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Clinical Research Article

Bone Material Strength Index Is Low in Patients With Cushing's Syndrome Even After Long-term Remission

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Abstract

Objective: Hypercortisolism in endogenous Cushing's syndrome (CS) results in decreased bone mineral density (BMD) and increased fracture risk. Although after remission BMD improves, the fracture rate remains elevated, suggesting that BMD may not adequately reflect fracture risk in this group. The aim was to evaluate bone material properties, another component of bone quality, using impact microindentation in patients with CS in remission.

Methods: Cross-sectional study in 60 CS patients and 60 age-, sex-, and BMD-matched controls at a tertiary referral center between 2019 and 2021. Bone material strength index (BMSi) was measured by impact microindentation using the OsteoProbe® device at the tibia. In addition, laboratory investigation, BMD, and vertebral fracture assessment were performed.

Results: By design, patients and controls were comparable for age (median age 56.5 years), sex (48 women), and BMD at the lumbar spine and femoral neck. They were also comparable regarding the number of fragility fractures (21 vs 27, $P = .22$). The median time of remission in patients was 6 years (range 1 to 41). Despite comparable BMD, BMSi was significantly lower in CS patients compared to controls (76.2 ± 6.7 *vs* 80.5 ± 4.9, $P < .001$). In CS patients, BMSi was negatively correlated with body mass index ($r = -0.354$, $P = .01$) but not related to the presence of fracture, physiological hydrocortisone replacement use, other pituitary insufficiencies, or time since remission.

Conclusion: Bone material properties remain altered in patients with endogenous CS, even after long-term remission. These abnormalities, known to be associated with fractures in other populations, may play a role in the persistent bone fragility of steroid excess.

Key Words: bone material properties, bone quality, bone fragility, fractures, impact microindentation, IMI, secondary osteoporosis

Cushing's syndrome (CS) is a rare endocrine condition characterized by endogenous glucocorticoid excess, resulting from ACTH-secreting pituitary adenoma, ectopic ACTH production, or autonomous adrenal cortisol overproduction. Osteoporosis and fragility fractures are a main clinical manifestation of the disease and a relevant cause of morbidity and mortality (1-3). The skeletal complications are characterized by decreased bone formation and increased bone resorption, resulting in bone loss and an increased fracture risk. At diagnosis, osteoporosis is observed in up to 50%, and fractures have been reported in 30% to 76% of patients (3, 4). Fractures typically also occur in patients with normal or only slightly decreased bone mineral density (BMD) (5), suggesting that in CS factors contributing to bone quality other than BMD are also affected and have to be taken into account when assessing fracture risk in this group. Moreover, following remission of hypercortisolism, BMD increases toward values in the normal range, but fracture risk seems to remain increased (6-9).

Factors contributing to bone quality include bone architecture on the macro and micro level and tissue material properties (10). The microarchitecture of cortical and trabecular bone at the radius and the tibia has been assessed in 30 patients with active endogenous CS using high-resolution peripheral quantitative computed tomography and found to be altered compared to healthy controls (11). Tissue material properties of bone, however, could only be assessed until recently ex vivo by using transiliac bone biopsy specimens. Since the introduction of the impact microindentation (IMI) technique, these properties have become measurable in humans in vivo (12). IMI is performed with the handheld device OsteoProbe® that imparts a single impact load to the bone surface and is approved for use at the tibia. By driving the

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probe into the bone surface, the resistance of bone tissue to a given mechanical challenge can be measured as the bone material strength index (BMSi). BMSi values are altered in patients with fragility fractures compared to patients without fractures, independently of BMD (13-16), and are strongly associated with material properties of subperiostal mineralized bone surface (17). In a study conducted in patients requiring exogenous glucocorticoid therapy for various underlying disorders, the BMSi was found to be significantly decreased already after 7 weeks of treatment (18).

To date, there is no data on BMSi measurements in patients with endogenous CS. Therefore, the aim of this study was to evaluate whether bone material properties, as assessed by IMI, are altered in patients with endogenous CS in remission compared to controls in order to provide additional information on bone health in this group.

Patients and Methods

Study Design

This is a cross-sectional study evaluating BMSi in men and women in remission after treatment for CS and in age-, sex-, and BMD-matched controls. All BMD ranges and patients with and without fragility fractures were included. Patients and controls were studied at the outpatient clinics of the Leiden University Medical Center (LUMC), the coordinating center of the European Reference Network for Rare Endocrine conditions, and a European reference center for pituitary and adrenal diseases.

The study was approved by the institutional medical ethical committee, and all subjects provided full, written informed consent. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patients in Remission After Treatment for CS

The whole cohort of patients aged 18 to 85 years with adult-onset CS that were in remission for at least 6 months and under periodical prospective follow-up at the outpatient clinic of the Department of Endocrinology of the LUMC since 1978 (19) were eligible to participate in the study. The cohort patients were included between June 2019 and August 2021; the inclusion period was prolonged due to the COVID-19 pandemic. The diagnosis of CS as well as treatment of these patients including surgery, preoperative cortisol-lowering treatment as needed, posttreatment hydrocortisone (HC) replacement therapy, and tapering when the axis improved followed the Endocrine Society clinical practice guidelines and their update by the Pituitary Society (20, 21). The state of remission was also established according to these guidelines and was defined both as insufficient cortisol secretion after treatment of hypercortisolism with the need for hydrocortisone replacement therapy and, in the case of presumed normalized cortisol secretion, as normal 24-hour urinary free cortisol (UFC), midnight salivary cortisol, and normal overnight 1 mg dexamethasone suppression test.

Controls

Men and women who had no history of CS or exogenous steroid excess served as controls. They were recruited from the outpatient clinics of the Center for Bone Quality or from the regional Fracture Liaison Service of the LUMC and were matched for age, sex, and BMD.

Exclusion criteria for both groups were: the presence of a metabolic bone disease other than osteoporosis, any untreated endocrine disorder, severe liver insufficiency or chronic kidney disease (stage IV or V), immobilization, a contraindication for IMI measurement (22), or inability to provide informed consent. In addition, the use of any treatment affecting bone metabolism, other than calcium and vitamin D, was also an exclusion criterion, with the exception of bisphosphonate (BP) or denosumab use in patients in remission after treatment for CS (CS patients). The presence or absence of fragility fractures was not considered in the selection process of controls.

Methods

A full medical history; use of medication including agents affecting bone metabolism, vitamin D supplementation, and dietary calcium intake; and a detailed fracture history with documentation of sites and dates of occurrence of fractures were obtained from all subjects. A fragility fracture was defined as any low-energy fracture, excluding those of the hands, feet, and skull. Data on clinical risk factors for fracture as used in the FRAX algorithm were obtained from all subjects (23). In CS patients, clinically relevant data regarding hypercortisolism, such as date of surgery, number of relapses, date of remission after last recurrence, HC dependency, and postsurgical pituitary hormone deficiencies were also retrieved from the patient files. Duration of HC replacement was defined as the period of time that patients required HC replacement; in those currently on HC replacement, duration was calculated from the initiation of replacement therapy until the date of IMI performance.

Laboratory Parameters

Serum calcium (albumin-corrected) and creatinine concentrations were measured by semiautomated techniques. Plasma intact PTH was measured using the Immulite 2500 (Siemens Diagnostics, Breda, The Netherlands) and serum 25-hydroxyvitamin D concentrations by the 25- hydroxyvitamin-vitamin D TOTAL assay (DiaSorin D.A./N.V., Brussels, Belgium). In CS patients, clinical remission was biochemically confirmed by the assessment of UFC, serum, and salivary cortisol. UFC was analyzed using an in-house LC-MS/MS method, calibrated using Cerilliant certified reference material C-106, cortisol 1 mg/mL in methanol. Twenty-four-hour cortisol concentrations below 150 nmol/24 hours were considered normal. Serum and salivary cortisol were analyzed using a Roche ECLIA Cortisol assay (second generation) on a Modular E170 immunoanalyzer (Roche Holding AG, Basel, Switzerland). In midnight saliva, cortisol levels below 7.5 nmol/L were considered normal (21). The cutoff limit for the dexamethasone suppression test was 50 nmol/L (21).

BMD

Areal BMD was assessed at the lumbar spine (L1-L4) and both hips using dual-energy X-ray absorptiometry (DXA) performed with the Hologic QDR Discovery A machine (Hologic, Bedford, MA, USA). The mean BMD values of the femoral neck were utilized for the analysis. Z-scores were

calculated using National Health and Nutrition Examination Survey III reference values compatible with the reference values of the Dutch population (24).

Vertebral Fracture Screening

At the time of inclusion, single energy X-ray lateral vertebral fracture assessment images of the spine (T4-L4) were obtained through DXA or conventional antero-posterior and lateral radiographs of the thoracic and lumbar spine, adhering to standardized protocols for vertebral deformity detection. These images were then evaluated independently by 2 experienced readers using the semiquantitative Genant method (25). All vertebral fractures were considered in the analysis.

IMI

BMSi was assessed in all patients using the handheld microindenter device (OsteoProbe® RUO, Active Life Scientific, CA, USA) by IMI at the midshaft of the tibia, as described previously (26). Briefly, patients were positioned in a supine position with the tibia externally rotated to align the flat surface of the medial tibia diaphysis in a horizontal position. The measurement site was defined as the mean distance between the medial malleolus and the distal apex of the patella. After disinfection and local anesthesia of the skin and periosteum with lidocaine 1%, the test probe was gently inserted into the skin until reaching the bone surface. Following at least 5 valid measurements, an additional 5 measurements were taken on a polymethylmethacrylate calibration phantom. BMSi was computed by the computer software. Three experienced operators conducted the measurements. Intraobserver and interobserver coefficients of variation in our center were found to be 2.2% and 1.6%, respectively.

Statistical Analysis

Results are presented as mean ± SD unless stated otherwise. Descriptive statistics were used to describe clinical and laboratory parameters. Normality assumptions were checked by inspection of histograms and tested by a Kolmogorov–Smirnov test. Differences in baseline characteristics between patients and controls were assessed using a paired *t*-test or Wilcoxon signed ranks test and McNemar's test for normally and not normally distributed continuous and for categorical variables, respectively. Conditional logistic regression was used to assess BMSi and BMD values adjusted for body mass index (BMI) to compare BMSi and BMD values between patients and controls. Correlations between BMSi values and patients' or disease characteristics were examined by Pearson's and Spearman's tests for normally and not normally distributed variables, respectively. A *P*-value <.05 was considered to be statistically significant. All analyses were performed using SPSS software for Windows (version 29.0; SPSS Inc., Chicago, IL, USA) and graphs were constructed with Graphpad Prism (version 8.0; GraphPad Software Inc., La Jolla, CA, USA).

Results

Sixty-one of 73 eligible patients with CS in remission agreed to participate (Fig. 1). The IMI measurement could not be performed in 1 patient due to excessive lower leg edema, resulting in 60 CS patients included in the study (Table 1). Of these, 48 were women and 12 were men, with a median age of 56.5

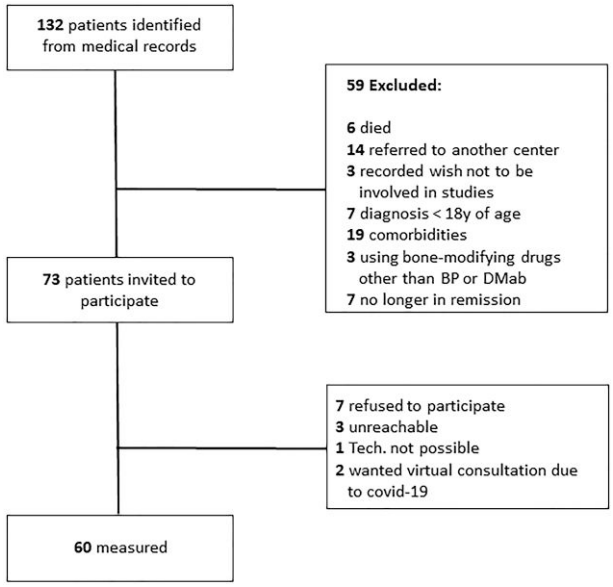

Figure 1. Patient flowchart.

Table 1. Disease-specific characteristics of patients with CS in remission

Characteristics	CS in remission
n	60
Age at diagnosis, years (range)	40.0 (18-66)
Time since diagnosis, years (range)	8.4 (1-42)
Origin of hypercortisolism, n (%)	
Pituitary	51 (85.0)
Adrenal	7 (11.7)
Ectopic	2 (3.3)
UFC at diagnosis (nmol/24 hours)[a]	646 (25-7792)
Recurrence before final remission, n (%)	21 (35.0)
Current HC replacement, n (%)	30 (50.0)
Current HC dosage, mg	20.0 (5-30)
Current hypopituitarism,[b] n (%)	
Any hypopituitarism	21 (41.2)
Hypothyroidism	19 (37.3)
Hypogonadism	12 (23.5)
GH deficiency	15 (29.4)
Current laboratory values	17.0 ± 3.0
Free T4[c] (pmol/L)	12.4 (0.1-78.5)
LH[d] (IU/L)	13.0 (0.1-101.5)
FSH[e] (IU/L)	18.8 ± 6.5

Values are expressed as median (ranges). Free T4 and IGF1 are expressed as mean ± SD.
Abbreviations: CS, Cushing's syndrome; HC, hydrocortisone; UFC, urinary free cortisol.
[a]UFC was available in 49 patients.
[b]Percentage expressed for all patients with pituitary hypercortisolism only.
[c]Free T4 reference range, 12-22 pmol/L.
[d]LH reference range, premenopausal 1-60 IU/L; postmenopausal 8-60 IU/L, men 2-9 IU/L.
[e]FSH reference range, premenopausal 2-21.5 IU/L; postmenopausal 26-135 IU/L, men 1.5-12.5.

years (range 25-76 years). The median age at diagnosis was 40.0 years (range 18-66 years). A corticotroph pituitary adenoma was the main cause of endogenous hypercortisolism in most patients (n = 51), followed by adrenal (n = 7) and ectopic origin (n = 2). The median duration of remission was 6.0 years (range 1-41 years). Twenty-one patients (35%) had recurrent hypercortisolism requiring reintervention before remission was achieved (range 1-4 recurrences). Among the 48 women, 30 (62.5%) were premenopausal and 18 were postmenopausal at the time of remission. The median time since menopause at the time of IMI measurement was 11.5 years (range 1-27 years).

After remission, 53 patients (88.3%) had been HC dependent, of which 30 patients (50%) were still using HC replacement at the time of the BMSi measurement. The median HC dosage was 20 mg per day (range 5-30 mg). Of the 51 patients treated for Cushing's disease, 21 (41.2%) also had other pituitary hormone deficiencies. Central hypothyroidism was reported in 19 (37.3%) and adequately substituted in all of them. Hypogonadotropic hypogonadism was present in 12 (23.5%) and GH deficiency in 15 patients (29.4%).

At the time of inclusion, 7 patients (11.7%) were using antiresorptive agents (5 oral BP, 1 IV BP, 1 denosumab) for a median duration of 37 months (range 13-55 months). Reasons for treatment with antiresorptive agents were osteoporosis or osteopenia with and without fractures (n = 5) and to decrease fracture risk in the presence of severe CS (n = 1). In 1 patient with normal BMD, antiresorptive treatment had been initiated due to the occurrence of multiple vertebral fragility fractures leading to transient paraparesis 3 years after remission of CS. None of the patients was using osteoanabolic agents.

Patients' characteristics and laboratory test results of the 60 CS patients (48 women) and 60 matched controls (48 women) are shown in Table 2. By design, the 2 groups were comparable for age, sex, and BMD at the lumbar spine (LS) and femoral neck (FN). They were also comparable regarding the number of fragility fractures. A significantly higher number of patients were using vitamin D compared to controls (n = 36 vs n = 20, $P = .006$), while there was no difference in the number of patients and controls using oral calcium supplements (n = 18 vs n = 12, $P = .225$). The majority of patients and controls had osteopenia (53.3% and 60%), while 23 patients and 19 controls had normal BMD, and 5 patients and controls had osteoporosis, respectively.

Table 2. Characteristics of patients with CS in remission and controls

	CS in remission (n = 60)	Controls (n = 60)	*P*-value
Age, years	56.5 (25-76)	56.5 (20-74)	.97
Male/female	12/48	12/48	1.00
LS BMD, g/cm²	0.96 ± 0.13	0.96 ± 0.13	.82
Z-score LS	0.3 ± 1.2	0.2 ± 1.2	.64
FN BMD, g/cm²	0.75 ± 0.12	0.74 ± 0.10	.70
Z-score FN	−0.1 ± 0.9	0.0 ± 0.9	.82
BMI, kg/m²	26.1 (18.7-43.0)	25.4 (14.7-36.5)	.09
Smoking, n (%)	11 (18.3)	11 (18.3)	1.00
Alcohol use ≥ 3 U/day, n (%)	4 (6.7)	8 (13.3)	.22
Calcium,[a] mmol/L	2.4 ± 0.1	2.3 ± 0.1	<.001
Creatinine,[b] umol/L	74.3 ± 15.6	69.0 ± 13.3	.05
25-OH vitamin D,[c] nmol/L	82.7 ± 29.6	71.1 ± 28.9	.03
PTH, pmol/L,[d] pmol/L	4.8 (2.4-13.8)	2.8 (1.0-7.5)	<.001
Fragility fracture any, n (%)[e]	21 (36.2)	27 (47.3)	.22
History of hip fracture, n (%)	0 (0.0)	5 (8.3)	.01
Prevalent vertebral fracture (radiological), n (%)[f]	15 (25.9)	6 (10.5)	.03
History of NHNV fracture, n (%)	11 (18.3)	20 (33.3)	.06
BMSi	76.2 ± 6.7	80.5 ± 4.9	<.001

Values are expressed as mean ± SD. Age, BMI, and PTH are expressed as median and ranges. Bold numbers indicate statistical significance.
Abbreviations: BMD, bone mineral density; BMI, body mass index; BMSi, bone material strength index; CS, Cushing's syndrome; FN, femoral neck; LS, lumbar spine; NHNV, nonhip nonvertebral.
[a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L.
[b]Creatinine reference range, 64-104 umol/L for males; 49-90 umol/L for females.
[c]25-OH vitamin D reference range, 50-250 nmol/L.
[d]PTH reference range, 0.7-8.0 pmol/L.
[e]Percentages were calculated for 58 patients and 57 controls.
[f]Vertebral fracture screening was available in 57 patients and 56 controls.

BMSi

BMSi values were significantly lower in CS patients compared to controls, 76.2 ± 6.7 vs 80.5 ± 4.9, $P < .001$ (Fig. 2), also after the exclusion of the 7 patients using antiresorptive agents ($P < .001$). BMSi values of the 2 patients with ectopic CS were very low, at 66.2 and 69.2 (*P*-value not available due to the low number of patients), while mean values of patients with pituitary and adrenal CS were comparable: 76.6 ± 7.0 vs 76.0 ± 3.1, $P = .686$. However, the findings of this study did not change when excluding the 2 patients with ectopic CS.

In CS patients, BMSi values negatively correlated with BMI (r = −0.354, $P = .006$), while in controls this correlation was absent (r = −0.135, $P = .304$). BMSi values did not correlate with age (r = 0.160, $P = .223$), LS BMD (r = 0.102, $P = .461$), or FN BMD (r = 0.077 $P = .557$) in patients or controls, although in controls there was a borderline significant weak correlation with age (r = −0.246, $P = .058$).

There was no correlation of BMSi with age at diagnosis (r = 0.184, $P = .159$), but the 2 lowest BMSi results (BMSi 60.7

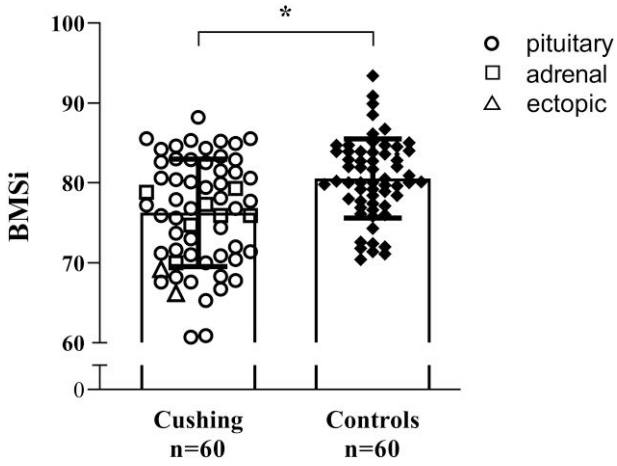

Figure 2. BMSi in Cushing patients and controls. Data are shown in box-whisker plots and statistical differences are displayed for BMSi. Boxes indicate the median and interquantile range. Bars indicate minimum and maximum values. *$P < .001$.
Abbreviations: BMSi, bone material strength index.

and 60.9; see Fig. 2) were found in the 2 patients who were the youngest at the time of diagnosis (18 and 20 years). There was no correlation of BMSi with UFC levels at diagnosis (r = 0.061, $P = .676$) or with the time since disease remission (r = −0.087, $P = .510$), including when stratified by remission intervals of 5 years (0-5 years, 6-10 years, 11-15 years, etc.).

BMSi values in CS (excluding the outliers with ectopic Cushing's) differed between women and men (75.6 ± 6.5 vs 80.5 ± 5.7, $P = .032$), which was not the case in female and male controls (80.1 ± 4.5 vs 82.0 ± 6.5, $P = .611$). BMSi values of the 30 women who achieved remission during premenopause did not significantly differ from those of the 18 women who achieved remission during postmenopause (74.8 ± 6.9 vs 76.6 ± 6.0, $P = .443$). HC dependency at the time of inclusion did not affect BMSi values: they were comparable in 30 patients who were HC dependent compared to those in 30 patients who were not HC dependent (75.9 ± 7.1 vs 76.5 ± 6.3, $P = .731$), also after adjusting for BMI ($P = .761$). BMSi values did not correlate with the daily HC dose used (r = −0.266, $P = .172$), nor did it correlate in the 57 patients using physiological doses up to 20 mg per day. However, the mean BMSi of the 3 patients using supraphysiological HC doses (>20 mg/day) was very low, 72.9 ± 8.7, and numerically strikingly lower than the mean BMSi of the patients using physiological doses, 76.8 ± 7.2 (*P*-value not available due to the low number of patients using supraphysiological doses). BMSi values did not differ between patients with and those without other pituitary hormone deficiencies (data not shown).

BMSi, BMD, and Fractures

Twenty-one patients (35%) with CS in remission had previously experienced 1 or more fragility fractures, including those detected on spine images. Of these, 15 had vertebral fractures, 6 had both vertebral and nonvertebral fractures, and 11 patients had nonvertebral fractures only. Of the 15 patients with vertebral fractures, 7 patients had multiple vertebral fractures (range 2-10). In total, 4 grade 3 vertebral fractures, 16 grade 2 vertebral fractures, and 21 grade 1 vertebral fractures were detected. Clinical vertebral fractures were diagnosed in only 2 patients, while in 13 they were detected radiologically.

Two CS patients and 3 controls, who had no other fragility fractures, did not undergo vertebral fracture screening and were consequently excluded from further analysis, as they could not be accurately categorized as "nonfractured." As a result, the final analysis included 58 CS patients and 57 controls.

The baseline characteristics and laboratory measurements of CS patients with and without fractures were comparable (see Table 3). BMSi did not differ between patients with and patients without fractures (77.0 ± 7.1 vs 75.9 ± 6.5, $P = .551$), also after adjusting for BMI ($P = .139$), sex ($P = .641$), or age (0.575) or when analyzing men and women separately (data not shown). BMSi did also not differ between the 15 patients with vertebral fractures and the 43 patients without vertebral fractures (77.7 ± 5.7 vs 75.8 ± 7.0, $P = .321$).

In contrast, in controls, BMSi was significantly lower in those with fragility fractures (n = 27) compared to those without fractures (n = 30) (77.7 ± 4.2 vs 82.9 ± 4.4, $P < .001$), also after adjusting for sex ($P < .001$) or age ($P < .001$). BMSi values of controls with fragility fractures were also comparable to BMSi values of CS patients with fragility fractures (77.7 ± 4.2 vs 77.0 ± 7.1, $P = .934$).

Table 3. Characteristics of CS patients with and without fragility fractures

	Fracture (n = 21)	No fracture (n = 37)	*P*-value
Age, years	56.0 (31-76)	56.0 (25-73)	.71
Male/female	5/16	6/31	.48
LS BMD, g/cm²	0.98 ± 0.12	0.95 ± 0.13	.34
Z-Score LS	0.6 ± 1.1	0.1 ± 1.3	.20
FN BMD, g/cm²	0.75 ± 0.12	0.74 ± 0.12	.85
Z-score FN	0.0 ± 0.8	−0.1 ± 1.0	.80
BMI, kg/m²	27.3 (18.7-43.0)	25.3 (19.7-39.4)	.09
Smoking, n (%)	4 (19.0)	7 (18.9)	.99
Alcohol use ≥3 U/day, n (%)	3 (14.3)	1 (2.7)	.09
Calcium,[a] mmol/L	2.4 ± 0.1	2.4 ± 0.1	.69
Creatinine,[b] umol/L	74.1 ± 12.4	74.9 ± 17.6	.85
25-OH vitamin D,[c] nmol/L	89.5 ± 37.2	78.9 ± 22.7	.20
PTH, pmol/L,[d] pmol/L	4.6 (3.1-13.8)	4.7 (2.4-11.0)	.63
UFC (nmol/24 hours)[e]	693 (60-7792)	641 (25-4991)	.50
BMSi	77.0 ± 7.1	75.9 ± 6.5	0.55

Values are expressed as mean ± SD. Age, BMI, PTH, and UFC are expressed as median and ranges. Bold numbers indicate statistical significance.
Abbreviations: BMD, bone mineral density; BMI, body mass index; BMSi, bone material strength index; FN, femoral neck; LS, lumbar spine; UFC, urinary free cortisol.
[a]Calcium (albumin-corrected) reference range, 2.15-2.55 mmol/L.
[b]Creatinine reference range, 64-104 umol/L for males; 49-90 umol/L for females.
[c]25-OH vitamin D reference range, 50-250 nmol/L.
[d]PTH reference range, 0.7-8.0 pmol/L.
[e]UFC was available in 20 patients with and 28 patients without fractures.

In CS patients, BMD did not differ between patients with and patients without fractures either at the LS or at the FN (LS: 0.98 ± 0.11 vs 0.95 ± 0.14, $P = .367$; FN 0.75 ± 0.12 vs 0.75 ± 0.12, $P = 0.951$). A DXA diagnosis of osteoporosis was established in only 1 of the patients with fractures, while 9 had osteopenia and 8 normal BMD, respectively.

All findings of this study did not change when excluding the 7 patients currently using antiresorptive agents.

Discussion

This study demonstrates that BMSi values, measured by IMI at the tibia, are significantly lower in patients in remission after treatment for CS compared to matched controls and are not correlated to BMD values. In this cross-sectional study, there is no difference in BMSi values in CS patients with compared to those without fragility fractures, which is in contrast to controls in whom BMSi values are lower in those with fractures. In addition, BMSi values are negatively correlated to BMI in CS patients only, which might reflect the severity of cortisol exposure during active disease. Low BMSi values, known to be associated with prevalent fractures in other populations, may thus at least in part play a role in the observed persistent bone fragility in patients treated for CS.

Osteoporosis and fragility fractures are a known complication of CS. Osteoporosis is present in up to 50% at diagnosis (3, 11, 27-29), and the reported prevalence of fragility fractures in CS in the literature is high, ranging from 30% to 76% (3, 4). As shown in several studies, BMD clearly

increases and usually returns to normal values after successful abrogation of cortisol excess (6-9, 30-34), although large-scale longitudinal studies are lacking. Also in our study, mean DXA Z-scores after a mean of 6 years after remission were in the normal range (+ 0.3 SD at the LS and −0.1 SD at the FN, respectively). Fracture risk is highest within the last 2 to 3 years before diagnosis of CS and seems to decrease after treatment of the disease (1, 9, 35, 36). However, scarce data indicate that fracture risk might remain increased up to 30 years after diagnosis (1, 3, 37).

This is to our knowledge the first study reporting BMSi values, measured by IMI, in patients with CS. Our results indeed show lower BMSi values in CS patients 6 years after remission, compared to matched controls, in the presence of comparable BMD values. Mean BMSi values of our CS cohort after long-term remission were comparably low to those measured in patients exposed to exogenous glucocorticoids, known to rapidly increase fracture risk, for various underlying disorders after 20 weeks of glucocorticoid-treatment (median BMSi 77.3) (18). Half of our cohort of CS patients was using oral HC replacement at a physiological dose at the time of measurement, and BMSi values did not significantly differ between those who did and those who did not. The median daily HC dose used in our cohort was 20 mg, which is expected not to have adverse glucocorticoid-related effects (38, 39), but BMSi values of the 3 patients using supraphysiological doses indeed were strikingly lower.

Interestingly, we found a negative relationship of BMSi with BMI in our CS cohort, meaning that patients with higher BMI had lower and thus worse BMSi values. Both higher BMI and lower BMSi could be consequences of more severe cortisol exposure during active disease. This is supported by the fact that there was no correlation between BMSi with BMI in controls, as in earlier published studies by our group performed in patients with primary osteoporosis with or without fractures and in patients with acromegaly (13, 40) and with the fact that the usually observed correlation of BMSi with age was not present, although data in the literature on the correlation of BMSi with BMI or age are conflicting (12). However, we found no correlation of BMSi with 24-hour UFC concentrations as a marker of disease severity at the time of diagnosis, although waist circumference, or the Cushing severity index, which was first described in 2000 and thus after our cohort started, would be better markers of disease activity (41). These clinical data have only inconsistently been recorded, and prospective studies designed to answer this question would be needed. There might also be a negative influence of adipose tissue itself on bone strength, as was suggested by a study performed in elderly Swedish women. That study reported an inverse relationship between BMSi and the amount of subcutaneous fat at the tibia, whole body fat mass, and BMI (42). A third explanation would be a more metrological one, hypothesizing that subjects with higher BMI have more adipose tissue at the tibia, which itself could interact with the IMI measurement since the needle has to stick through a greater amount of tissue and thereby possibly generates lower BMSi values. However, the latter 2 hypotheses would also apply to controls, among whom no correlation of BMSi with BMI was observed.

Also, after adjusting for BMI, BMSi but also BMD values did not differ between CS patients with prevalent fractures and those without. Thirty-five percent of our CS patients had fractures, which is in accordance with previously reported rates (3, 7, 37). Vertebral fractures were present in 20% of our cohort. However, the majority of those fractures had been detected radiologically only, and routine screening for vertebral fractures was not conducted for all patients at the time of diagnosis. Therefore, it cannot be ruled out that some vertebral fractures occurred during active disease or shortly after remission, when BMSi may have been different. Furthermore, some patients had been treated with antiresorptive agents during active but also remitted disease, which might have influenced their BMSi values. Although CS patients using antiresorptive agents at the time of IMI measurement had comparable BMSi values to those using none, another study performed by our group demonstrated that antiresorptive treatment, given for a mean period of 2 years, increases BMSi values in patients with low bone mass at risk for fracture (26). In contrast, in control subjects, BMSi was lower in patients with fractures compared to those without fractures. This is in accordance with the results of many earlier studies, in which subjects with fractures (hip, vertebral, nonhip-nonvertebral) had lower BMSi values compared to adequate controls without fractures (13-16, 43-45). These earlier studies suggest that BMSi measured at the tibia is associated with increased bone fragility at all relevant skeletal sites. Strikingly, the mean BMSi values of our whole CS cohort were in the range or even lower than those of most reported fracture groups and also were lower than the very recently published mean reference values of healthy women and men of comparable age from Australia, Europe, and the United States (mean BMSi 81.3) (46). The finding that female CS patients had lower BMSi values than male CS patients is somewhat surprising. Some earlier studies on bone health in CS patients revealed rather higher BMD values and a lower fracture rate in female compared to male patients (6, 9, 28, 47, 48). In addition, most earlier studies on BMSi did not show any differences between women and men (12). However, the aforementioned very recent publication on reference intervals in healthy adults also including healthy participants from our center reported lower BMSi for women (79.0 ± 9.1) than for men (84.4 ± 6.9), which might very well also apply to our CS patients, independently of their disease (46). Furthermore, a very recent study in patients with adrenal (subclinical) CS also detected an increased fracture incidence in postmenopausal women (49).

Our data thus highlight that the follow-up of patients with CS—not only of those with active disease but also of those in remission—should focus on bone health as well. In addition to fracture history, DXA BMD measurement, and ensuring adequate calcium and vitamin D substitution when necessary, a vertebral fracture screening using vertebral fracture assessment or spine radiographs should also be performed in all patients, regardless of disease activity. Vertebral fractures, which have been shown to be associated with high morbidity and mortality (50-54), are highly prevalent in this disease, and only half of patients present with clinical symptoms (4). Bone material properties rather than BMD might be affected after remission of the disease, as suggested by our study. The lowest BMSi values were observed in patients with ectopic CS, in patients with early onset of disease, and in those using supraphysiological HC doses, all of which are groups at risk for more severe bone involvement (2, 4, 20, 55). IMI might be a valuable additional tool in the evaluation of bone fragility in CS patients.

Our study has some limitations. First, as referenced in the Methods section, the microindentation technique is relatively

novel, and reliable results can only be obtained with proper training. However, in this study, only experienced operators performed the measurements, and the inter- and intraobserver measurements were excellent. Another limitation is that our CS cohort was heterogenous with respect to time and severity of steroid exposure. Some data, such as the number of recurrences, HC use, or other pituitary hormone deficiencies, had to be collected retrospectively in some patients. However, we included a large number of participants with this rare disease, with an expected female-to-male ratio of 5:1 and a broad age range at a tertiary referral center, providing a representative sample of the whole CS in remission population in clinical practice. Furthermore, the cohort has been well documented and followed prospectively since 1978 at the LUMC, a European reference center for pituitary and adrenal diseases. There were no missing data apart from vertebral fracture screening and 24-hour urinary cortisol for some patients at the time of CS diagnosis. Another limitation is the study's cross-sectional design, necessitated by the rarity of the disease. This design did not allow for the evaluation of BMSi at the time or prior to fracture occurrence, and it led to the inclusion of some CS patients treated with antiresorptive agents, as described previously. However, the study provides valuable information on the long-term bone sequalae of the disease. Assuming an increase in BMSi in CS patients treated with bone agents, the difference in BMSi between patients and treatment-naive controls is even more remarkable. Moreover, repeated analysis in only treatment-naive CS patients did not change results.

In conclusion, we show that BMSi values are lower in patients with endogenous CS compared to matched controls even after long-term remission and are inversely correlated with BMI in patients only. Furthermore, BMSi values are low independent of the presence of fractures, BMD values, ongoing HC use, or other pituitary hormone deficiencies. Our data thus indicate that tissue-level properties of bone are permanently impaired also after treatment of the disease. These abnormalities, which are known to be associated with fractures in other populations, may, at least in part, contribute to the persistent bone fragility in treated CS. This underscores the importance of comprehensive bone health assessment in patients with CS, not only at the time of diagnosis but also after long-term remission. Further studies on BMSi in CS patients with active disease are warranted.

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Author Contributions

M.S. (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Methodology [equal], Investigation [equal], Project administration [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]); P.J.C.S. (Data curation [equal], Formal analysis [equal], Writing—review & editing [supporting]); L.E.H.B. (Resources [equal], Writing—review & editing [supporting]); N.R.B. (Conceptualization [equal], Methodology [equal], Resources [equal], Supervision [supporting], Writing—review & editing [supporting]); F.M.H. (Resources [equal], Writing—review & editing [supporting]); M.F.N. (Resources [equal], Writing—review & editing [supporting]); F.D.V. (Resources [equal], Writing—review & editing [supporting]); E.M.W. (Conceptualization [equal], Investigation [equal], Methodology [equal], Resources [equal], Writing—review & editing [supporting]); A.M.P. (Conceptualization [equal], Methodology [equal], Resources [equal], Supervision [supporting], Writing—review & editing [supporting]); N.M.A.-D. (Conceptualization [lead], Data curation [equal], Formal analysis [equal], Investigation [lead], Methodology [equal], Project administration [equal], Resources [lead], Supervision [lead], Visualization [equal], Writing—original draft [supporting], Writing—review & editing [supporting]

Disclosures

M.S., P.J.C.S., L.E.H.B., N.R.B., F.M.H., M.F.N., F.V., E.M.W., and A.M.P. declare that they have no conflicts of interest. N.M.A.D. is an unpaid member of the Scientific Board of Active Life Scientific, manufacturer of OsteoProbe®. N.M.A.D. is an editorial board member for *The Journal of Clinical Endocrinology & Metabolism* and played no role in the journal's evaluation of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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