CLINICAL STUDY

Bone loss determined by quantitative ultrasonometry correlates inversely with disease activity in patients with endogenous glucocorticoid excess due to adrenal mass

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Abstract

Objective: Glucocorticoid excess is widely recognized as one of the most important causes of bone loss. The mechanism of glucocorticoid-induced osteoporosis is presumably multifactorial, and consists of the loss of organic and non-organic compounds. Efforts have been made to develop simple physical methods for the assessment of bone tissue for the screening of subjects at high risk of osteoporosis, without the use of radioactive sources or ionizing radiation. Quantitative ultrasonometry (QUS) has been suggested as a useful method for monitoring patients undergoing glucocorticoid therapy, which is the most common cause of glucocorticoid excess. QUS appears to detect more structural bone changes than the traditional methods and allows assessment of bone density and elasticity, both characteristics influenced by organic and non-organic bone compounds. However, the use of QUS has not yet been extensively investigated in subjects with endogenous cortisol excess. The aim of this study was to evaluate the usefulness and predictive power of QUS in assessing bone loss in subjects with differing degrees of endogenous cortisol excess due to adrenal mass.

Design: Thirty-four patients (20 women and 14 men) aged between 21 and 59 years were evaluated; fifteen (9 women and 6 men; median age, 42 years) were affected by overt Cushing's syndrome (CS) and nineteen (11 women and 8 men; median age, 44 years) by subclinical CS, defined as lacking clinical signs of hormone excess despite the presence of at least two abnormalities in hypothalamic–pituitary–adrenal axis function, as assessed by routine endocrine tests. All women included were eumenorrhoeic.

Methods: QUS measurement of amplitude-dependent speed of sound was performed on the 2nd to 5th proximal phalanges of the non-dominant hand using a DBM Sonic 1200R bone profiler (Igea S.r.l., Italy). The results were compared with bone density assessed on lumbar vertebrae (L1–L4) and femoral neck sites by dual-energy X-ray absorptiometry (DEXA).

Results: A strongly significant bone loss was detected by finger QUS measurement when the patients were considered either all together or as two subgroups ($P < 0.001$, all). The bone density decrease in the fingers was similar to that found at the lumbar spine and femoral neck by the DEXA technique. Lumbar and finger Z-scores correlated inversely with 24 h urinary free cortisol (UFF) excretion ($P < 0.01$, both). Finger Z-scores also correlated inversely with the estimated duration of subclinical CS ($P < 0.05$). Concerning disease activity, only UFF was confirmed by multivariate analysis to be an independent factor influencing bone loss ($P < 0.05$). A positive correlation between the results of the two techniques was found in controls ($P < 0.05$) but not in patients. The lack of correlation between the two techniques in patients can probably be attributed to the different parameters of bone alteration measured by the techniques.

Conclusions: The detection of bone loss in subclinical CS similar to that in overt CS suggests that all subjects with endogenous cortisol excess should be evaluated for bone mass. QUS measurement appears to be a reliable, radiation-free, simple and fast tool for the identification of bone alteration in subjects with endogenous cortisol excess.

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Introduction

The early diagnosis of osteoporosis is a very important objective since no treatment is currently able to restore

lost bone mass completely and, in particular, the continuity of bone trabeculae (1). Since it was first described by Harvey Cushing in 1932 (2), the syndrome of glucocorticoid excess has been recognized

as one of the most important causes of bone loss. Osteoporosis and its related fractures have been widely reported in patients with endogenous glucocorticoid excess and they feature significantly in the morbidity associated with Cushing's syndrome (CS) (1, 3–6), as well as in subjects undergoing chronic glucocorticoid treatment with or without other clinical features of cortisol excess (1). Recently, a subtle cortisol over-production has been described in an increasing number of subjects with incidentally detected adrenal mass without clear clinical signs of cortisol excess (7–16). This pathological entity (subclinical CS) seems to be more frequent than overt CS and a decrease in bone mineral density (BMD) and alterations in bone turnover markers have been previously reported in patients with this disorder (5, 6, 17).

Different methods of bone mass evaluation are currently adopted, and dual-energy X-ray absorptiometry (DEXA) is considered the gold standard for BMD measurement in patients with steroid excess (18). However, ultrasound instruments using attenuation and/or velocity propagation determinations, not based on radioactive sources or ionizing radiation, have been developed as a screening test for subjects at high risk of osteoporosis (19, 20). Quantitative ultrasonometry (QUS) is commonly employed for the screening of menopausal osteoporosis (20–25) and has been suggested for use in monitoring patients undergoing glucocorticoid therapy, which represents the most common type of glucocorticoid excess (26). QUS may be more sensitive to structural bone changes than the traditional methods (21, 23, 26) because ultrasound velocity depends on bone density and elasticity, both of which are influenced by organic and non-organic bone compounds (21, 23, 24). For this reason QUS could prove a useful option for monitoring subjects with glucocorticoid excess, because the mechanism of glucocorticoid-induced osteoporosis is presumably multifactorial and consists in the loss of both organic and non-organic compounds (1).

To the best of our knowledge, no previous studies have extensively investigated the use of QUS in subjects with endogenous cortisol excess. The aim of the present cross-sectional study was to evaluate the usefulness of QUS and its predictive power in assessing bone loss in patients with differing degrees of endogenous cortisol excess due to adrenal tumours. BMD was measured by QUS using the amplitude-dependent speed of sound (Ad-SoS) determination at proximal phalanges, a site known to undergo early important morpho-structural changes associated with bone mass resorption (27, 28). The results of QUS measurement were compared with DEXA measurement performed at two sites: the lumbar spine and the femoral neck, in order to obtain more complete information on bone mass in these subjects.

Patients and methods

Patients

Thirty-four patients (20 women and 14 men; age range, 21–59 years; median, 43 years) with adrenal masses, were evaluated after their informed consent had been obtained. A control group of 76 healthy subjects matched for age and sex, was used for both endocrine and BMD evaluation. Neither cases nor controls were under any treatment known to interfere with skeletal or mineral metabolism. Their usual intake of coffee did not exceed four cups a day; there were two mild smokers (5–10 cigarettes per day) among the patients and five among the controls. Kidney and liver functions were normal in all subjects. All 20 female patients included in this study were of child-bearing age and eumenorrhoeic (cycles every 25–35 days).

Overt Cushing's syndrome Nine women and six men, aged 21–50 years (median, 42 years) and affected by Cushing's syndrome, participated in this study. The diagnosis of Cushing's syndrome was made on the basis of standardized clinical, hormonal and radiological criteria (29, 30).

Subclinical Cushing's syndrome Eleven women and eight men, aged 25–59 years (median, 44 years), entered this study. Criteria for the diagnosis of subclinical hypercortisolism (16) were adopted in accordance with the recommendations of the National Italian Study Group on Adrenal Tumours (31, 32). They included a lack of specific clinical signs of steroid excess in an 'incidentally' detected adrenal mass, associated with at least two abnormalities of hypothalamic–pituitary–adrenal axis function, assessed by routine tests (16, 31, 32). Failure to suppress serum cortisol to below 3 µg/dl (83 nmol/l) by low-dose dexamethasone (DXM) test was a mandatory inclusion criterion.

The two subgroups of patients (overt and subclinical CS) were not different in terms of BMI, men/women ratio, tumour size or age (Table 1).

Materials and methods

At study entry, all subjects underwent an evaluation of their hypothalamo–pituitary–adrenal axis function and bone densitometry. The following endocrine evaluations were performed: baseline serum cortisol (F) and plasma ACTH at 0800, 1600 and 2400 h (mean of at least two samples taken on different days), 24 h excretion of urinary free cortisol (UFF), a low-dose 2 mg DXM suppression test (orally, 0.5 mg four times a day for 2 days with measurement of serum cortisol and other steroids at 0800 h the following morning; UFF was also determined). Other parameters considered for the assessment of disease activity in patients with

Table 1 Clinical and biochemical features of patients and controls.

	Patients			Controls (n = 76)
	All (n = 34)	Overt CS (n = 15)	Subclinical CS (n = 19)	
Men/Women	14/20	6/9	8/11	30/46
BMI (kg/m ²)	29.7±5.8	31.3±5	28.14±6	28.6±4.9
Time since diagnosis (months)*	11.5±8.2	4.72±2.2 ^a	18±6.5	—
Age (years)	43.6±10.3	41.73±10.2	44.5±9.8	46.5±13.7
Calcium (mg/dl)	9.24±0.46	9.26±0.51	9.23±0.42	9.10±0.6
Phosphorus (mg/dl)	3.63±0.59	3.86±0.73	3.46±0.4	3.7±0.7
Albumin (g/dl)	4.30±0.5	4.28±0.4	4.31±0.4	4.25±0.65
Urinary calcium (mg/24 h)**	343±59	351±46	328±38	268±51
Alkaline phosphatase (U/l)	172±78	176±83.7	164±39	169±48
F 0800 h (μg/dl)	21.8±8.0 ^{aa}	25.4±8.1 ^{aa}	19.1±7.1 ^b	16.3±4.64
F average (μg/dl)	16.18±6.27 ^{aa}	22.11±5.3 ^{aa}	12.44±3.16 ^{aa}	9.99±2.24
F% ratio	71.7±46.6 ^{aa}	97.9±37 ^{aa}	53.6±44.6 ^{aa}	24.96±15.95
F post-DXM (μg/dl)	7.86±10.1 ^{aa}	13.25±13.7 ^{aa}	4.39±4.89 ^{aa}	1.43±0.52
UFF (μg /24 h)	297.6±393 ^{aa}	512.6±550 ^{aa}	162.8±131 ^{aa}	83.3±52.9
UFF post-DMX (μg /24 h)	357±685 ^{aa}	706±555 ^{aa}	57.8±34 ^{aa}	11±5
ACTH (pg/ml)	8.98±6.7 ^a	9±6.1 ^b	8.95±7 ^a	12.5±4.5

Data are expressed as means ± s.d. BMI, body mass index. F average was determined as (F 0800 h + F 1600 h + F 2400 h)/3, and F% ratio as F 0800 h/F 2400 h × 100. UFF, urinary free cortisol; *significance is referred vs subclinical CS group; **corrected for creatinine excretion. Factors for converting conventional units to SI units: F: 1 μg/dl = 27.59 nmol/l; UFF: 1 μg/24 h = 2.759 nmol/24 h; ACTH: 1 pg/ml = 0.2202 pmol/l; Significance differences vs controls: ^{aa}*p* < 0.0001; ^a*p* < 0.001; ^b*p* < 0.05.

adrenal mass were daily average cortisol, calculated as (F 0800 h + F 1600 h + F 2400 h)/3, and cortisol percent ratio (F% ratio) expressing circadian rhythm abnormalities, calculated as (F 2400 h/F 0800 h) × 100. To define the reference range for each variable determined, the means ± 2 s.d. were calculated in the control group. None of the participants showed any alteration in androgen/oestrogen levels (at least two different measurements on two different days were performed); this allowed us to exclude a mixed steroid hyperactivity of the tumour (data not shown).

Serum calcium, phosphorus, creatinine and alkaline phosphatase were determined in the morning (0800 h) after at least 8 h fasting. Urinary calcium and phosphorus were determined in 24 h urine collections, correcting the values for creatinine excretion.

Bone densitometry

Ultrasound evaluation of bone density was performed using a DBM Sonic 1200R bone profiler (Igea S.r.l. Carpi, Mo, Italy). The sound frequency employed was 1.25 MHz, and the Ad-SoS was determined. QUS measurements were carried out on the 2nd to 5th proximal phalanges of the non-dominant hand and the mean value per person was calculated. Measurements were always performed by the same operator and the coefficient of variation (CV) was 0.73%, determined by repeated measurements in a subgroup of 12 subjects (3 measurements per person on 3 different days). The CV is in accordance with a study previously published on a large population sample, where its value was 0.75% (33). Ad-SoS results are expressed as m/s and as T- and Z-scores; the latter two were calculated with the

software provided by manufacturer using measurements obtained in an Italian population sample as a reference database.

Lumbar spine (L1–L4) and femoral neck BMD values were measured by DEXA, using a Hologic QDR 1000 densitometer (Hologic, Inc., Waltham, MA, USA). Individual BMD values were expressed as g/cm² and as T- and Z-scores. The reference population adopted was the international pooled sample provided by the manufacturer; their data, in fact, did not differ significantly from those obtained on a local sample in a study performed when the machine was set up (34).

Assays

All hormone assays were performed in the same laboratory using commercially available kits. F and UFF were tested using an Immulite solid phase chemiluminescent enzyme immunoassay (DPC Los Angeles, CA 90045–5597, USA); ACTH was tested using a double-antibody ¹²⁵I radioimmunoassay (DPC Los Angeles). Serum and urinary calcium, phosphorus, creatinine and circulating alkaline phosphatase were assayed in the same laboratory using standard methods.

Statistical analysis

The results are expressed as means ± s.d. Student's unpaired *t*-test was used to compare the means between the groups. The non-parametric method (Mann–Whitney *U* test) was used when Wilk–Shapiro's test was not consistent with the Gaussian distribution of the data. Linear and multiple regression

Table 2 Bone mass measured at different skeletal sites in patients and controls.

	Patients (n = 34)			Controls (n = 76)
	All	Overt CS (n = 15)	Subclinical CS (n = 19)	
Proximal finger Ad-SoS (m/s)	1956±135 ^{aa}	1941±126 ^{aa}	1968±139 ^{aa}	2103±43
Proximal finger Z-score	-1.69±1.6 ^{aa}	-2.0±1.6 ^{aa}	-1.48±1.5 ^{aa}	-0.11±0.7
Lumbar spine BMD (g/cm ²)	0.9±0.27 ^{aa}	0.87±0.15 ^{aa}	0.93±0.16 ^{aa}	1.05±0.1
Lumbar spine Z-score	-1.13±1.4 ^{aa}	-1.4±1.4 ^{aa}	-0.86±1.3 ^{aa}	-0.14±0.05
Femoral neck BMD (g/cm ²)	0.8±0.15 ^{aa}	0.75±0.12 ^{aa}	0.83±0.09 ^{aa}	0.93±0.105
Femoral neck Z-score	-0.73±1.02 ^{aa}	-0.96±1.03 ^{aa}	-0.5±1.01 ^{aa}	0.06±0.02

Bone ultrasonometry was used to measure the proximal phalanges of non-dominant hands. Ad-SoS, amplitude dependent speed of sound, BMD, bone mineral density measured by DEXA technique at lumbar spine (L1–L4) and femoral neck. ^{aa}*P* < 0.001 vs controls.

analyses were used to look for the determinants of bone mass. Significance was retained for *P* < 0.05.

subgroup (*P* < 0.001) (Table 1). The latter were advised to undergo surgical treatment.

Results

The clinical and hormonal characteristics of the patients are shown in Table 1. When all patients were considered together they had significantly higher morning F, UFE, average F, F% ratio, and significantly lower ACTH levels than controls. Moreover, all patients displayed a smaller F decrease after DXM administration. Considering the two subgroups separately (overt vs subclinical CS), the only significant difference was found in UFE baseline and post-DMX levels; both were higher in overt CS patients (*P* < 0.05). On the other hand, serum calcium, phosphorus and alkaline phosphatase and urinary calcium and phosphorus did not differ significantly between patients and controls.

The estimated duration of the disease, i.e. the period of time between the diagnosis of cortisol excess and study recruitment, was significantly longer in the subclinical CS than in the overt CS

Bone densitometry

A strongly significant bone loss was detected by finger Ad-SoS measurement both when the patients were considered together and as two subgroups, compared with healthy sex- and age-matched controls (*P* < 0.001, all). Lumbar BMD measured by the DEXA technique was significantly lower in patients when considered together and as two subgroups (*P* < 0.001, all). A smaller decrease was found in femoral neck BMD in the whole group of patients and in the subgroups (*P* < 0.001, all) (Table 2, Fig. 1). The same significant differences were found when the values were corrected by sex and age (Z-score, Table 2).

When considering the patients individually, the T-scores determined by the DEXA technique were between -1 s.d. and -2.5 s.d. in nine (26%) and < -2.5 s.d. in another nine patients at least at one investigated site, thus fulfilling the WHO criteria for osteopenia and osteoporosis, respectively (35). In particular, the

Figure 1 Bone mineral density decrease expressed as the T-score at different skeletal sites in the subgroups of patients with overt Cushing's syndrome (CS), subclinical CS, and controls. The determinations at lumbar spine (L1–L4) and femoral neck sites were made using the DEXA technique, whereas quantitative ultrasonometry was employed at the proximal phalanges. Standard deviation (*P* < 0.001), vs each control.

alteration in BMD (a decrease by at least 1 S.D.) was found in 59% of subjects with overt CS and in 47% of those with subclinical CS; nevertheless this difference was not significant ($P = 0.726$, 95% confidence interval (CI): -0.458 to 0.218).

In addition, recent fractures were reported in five patients, three of whom were in the subclinical CS group (1 vertebral, 1 ankle and 1 humeral fracture), and two in the overt CS group (1 radial and 1 vertebral fracture). Using Pearson's test, BMI was significantly lower in the group of patients with fractures ($P < 0.05$), while no difference was found in terms of age, disease activity and estimated disease duration between the group of patients with or without fractures.

The Z-score determined by QUS correlated negatively with UFF in all patients ($\beta: -0.01$; S.E. = 0.002 , $P < 0.01$). A similar correlation was found between lumbar Z-score and UFF values in all patients ($\beta: -0.012$; S.E. = 0.0025 , $P < 0.01$). Another significant inverse correlation was found between QUS Z-score and the estimated duration of the disease in subclinical CS patients ($\beta: -1.198$; S.E. = 0.54 , $P < 0.05$), who were characterized by longer duration of the disease. Since several factors can affect the relationship between BMD and endocrine parameters, a multivariate analysis of the data was performed using age, sex, BMI and estimated disease duration as covariants. The only correlation confirmed as significant by multivariate analysis was between finger ultrasound Z-score and UFF when all patients were considered as a single group ($\beta: -0.01$; S.E. = 0.003 , $P < 0.05$). On the other hand, no significant correlation was found between BMD as measured by DEXA and patient variables using multivariate analysis. No correlation was found between the two techniques used in patients, while they correlated in controls ($\beta: 0.015$; S.E. = 0.013 , $P < 0.05$).

Discussion

Although QUS has been suggested as a useful monitoring technique for subjects with glucocorticoid excess because of its capacity to detect the structural characteristics of bone (26), this possible application has yet to be extensively investigated. The present study aims to contribute to this topic.

A significant alteration in bone density has been found in patients with endogenous cortisol excess by both DEXA measurements and QUS. Using the DEXA technique, BMD impairment was expressed to the greatest extent at the lumbar spine, an early principal target of glucocorticoid-induced bone damage, given its prevalent trabecular structure (1) (Table 2, Fig. 1). Nevertheless, the most important bone impairment was detected by Ad-SoS at the proximal phalanges, which are also known to undergo early changes associated with bone resorption because of their high trabecular compound content (19, 27, 28). The more significant

bone alteration detected by QUS compared with that detected by DEXA in this study probably mirrors the different parameters considered by the techniques and the multifactorial origin of steroid-induced bone loss. However, the two techniques of BMD assessment have previously been found to correlate with each other at different measurement sites in children and postmenopausal women (21, 23, 36) and a positive correlation was found in healthy controls in this study.

As the WHO criteria for osteopenia and osteoporosis had not yet been validated for bone QUS, this terminology has not been used to describe ultrasound results in our patients; however, some authors have suggested a T-score of between -1 and -3.2 S.D. as indicative for osteopenia and a T-score < -3.2 S.D. as indicative for osteoporosis (33). If such indices were adopted, 12/34 patients would have been classed as osteopenic and 10 as having osteoporosis, compared with 9/34 patients osteopenic and 9/34 osteoporotic subjects identified using the DEXA technique.

Concerning steroid production abnormalities, a great variability in cortisol excess was found in patients with overt and subclinical CS. In the subgroup of patients with subclinical CS, alterations in cortisol secretion ranged from mild to completely pathological and biochemically similar to those seen in overt CS (16). Among the endocrine parameters considered as factors of disease activity, only UFF was found as an independent factor influencing bone density when detected by DEXA at the lumbar site or by QUS at the fingers. A similar finding – simple negative correlation between DEXA lumbar and femur BMD and UFF – has been previously documented in eumenorrhoeic women with overt Cushing's syndrome (6). The estimated duration of cortisol excess correlated negatively with QUS-determined bone density only in subclinical CS patients, who were characterized by longer duration of the disease. These findings are in accordance with previous observations describing the degree of bone loss related to dose and duration of glucocorticoid therapy (1, 37). Also a protracted low-dose treatment (37), presumably not associated with clear signs and symptoms of Cushing's syndrome, has been found to decrease BMD. Nevertheless, Chiodini *et al.* (6) found no correlation between BMD decrease at several skeletal sites, determined by DEXA, and estimated duration of active Cushing's disease in 18 eumenorrhoeic women. However, our findings suggest that the amount of endogenous cortisol aside, the duration of its excess seems to be an important factor in the determination of bone loss. The well-known protective influence of a higher BMI on fracture risk has also been seen in subjects with endogenous cortisol excess in this study.

Factors other than bone mineral content can influence bone state in patients with cortisol excess. In fact, generalized wasting of connective tissue is known to occur in Cushing's syndrome (18, 38, 39). In bone, the long-term exposure of the osteoblasts to

glucocorticoids decreases cell proliferation and protein synthesis (18), including type I collagen, osteocalcin, bone sialoprotein, alkaline phosphatase, collagenase and others (18). Reduced collagen synthesis has also been shown in patients with subclinical CS by Sartorio *et al.* (5). Wasting of connective tissue could be an important mechanism contributing to fracture risk in patients with cortisol excess and might partly explain the difference between the DEXA and QUS measurements in this study. Osteoporosis has been defined according to BMD criteria in postmenopausal women (mineral content corrected for skeletal area examined) (35); nevertheless, this definition cannot be used for other types of osteoporosis, as fractures occurring at higher levels of bone density detected by the DEXA technique have been reported in glucocorticoid-induced osteoporosis (40). In the light of these findings, QUS bone density determination may have some advantages over DEXA in the assessment of bone loss in patients with glucocorticoid excess. In fact, it seems to assess bone density and elasticity, characteristics influenced by bone mass, trabecular and cortical distribution and by organic and non-organic bone compounds (21, 23, 24).

The alterations in bone metabolism are probably part of the negative metabolic situation in subjects with endogenous cortisol excess, even when bone loss has not been found by all authors (41). The result of this study suggests that both the grade and duration of endogenous cortisol excess may be an important determinant of the morbidity in these subjects.

In conclusion, the alterations in bone turnover (5, 17, 39) and bone density in subjects with subclinical CS are similar to those found in overt CS. These findings suggest that all subjects with any grade of endogenous cortisol excess should be evaluated for bone mass density. The amplitude-dependent speed of sound measurement of BMD at the proximal phalanges of the non-dominant hand has been found to be as effective as the conventional DEXA technique in determining glucocorticoid-induced bone loss. Considering that subjects with adrenal mass must undergo periodic follow-ups that include endocrine and radiological evaluation, as well as bone density monitoring, QUS appears to be a reliable, radiation-free, inexpensive and very fast diagnostic tool for identifying subjects with bone alteration due to endogenous cortisol excess. One question that remains unanswered is whether QUS should be used to monitor patients during the disease or after its treatment.

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