RESEARCH

Prednisolone is associated with a worse bone mineral density in primary adrenal insufficiency

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Abstract

Context: Patients with primary adrenal insufficiency (PAI) or congenital adrenal hyperplasia (CAH) receive life-long glucocorticoid (GC) therapy. Daily GC doses are often above the physiological cortisol production rate and can cause long-term morbidities such as osteoporosis. No prospective trial has investigated the long-term effect of different GC therapies on bone mineral density (BMD) in those patients.
Objectives: To determine if patients on hydrocortisone (HC) or prednisolone show changes in BMD after follow-up of 5.5 years. To investigate if BMD is altered after switching from immediate- to modified-release HC.
Design and patients: Prospective, observational, longitudinal study with evaluation of BMD by DXA at visit1, after 2.2 ± 0.4 (visit2) and after 5.5 ± 0.8 years (visit3) included 36 PAI and 8 CAH patients. Thirteen patients received prednisolone (age 52.5 ± 14.8 years; 8 women) and 31 patients received immediate-release HC (age 48.9 ± 15.8 years; 22 women). Twelve patients on immediate-release switched to modified-release HC at visit2.
Results: Prednisolone showed significantly lower Z-scores compared to HC at femoral neck (−0.85 ± 0.80 vs −0.25 ± 1.16, $P < 0.05$), trochanter (−0.96 ± 0.62 vs 0.51 ± 1.07, $P < 0.05$) and total hip (−0.78 ± 0.55 vs 0.36 ± 1.04, $P < 0.05$), but not at lumbar spine, throughout the study. Prednisolone dose decreased by 8% over study time, but no significant effect was seen on BMD. BMD did not change significantly after switching from immediate- to modified-release HC.
Conclusions: The use of prednisolone as hormone replacement therapy results in significantly lower BMD compared to HC. Patients on low-dose HC replacement therapy showed unchanged Z-scores within the normal reference range during the study period.

Key Words
- bone mineral density
- hydrocortisone
- prednisolone
- modified-release hydrocortisone
- congenital adrenal hyperplasia

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Introduction

In states of adrenal insufficiency (AI), such as primary adrenal insufficiency (PAI) and congenital adrenal hyperplasia (CAH), glucocorticoids (GCs) are given in low doses as hormone replacement therapy. However, the daily intake of GCs in PAI and CAH still tends to be more than the total daily endogenous cortisol secretion seen in healthy subjects (5–10 mg of cortisol/m^2 of body surface area/day) (1) possibly triggering long-term morbidities such as osteoporosis. GC-induced decline in bone formation is associated with an increased fracture risk related both to the dose and duration of GC treatment (2). Recent studies on bone mineral density (BMD) in PAI and CAH patients showed different results. This may be not only due to the often small patient numbers and the

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cross-sectional design, but also due to different disease durations, different GC type and daily dosing. This is highlighted by reduced BMD in all PAI patients in some studies (3, 4); however, other studies reported reduced BMD only in men (7, 8) or only in postmenopausal women (5, 6). Other studies did not find sex differences in BMD (9, 10, 11). In CAH patients, some studies reported reduced BMD in all or some subpopulations of CAH patients (18, 19, 20, 21, 22, 23, 24, 25, 26), whereas others described no significant differences in BMD between patients with CAH and controls (11, 12, 13, 14, 15, 16, 17).

Longitudinal studies are scarce: a study including 15 patients with CAH reported an increase in L1-L4 BMD but a decrease of femoral neck BMD after 8–10 years (26); another study including 6 PAIs and 6 secondary AIs also showed a mixed response (27). Recently, we showed that a dose reduction in daily HC dose resulted in an increase in lumbar spine and total hip BMD in a large cohort of PAI and CAH (28). However, no longitudinal study has examined the effect of different GCs in detail.

Prednisolone is used in approx. 5% of patients with AI as hormone replacement therapy (29). The Endocrine Society Clinical Practice guideline suggests prednisolone (3–5 mg/day) as an alternative to hydrocortisone (HC), particularly in patients with reduced compliance (30); however, data comparing the safety and efficacy of prednisolone and HC over time are very scarce. Recently, it was demonstrated that AI patients on prednisolone possess a lipid profile, which is associated with a higher risk for cardiovascular disease compared to patients on HC (31). Our group showed in a cross-sectional study setting that AI patients on prednisolone present a worse BMD compared to HC (11).

The aim of this study was to determine if patients on HC or prednisolone replacement therapy show changes in BMD after follow-up of 5.5 years and to investigate if BMD alters after switching from immediate- to modified-release HC.

Materials and methods

Design and subjects

This was a 5-year prospective and observational study including patients from one large endocrine outpatient clinic. Inclusion criteria were PAI or CAH verified by review of the medical records. Patients under the age of 18 years, patients diagnosed with bone metastasis, AI due to long-term pharmacological GC treatment, adrenocortical carcinoma and patients with less than 12-month duration of disease were excluded. The study was approved by the *Ethikkommission der Charité – Universitätsmedizin Berlin* (EA1/027/10), and all patients gave written informed consent prior to participation.

At study enrolment (visit1), after 2 and 5 years (visit2 and visit3, respectively), BMD was assessed by DXA scan, and all clinical data, including hormone replacement therapies, DHEA and bisphosphonate therapies and changes were documented. Fracture histories were ascertained by self-report, and patients were questioned about fractures they had between 2010 and 2017. Patients were seen every 4–6 months by two experienced endocrinologists (M V and M Q). The endocrinologists had the aim to optimize GC replacement therapy according to best clinical practice guidelines (32, 33, 34).

Of the included 70 patients, 26 patients had an incomplete data set after 5 years or refused to have a DXA scan at follow-up visits, and therefore, were excluded. For final analysis, there were 36 patients suffering from PAI (28 women) and 8 patients with CAH (6 men; salt-wasting $n=7$, simple virilizing $n=1$). By the end of 2012, modified-release HC became available for the treatment of patients with AI in Germany. From that time point onwards, AI patients were free to choose which HC regimen they wanted to have. At visit2, 12 of 31 patients on immediate-release HC treatment (nine PAIs and three CAHs) decided to switch to modified-release HC (Fig. 1). Due to the different pharmacological profiles between

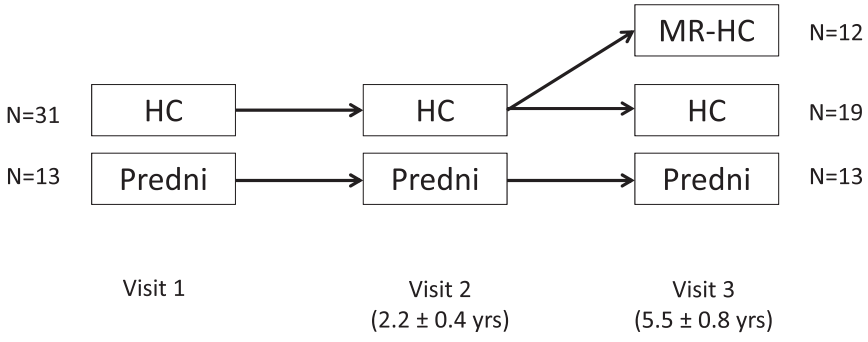

Figure 1
Study design. HC, hydrocortisone; MR-HC, modified-release hydrocortisone; predni, prednisolone.

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immediate- and modified-release HC, we decided to separately analyze those two groups. For analysis, the dose of GC was converted into milligrams of HC equivalent (1 mg prednisolone = 6 mg HC) (35). The daily HC equivalent intake was corrected for body surface area.

Measurements

Blood was drawn at visit1 (baseline) and at follow-up visits (visit 2 and 3) for measurement of the following parameters: serum calcium (Ca; Roche Diagnostics GmbH), phosphorus (P; Roche Diagnostics GmbH), alkaline phosphatase (AP; Roche Diagnostics GmbH), parathyroid hormone (PTH; Elecsys-PTH, Roche Diagnostics GmbH) and 25-hydroxyvitamin D3 (25(OH) D3; IDS-ISYS immunoassay; Immunodiagnostic Systems (IDS) GmbH, Frankfurt, Germany).

BMD was measured using dual energy X-ray absorptiometry (DXA) (GE Lunar DPX-NT, Madison, WI, USA) at total hip, lumbar spine (L1-L4), femoral neck and greater trochanter. The manufacturer's standard scan and positioning protocols were adhered to. Spine phantom quality control scans were performed daily during the study, and phantom data were entered into a Shewhart chart with a previously established baseline (the mean of ten initial control scans). The control scans were always within ±1.5% from the initial mean, and the precision error was s.d. 0.004 g/cm^2, CV 0·414% or below. Scans were analyzed using GE-Lunar software version enCORE 2004. The results were transformed into Z-scores using sex- and age-matched reference data provided by the manufacturer originating from an American population.

Statistical analysis

BMD is expressed by Z-scores, which represent age- and gender-adjusted standard deviation score (SDS), which are presented as means with 95% CIs. This was derived from quality controls performed according to manufacturer´s standards, as described in the manuals of standard operating procedures. CI different from zero indicates Z-scores statistically different from the reference population. A T-score value below −2.5 s.d. was defined as osteoporosis.

Comparisons between two groups were made using the unpaired *t*-test when values were normally distributed. Otherwise, the Mann–Whitney rank-sum test was used. When continuous variables were compared in all three groups, one-way ANOVA was used for normal distributions. In cases of non-normal distribution the Kruskal–Wallis test was performed. Chi-square was used for calculations of frequency. When the expected frequency was small ($n<5$), the Fisher's exact test was used. Analyses were performed using the statistical software package SPSS, version 22.0 (SPSS Inc). Significance was accepted if $P<0.05$.

Results

Clinical data of our study cohort at visit 1 is shown in Table 1. There were no significant differences in age, BMI, duration of disease, calcium, phosphorus, alkaline phosphatase, 25-hydroxyvitamin D3 or parathyroid hormone between AI patients on HC or prednisolone therapy (Table 1). Furthermore, there were no significant differences in BMI, serum calcium, serum phosphate, alkaline phosphatase, parathyroid hormone and 25-hydroxyvitamin D3 between the treatment groups during the whole study period (data not shown).

Patients on prednisolone showed significantly lower Z-scores at femoral neck (−0.85±0.80 vs −0.25±1.16, $P<0.05$), trochanter (−0.96±0.62 vs 0.51±1.07, $P<0.05$) and total hip (−0.78±0.55 vs 0.36±1.04, $P<0.05$) at all visits during the study period (Fig. 2 and Table 2). However, Z-scores at lumbar spine (L1-L4) were not significantly different between the treatment groups at visit1 and throughout the study. Also T-scores were significant lower at femoral neck, trochanter and at total hip, but not at lumbar spine, in AI patients on prednisolone compared to patients on HC (Table 2).

Prednisolone doses decreased by 8% over study time (mean 5.47–5.05 mg/day), but no significant effect was seen on BMD. HC doses remained unchanged during the study period, and BMD did not alter during the study period in subjects on HC medication. Furthermore, BMD did not change significantly after switching from immediate- to modified-release HC in AI patients (visit3 vs visit2) (Fig. 2 and Table 2). There was no significant difference in Z-scores or T-scores between immediate- and modified-release HC at visit3 (Table 2).

The diagnosis of osteoporosis was not significantly different among the three treatment groups (Table 3). Also the frequency of bisphosphonate therapy or DHEA therapy was not significantly different between the groups. Several fractures appeared throughout the study period from 2010 to 2017, showing the highest number of fractures in the prednisolone group (Table 3). Fractures associated with road traffic accidents or skiing accidents are usually not regarded as osteoporosis associated fractures and

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Table 1 Clinical data at visit1 (study start) in patients with PAI or CAH separated into different GC groups.

	Visit 1		
	Always on immediate-release HC	Immediate-release HC (switch to modified-release HC at visit 2)	Prednisolone
n (PAI/CAH)	19 (17/2)	12 (9/3)	13 (10/3)
Sex (men/women)	6/13	3/9	5/8
Postmenopausal status (women)	9/13	4/9	5/8
Age (years)	49.6 ± 16.9	47.8 ± 14.5	52.5 ± 14.8
BMI (kg/m²)	26.3 ± 4.9	24.3 ± 2.2	26.5 ± 4.6
Duration of disease (years)	19.2 ± 11.6	17.4 ± 11.3	23.5 ± 11.8
Daily HC-equivalent dose (mg)	24.2 ± 6.1	21.1 ± 5.1	***32.7*** ± ***11.3***[a,bb]
GC dose per body surface (mg/m²)	13.2 ± 3.1	12.1 ± 2.9	***17.9*** ± ***5.7***[a,b]
Calcium (2.15–2.65 mmol/L)	2.32 ± 0.08	2.33 ± 0.07	2.26 ± 0.1
Phosphorus (0.8–1.5 mmol/L)	0.90 ± 0.17	0.90 ± 0.17	0.9 ± 0.1
Alkaline phosphatase (35–104 U/L)	54.7 ± 12.4	52.3 ± 14.2	55.5 ± 15.4
Parathyroid hormone (11–67 pg/mL)	35.6 ± 27.5	36.1 ± 9.6	32.4 ± 14.5
25-Hydroxyvitamin D_3 (50–250 nmol/L)	61.4 ± 22.7	62.7 ± 27.3	52.8 ± 19.1

Means ± S.D. For analysis, the dose of GC was converted into milligrams of HC equivalent (1 mg prednisolone = 6 mg hydrocortisone). When continuous variables were compared in all three groups, one-way ANOVA was used for normal distributions. In cases of non-normal distribution the Kruskal–Wallis test was performed. Chi-square was used for calculations of frequency. When the expected frequency was small ($n < 5$), the Fisher's exact test was used. [a]$P < 0.05$ to immediate-release HC; [b]$P < 0.05$, [bb]$P < 0.01$ to immediate-release HC (switch to modified-release HC at visit2).CAH, congenital adrenal hyperplasia; GC, glucocorticoid; HC, hydrocortisone; PAI, primary adrenal insufficiency.

might be excluded. This would results in fractures in 2 of 19 patients in the immediate-release HC group, 0 of 12 in the modified-release HC group and 3 of 13 patients in the prednisolone group. Even when analyzing all patients on HC ($n = 31$) vs patients on prednisolone ($n = 13$), there was no significant difference in frequency of fractures ($P = 0.144$).

Discussion

GC therapy using pharmacological doses has a detrimental effect on bone, which is known to be strongly dose dependent. Also in hormone replacement, it is known that doses of 30 mg HC daily have negative effects on bone, and a dose-relationship was described (4). However, at low doses of 20 mg HC daily, no differences in BMD could be observed compared to healthy controls (11). Recently, we were able to show that a dose reduction in even low daily HC doses resulted in an increase in lumbar spine and total hip BMD in a large cohort of PAI and CAH (28). This strongly suggests that the previously seen detrimental effects of GC replacement therapy in PAI were primarily a consequence of supraphysiological GC exposure. These data emphasize the need to aim for the lowest possible HC replacement dose in AI patients that maintains health and avoids the development of long-term adverse effects such as decreased BMD.

In contrast to the predominant use of HC by patients in Europe and the United States, many PAI patients in other regions of the world are prescribed oral prednisolone in view of its ease of administration, low cost and easy availability (36), however, often in too high doses, e.g. 7.5 mg/day (37). A dose of 5 mg/day prednisolone, given to healthy volunteers, has been shown to significantly impair markers of bone formation and, to a lesser extent, bone resorption markers (38). Recent data suggest that prednisolone is deleterious for BMD even when used in low doses for hormone replacement therapy in AI (11, 18, 28, 36, 39).

This study is the first prospective and longitudinal study in AI investigating the impact of prednisolone on BMD over a longer period of more than 5 years. We found significantly reduced BMD at femoral neck,

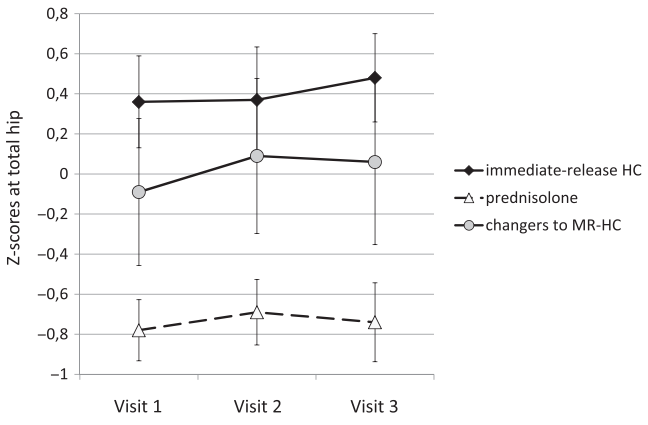

Figure 2
Z-scores at total hip of patients with primary adrenal insufficiency on immediate-release or MR-HC or prednisolone over the study period of 5.5 years. Means ± S.E.M. HC, hydrocortisone; MR-HC, modified-release hydrocortisone; predni, prednisolone.

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Table 2 Bone mineral density (BMD) at (a) visit1 (start of the study), at (b) visit2 (after 2.2 ± 0.4 years) and at (c) visit3 (after 5.5 ± 0.8 years) in patients with PAI or CAH divided into different GC groups.

	Always on immediate-release HC	Immediate-release HC (switch to modified-release HC at visit 2)	Prednisolone
(a) Visit1			
Z-score lumbar spine (L1-L4)	−0.26 ± 0.89	−0.51 ± 1.12	−0.58 ± 1.14
Z-score femoral neck	0.25 ± 1.16	−0.07 ± 1.20	***−0.85 ± 0.80***^{aa,b}
Z-score greater trochanter	0.51 ± 1.07	0.18 ± 1.53	***−0.96 ± 0.62***^{aa,b}
Z-score total hip	0.36 ± 1.04	−0.09 ± 1.27	***−0.78 ± 0.55***^{aa}
T-score lumbar spine (L1-L4)	−0.84 ± 1.10	−0.91 ± 1.21	−1.12 ± 1.30
T-score femoral neck	−0.32 ± 1.30	−0.61 ± 1.27	***−1.48 ± 1.02***^{a}
T-score greater trochanter	0.26 ± 1.09	−0.08 ± 1.41	***−1.20 ± 0.80***^{b,aaa}
T-score total hip	−0.03 ± 1.14	−0.52 ± 1.20	***−1.27 ± 0.74***^{aa}
(b) Visit2			
Z-score lumbar spine (L1-L4)	−0.22 ± 0.86	−0.40 ± 1.35	−0.42 ± 1.16
Z-score femoral neck	0.26 ± 1.20	−0.12 ± 1.19	***−0.62 ± 0.64***^{a}
Z-score greater trochanter	0.43 ± 1.34	0.28 ± 1.61	***−0.81 ± 0.72***^{aa,b}
Z-score total hip	0.34 ± 1.15	0.09 ± 1.34	***−0.69 ± 0.59***^{aa,b}
T-score lumbar spine (L1-L4)	−0.87 ± 0.97	−0.84 ± 1.33	−1.09 ± 1.24
T-score femoral neck	−0.44 ± 1.37	−0.73 ± 1.16	***−1.45 ± 0.74***^{a}
T-score greater trochanter	−0.28 ± 2.36	−0.03 ± 1.44	***−1.21 ± 0.76***^{b}
T-score total hip	−0.19 ± 1.24	−0.38 ± 1.25	***−1.36 ± 0.68***^{aa,b}
(c) Visit3			
Z-score lumbar spine (L1-L4)	−0.14 ± 1.16	−0.39 ± 1.47	−0.18 ± 0.59
Z-score femoral neck	0.26 ± 0.99	−0.30 ± 1.22	***−0.71 ± 0.73***^{aa}
Z-score greater trochanter	0.46 ± 1.13	0.11 ± 1.90	***−1.07 ± 0.72***^{aa}
Z-score total hip	0.48 ± 0.96	0.06 ± 1.43	***−0.74 ± 0.71***^{aa}
T-score lumbar spine (L1-L4)	−0.67 ± 1.04	−0.89 ± 1.47	−1.15 ± 1.02
T-score femoral neck	−0.55 ± 1.42	−1.11 ± 1.43	***−1.89 ± 0.74***^{aa}
T-score greater trochanter	0.06 ± 1.24	−0.38 ± 1.73	***−1.91 ± 0.84***^{b,aaa}
T-score total hip	−0.16 ± 1.20	−0.50 ± 1.47	***−1.58 ± 0.84***^{aa,b}

Z-scores represent age and gender-adjusted SDS. Means ± SD. When continuous variables were compared in all three groups, one-way ANOVA was used for normal distributions. In cases of non-normal distribution the Kruskal-Wallis test was performed.
$^{a}P<0.05$, $^{aa}P<0.01$, $^{aaa}P<0.001$ to immediate-release HC; $^{b}P<0.05$, $^{bb}P<0.01$ to immediate-release HC (switch to modified-release HC at visit2).
CAH, congenital adrenal hyperplasia; GC, glucocorticoid; HC, hydrocortisone; PAI, primary adrenal insufficiency.

greater trochanter and total hip, but not at lumbar spine, in patients on a long-term replacement therapy with prednisolone compared to those on HC. Surprisingly, those patients did not display any difference in BMI suggesting that the bone, especially femur and hip, is more sensitive to this long-acting synthetic GC than other tissues and a better long-time marker for GC exposure. The often used relative biological potency and conversion rate of HC and prednisolone was calculated using the ACTH-suppressive effect (40) resulting in a 1:4 conversion rate. However, the effects of prednisolone on growth in CAH children result in a suggested conversion rate of 6–8:1 (35).

Our data endorse cross-sectional data from Swedish CAH patients who presented lower femoral and lumbar BMD and more severe fractures when receiving prednisolone (39). Also in a cohort from India with 41 PAI patients, prednisolone resulted in a decreased BMD (36). In our study, the two treating physicians tried to reduce the prednisolone doses during the study period. They achieved a dose reduction of 8%, and it was documented that a further reduction was often not tolerated from patient´s side. However, the aim should be a further dose reduction to 3–5 mg/day in those patients who are not willing to change prednisolone. Due to the long study period, we were able to document all fractures occurring during that time (2010 to 2017). It is noteworthy that the worst fractures appeared in the prednisolone group; however, the frequency of fractures was not statistically different between HC-treated patients and patients receiving prednisolone. However, this might be also due to our small cohort size and further investigations are warrant in larger cohorts.

In our study, we showed that HC replacement therapy seems to be safe regarding bone health when used in standard replacement doses. BMD remained stable over a long treating period of 5.5 years. Also the change from immediate-release to modified-release HC seemed to be safe and did not worsen BMD. In the group of patients with modified-release HC no spontaneous fractures appeared during the study period. In a recent, longitudinal study,

Table 3 Clinical data, concomitant medications and fractures in patients with adrenal insufficiency separated into different glucocorticoid replacement groups during study period 2010–2017.

	Immediate-release HC ($n = 19$)	**Modified-release HC** ($n = 12$)	**Prednisolone** ($n = 13$)
Diagnosis osteoporosis	4/19	3/12	4/13
Therapy with bisphosphonate	4/19	1/12	3/13
DHEA therapy (women)	5/13	3/9	5/8
Sex steroid HRT (women)	3/13	2/9	1/8
Fractures during study period 2010–2017	2/19: - Radius fracture 12-2011 - Hand wrist fracture 7-2010 and ulnar fracture 10-2011	1/12: - Os pubis fracture due to motorcycle accident 6-2010	4/13: - Metatarsal fracture 5-2015 - Lumbal vertebral body 2 fracture 11-2015 - Lumbal vertebral body 8 and 11 fracture 2010 - Proximal femur fracture left 3-2013 due to skiing accident

Chi-square was used for calculations of frequency. When the expected frequency was small ($n < 5$), the Fisher's exact test was used.
HC, hydrocortisone; HRT, hormone replacement therapy.

14 patients with secondary AI (ten females: median age 55 years, range 31–77 years) who had used conventional GC regimens for at least 12 months were changed to modified-release HC (41). After 24 months of treatment with modified-release HC, a significant increase in BMD values at lumbar spine and femoral neck was observed, without statistically significant change in total hip BMD.

Our study has a number of limitations. In general, the patient cohort was heterogeneous in terms of etiology of PAI and forms of CAH. Subgroups were too small for further subanalysis of PAI and CAH. Also the presence or absence of bisphosphonate and/or DHEA therapy might play a role as confounder, especially due to the fact that the lowest proportion of patients on bisphosphonate therapy occurred in the modified-release HC group and the highest proportion of patients on DHEA therapy occurred in the prednisolone group. Furthermore, this study was not randomized and consisted of only 44 patients. We cannot exclude influences of previous GC replacement regimens before study inclusion on the current BMD status of your cohort, especially episodes in the 1990s and 2000s – years when higher doses of GC replacement therapy were commonly used.

In summary, we showed that prednisolone seems to be worse for BMD at femur and hip in doses commonly used in hormone replacement therapy for AI (e.g. 5 mg/day). Therefore, patients should be switched either to HC therapy or prednisolone should be at least further reduced to doses of 3–4 mg/day.

Declaration of interest
Marcus Quinkler and Kathrin Zopf received honoraria for talks and consultancy from Viropharma/Shire; Tina Kienitz received honoraria for consultancy from Viropharma/Shire.

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