View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by RERO DOC Digital Library

RHEUMATOLOGY
doi:10.1093/rheumatology/ket228
Advance Access publication 23 July 2013

Original article

Primary male osteoporosis is associated with enhanced glucocorticoid availability

Spyridon Arampatzis[1,2], Andreas Pasch[2], Kurt Lippuner[1] and Markus Mohaupt[2]

Abstract

Objective. While systemic glucocorticoids compromise bone metabolism, altered intracellular cortisol availability may also contribute to the pathogenesis of primary male osteoporosis (MO). The objective of this study was to assess whether intracellular cortisol availability is increased in MO due to a distorted local cortisol metabolism.

Methods. Forty-one patients with MO were compared with age- and BMI-matched non-osteoporotic subjects after excluding overt systemic hypercortisolism ($N = 41$). Cortisol, cortisone and the respective tetrahydro-, 5α-tetrahydro- and total cortisol metabolites were analysed by GC-MS in 24 h urine. Apparent 11β-hydroxysteroid dehydrogenase (11β-HSD) enzyme activities, excretion of cortisol metabolites and calcium, and fractional urinary calcium excretion were assessed and related to BMD.

Results. Fractional and total urinary calcium excretion negatively correlated with BMD at all ($P < 0.05$) and at three of five ($P < 0.05$) measurement sites, respectively. While systemic cortisol was unchanged, apparent 11β-HSD enzyme activity in MO patients ($P < 0.01$) suggested increased intracellular cortisol availability. Total and fractional urinary calcium excretion was higher, with apparent 11β-HSD enzyme activities consistent with an enhanced intracellular cortisol availability ($P < 0.05$).

Conclusion. Apparent 11β-HSD enzyme activities consistent with increased intracellular cortisol availability correlated with urinary calcium loss and reduced bone mineral density in MO. The changes in 11β-HSD activity were associated with both the fractional calcium excretion, suggesting altered renal calcium handling, and the absolute urinary calcium excretion. Both mechanisms could result in a marked bone calcium deficiency if insufficiently compensated for by intestinal calcium uptake.

Key words: idiopathic osteoporosis, male, cortisol, 11β-hydroxysteroid dehydrogenase, bone mineral density, gas chromatography–mass spectrometry.

CLINICAL SCIENCE

Introduction

Primary male osteoporosis (MO) contributes significantly to the overall public health burden [1] and is a major predictor of future fractures and hip fracture-related morbidity and mortality in men [2]. Compared with osteoporosis in women, MO is even less recognized and treated in men, including those with a history of fractures. The diagnosis is made after exclusion of secondary causes of osteoporosis, such as hypogonadism, alcohol abuse, vitamin D deficiency and use of exogenous glucocorticoids [3–5].

While treatment with exogenous glucocorticoid hormones and endogenous cortisol excess syndromes are well-known causes of osteoporosis, the contribution of altered intracellular cortisol (F) availability is less well defined. Recent work has indicated enhanced F secretion in subjects with osteoporosis likely due to mild autonomous F excess in some individuals [6]. Furthermore, subclinical hypercortisolism was associated with osteoporosis and vertebral fractures in participants referred for suspected osteoporosis [7]. Likewise, an enhanced intracellular F may contribute to osteoporosis, an assumption supported by the presence of gene polymorphisms in 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), a primary glucocorticoid regulatory enzyme associated with low BMD and fracture risk in postmenopausal women without hypercortisolism [8].

[1]Department of Osteoporosis and [2]Department of Nephrology and Hypertension, Inselspital, University Hospital Bern and University of Bern, Berne, Switzerland.

Submitted 13 December 2012; revised version accepted 9 May 2013.

Correspondence to: Markus Mohaupt, Department of Nephrology and Hypertension, University of Bern, CH-3010 Berne, Switzerland. E-mail: markus.mohaupt@insel.ch

© The Author 2013. Published by Oxford University Press on behalf of the British Society for Rheumatology. All rights reserved. For Permissions, please email: journals.permissions@oup.com

F binds to both the glucocorticoid and the mineralocorticoid receptor. Intracellular glucocorticoid availability is effectively controlled on the pre-receptor level by the enzyme 11β-HSD type 2 (11β-HSD2), catalyzing the degradation of hormonally active F to inactive cortisone (E). In contrast to 11β-HSD2, the low-affinity enzyme 11β-HSD1, though bidirectional, predominantly converts E to F as an oxoreductase and is expressed in human adult bone and in cultured primary osteoblasts [9, 10].

The enzyme 11β-HSD2 is highly expressed in the kidney [11], the colon [12] and in fetal bone [13] as well as in human osteosarcoma cells [10] and at low levels in adult osteoblasts [9]. Likewise, 11β-HSD1 is the predominant isozyme expressed in normal adult osteoblasts and osteoclasts, with both reductase (cortisone-to-cortisol conversion) and dehydrogenase (cortisol-to-cortisone conversion) activities [9], and determines their response to glucocorticoids [14]. In most studies elevated circulating glucocorticoid concentrations and higher bone turnover markers are associated with greater bone loss in healthy men [15–17]. Since bone-specific responses to glucocorticoids appear to be correlated with serum E but not F levels [18], the presence of a local activator of inactive E, i.e. 11β-HSD1, is conceivable. Glucocorticoid receptor activation is known to directly promote osteoporosis, while increased glucocorticoid availability through inhibition of 11β-HSD2 leads to enhanced urinary calcium excretion [19–22]. Given the availability of the glucocorticoid receptor in bone-forming osteoblasts [23], a slightly dysregulated and thus altered F availability could be causally related to MO. In line with this reasoning, we hypothesized that integrated 11β-HSD type 1 and type 2 activity is shifted as a net effect towards higher intracellular F concentrations in patients with MO when compared with age- and BMI-matched non-osteoporotic male subjects.

Material and methods

Study population and study design

Patients were selected from the Department of Nephrology and Hypertension and from the Department of Osteoporosis, University Hospital Bern, Bern, Switzerland. Between 2005 and 2010, more than 3500 male patients were referred for densitometric evaluation after kidney stone disease or for suspected osteopenia/osteoporosis or both. Of these, 559 (16%) consecutive male outpatients underwent further complete bone metabolic workup due to either renal stone disease and/or osteopenia/osteoporosis in our department. In this retrospective case–control study, patients with complete datasets and a diagnosis of MO were included for analysis and matched for gender, age and BMI (patient allocation is illustrated in Fig. 1). MO was defined as a fragility fracture or a densitometric T-score $\leqslant -2.5$ S.D. at any measured site (see section on BMD measurements) without symptoms of overt systemic hypercortisolism and after exclusion of secondary causes for osteoporosis, such as a history of or current alcohol abuse, hypogonadism with low serum testosterone levels (defined as <11 nmol/l at <60 years, <7 nmol/l at >60 years), primary hyperparathyroidism (pHPT, defined as serum intact PTH $\geqslant 66$ ng/l) and other endocrine disorders, vitamin D deficiency (defined as serum 25-OH vitamin D <75 nmol/l), hyperthyroidism (TSH <0.1 μIU/ml), malabsorption, liver/coeliac disease, any malignant disease including monoclonal gammopathy of unknown significance and use of certain medication or substances (licorice, lithium, anticonvulsants, oral anticoagulants, any previous bone antiresorptive treatment, glucocorticoids, diuretics or chemotherapy/radiotherapy). Furthermore, patients with reduced creatinine clearance determined by the modification of diet in renal disease (MDRD)-derived estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 were excluded. Applying these strict criteria, 41 patients with MO were selected for this case–control study.

Based on the same strict exclusion criteria, 41 non-osteoporotic patients (CON) with a densitometric T-score above -2.5 S.D. at all measured sites, referred to us during the same time period for osteoporosis evaluation and 1:1 matched for BMI and age, were extracted from the same database and served as control group. Medical history, radiological records and physical examination results were available for all patients included.

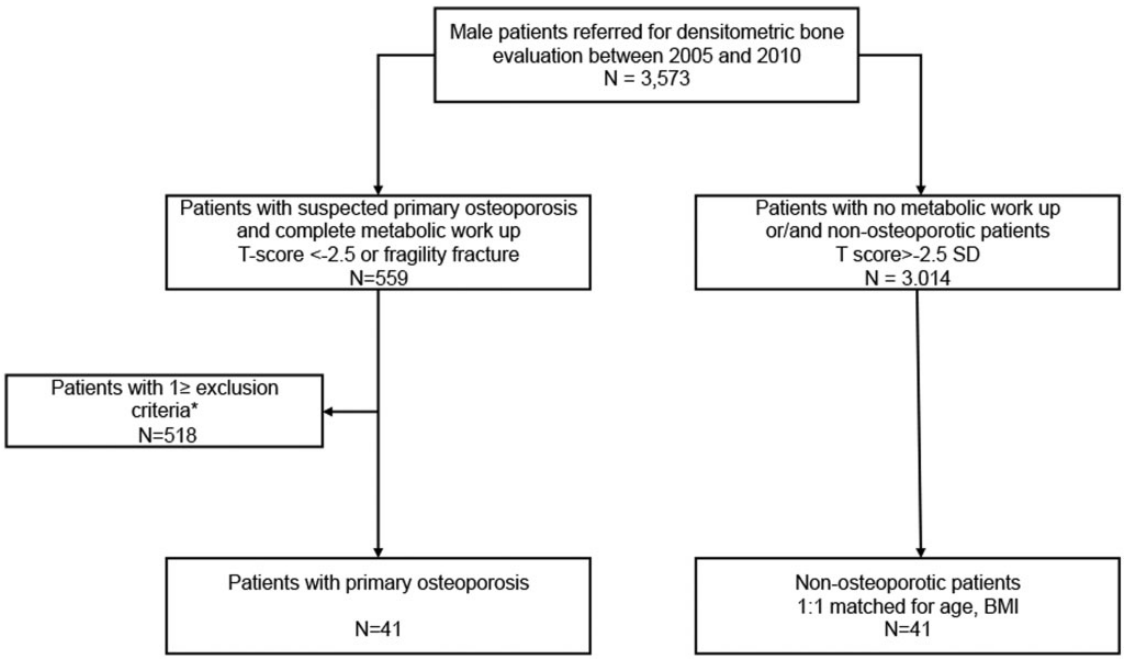
For the subjects serving as controls, written consent was obtained according to the Declaration of Helsinki and was approved by the local institutional review board of the University of Bern conforming to standards currently applied in Switzerland. No ethical approval and consent was required for the retrospective analysis of the patients with overt osteoporosis or fragility fractures. All individuals with a complete sampled dataset were anonymously included in a database. A complete dataset comprised basic patient demographics, information on current medication, fracture history and diseases associated with osteoporosis. Prospectively collected information on vertebral and non-vertebral fractures was retrospectively analysed after extraction from medical and radiological records. Body weight and height were measured on a standard beam scale without shoes and heavy clothing and using a Harpenden stadiometer as the average of two consecutive measurements, respectively. BMI was calculated as kilograms per square metre.

Serum creatinine was used to calculate eGFR. Total and ionized calcium, phosphate, 25-OH vitamin D, 1,25-OH_2 vitamin D, intact PTH and alkaline phosphatase in serum samples and creatinine, calcium, sodium and phosphate in 24 h urine samples were measured using standard laboratory methods. All urinary measurements were corrected for urinary creatinine concentrations. The fractional excretion of calcium (FE_{Ca}) was calculated as $(\text{urine/serum})_{\text{calcium}}/(\text{urine/serum})_{\text{creatinine}}$.

Urinary steroid hormone analysis by GC-MS

Urinary excretion of steroid hormone metabolites was determined by GC-MS as reported by Shackleton and by our group [24, 25]. The 24 h urinary excretion of tetrahydroaldosterone, F, E, tetrahydro-F (THF), 5α-THF,

Fig. 1 Patients allocation.



*Patients with one or more of the following exclusion criteria: history of or current alcohol abuse, hypogonadism with low serum testosterone levels, pHPT and other endocrine disorders (pHPT defined as serum intact PTH $\geqslant$66 ng/l), vitamin D deficiency (defined as serum 25-OH vitamin D $<$75 nmol/l), hyperthyroidism (defined as TSH $<$0.1 μIU/ml), malabsorption, liver/coeliac disease, any malignant disease including monoclonal gammopathy of unknown significance, use of certain medication or substances (licorice, lithium, anticonvulsants, oral anticoagulants, any previous bone antiresorptive treatment, glucocorticoids, diuretics or chemotherapy/radiotherapy) and reduced creatinine clearance determined with eGFR $<$60 ml/min/1.73 m^2.

THE and total F metabolites consisting of E, THE, α-cortolone, β-cortolone, 20α-dihydro-E (20α-DHE), 20β-DHE, F, THF, α-cortol, β-cortol, 20α-DHF, 20β-DHF, 6β-OH-F and 18-OH-F were measured. The apparent 11β-HSD2 and 11β-HSD1 enzyme activity was assessed by calculating the urinary F/E ratio and the corresponding ratio of their TH metabolites (THF + 5α-THF)/THE. High ratios of the apparent activities for urinary F/E and (THF + 5α-THF)/THE indicate high substrate and low product concentrations and reflect low 11β-HSD2 enzyme activity. In addition, a high ratio of (THF + 5α-THF)/THE also indicates a high apparent 11β-HSD1 activity, which acts as an oxoreductase converting E to F. Excretion of total F metabolites was determined to obtain a measure for systemic F production and to exclude alterations of the availability of the substrates used to calculate changes in enzyme activity as a confounding variable. Ratios for the excretion of total F metabolites as related to the ratios of steroid hormone metabolites indicating apparent enzyme activities were produced to correct for substrate and product determined variations in enzymatic activities. To adjust for variations in urine collection, urinary excretion of steroid hormone metabolites was normalized to micrograms of steroid hormone metabolite per millimole of urinary creatinine excretion.

BMD measurements

Routine clinical measurement of BMD was performed at the lumbar spine (LS), the non-dominant femoral neck (FN), the proximal femur (PF) and the distal tibial diaphysis (T-DIA) and epiphysis (T-EPI) using DXA (Hologic QDR 4500A, Hologic, Bedford, MA, USA). Scans were performed according to the manufacturer's guidelines. The standardized procedure for tibial measurements was published previously by our group [26, 27].

BMD was expressed in grams per square centimetre of hydroxyapatite and as T-scores (s.d. from the mean of a healthy young reference population). The National Health and Nutrition Examination Survey III (NHANES III) database [28, 29] served as reference for all hip sites, and the manufacturer's normative database was used as reference for the LS after analysis according to the International Society for Clinical Densitometry rules ([30], official positions 2007; available at http://www.iscd.org/Visitors/positions/officialPositionsText.cfm). For tibial BMD, the local Bern normative database served as reference [31]. Quality control was performed daily (anthropometric spine phantom supplied by the manufacturer) with an overall precision error of 0.3% *in vitro* and a mean precision error in our hands of 1.1% *in vivo*.

Statistical analysis

Data are expressed as means ± S.D. unless otherwise stated. Normal distribution was assessed by the D'Agostino–Pearson omnibus normality test. First-order linear regression modelling and correlations as well as a multivariate linear regression model were chosen to analyse the relationship between BMD, FE_{Ca}, ionized serum calcium, measured steroid hormone metabolites and apparent enzyme activities. χ^2 testing was used to identify differences between categorical and dichotomized data. All analyses were performed using SYSTAT version 12 (SPSS, Inc., Chicago, IL). Significance was assigned at $P < 0.05$.

Table 1 Biochemical markers

	CON	MO
Serum		
Total calcium, mmol/l	2.35 (0.09)	2.31 (0.09)
Ionized calcium, mmol/l	1.22 (0.03)	1.20 (0.04)
Phosphate, mmol/l	1.02 (0.11)	0.98 (0.16)
Alkaline phosphatase, U/l	74 (57)	71 (21)
Intact PTH, ng/l	40 (12)	42 (13)
25-OH vitamin D, nmol/l	135 (36)	167 (17)
1,25-$(OH)_2$ vitamin D, pmol/l	112 (33)	114 (38)
$eGFR_{MDRD}$, ml/min/1.73 m^2	89 (13)	91 (18)
24 h urinary		
Creatinine, mmol/24 h	14909 (3187)	14490 (4095)
Phosphate, mmol/24 h	33 (11)	30 (10)
Sodium, mmol/24 h	196 (78)	202 (54)
BMD		
Lumbar spine (LS)	−1.0 (1.1)[a]	−2.5 (1.1)
Proximal femur (PF)	−1.1 (0.9)[a]	−1.8 (0.7)
Femoral neck (FN)	−0.5 (0.7)[b]	−1.2 (0.8)
Tibial diaphysis (T-DIA)	+0.2 (0.9)[c]	−0.5 (1.7)
Tibia epiphysis (T-EPI)	−0.9 (0.7)[d]	−1.7 (1.0)

Biochemical markers, mean (S.D.) and BMD of all scanned bone regions as T-scores (S.D. from the mean of a healthy young reference population) are given. [a]$P < 0.0001$. [b]$P = 0.0001$. [c]$P = 0.002$. [d]$P = 0.0002$.

Results

Clinical characteristics of patients

Control subjects and patients were matched for age (55 ± 2 and 56 ± 3 years, respectively) and BMI (26 ± 1 *vs* 25 ± 1 kg/m^2). The prevalence of diabetes mellitus type II (5% *vs* 10%), alcohol abuse (none) and smoking (10% vs 7%) were rare and equally distributed between patients with and without osteoporosis. Also the combined use of calcium plus 25-OH vitamin D supplementation (2% *vs* 2%) was balanced between both groups.

Biochemical parameters

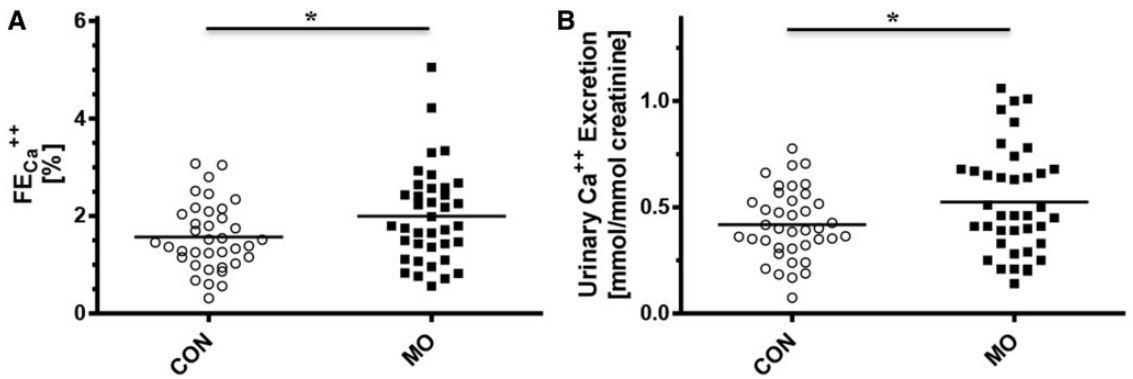
As shown in Table 1, patients with MO did not differ significantly from CON with regard to renal function, 25-OH- and 1,25-$(OH)_2$ vitamin D levels, intact PTH, alkaline phosphatase, serum phosphate and total and ionized calcium. Similar urinary sodium and phosphate excretion in both groups indicate the absence of substantial nutritional differences. However, the FE_{Ca} ($P = 0.0318$, Fig. 2A) and absolute urinary excretion of calcium corrected for creatinine ($P = 0.0307$, Fig. 2B) were significantly higher in patients with MO.

Relationship of urinary absolute and fractional excretion of calcium to BMD

As expected by design, BMD T-scores measured by DXA were significantly lower at all skeletal sites (LS, FN, PF, T-DIA and T-EPI) in patients with MO than in the CON group (Table 1). As direct intestinal calcium uptake and calcium distribution are difficult to assess, indirect parameters such as markers of nutrition, vitamin D and PTH regulation as well as systemic serum calcium concentrations were applied without obvious major differences between the two groups.

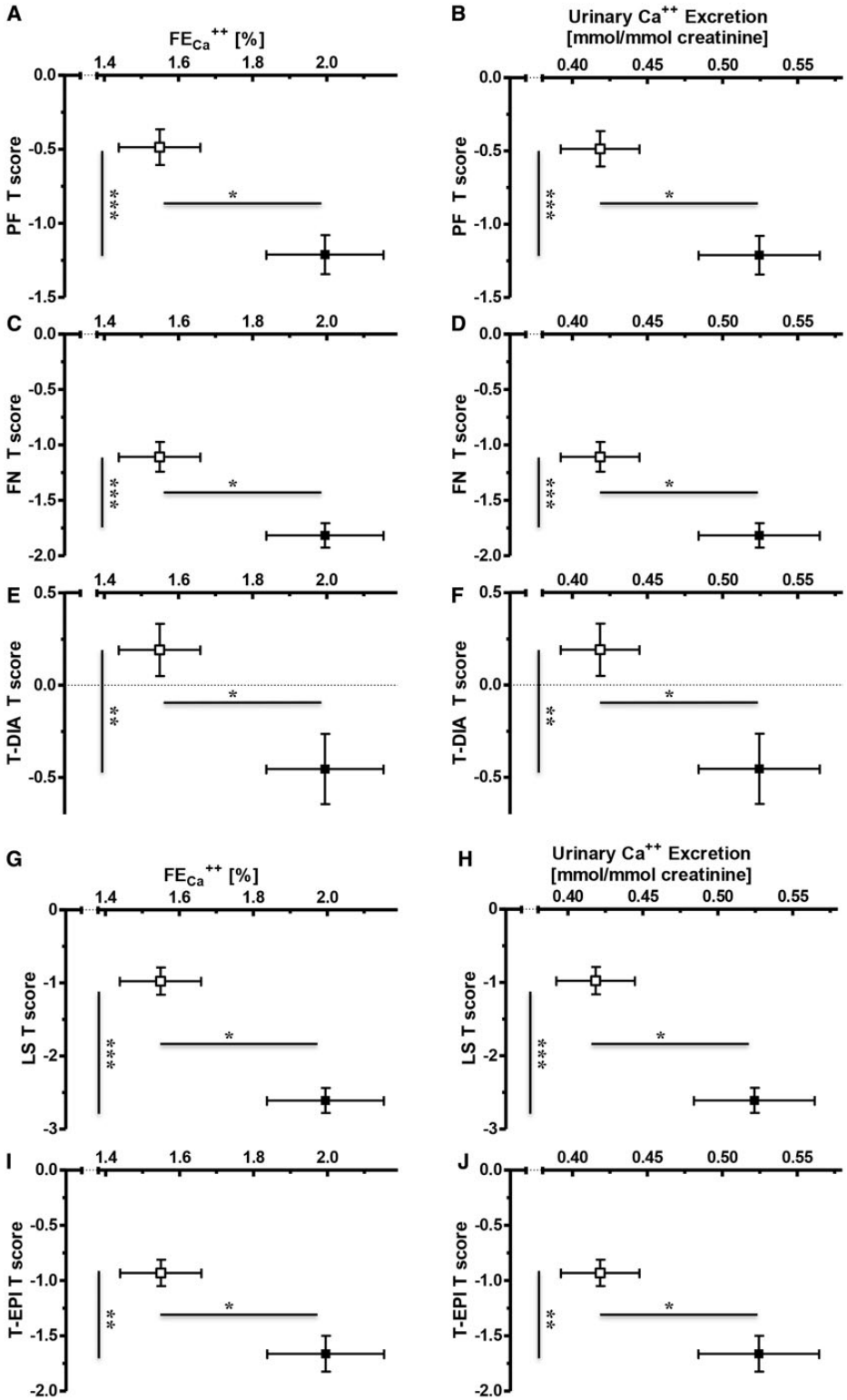
Significant negative correlations were found between $FE_{Ca^{++}}$ and BMD at all measurement sites (Fig. 3A; PF T-score, $r^2 = 0.0535$, $P = 0.0473$; C, FN T-score,

Fig. 2 Urinary calcium loss.



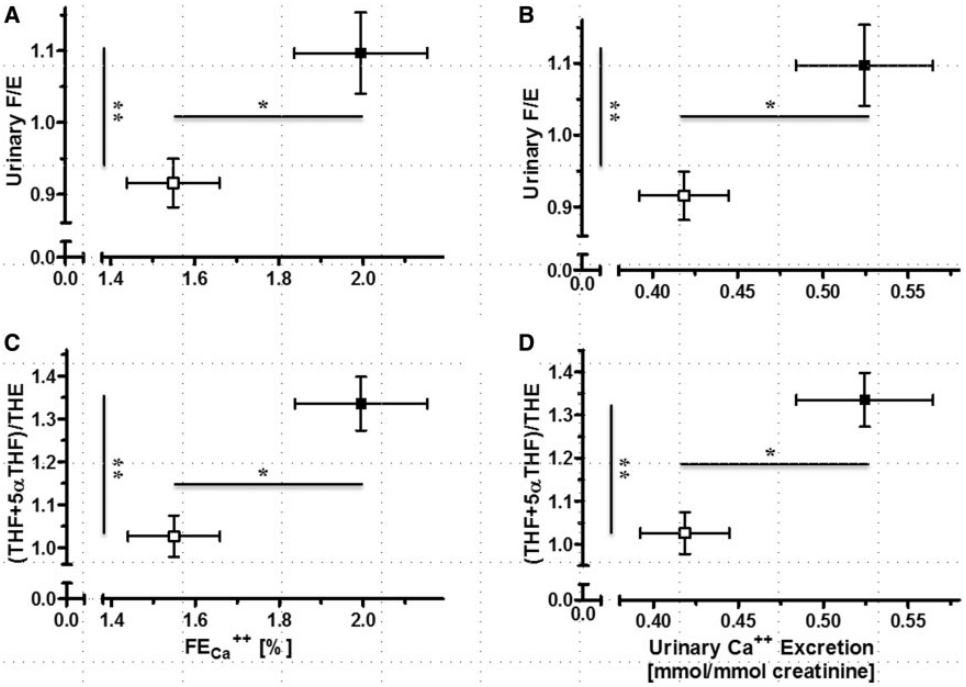
Urinary Ca^{++} loss in men with primary male osteoporosis (MO, filled square) and nonosteoporotic patients (CON, open circle). (**A**) The fractional excretion of Ca^{++} ($FE_{Ca^{++}}$, $P = 0.0318$) and (**B**) the absolute urinary Ca^{++} excretion corrected for creatinine ($P = 0.0307$) were higher in patients with MO. Means are specified by the black line. *$P < 0.05$.

Fig. 3 Urinary calcium loss and BMD.



The relation of BMD to $FE_{Ca^{++}}$ ($P = 0.0232$ between both groups) is shown in (**A**) the proximal femur (PF; $P < 0.0001$), (**C**) the femoral neck (FN; $P < 0.0001$), (**E**) the distal tibial diaphysis (T-DIA; $P = 0.0071$), (**G**) the lumbar spine (LS; $P < 0.0001$) and (I) distal tibial epiphysis (T-EPI; $P = 0.0005$) and to absolute urinary Ca^{++} excretion ($P = 0.0307$ between both groups) in (**B**) PF, (**D**) FN, (**F**) T-DIA, (**H**) LS and (**J**) T-EPI. CON is indicated by open circles and MO by filled squares. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

Fig. 4 Urinary calcium loss was related to apparent enzyme activities of 11β-HSD.



The correlation of FE_{Ca} to urinary F/E is shown in (**A**) ($r^2 = 0.0740$, $P = 0.0297$), to (THF + 5α-THF)/THE in (**C**) ($r^2 = 0.0527$, $P = 0.0443$), and of absolute urinary Ca^{++} excretion to urinary F/E in (**B**) ($r^2 = 0.0623$, $P = 0.0275$) and to (THF + 5α-THF)/THE in (**D**) FE_{Ca} ($r^2 = 0.0465$, $P = 0.0498$). Cortisol (**F**), cortisone (**E**), tetrahydro-F (THF), tetrahydro-E (THE). CON is indicated by open circles and MO by filled squares. $^{**}P < 0.01$, $^{*}P < 0.05$.

$r^2 = 0.0389$, $P = 0.0315$; E, T-DIA T-score, $r^2 = 0.1306$, $P = 0.0020$; G, LS T-score, $r^2 = 0.0956$, $P = 0.0074$; and I, T-EPI T-score, $r^2 = 0.1337$, $P = 0.0016$) and between urinary Ca^{++} excretion at some sites (Fig. 3B; PF T-score, $r^2 = 0.0277$, $P =$ NS; D, FN T-score, $r^2 = 0.0180$, $P =$ NS; F, T-DIA T-score, $r^2 = 0.0535$, $P = 0.0491$; H, LS T-score, $r^2 = 0.0785$, $P = 0.0143$; and J, T-EPI T-score, $r^2 = 0.1089$, $P = 0.0041$).

Mechanisms of increased renal calcium loss: altered intracellular cortisol availability

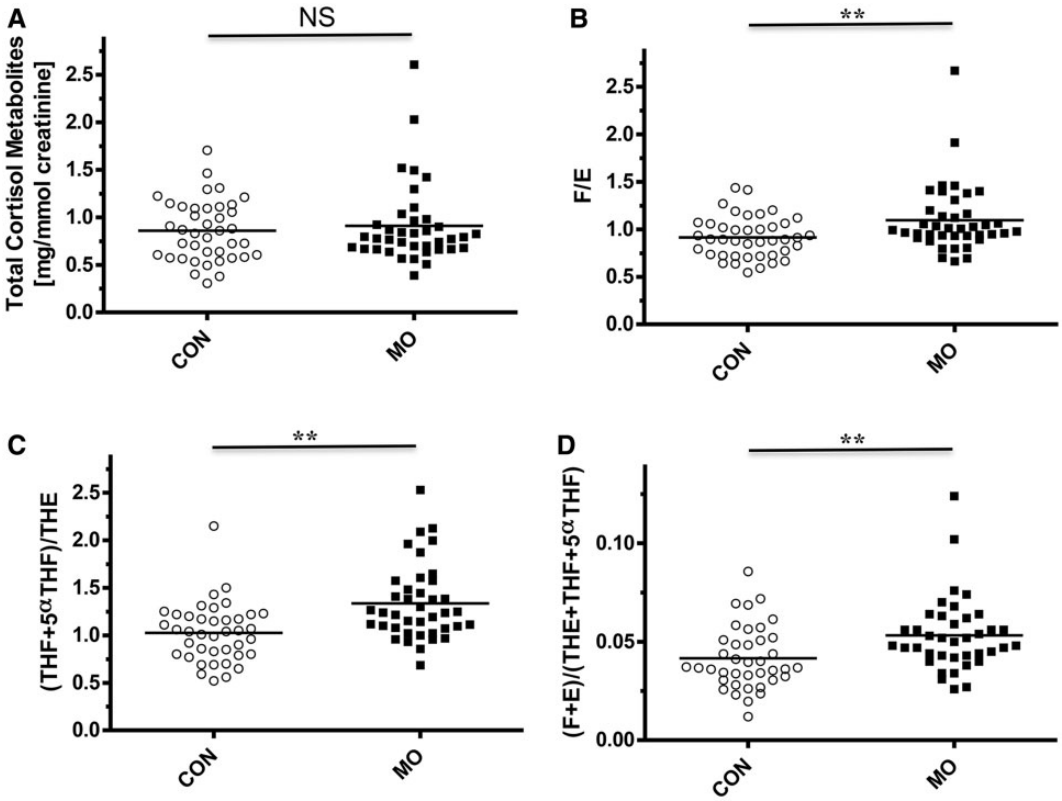
Urinary F/E was positively correlated with $FE_{Ca^{++}}$ (Fig. 4A, $r^2 = 0.0793$, $P = 0.0125$) and with absolute calcium excretion (Fig. 4B, $r^2 = 0.0522$, $P = 0.0443$), suggesting a renal contribution to the bone calcium deficit. Similar observations were made for the ratio (THF + 5α-THF)/THE, and although the degree of correlation was lower, it was statistically significant (Fig. 4C; $FE_{Ca^{++}}$, $r^2 = 0.0410$, $P = 0.0449$; and D, absolute calcium excretion, $r^2 = 0.0493$, $P = 0.0478$). Systemic hypercortisolism in patients with MO was excluded by assessing the urinary excretion of total F metabolites, which did not differ between groups (Fig. 5A). Renal cortisol degradation was compromised in patients with MO, as indicated by the urinary F/E ratio (Fig. 5B). Similarly, the ratio of (THF + 5α-THF)/THE suggested enhanced intracellular cortisol availability (Fig. 5C). The difference between the ratio (F + E)/(THF + 5α-THF + THE) in the two groups indicated that the renal effect appeared to be prevalent. The availability of total F metabolites did not affect the apparent urinary activities F/E (THF + 5α-THF)/THE or (F + E)/(THF + 5α-THF + THE) (data not shown).

Discussion

Our study provides first-time evidence that MO is associated with enhanced calcium loss via the kidney due to dysregulation of the 11β-HSD enzyme system, resulting in enhanced local F availability. We demonstrated a compromised F metabolism via the 11β-HSD1 and 11β-HSD2 enzyme systems strongly related to low BMD in patients with MO, and also that these systems play a role in calcium handling. Taken together, the strong relationship of BMD and urinary F/E to FE_{Ca} suggests that a major determinant in renal calcium handling is the renal F metabolism via 11β-HSD2. This then controls the availability of F and its tubular effects on renal calcium balance, such as provided by the FE_{Ca}. Given the intracellular location of the glucocorticoid receptor, systemic hypercortisolism is not a prerequisite for enhanced receptor activity, which would also be due to altered intracellular F metabolism.

Bone is a known glucocorticoid target organ. It has been shown that glucocorticoids increase the lifespan of osteoclasts, which harbour only 11β-HSD1 [9, 32]. In contrast, osteoblast activity is compromised by glucocorticoids. These cells are known to host both 11β-HSD1 and 11β-HSD2 [10]. As a result, the glucocorticoid-induced

Fig. 5 Intracellular but not systemic F availability was enhanced in MO.



Urinary excretion of total cortisol metabolites (**A**) was unchanged between CON and patients with MO, whereas the ratio of (**B**) urinary F/E ($P = 0.0069$) and of (**C**) (THF + 5α-THF)/THE ($P = 0.0002$) indicated an enhanced intracellular local F availability in patients with MO. This effect seems to affect the F and E ratio more, indicating renal metabolism of F, (**D**) (F + E)/(THF + 5α-THF + THE) ($P = 0.0037$). Means are specified by the black line. $^{**}P < 0.01$. NS: not significant.

activation of osteoclasts and inactivation of osteoblasts results in a negative bone calcium balance that ultimately leads to osteoporosis.

BMD is an established marker of bone health and a surrogate marker of bone mass that is routinely used for the clinical assessment of fracture risk. In line with this, numerous static and dynamic bone histomorphometric parameter-based studies have demonstrated that hypercalciuric patients, e.g. hypercalciuric renal stone-formers, carry the highest risk of bone disease [31, 33–35]. The effects in our study were not related to systemic glucocorticoid availability, but to the integrated apparent enzyme activity of both 11β-HSDs, which are modulators of local intracellular F availability. Our findings indicate two mechanisms related to calcium excretion. First, the increased cellular F availability releases calcium from the bone, which leads to enhanced urinary calcium excretion. Second, the increased intracellular F availability alters renal calcium handling by promoting FE_{Ca}, as has been described in healthy volunteers undergoing inhibition of 11β-HSD2 activity by treatment with glycyrrhetinic acid, which led to high F availability [36]. This promotes osteoporosis by enhanced calcium loss. The calcium loss could be primarily renal (i.e. a renal calcium leak), resulting in a negative calcium balance. As the elevated renal FE_{Ca} is accompanied by normal serum calcium concentrations, the enhanced renal loss must be complemented by release of calcium from enhanced intestinal reabsorption or bone loss, as manifested in osteoporosis. As hormonal adaptation by altered vitamin D and/or PTH was not observed, a role for glucocorticoids appears to be even more likely.

The ensuing calcium deficiency in the main calcium storage organ, bone, might be further augmented by reduced vitamin D stores leading to impaired intestinal calcium absorption, or by dysregulated calcium distribution throughout the body. Of note, nutritional indicators, serum vitamin D, PTH and circulating calcium and phosphate concentrations were not different between osteoporosis patients and controls in our study. As this finding was based on steroid metabolite measurements in the urine, it is not clear at first glance which organ system is primarily involved in calcium handling (i.e. intestine, bone or kidney) and, as such, is responsible for the osteoporotic state. The alternative scenario of surplus calcium derived from enhanced intestinal calcium

absorption excreted by the kidney was not assessed by our study.

Intestinal calcium uptake is regulated via the activity of the calcium channel TRPV6. The expression of TRPV6 is promoted by high 1,25-$(OH)_2$ vitamin D concentrations via activation of the vitamin D receptor. Glucocorticoids, on the contrary, impair vitamin D receptor activity and induce a state of calcium malabsorption. Given the enhanced activity of local glucocorticoids seen in our patients with MO, a primary state of calcium hyperabsorption causing enhanced renal calcium loss is unlikely. In line with this, the acute systemic inhibition of 11β-HSD2 by licorice decreased ionized serum calcium concentrations in healthy subjects [36].

Among the shortcomings of our study is the lack of direct measurements of bone 11β-HSD activity. Furthermore, no standardized dietary protocols were included to correct for nutritional calcium intake. We used a cross-sectional design, which shows association and not causality. Finally, this study was conducted on a highly selected patient population, and the findings in this patient population may not be generalizable to the population as a whole.

Among the strengths of our study is the homogeneity of the case and control cohorts. They were of similar age, came from the same geographic area and had similar BMIs. We also confirmed bone mineral abnormalities after strict exclusion of possible secondary causes. Fragility fractures were assessed and confirmed by radiological methods. Calibrated DXA measurements were performed by a small number of highly qualified and experienced technicians with high reproducibility. As renal function was normal in all participants and age was meticulously matched between both cohorts, determination of 11β-HSD activity was valid [37, 38]. Furthermore, we excluded major dependencies of the apparent activities as calculated by the ratio of substrate to product from the availability of total F metabolites.

In conclusion, the alteration in 11β-HSD increases intracellular F availability. In patients with MO, the overall effect is enhanced FE_{Ca}, suggestive of disturbed renal calcium handling. Furthermore, the absolute excretion of calcium was more compatible with delivery to the kidneys from other sources, including enhanced intestinal reabsorption, increased bone losses, or both, as present in MO. Enhanced local glucocorticoid availability is a previously unrecognized risk factor associated with osteoporosis. These findings suggest that novel therapeutic options such as inhibitors of 11β-HSD1 or activators of 11β-HSD2 might be of benefit in patients with MO.

Rheumatology key messages

- In primary MO, local glucocorticoid availability is enhanced due to dysregulated 11β-HSD enzyme activity.
- In primary MO, enhanced renal calcium loss is associated with disturbed 11β-HSD activity.

Acknowledgements

S.A. was supported by a research grant from the Hellenic Nephrology Society and M.G.M. was supported by grants from the Swiss National Foundation via the nccr kidney.ch and by a personal grant (320030_135596).

Disclosure statement: The authors have declared no conflicts of interest.

References

1 Feldstein AC, Nichols G, Orwoll E *et al*. The near absence of osteoporosis treatment in older men with fractures. Osteoporos Int 2005;16:953–62.

2 Kiebzak GM, Beinart GA, Perser K *et al*. Undertreatment of osteoporosis in men with hip fracture. Arch Intern Med 2002;162:2217–22.

3 Diamond T, Smerdely P, Kormas N *et al*. Hip fracture in elderly men: the importance of subclinical vitamin D deficiency and hypogonadism. Med J Aust 1998;169: 138–41.

4 Kelepouris N, Harper KD, Gannon F *et al*. Severe osteoporosis in men. Ann Intern Med 1995;123:452–60.

5 Scane AC, Francis RM, Sutcliffe AM *et al*. Case-control study of the pathogenesis and sequelae of symptomatic vertebral fractures in men. Osteoporos Int 1999;9:91–7.

6 Kann P, Laudes M, Piepkorn B *et al*. Suppressed levels of serum cortisol following high-dose oral dexamethasone administration differ between healthy postmenopausal females and patients with established primary vertebral osteoporosis. Clin Rheumatol 2001;20:25–9.

7 Chiodini I, Mascia ML, Muscarella S *et al*. Subclinical hypercortisolism among outpatients referred for osteoporosis. Ann Intern Med 2007;147:541–8.

8 Hwang JY, Lee SH, Kim GS *et al*. HSD11B1 polymorphisms predicted bone mineral density and fracture risk in postmenopausal women without a clinically apparent hypercortisolemia. Bone 2009;45:1098–103.

9 Cooper MS, Walker EA, Bland R *et al*. Expression and functional consequences of 11beta-hydroxysteroid dehydrogenase activity in human bone. Bone 2000;27: 375–81.

10 Bland R, Worker CA, Noble BS *et al*. Characterization of 11beta-hydroxysteroid dehydrogenase activity and corticosteroid receptor expression in human osteosarcoma cell lines. J Endocrinol 1999;161:455–64.

11 Mercer WR, Krozowski ZS. Localization of an 11 beta hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. Endocrinology 1992;130: 540–3.

12 Stewart PM, Whorwood CB, Mason JI. Type 2 11 beta-hydroxysteroid dehydrogenase in foetal and adult life. J Steroid Biochem Mol Biol 1995;55:465–71.

13 Condon J, Gosden C, Gardener D *et al*. Expression of type 2 11beta-hydroxysteroid dehydrogenase and corticosteroid hormone receptors in early human fetal life. J Clin Endocrinol Metab 1998;83:4490–7.

14 Cooper MS, Blumsohn A, Goddard PE *et al*. 11beta-hydroxysteroid dehydrogenase type 1 activity predicts the effects of glucocorticoids on bone. J Clin Endocrinol Metab 2003;88:3874–7.

15 Cooper MS, Syddall HE, Fall CH *et al*. Circulating cortisone levels are associated with biochemical markers of bone formation and lumbar spine BMD: the Hertfordshire Cohort Study. Clin Endocrinol 2005;62:692–7.

16 Dennison E, Hindmarsh P, Fall C *et al*. Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. J Clin Endocrinol Metab 1999;84:3058–63.

17 Bauer DC, Garnero P, Harrison SL *et al*. Biochemical markers of bone turnover, hip bone loss, and fracture in older men: the MrOS study. J Bone Miner Res 2009;24:2032–8.

18 Pierotti S, Gandini L, Lenzi A *et al*. Pre-receptorial regulation of steroid hormones in bone cells: insights on glucocorticoid-induced osteoporosis. J Steroid Biochem Mol Biol 2008;108:292–9.

19 Chhokar VS, Sun Y, Bhattacharya SK *et al*. Hyperparathyroidism and the calcium paradox of aldosteronism. Circulation 2005;111:871–8.

20 Ferrari P. Cortisol and the renal handling of electrolytes: role in glucocorticoid-induced hypertension and bone disease. Best Pract Res Clin Endocrinol Metab 2003;17:575–89.

21 Lems WF, Van Veen GJ, Gerrits MI *et al*. Effect of low-dose prednisone (with calcium and calcitriol supplementation) on calcium and bone metabolism in healthy volunteers. Br J Rheumatol 1998;37:27–33.

22 Suzuki Y, Ichikawa Y, Saito E *et al*. Importance of increased urinary calcium excretion in the development of secondary hyperparathyroidism of patients under glucocorticoid therapy. Metabolism 1983;32:151–6.

23 Beavan S, Horner A, Bord S *et al*. Colocalization of glucocorticoid and mineralocorticoid receptors in human bone. J Bone Miner Res 2001;16:1496–504.

24 Shackleton CH. Mass spectrometry in the diagnosis of steroid-related disorders and in hypertension research. J Steroid Biochem Mol Biol 1993;45:127–40.

25 Shojaati K, Causevic M, Kadereit B *et al*. Evidence for compromised aldosterone synthase enzyme activity in preeclampsia. Kidney Int 2004;66:2322–8.

26 Ruetsche AG, Kneubuehl R, Birkhaeuser MH *et al*. Cortical and trabecular bone mineral density in transsexuals after long-term cross-sex hormonal treatment: a cross-sectional study. Osteoporos Int 2005;16:791–8.

27 Casez JP, Troendle A, Lippuner K *et al*. Bone mineral density at distal tibia using dual-energy X-ray absorptiometry in normal women and in patients with vertebral osteoporosis or primary hyperparathyroidism. J Bone Miner Res 1994;9:1851–7.

28 Looker AC, Wahner HW, Dunn WL *et al*. Updated data on proximal femur bone mineral levels of US adults. Osteoporos Int 1998;8:468–89.

29 Looker AC, Orwoll ES, Johnston CC Jr *et al*. Prevalence of low femoral bone density in older U.S. adults from NHANES III. J Bone Miner Res 1997;12:1761–8.

30 Lewiecki EM, Gordon CM, Baim S *et al*. International Society for Clinical Densitometry 2007 adult and pediatric official positions. Bone 2008;43:1115–21.

31 Jaeger P, Lippuner K, Casez JP *et al*. Low bone mass in idiopathic renal stone formers: magnitude and significance. J Bone Miner Res 1994;9:1525–32.

32 Jia D, O'Brien CA, Stewart SA *et al*. Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density. Endocrinology 2006;147:5592–9.

33 Trinchieri A, Nespoli R, Ostini F *et al*. A study of dietary calcium and other nutrients in idiopathic renal calcium stone formers with low bone mineral content. J Urol 1998;159:654–7.

34 Steiniche T, Mosekilde L, Christensen MS *et al*. A histomorphometric determination of iliac bone remodeling in patients with recurrent renal stone formation and idiopathic hypercalciuria. APMIS 1989;97:309–16.

35 Pasch A, Frey FJ, Eisenberger U *et al*. PTH and 1.25 vitamin D response to a low-calcium diet is associated with bone mineral density in renal stone formers. Nephrol Dial Transplant 2008;23:2563–70.

36 Ferrari P, Bianchetti MG, Sansonnens A *et al*. Modulation of renal calcium handling by 11 beta-hydroxysteroid dehydrogenase type 2. J Am Soc Nephrol 2002;13:2540–6.

37 Henschkowski J, Stuck AE, Frey BM *et al*. Age-dependent decrease in 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) activity in hypertensive patients. Am J Hypertens 2008;21:644–9.

38 Draper N, Stewart PM. 11beta-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. J Endocrinol 2005;186:251–71.