quality. Of these, suppression of bone formation and increased rate of apoptosis of osteoblasts and osteocytes are considered major determinants of the deleterious effect of glucocorticoids on bone.

In osteoblasts, glucocorticoids inhibit Wnt signaling (3, 4), a pathway essential for osteoblast proliferation, differentiation, and survival; enhance the expression of receptor activator of nuclear factor-κ B-ligand (RANKL); and reduce that of osteoprotegerin promoting also osteoclast activity (5, 6). In recent years, the osteocyte-derived protein sclerostin has emerged as a key inhibitor of the Wnt signaling pathway in osteoblasts. In rodents, glucocorticoids stimulated

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Abbreviations: CTX, β-C-terminal telopeptide; FGF23, fibroblast growth factor 23; P1NP, Amino-terminal propeptide of type 1 procollagen; RANKL, receptor activator of nuclear factor-κ B-ligand.

the expression of *SOST*, the gene encoding sclerostin (3), and a neutralizing antibody against sclerostin prevented glucocorticoid-induced bone loss (7). These findings suggest that glucocorticoids may exert their action on bone formation by stimulating the production of sclerostin. In the present study, we tested this hypothesis in humans. For this, we studied patients with endogenous hypersecretion of cortisol because in glucocorticoid-treated patients, disease-related factors may have independent effects on bone metabolism and fragility and may also affect the synthesis of sclerostin (8, 9).

Patients and Methods

Patients

We studied 21 consecutive patients with endogenous hypercortisolism. The diagnosis and causes of hypercortisolism were established as previously described (10). Fifteen patients had ACTH-producing pituitary adenomas, two had adrenal adenomas, and four had ectopic ACTH production (two of unknown origin, one neuroendocrine tumor, and one metastatic melanoma). Nonfasting blood samples were obtained from all patients at different times of the day, mainly in the afternoon.

In 12 of the 21 patients, blood samples were also obtained at various time intervals after surgical treatment and achievement of biochemical remission. Eight of these patients were treated by transsphenoidal surgery and four by bilateral adrenalectomy. Remission was established by an adequate suppression of plasma cortisol after 1 mg dexamethasone overnight and disappearance of clinical signs and symptoms of hypercortisolism postoperatively (10). At the time of blood collection, eight of the 12 patients were receiving substitution therapy with hydrocortisone (n = 7) or dexamethasone (n = 1), and two patients used L-T_4 for secondary hypothyroidism.

Preoperative levels of sclerostin and bone turnover markers were compared with those of 21 age- and gender-matched healthy volunteers who were recruited by advertisements. None of these individuals used any medication or had any illness requiring medical attention. Similar to patients, nonfasting blood samples were obtained from controls in the afternoon.

All studied subjects (healthy and patients) had normal renal function and serum calcium and phosphate concentrations.

Biochemical measurements

Creatinine was measured by semiautomated techniques. Intact PTH was measured by the Immulite 2500 (Siemens Diagnostics, Breda, The Netherlands). Amino-terminal propeptide of type 1 procollagen (P1NP) and β-C-terminal telopeptide (CTX) were determined by the E-170 system (Roche BV, Woerden, The Netherlands). Sclerostin was measured by an electrochemiluminescence assay (MSD 96-well MULTI-ARRAY Human Sclerostin Assay; Meso Scale Discovery, Gaithersburg, MD), as previously described (11). With this assay, no sclerostin could be detected in the serum of 19 patients with sclerosteosis, whereas sclerostin was detectable in serum of 77 healthy individuals. In previous studies, we measured sclerostin in serum, whereas in the present study, we used EDTA plasma samples due to their availability. Because of earlier reported differences in sclerostin levels in plasma and serum (12), we first measured sclerostin levels in simultaneously obtained serum and plasma samples from 26 individuals with serum sclerostin levels ranging from 16.2–94.7 pg/ml. Compared with serum, sclerostin levels were on average 3.6-fold higher in plasma, but the two values were highly and significantly correlated (r = 0.91; $P = 0.001$).

Fibroblast growth factor 23 (FGF23) levels, as an independent index of osteocyte function, were measured with the Intact FGF23 ELISA kit (Kainos Laboratories, Tokyo, Japan). The intra- and interassay coefficients of variation were 9 and 11%, respectively.

Statistical analysis

Values are reported as mean ± SD, unless otherwise stated. Differences between patients and controls were assessed by *t* test. Of the 12 patients who were studied during biochemical remission, differences in sclerostin levels and other biochemical markers before and after surgical treatment were analyzed by paired *t* tests. Correlations between markers and changes in markers were assessed by Pearson's correlation. Normality of distribution was assessed by Shapiro-Wilk tests. Levels of CTX and FGF23 were log transformed because of skewness. Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL). A *P* value <0.05 was considered significant.

Results

Baseline biochemistry

Group characteristics and relevant biochemical parameters at baseline in patients and controls are shown in Table 1. Of the 21 patients with endogenous hypercortisolism included in the study, 16 were female (11 premenopausal and five postmenopausal) and five were male. Mean age was 41.3 ± 12.7 yr, mean weight 86.6 ± 17.1 kg, and mean body mass index 31.2 ± 6.6 kg/m^2.

Mean plasma sclerostin levels were significantly lower in patients compared with controls (112.0 ± 49.0 *vs.* 207.2 ± 48.4 pg/ml, $P < 0.001$), whereas there were no significant differences between the two groups in levels of P1NP, CTX, or FGF23 (Table 1).

Mean afternoon cortisol concentrations of patients was 0.63 ± 0.31 μmol/liter, and there was no relationship between plasma cortisol and sclerostin levels(r = −0.28;

TABLE 1. Demographics and baseline biochemical parameters of patients with endogenous hypercortisolism and age- and-gender matched healthy controls

	Controls	Patients	*P* value
Male/female	5/16	5/16	
Age (yr)	41.6 ± 11.8	41.3 ± 12.7	0.66
P1NP (ng/ml)	44.9 ± 14.3	36.9 ± 21.9	0.12
CTX (pg/ml)	224 ± 144	229 ± 224	0.94
Sclerostin (pg/ml)	207 ± 48	112 ± 49	<0.001
FGF23 (pg/ml)	37.5 ± 16.9	42.8 ± 12.1	0.25
Creatinine (μmol/liter)	68.6 ± 12.8	66.9 ± 13.8	0.62

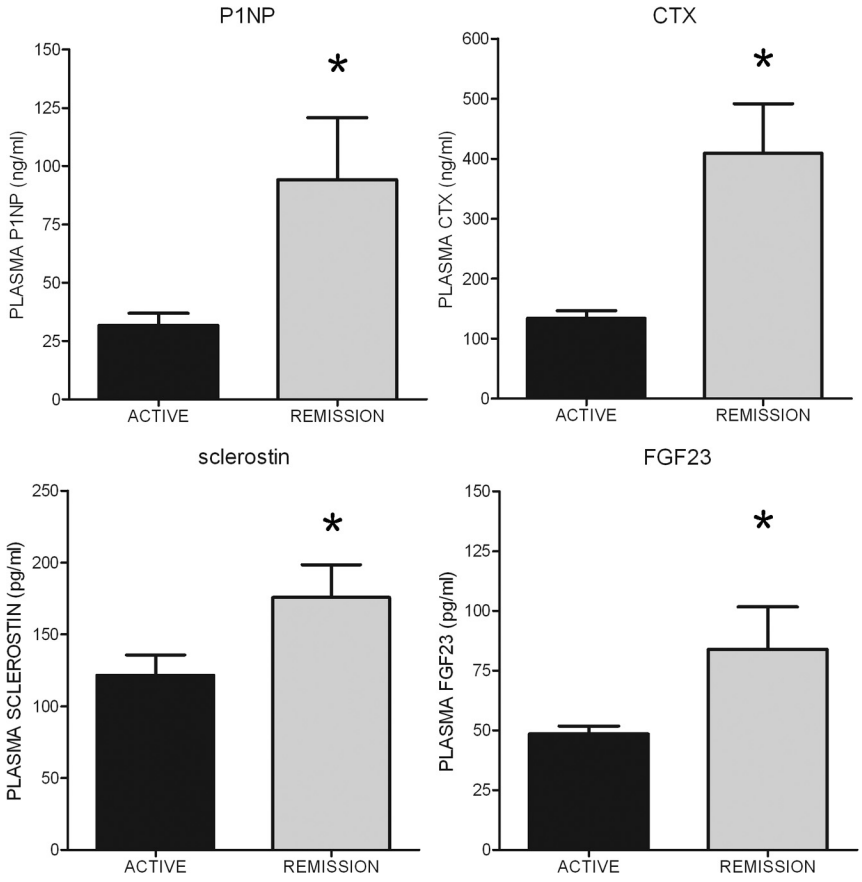

FIG. 1. Mean (SEM) levels of biochemical markers of bone turnover, sclerostin, and FGF23 in patients with endogenous hypercortisolism at baseline (*black bars*) and during biochemical remission (*gray bars*). *, $P < 0.05$.

$P = 0.31$) or P1NP (r = 0.45; $P = 0.09$); however, plasma cortisol concentrations were significantly correlated with serum CTX levels (r = 0.65; $P = 0.009$). Serum levels of CTX and P1NP were also significantly correlated in these patients (r = 0.55; $P = 0.03$).

Changes in biochemical parameters after treatment

Mean plasma cortisol concentration was 0.18 ± 0.18 μmol/liter in the 12 patients who were followed after surgical treatment. Changes of all studied biochemical parameters during remission of the disease are shown in Fig. 1. Remission was associated with a significant increase in mean sclerostin levels (from 121.4 ± 46.5 to 175.8 ± 78.5 pg/ml; $P = 0.003$). Similarly, P1NP levels increased from 31.7 ± 18.2 to 94.2 ± 92.2 ng/ml ($P = 0.037$), and CTX levels increased from 134.2 ± 44 to 409.2 ± 285 pg/ml ($P = 0.005$). Plasma FGF23 levels also increased during biochemical remission from 44.2 ± 12.2 to 84.0 ± 58.8 pg/ml ($P = 0.017$). Serum creatinine and plasma PTH concentrations, which may affect circulating sclerostin did not change significantly (68.8 ± 14.7 to 71.8 ± 14.1 μmol/liter, $P = 0.59$; and 4.9 ± 2.3 to 4.3 ± 2.1 pmol/liter, $P = 0.60$, respectively).

The median time of blood sampling after surgical treatment was 3 months (range 1 wk to 18 months). There was a positive correlation between the percent changes in plasma sclerostin levels and time of surgery (r = 0.68; $P = 0.015$). There was no correlation between sclerostin levels and changes of bone turnover markers.

Discussion

Since its discovery a decade ago, sclerostin has emerged as a key negative regulator of bone formation, and glucocorticoids were reported to stimulate the expression of the *SOST* gene, which encodes sclerostin (3). Contrary to this finding, we show here that plasma sclerostin levels are significantly decreased in patients with endogenous hypercortisolism and increase after surgical treatment and achievement of clinical and biochemical remission.

In bone, sclerostin is exclusively produced by osteocytes (13), cells that are directly affected by glucocorticoids that promote their apoptosis (1). Recent evidence suggests that glucocorticoids may also induce autophagy in osteocytes (14) and/or may increase oxidative stress (15). We, therefore, propose that the decreased sclerostin levels of patients with hypercortisolism are due to decreases in osteocyte function and/or number rather than to a direct, inhibitory, effect of glucocorticoids on sclerostin production. The increases in sclerostin levels during biochemical remission may be due either to the recovery of the function of the osteocytes after the cessation of the metabolic stress or to an increase in the number of osteocytes after the removal of the apoptotic effect of glucocorticoids.

To further explore this notion, we measured circulating FGF23 in our patients before and after treatment. FGF23 is also produced by osteocytes, but its production is not affected by glucocorticoids (3). It can, therefore, be considered as an independent parameter of the function and/or number of osteocytes. The observed significant increases in the levels of FGF23 after treatment strongly support the hypothesis that the increases in sclerostin levels represent a restoration of osteocyte numbers and/or function. Consistent with this hypothesis is also the observed increase in the biochemical markers of bone turn-

over. Improvement in osteocyte number and/or function may be responsible for the reversibility of the fracture risk after discontinuation of glucocorticoid therapy (16).

Bone loss after the exogenous administration of glucocorticoids is biphasic with an initial rapid phase occurring during the first months of treatment followed by a prolonged phase of much slower bone loss. It has been postulated that during the initial phase, an imbalance between bone resorption and bone formation, in favor of resorption, is the predominant pathogenetic factor for bone loss, whereas during the second, chronic, phase, reduced bone formation and osteocyte apoptosis are the main determinants of bone strength (17). Our findings in patients with chronic endogenous hypercortisolism are consistent with this sequence of events. Thus, in the presence of chronic glucocorticoid excess, sclerostin does not seem to affect bone formation, but its values in blood may be a measure of the function and/or number of osteocytes. However, sclerostin may very well be directly involved in the initial phase of bone loss induced by glucocorticoids. This could not be studied in our patients, but we previously showed that treatment of a patient with sclerostin deficiency with prednisone reduced both resorption and formation markers, and we suggested that sclerostin plays an important role in the modulation of bone resorption by glucocorticoids, whereas it does not affect their action on bone formation (18). Furthermore, mice treated with dexamethasone were protected from glucocorticoid-induced bone loss, when they were simultaneously treated with a neutralizing antibody against sclerostin (7). In these mice, the protective effect of the sclerostin antibody was mainly due to the stabilization of bone resorption, with no evident effect on bone formation. Sclerostin is thought to act predominantly as an inhibitor of bone formation, but a recent study has shown that sclerostin can also stimulate osteoclast differentiation and function by a RANKL-mediated mechanism in osteocytes (19). In addition, inhibition of RANKL by osteoprotegerin reduces the rate of osteocyte apoptosis in glucocorticoid-treated mice (20). Thus, although we found no evidence for a role of sclerostin in glucocorticoid-induced suppression of bone formation during longstanding endogenous hypercortisolism, sclerostin may play a role in the early phase of glucocorticoid-induced bone loss. Additional studies are needed to address these questions.

In conclusion, in this study, we found that patients with endogenous hypercortisolism have decreased circulating sclerostin levels, which increase during biochemical remission of the disease, and we propose that these changes in sclerostin levels are due to the detrimental effect of glucocorticoids on osteocytes rather than to a direct effect of glucocorticoids on sclerostin synthesis.

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