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Glucocorticoids and sarcoidosis: a longitudinal study on the effects on cortical and trabecular bone

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Abstract. *Background:* Glucocorticoid induced osteoporosis is a well-known side effect of glucocorticoid treatment. In sarcoidosis the impact on bone by glucocorticoid treatment is complex due to hormonal disturbances of calcium and vitamin-D, which by itself may cause bone loss. In this study we aimed to investigate the longitudinal impact of glucocorticoids on cortical and trabecular bone in patients with mild, recently diagnosed sarcoidosis. *Methods:* Ten patients (8 females; mean age 44 (±13)) were studied during one year of glucocorticoid treatment. The assessment of mainly cortical to purely trabecular bone was made by dual X-ray absorptiometry (DXA) of the spine and hip, quantitative ultrasound of the calcaneus, and magnetic resonance relaxometry of the spine and calcaneus. Bone and hormonal measurements were performed at baseline, after 3, 6, and 12 months, and baseline, 3 weeks and 3 months, respectively. *Results:* DXA of the spine, decreased from baseline at 6 months (P=0.01). R2' of the calcaneus decreased with time (B: -3.6;P=0.03). In the females (n=8) there was a significant decrease in DXA of the spine when comparing 3 months and 6 months (P=0.03), and 3 months and 12 months (P=0.02) and a decrease in R2'of the calcaneus from baseline to 12 months (P=0.01). There was no change in hormonal levels. *Conclusion:* Treatment of initial mild sarcoidosis with dose tapered glucocorticoid therapy only mildly affects the final trabecular and cortical bone and hormone levels. Dose tapering is an important part in glucocorticoid therapy, likely contributing to the mild effects on bone observed in this study. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 63-69)*

Key words: glucocorticoids, osteoporosis, sarcoidosis, DXA, MRI, relaxometry, ultrasound

Introduction

Glucocorticoids (GC) are central in the treatment of autoimmune and inflammatory diseases, widely used in respiratory, bowel, and rheumatoid diseases, as well as for immunosuppression. Although essential in clinical practice, the range of side effects accompanying GC treatment, may limit its use. Especially the effects of GC on bone may be deleterious, and GC induced osteoporosis (GIO) is one of the most common causes of secondary osteoporosis (1).

GIO mainly affects trabecular bone (2,3). Trabecular bone is believed to have a supporting function with a greater biomechanical competence than cortical bone. Since the surface of trabecular bone is

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much greater than that of cortical bone a rapid bone loss, first observed in trabecular bone is rational. Bone trabeculae that have become disconnected or interrupted do not re-establish (4). In addition, several small trabeculae have a greater biomechanical competence than a few thick, even if the amount of trabecular bone mineral is equal (5). A loss in trabecular bone mineral should therefore theoretically be more severe than a loss in cortical bone.

GC therapy is the most common treatment for sarcoidosis (6). Sarcoidosis is a granulomatous inflammatory disease of unknown aetiology. The sarcoid granulomas may affect any organ, however, pulmonary involvement, intra-thoracic lymph-node enlargement and skin and ocular engagement occur in more than 90% of patients with sarcoidosis (6). Patients with sarcoidosis are often relatively unaffected by their disease, whereby weighing the risks against benefits for GC therapy arises.

The effects on bone by GC therapy in patients with sarcoidosis is complex. The sarcoid granulomas convert 25-OH vitamin D (25-vitD) to the active form 1.25OH_2vitD (1.25-vitD), which in turn may cause hypercalcemia (7–9). Elevated levels of vit-D as well as hypercalcemia may restrict the use of ordinary supplements used in GIO such as calcium and vit-D. Besides, the metabolic disturbances by themselves may cause a loss in bone mineral. In this study we aimed to increase the understanding of GC treatment on cortical and trabecular bone in sarcoidosis. In order to exclude the confounding effect and impact on bone by underlying disease we solely focused on patients unaffected by their disease in their daily life.

Material and methods

Patients

Patients were prospectively and consecutively recruited from the department of respiratory medicine, Karolinska University Hospital. Inclusion criterion was patients with recently diagnosed sarcoidosis, unaffected in activities of daily life by their disease. Ten patients in total (8 female (4 postmenopausal)/2male; mean age 44 (±13)) with recently (<2 weeks) diagnosed sarcoidosis were recruited. All patients had biopsy-verified sarcoidosis in the lungs. The patients recruited did not take any medications of importance for this study (eg bisphosphonates). However, one postmenopausal participant was taking 2 mg estradiol a day as hormone replacement therapy. Informed consent from each participant and ethical approval from the regional ethical review board was obtained.

Patient dropouts in the evaluation of bone mineral density (BMD) and lab measurements are included in the tables in results. Magnetic resonance (MR) measurement data were lost due to storage failure in one patient, and the pulse-sequence to obtain R2' of calcaneus was not functional at baseline (n=3) and 3-month follow-up (n=1). One of the patients (male, aged 38) was too tall (198 cm) for dual x-ray absorptiometry (DXA), and the forefoot was excluded from the examination field. It was also impossible to obtain measurements of the calcaneus using quantitative ultrasound (QUS) in this individual due to the heel diameter. Further dropouts are specified in the tables.

Medication and Clinical Parameters

All participants were assigned a standardised regimen of GC therapy. The dose was 60 mg prednisolone, every other day, with conventional dose tapering with time. The prednisolone dose as well as total time of treatment is presented in Table 1.

Body weight, waist-hip ratio, body fat impedance and blood pressure were measured at baseline, after 2 weeks and 3 months. Laboratory measurements were analysed at the department of clinical chemistry, Karolinska University Hospital. Plasma cholesterol, triglycerides, glucose, total calcium, albumin, parathormone (PTH), 25-vitD and 1.25vitD were analysed using routine methods. Analyses were done at baseline, after 2 or 3 weeks,

Table 1. Prednisolone scheme

Prednisolone Dose (mg)	Duration
60	2 weeks
50	2 weeks
40	2 weeks
30	3 months
20	6 months
10	1 month
5	1 month

The dose above was taken every other day, for the duration specified

and 3 months of prednisolone treatment. Normal reference ranges used in this study are: total calcium 2.20-2.60 mmol/l, albumin 40-51 g/l and PTH 10-65 ng/ml.

Bone Measurement

All bone measurements were evaluated at baseline and after 3, 6 and 12 months of GC treatment. For bone measurement DXA of the whole body, spine and hip (Hologic QDR 2000, Hologic inc., Waltham, Massachusetts, USA), and broadband ultrasound attenuation (BUA) of right the calcaneus using a Lunar Achilles (Lunar Corp, Wisconsin, USA) was used. Trabecular bone structure of the spine and right calcaneus was evaluated using MR relaxometry (MR-R). A 1.5 T MRI scanner (GE Signa Echo speed, Wisconsin, USA) was used.

Technical Description of MR Relaxometry

A magnetic inhomogeneity can be observed at the interface between two materials with different magnetic properties, such as bone marrow and trabecular bone. The magnetic inhomogeneity disturbs the coherence of magnetic spins in gradient echo imaging, resulting in decreased signal in areas with different magnetic susceptibility. The amount of signal in a volume element (voxel) of the MR image is affected by echo time (TE) and by the strength of the inhomogeneity. By making several images with different TE the rate of the transaxial signal decay (R2*) can be calculated (Figure 1). R2* is greater in areas with a lot of trabecular bone (10). The measure has been shown to correlate with vertebral biomechanical strength in vitro (11) and it can discriminate between osteoporotic and normal individuals (12). In this study we used nine echoes with different TE to calculate R2* of the lumbar spine. The first TE was 9.0 ms and then a step size of 4.65 ms was used in order to avoid effects of chemical shift (13). The initial echo was set to 9.0 ms instead of 9.3 ms due to a previously shown time shift in the GE scanner (13). In addition the trabecular bone of the calcaneus was studied using a technique developed by Ma et al (14), enabling separation of R2* into irreversible signal decay (R2) and reversible signal decay (R2'). R2 is affected by the relative amount of free water, protein, fat and carbohydrate in the voxel and R2' is affected

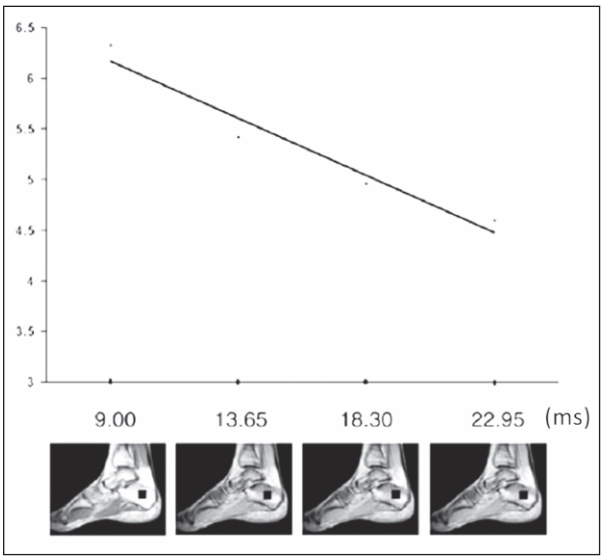

Fig. 1. The MRI signal decay in calcaneus as a function of echo time. The region of interest is shown as a black box. The denser the trabecular structure is, the faster the MRI signal decays. This results in a steeper slope of the regression line in the graph and it will be recorded as a greater R2'

by magnetic inhomogeneity caused by imperfections in the magnetic field of the MR scanner (extrinsic) or from magnetic susceptibility (intrinsic), such as from trabecular bone. Theoretically R2' should be more sensitive than R2*. The relaxation rate was calculated on a voxel by voxel basis with an in-house developed software. Voxels with the correlation coefficient of the regression line below 0.9 were discarded in order to reduce the influence of noise.

Statistics

SPSS 22.0 was used for statistical analysis. Descriptive statistics are presented as means (±SD). Clinical parameters were non-parametric, and the Wilcoxon signed rank test was used for analysis. All bone measurement variables were parametric and were modelled as dependent variables in multiple linear regression analysis, with time as an independent variable, and additionally age and gender added to the model as "controlling" independent variables. ANOVA with repeated measurements, was used for analysis of bone measurements over time, and was done for the whole cohort (n=10), and in order to homogenize data, for the females only (n=8). $P<0.05$ was set as the threshold of statistical significance.

Results

At baseline two postmenopausal women had osteoporosis of the lumbar spine, and three (two postmenopausal women) were osteopenic according to the reference material provided by the manufacturers, except for MR-R where no widely accepted published reference material exists. Multiple linear regression analysis for bone measurements were non-significant over the time range from baseline to 12 months, except for MR-R calcaneus which showed a significant decrease of R2' during the study (B: -3.6; P=0.03); the regression analyses are shown in Table 2A. Average BMD of whole body, spine, hip and calcaneus before treatment, as well as BUA, speed of sound (SOS) and stiffness together with the relaxation rate of spine and calcaneus, for the whole cohort (n=10) and for the females (n=8), are shown in Table 2B. ANOVA with repeated measures for the whole cohort showed a significant decrease in BMD of the spine after 6 months (P=0.01) (Table 2B). In the female cohort (n=8) a significant difference was seen in BMD of the spine between 3 months vs 6 months (P=0.03) and 3 months vs 12 months (P=0.02) (Table 2B). R2' of the calcaneus decreased between baseline and 12 months (P=0.01).

All patients had normal albumin corrected serum calcium, PTH, glucose, lipids and blood pressure at baseline. 1.25-Vit D was increased and 25-vit D was decreased. During treatment and at every control point there were no significant changes in metabolic parameters, except for an increase in total cholesterol and body weight and fat. Cholesterol levels were significantly increased after two weeks and three months compared to baseline (P<0.05). Body weight increased after three months, and the increase showed to be significant compared to that after two weeks of treatment (P<0.05). Body fat impedance was significantly higher after three months compared to baseline (P<0.05). In average the patients gained 4.7 kg in weight during the time of treatment (Table 3).

Table 2 A. Change in bone mineral density over time

Bone Measurements	Time, Regression Coefficient B	P-value
DXA Whole body (BMD)	0.01	ns
DXA Spine (L1-L4) (BMD)	0.00	ns
QUS Calcaneus (BUA)	0.05	ns
QUS Calcaneus (SOS)	3.4	ns
QUS Calcaneus (Stiffness)	3.8	ns
MR-R Spine (R2*)	-2.6	ns
MR-R Calcaneus (R2')	-3.6	0.03

A linear regression analysis was performed with the bone measurements as a dependent variable, and time as an independent variable; additional independent controlling variables were age and gender. Values are given as the regression coefficient B. DXA=dual x-ray absorptiometry, ns=not significant, QUS=quantitative ultrasound, MR-R= magnetic resonance relaxometry

Discussion

Our results suggest that the treatment of initial mild sarcoidosis with dose tapered GC therapy only mildly affects the final trabecular and cortical bone and hormone levels. This is most likely due to the rapid dose tapering of GC, suggesting its importance in GC therapy. Previous studies have shown that GC induced bone loss generally plateaus after about one year of GC treatment (18). In our study, after 12 months of treatment there was only a significant decrease in trabecular bone structure of calcaneus, identified using MRI, while the other modalities could not detect any significant decrease in bone parameters. When homogenizing the material into a female cohort also a small, but significant, reduction of BMD of the spine (-2.5%) could be observed. This is considerably less than the 4.6% reduction that has previously been observed in healthy men (19).

The effects of GC therapy on bone in patients with sarcoidosis are scarcely investigated. Montemurro et al followed 35 patients with sarcoidosis, and reported a similar time course for BMD loss to that of other diseases such as asthma and rheumatoid

Table 2 B. Change in bone mineral density over time

	Unit	Baseline	3 months	6 months	12 months	P-value
Whole cohort (n=10)						
DXA Whole body (BMD)	g/cm²	1.163 (0.157) [10]	1.179 (0.141) [8]	1.169 (0.162) [10]	1.175 (0.150) [10]	ns
DXA Spine (L1-L4) (BMD)	g/cm²	1.117 (0.238) [10]	1.102 (0.231) [8]	1.084 (0.224) [10]	1.093 (0.215) [10]	<0.05 (6 months vs baseline)
QUS Calcaneus (BUA)	dB/Hz	116.4 (15.8) [9]	111.4 (18.9) [8]	112.0 (15.4) [8]	113.8 (15.5) [9]	ns
QUS Calcaneus (SOS)	m/s	1533 (46) [9]	1526 (41) [8]	1524 (39) [8]	1539 (60) [9]	ns
QUS Calcaneus (Stiffness)	a.u.	86.4 (22.8) [9]	81.4 (23.0) [8]	81.3 (20.1) [8]	86.7 (25.7) [9]	ns
MR-R Spine (R2*)	ms^{-1} [10]	63.4 (11.4) [9]	60.1 (5.5) [9]	64.1 (9.9) [9]	58.4 (9.7)	ns
MR-R Calcaneus (R2')	ms^{-1}	68.4 (17.0) [7]	61.9 (17.8) [8]	62.6 (15.4) [10]	59.2 (14.0) [8]	ns
All females (n=8)						
DXA Whole body (BMD)	g/cm²	1.178 (0.166) [8]	1.182 (0.176) [8]	1.179 (0.173) [8]	1.187 (0.158) [8]	ns
DXA Spine (L1-L4) (BMD)	g/cm²	1.137 (0.240) [8]	1.135 (0.229) [8]	1.112 (0.219) [8]	1.109 (0.213) [8]	<0.05 (3 months vs 6 months; 3 months vs 12 months)
QUS Calcaneus (BUA)	dB/Hz	117.1 (16.5) [8]	112.9 (19.9) [8]	113.6 (15.9) [8]	114.1 (16.6) [8]	ns
QUS Calcaneus (SOS)	m/s	1529 (44) [8]	1530 (42) [8]	1528 (40) [8]	1534 (57) [8]	ns
QUS Calcaneus (Stiffness)	a.u.	85.9 (22.9) [8]	83.6 (23.9) [8]	83.6 (20.5) [8]	85.3 (25.6) [8]	ns
MR-R Spine (R2*)	ms^{-1}	63.9 (12.8) [8]	60.5 (6.3) [8]	63.5 (7.6) [8]	60.2 (9.8) [8]	ns
MR-R Calcaneus (R2')	ms^{-1}	70.4 (14.9) [7]	67.5 (20.2) [8]	68.4 (12.3) [8]	59.5 (17.6) [8]	<0.05 (baseline vs 12 months)

ANOVA with repeated measures was performed first for the whole cohort (n=10), and subsequently for all females (n=8). Values are reported given as means (±SD) [number of patients]; significance values are given. a.u.=arbitrary unit, DXA=dual x-ray absorptiometry, ns=not significant, QUS=quantitative ultrasound, MR-R= magnetic resonance relaxometry.

Table 3. Change in clinical parameters over time

Clinical Parameters	Baseline	After 3 weeks (3W)	After 3 months (3M)	P-value
Body Weight, kg	79.0 (7.5)	78.7 (7.3)	80.2 (7.4)	<0.017 (3W vs 3M)
Body Fat, Bioimpedance, %	37.0 (1.4)	37.3 (1.5)	38.6 (2.1)	<0.05 (Baseline vs 3M)
Waist/Hip Ratio	0.84 (0.02)	0.86 (4.0)	0.84 (0.01)	ns
Systolic Blood Pressure, mmHg	126 (7)	127 (5)	124 (6)	ns
Diastolic Blood Pressure, mmHg	76 (4)	76 (4)	79 (4)	ns
P-Glucose, mmol/L	4.6 (0.1)	4.5 (0.1)	4.5 (0.2)	ns
P-Total Cholesterol, mmol/l	5.3 (0.3)	6.0 (0.3)	5.9 (0.4)	<0.05 (Baseline vs 3w; Baseline vs 3m)
P-Triglycerides, mmol/L	1.8 (0.4)	1.8 (0.2)	1.7 (0.2)	ns
S-Ca corrected for albumin, mmol/l	2.30 (0.11)	2.39 (0.08)	2.34 (0.11)	ns
S-PTH ng/ml	25.8 (21.4)	28.7 (16.5)	36.2 (29.8)	ns
S-1.25Vit D pg/ml	50.1 (11.4)	43.4 (13.4)	48.3 (0.2)	ns
S-25vit D ng/ml	19.5 (8.1)	19.1 (7.7)	19.5 (5.3)	ns

Values are given as means (standard error of the mean). Results represent that of 9 participants and comparisons over time were made with Wilcoxon signed rank tests. BT=before treatment, 3W=after 3 weeks, 3M=after 3 months, ns=not significant.

arthritis after one year (15). Rizzato et al reported that BMD loss was more frequent in patients with sarcoidosis on GC treatment, compared to other groups (16). In another study Rizzato et al reported a normal average BMD in 22 patients suffering from sarcoidosis less than 20 months (17).

The lumbar vertebral body consists primarily of trabecular bone, 80% (20). When obtaining the lumbar spine BMD using DXA the spinal processes are included in the measurement. The spinal processes contain mainly cortical bone (21). As osteoporosis is a surface process, the trabecular bone should be more severely affected than the cortical bone during periods with a negative bone balance. The decrease should therefore theoretically be great in calcaneus, which consists of 95% trabecular bone and it should also be more easily detected with the use of QUS and MR-R, two methods that primarily measure the trabecular bone.

Our study shows that the final GC effects on bone in otherwise healthy sarcoidosis patients are mildly changed with dose tapering GC therapy. This emphasizes the importance of rapid dose tapering as well as GC therapy every other day. Most bone measurements were at their lowest value at 3-6 months of therapy, and recovered at 12 months. Even though a quick recovery in BMD values was seen in the whole cohort, this was not the case in MR-R of the calcaneus and DXA of the spine in the female cohort. This indicates that trabecular bone might have been lost and that bone protective treatment such as bisphosphonate therapy might be of value, at least during the initial phase of high dosed GC treatment in sarcoidosis patients. However larger studies, including both men and women, are warranted to study the impact of dose tapered GC therapy on bone, as well as bone recovery, in patients with sarcoidosis. Further, it is important to emphasize that we chose to focus on patients who were unaffected of their disease, which much likely contributed to the final mild effect on BMD status of our participants. After starting GC therapy 1.25-VitD decreased as excepted and 25-vitD remained low. The normal lab values may be due to the recently diagnosed state of our participants, and may as well have impacted on the final BMD.

The strengths of our study include solely studying patients with sarcoidosis unaffected by their disease. By doing so we minimised the risk of confounding effects on BMD. The use of different techniques for assessment of trabecular and cortical BMD is another strength. Limitations include a small cohort, and dropouts from certain BMD and lab measurements. Although a small cohort, we nonetheless believe our results are of importance, as they provide data in a minimally investigated, yet important, topic.

Our results suggest the importance of tapered GC therapy on reducing the effects on final BMD in

sarcoidosis patients. Further research is warranted to increase the understanding of bone status in patients with sarcoidosis.

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