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ORIGINAL ARTICLE

The effects of cortisol and adrenal androgen on bone mass in Asians with and without subclinical hypercortisolism

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Abstract

Summary Analyses using the largest Korean cohort of adrenal incidentaloma (AI) revealed that subtle cortisol excess in premenopausal women and reduced dehydroepiandrosterone-sulfate (DHEA-S) in postmenopausal women and men are associated with bone mineral density (BMD) reduction in Asian patients with subclinical hypercortisolism (SH).

Introduction Few studies evaluated bone metabolism in Asians with SH. We investigated associations of cortisol and DHEA-S, an adrenal androgen, with BMD in Asians with AI, with or without SH.

Methods We used cross-sectional data of a prospective multicenter study from Korea. We measured BMD, bone turnover markers, cortisol levels after 1-mg dexamethasone suppression test (1-mg DST), DHEA-S, and baseline cortisol to DHEA-S ratio (cort/DHEA-S) in 109 AI patients with SH (18 premenopausal, 38 postmenopausal women, and 53 men) and 686 with non-functional AI (NFAI; 59 premenopausal, 199 postmenopausal women, and 428 men).

Results Pre- and postmenopausal women, but not men, with SH had lower BMDs at lumbar spine (LS) than those with NFAI (P = 0.008~0.016). Premenopausal women with SH also had lower BMDs at the hip than those with NFAI (P = 0.009~0.012). After adjusting for confounders, cortisol levels after 1-mg DST demonstrated inverse associations with BMDs at all skeletal sites only in premenopausal women (β = − 0.042~− 0.033, P = 0.019~0.040). DHEA-S had positive associations with LS BMD in postmenopausal women (β = 0.096, P = 0.001) and men (β = 0.029, P = 0.038). The cort/DHEA-S had inverse associations with LS BMD in postmenopausal women (β = − 0.081, P = 0.004) and men (β = − 0.029, P = 0.011). These inverse associations of cort/DHEA-S remained significant after adjusting for cortisol levels after 1-mg DST (β = − 0.079~− 0.026, P = 0.006~0.029). In postmenopausal women, the odds ratios of lower BMD by DHEA-S and cort/DHEA-S was 0.26 (95% CI, 0.08–0.82) and 3.40 (95% CI, 1.12–10.33), respectively.

Conclusion Subtle cortisol excess in premenopausal women and reduced DHEA-S in postmenopausal women and men may contribute to BMD reduction in Asians with SH.

Keywords Bone mineral density · Bone turnover marker · Cortisol · Dehydroepiandrosterone-sulfate (DHEA-S) · Subclinical hypercortisolism

Introduction

Adrenal incidentaloma (AI) is defined as an adrenal mass discovered during a radiologic examination that is performed for reasons unrelated to adrenal diseases. AIs have become a common clinical problem given the advances in imaging technologies [1]. Most AIs are associated with non-functional AI (NFAI) without any clinically relevant hormonal hyperfunction. However, there is an increasing number of patients with subclinical hypercortisolism (SH) with subtle cortisol excess but without a Cushing's phenotype (i.e., striae rubrae, proximal myopathy, facial plethora, easy bruising) [2–4].

The possible chronic consequences of SH, including hypertension, glucose intolerance or type 2 diabetes mellitus, obesity, and dyslipidemia are being closely investigated [2]. In addition to these, several studies have demonstrated that bone health deteriorates in SH, with decreased bone mineral density (BMD) at the lumbar spine (LS) and possibly at the femur. There is also impaired bone formation according to

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bone turnover markers (BTMs), and increased risk of vertebral fractures (VFs) [2, 4]. However, these previous studies have primarily been performed in Caucasian populations, despite the skeletal effect of cortisol excess may vary across ethnic groups [5] given the ethnicity-associated polymorphism in the glucocorticoid receptor (GR) [6] and in the enzyme 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) [7, 8]. Moreover, one study in Caucasians showed that VFs were independently associated with the ratio of baseline cortisol to dehydroepiandrosterone-sulfate (DHEA-S) (cort/DHEA-S) in women with SH or overt hypercortisolism [9]. This finding suggests that a reduction in adrenal DHEA-S secretion due to decreased adrenocorticotrophic hormone (ACTH) levels may also contribute to skeletal damage in patients with SH [2, 4]. To the best of our knowledge, however, no prior studies have evaluated the association of cort/DHEA-S with BMD in Asian patients with SH. Therefore, this study examined the associations of subtle cortisol excess and reduced adrenal DHEA-S secretion with BMD in 109 AI patients with SH (18 premenopausal women, 38 postmenopausal women, and 53 men), and in 686 NFAI patients (59 premenopausal women, 199 postmenopausal women, and 428 men) from the largest Korean cohort of AIs.

Materials and methods

Study population

The study population was based on the cross-sectional data "Co-work Of Adrenal Research (COAR)" study (clinicaltrial.gov No. NCT01382420). This was a randomized, parallel-group, multicenter, and open-labeled trial conducted at the Asan Medical Center (AMC), Samsung Medical Center (SMC), and Konkuk University Medical Center (KUMC) in Korea. Between July 2011 and June 2014, we recruited 1059 consecutive patients who were newly diagnosed with AIs. The diagnosis of AI was based on the detection of adrenal masses (≥ 1 cm) on computed tomography scans performed for unrelated diseases. We measured the following levels: basal morning ACTH and cortisol; 24-h urinary free cortisol (UFC); cortisol levels after 1-mg dexamethasone suppression test (1-mg DST); DHEA-S; 24-h urinary fractionated metanephrine; plasma renin activity; and plasma aldosterone levels [1].

We excluded patients who were suspected of having adrenal Cushing's syndrome (adrenal CS; $n = 20$), Cushing's disease (CD; $n = 1$), primary aldosteronism ($n = 61$), pheochromocytoma ($n = 60$), congenital adrenal hyperplasia ($n = 3$), adrenal carcinoma ($n = 10$), adrenal metastasis ($n = 4$), adrenal tuberculosis ($n = 2$), or pseudo-Cushing's syndrome ($n = 4$). We also excluded patients with a history of drug use in their lifetime that can influence the results of cortisol levels after 1-mg DST or bone metabolism (e.g., steroid, oral contraceptive pills, CYP3A4 inducers, thyroid hormone, bisphosphonate, or teriparatide). Patients were also excluded if they had history of a disease (such as hyperthyroidism) that could affect bone metabolism ($n = 75$). Subjects with another benign adrenal mass ($n = 24$) were excluded. The remaining 795 subjects were therefore eligible for inclusion. SH was diagnosed as cortisol levels after 1-mg DST > 138.0 nmol/L or cortisol levels after 1-mgDST > 61.0 nmol/L plus ACTH < 2.2 pmol/L or DHEA-S < 2.17 μmol/L in men or < 0.95 μmol/L in women, as described [10]. The 795 patients included 109 with AI plus SH (18 premenopausal women, 38 postmenopausal women, and 53 men) and 686 with NFAI (59 premenopausal women, 199 postmenopausal women, and 428 men) (Supplemental Fig. 1).

Patient information was collected using an interviewer-assisted questionnaire. The questionnaire included questions regarding smoking habits (current smoker or non-smoker), alcohol intake (≥ 3 U/day or < 3 U/day), regular outdoor exercise (≥ 30 min/day or < 30 min/day), history of medication use, previous medical or surgical procedures, and reproductive status (including menstruation). Height (cm) and weight (kg) were measured using a standardized protocol while subjects wore light clothing without shoes. Body mass index (BMI; kg/m^2) was calculated from their height and weight.

This study was approved by the Institutional Review Boards of AMC, SMC, and KUMC. Written informed consent was provided by all enrolled participants.

Hormonal measurements

The ACTH levels were measured with the immunoradiometric assay (IRMA) using the ELSA-ACTH kit (Cisbio bioassay, Codolet, France) on a Cobra II Gamma Counter (Packard Instrument Company, Meriden, CT, USA) at the AMC, and by IRMA using the ELSA-ACTH kit (Cisbio Bioassays, Codolet, France) on a RALS Gamma Counter GAMMA-10 (Shin Jin Medics Inc., Seoul, Republic of Korea) at the SMC. The ACTH levels were also measured with the IRMA using the ELSA-ACTH kit (Cisbio bioassay, Codolet, France) on a Wallac Wizard 1470 Gamma Counter (Perkin Elmer, Waltham, MA, USA) at the KUMC. The serum cortisol and UFC levels (after dichloromethane extraction) were measured as follows: with radioimmunoassay (RIA) using the Coat-A-Count® Cortisol kit (Siemens Healthcare Diagnostics, Los Angeles, CA, USA) on a Cobra II Gamma Counter (Packard Instrument Company) at the AMC; by RIA using the Cortisol RIA kit (Beckman Coulter) on a RALS Gamma Counter GAMMA-10 counter (Shin Jin Medics Inc.) at the SMC; and by RIA using the CORT-CT2 kit (Cisbio bioassay) on a Wallac Wizard 1470 Gamma Counter (Perkin Elmer) at the KUMC. The DHEA-S level was measured as follows: by RIA using the Coat-A-

Count® DHEA-SO_4 kit (Siemens Healthcare Diagnostics) on a Cobra II Gamma Counter (Packard Instrument Company) at the AMC; by RIA using the DHEA-S RIA CT kit (Asbach Medical Products, Obrigheim, Germany) on a RALS Gamma Counter GAMMA-10 (Shin Jin Medics Inc.) at the SMC; and by chemiluminescent immunoassay on a ADVIA Centaur system (Siemens Healthcare Diagnostics) at the KUMC. The intra- and inter-assay coefficients of variation (CV) for all assays were ~ 5% and 10%, respectively. We performed the 1-mg DST twice and used the mean value for analysis in those with a value > 50.0 nmol/L [10].

Assessment of BMD, BTMs, and fracture

The areal BMD (g/cm^2) was measured as follows: at the LS (L1–L4) and proximal femur (femoral neck (FN) and total femur (TF)) using dual energy X-ray absorptiometry (DXA) with Lunar equipment (Prodigy, software version 9.30.044; GE Healthcare, Madison, WI, USA) at the AMC; Hologic equipment (HOLOGIC Discovery W, software version 3.3.0.1; Hologic Inc., Bedford, MA, USA) at the SMC; and Hologic equipment (HOLOGIC Discovery W, software version 12.6; Hologic Inc.) at the KUMC. The precision of the equipment was presented as the CV. The CV values for LS were 0.67%, 1.00%, and 1.00% at the AMC, SMC, and KUMC, respectively. The CV values for the FN were 1.25%, 1.00%, and 1.00% at the AMC, SMC, and KUMC, respectively.

For the BTM measurements, the morning blood samples were obtained after 12 h of fasting and analyzed clinically at each medical center. The serum C-terminal telopeptide of type I collagen (CTX) levels were measured as follows: using electrochemical-luminescence immunoassay (ECLIA) with a β-CrossLaps kit (Roche Diagnostics, Basel, Switzerland) on a Cobas-6000 analyzer (Roche Diagnostics) with intra- and inter-assay CV of 1.0–4.6 and 1.6–4.7%, respectively, at the AMC; using CROSSL kit (Roche Diagnostics) on a Roche Modular Analytics E170 module (Roche Diagnostics) with intra- and inter-assay CV of 1.2–4.7% and 1.5–5.7%, respectively, at the SMC; and using a β-CrossLaps kit on a Cobas-8000 analyzer (Roche Diagnostics), with intra- and inter-assay CV of 1.0–4.6% and 1.6–4.7%, respectively, at the KUMC. Serum bone-specific ALP (BSALP) levels were measured as follows: by enzyme immunoassay using a MicroVue BAP kit (Quidel Corp., San Diego, CA, USA) on a Model 550 Microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) with intra- and inter-assay CV of 3.6 and 4.4%, respectively, at the AMC; by CLIA using Unicel DxI 800 Immunoassay kit (Beckman Coulter, Brea, CA, USA), with intra- and inter-assay CV of 1.5–2.6% and 3.3–5.9%, respectively, at the SMC; and with intra- and inter-assay CV of 3.6 and 4.4%, respectively, at the KUMC.

Lateral thoracolumbar radiographs were obtained from all participants in order to determine the morphological VF. The VF was assessed according to the recommendations of the Working Group on Vertebral Fractures [11] and quantitatively defined as a > 20% reduction in any vertebral height measurement (i.e., anterior, middle, or posterior) [12]. Non-VFs at major osteoporosis-related locations (defined as the hip, distal radius, and proximal humerus) were assessed using a self-administered questionnaire. Patients were excluded if they suffered fractures that were clearly caused by major trauma, such as motor vehicle accidents or falls from greater than standing height. Therefore, low-trauma fractures were only included if the report was definite.

Statistical analyses

There are known differences in BMD according to sex and menopausal status. Therefore, the analyses were performed separately according to sex and menopausal status. Data are presented as means ± standard deviations (SD), medians (interquartile range), or numbers (percent) unless otherwise specified. The baseline characteristics were compared using a Student *t* test or the Mann-Whitney *U* test for continuous variables. The chi-square test was used for categorical variables. We estimated the multivariable-adjusted least-square mean levels (95% confidence intervals (CI)) of BMD according to absence or presence of SH. We compared these mean levels using analysis of covariance after adjusting for the following potential confounders: centers for BMD measurements, age, sex, BMI, current smoking, alcohol use, and regular outdoor exercise. The associations of cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S with BMD and BTM were investigated through multiple linear regression analyses after adjusting for potential confounders. In these analyses, cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S were log-transformed because of their skewed distributions. Collinearity statistics analysis, such as the variance inflation factor (VIF) of the variables, was performed to detect multicollinearity (with VIFs < 10 indicating no multicollinearity). We sought to analyze the differences in the magnitudes of the associations of cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S with BMD according to different skeletal sites. To do so, we compared the corresponding regression coefficients using a previously reported equation, which is an extension of the *t* test with β-coefficients and standard error [13]. We performed multiple logistic regression analyses to generate odds ratios (ORs) and 95% CI, which compared the odds of lower BMD according to cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S levels after adjusting for confounding factor. We defined lower BMD by a Z-score ≤ − 2.0 for premenopausal women and men aged < 50 years, or T-score ≤ − 2.5 for postmenopausal women and men aged ≥ 50 years [14]. The ability to use cortisol levels

after 1-mg DST, DHEA-S, or cort/DHEA-S for predicting lower BMD was quantified using the area under the receiver operating characteristic (ROC) curve (AUC). We also compared the clinical characteristics of subjects with SH in this study to those from a previous Italian study regarding BMD ($n = 85$) [15]. For continuous variables, Student's t test assuming non-equal variance was conducted using mean values with SD. For categorical variables, chi-square test using numbers with percentages was conducted. This study used SPSS statistical software version 18.0 (SPSS Inc., Chicago, IL, USA). P values < 0.05 were considered significant.

Results

Baseline subject characteristics

The clinical characteristics of 77 premenopausal women (18 SH and 59 NFAI), 237 postmenopausal women (38 SH and 199 NFAI), and 481 men (53 SH and 428 NFAI) are shown in Table 1. Postmenopausal women and men with SH had larger adrenal masses than did those with NFAI ($P < 0.001$~0.004). Regardless of sex and menopausal status, cortisol levels after 1-mg DST and cort/DHEA-S levels were significantly higher in patients with SH than they were in patients with NFAI ($P < 0.001$~0.003), while ACTH and DHEA-S were lower ($P < 0.001$~0.002). Among cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S, only cortisol levels after 1-mg DST was significantly higher in premenopausal SH patients than in postmenopausal SH patients ($P = 0.008$) (Table 1).

BMD and fracture according to SH status

Premenopausal women with SH had significantly lower BMD at LS (by 9.1%, $P = 0.008$), FN (by 9.5%, $P = 0.012$), and TF (by 8.9%, $P = 0.009$) than did those with NFAI, while postmenopausal women with SH had significantly lower BMD only at LS (by 7.1%, $P = 0.016$) (Fig. 1). The BMDs at all skeletal sites did not differ between men with SH and NFAI.

There were no significant differences in the prevalence of fracture between premenopausal women with SH (0 of 18, 0.0%) and NFAI (1 of 59, 1.7%, $P = 0.578$), postmenopausal women with SH (0 of 38, 0.0%) and NFAI (8 of 199, 4.0%, $P = 0.209$), or men with SH (0 of 53, 0.0%) and NFAI (3 of 428, 0.7%, $P > 0.999$) (Table 1).

Association of cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S with BMD and BTMs

In premenopausal women, cortisol levels after 1-mg DST had negative associations with BMDs at all skeletal sites ($P = 0.019$~0.040), while both DHEA-S and cort/DHEA-S showed no associations ($P = 0.155$~0.344) (Table 2). In postmenopausal women, both DHEA-S and cort/DHEA-S showed positive ($P = 0.001$) and negative ($P = 0.004$) associations only with LS BMD, respectively, while cortisol levels after 1-mg DST showed no association ($P = 0.138$). There was no association of cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S with BTMs in both pre- and postmenopausal women. In premenopausal women, there were no differences in the magnitudes of inverse associations between cortisol levels after 1-mg DST and BMDs among the different skeletal sites ($P = 0.643$~0.937). In contrast, the magnitudes of positive association of DHEA-S ($\beta = 0.096$ vs. 0.005, $P = 0.014$) and inverse association of cort/DHEA-S ($\beta = -0.081$ vs. -0.001, $P = 0.019$) with LS BMD were larger than those with FN BMD in postmenopausal women.

For men, higher cortisol level after 1-mg DST was not significantly associated with either BMDs at all skeletal sites or BTMs (Table 2). The DHEA-S ($P = 0.038$) and cort/DHEA-S ($P = 0.011$) had positive and inverse associations with only LS BMDs, respectively. There was no association observed between DHEA-S, cort/DHEA-S, and BTMs. There were no differences in the magnitudes of associations between cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S and BMDs with regard to different skeletal sites.

In order to clarify the independent associations of DHEA-S or cort/DHEA-S with BMDs regardless of cortisol levels after 1-mg DST, we performed multiple linear regression analyses. These analyses included either both cortisol levels after 1-mg DST and DHEA-S or both cortisol levels after 1-mg DST and cort/DHEA-S (Table 3). In premenopausal women, cortisol levels after 1-mg DST was inversely associated with LS BMD even after adjusting for DHEA-S ($P = 0.036$, VIF = 2.029 for cortisol levels after 1-mg DST and 1.932 for DHEA-S) or cort/DHEA-S ($P = 0.028$, VIF = 2.348 for cortisol levels after 1-mg DST and 2.264 for cort/DHEA-S) without multicollinearity. In premenopausal women, cortisol levels after 1-mg DST was also inversely associated with TF BMD even after adjusting for DHEA-S ($P = 0.025$, VIF = 2.029 for cortisol levels after 1-mg DST and 1.932 for DHEA-S) without multicollinearity. In postmenopausal women, DHEA-S ($P = 0.002$, VIF = 1.081 for cortisol levels after 1-mg DST and 1.133 for DHEA-S) was positively associated and cort/DHEA-S ($P = 0.006$, VIF = 1.183 for cortisol levels after 1-mg DST and 1.105 for cort/DHEA-S) was inversely associated with LS BMD without multicollinearity. In men, cort/DHEA-S was inversely associated with LS BMD without multicollinearity ($P = 0.029$, VIF = 1.156 for cortisol levels after 1-mg DST and 1.336 for cort/DHEA-S). There were no independent associations of DHEA-S or cort/DHEA-S with BMDs at the hip in postmenopausal women and men regardless of cortisol levels after 1-mg DST.

Table 1 Baseline subject characteristics

Group	Premenopausal women (*N* = 77)			Postmenopausal women (*N* = 237)			Men (*N* = 481)		
	NFAI (*N* = 59)	SH (*N* = 18)	*P*	NFAI (*N* = 199)	SH (*N* = 38)	*P*	NFAI (*N* = 428)	SH (*N* = 53)	*P*
Age (years)	*42.5 ± 6.2*	*38.6 ± 6.1*	*0.020*	59.4 ± 7.4	59.7 ± 7.4	0.844	55.3 ± 9.6	56.9 ± 9.7	0.245
Height (cm)	160.9 ± 4.9	160.0 ± 4.5	0.471	156.6 ± 4.9	155.6 ± 4.3	0.269	169.2 ± 6.0	169.4 ± 5.9	0.862
Weight (kg)	67.7 ± 13.6	59.3 ± 11.3	0.132	61.4 ± 9.7	61.0 ± 8.8	0.841	74.3 ± 10.9	72.0 ± 11.9	0.149
BMI (kg/m^2)	25.1 ± 5.4	23.2 ± 4.5	0.180	25.1 ± 3.7	25.1 ± 3.1	0.894	25.9 ± 3.2	25.0 ± 3.7	0.057
Regular exercise	11 (18.6%)	4 (22.2%)	0.737	54 (27.1%)	12 (31.6%)	0.576	126 (29.4%)	14 (26.9%)	0.706
Current smoking	3 (5.1%)	1 (5.6%)	0.937	9 (4.5%)	1 (2.6%)	0.595	137 (32.0%)	18 (34.6%)	0.704
Drinking	1 (1.7%)	0 (0.0%)	0.578	2 (1.0%)	0 (0.0%)	0.535	29 (6.8%)	2 (3.8%)	0.417
Fracture	1 (1.7%)	0 (0.0%)	0.578	8 (4.0%)	0 (0.0%)	0.209	3 (0.7%)	0 (0.0%)	> 0.999
Adrenal mass									
Site			0.310			0.295			0.660
Bilateral	2 (3.4%)	0 (0.0%)		9 (4.5%)	2 (5.3%)		63 (14.7%)	10 (18.9%)	
Left	41 (69.5%)	10 (55.6%)		126 (63.3%)	19 (50.0%)		242 (56.5%)	30 (56.6%)	
Multiple lesions	1 (1.7%)	0 (0.0%)	0.578	15 (7.5%)	2 (5.3%)	0.619	71 (16.6%)	12 (22.6%)	0.699
Diameter (cm)	1.7 [1.3; 2.4]	2.1 [1.5; 2.6]	0.118	*1.6* [*1.2*; *2.2*]	*2.1* [*1.5*; *2.8*]	*0.004*	*1.6* [*1.2*; *2.1*]	*2.2* [*1.7*; *2.8*]	*< 0.001*
ACTH (pmol/L)	*5.3* [*3.0*; *6.2*]	*3.2* [*1.9*; *3.6*]	*0.002*	*6.6* [*3.3*; *8.4*]	*4.2* [*1.8*; *5.2*]	*0.001*	*6.8* [*4.4*; *10.2*]	*3.4* [*1.7*; *4.9*]	*< 0.001*
Cortisol (nmol/L)	295.7 [195.3; 363.6]	314.0 [236.7; 366.9]	0.302	318.9 [229.0; 408.3]	323.9 [218.0; 401.0]	0.706	342.1 [267.6; 419.3]	325.5 [251.1; 408.3]	0.661
DHEA-S (μmol/L)	*2.7* [*1.5*; *3.9*]	*0.7* [*0.3*; *0.8*]	*< 0.001*	*1.6* [*0.9*; *2.1*]	*1.1* [*0.3*; *0.6*]	*< 0.001*	*2.6* [*1.7*; *3.7*]	*1.4* [*0.9*; *2.0*]	*< 0.001*
Cortisol levels after 1-mg DST (nmol/L)	*39.5* [*27.6*; *41.4*]	*198.1* [*116.7*; *298.0*]*	*< 0.001*	*37.8* [*27.6*; *44.1*]	*138.2* [*77.0*; *161.4*]	*< 0.001*	*30.4* [*27.6*; *41.4*]	*110.4* [*82.7*; *157.3*]	*< 0.001*
Cort/DHEA-S	*0.1* [*0.1*; *0.2*]	*0.8* [*0.4*; *0.9*]	*< 0.001*	*0.3* [*0.1*; *0.4*]	*0.7* [*0.3*; *0.8*]	*< 0.001*	*0.1* [*0.1*; *0.2*]	*0.3* [*0.2*; *0.3*]	*0.003*
24-h UFC (nmol/day)	140.0 [82.3;168.2]	145.9 [94.4;172.0]	0.287	152.7 [74.8;156.6]	151.0 [82.9;128.6]	0.141	132.5 [91.6;190.7]	142.4 [99.4;222.5]	0.269
LS BMD (g/cm^2)	*1.108 ± 0.160*	*0.993 ± 0.131*	*0.007*	*0.993 ± 0.172*	*0.920 ± 0.146*	*0.020*	1.107 ± 0.186	1.088 ± 0.188	0.528
FN BMD (g/cm^2)	*0.900 ± 0.153*	*0.812 ± 0.107*	*0.028*	0.797 ± 0.130	0.775 ± 0.127	0.380	0.898 ± 0.144	0.871 ± 0.135	0.229
TF BMD (g/cm^2)	*0.972 ± 0.137*	*0.865 ± 0.096*	*0.003*	0.887 ± 0.148	0.863 ± 0.104	0.387	1.013 ± 0.150	0.964 ± 0.127	0.039

Significant results (P<0.05) are in italics

Values are expressed as means ± standard deviations or medians with interquartile ranges, or numbers with percentages, unless otherwise specified

P values were generated by a Student *t* test or the Mann-Whitney *U* test for continuous variables, and the chi-square test for categorical variables. *$P < 0.05$ compared to postmenopausal women with SH

NFAI, non-functional adrenal incidentaloma; *SH*, subclinical hypercortisolism; *BMI*, body mass index; *ACTH*, adrenocorticotrophic hormone; *DHEA-S*, dehydroepiandrosterone-sulfate; *1-mg DST*, 1-mg dexamethasone suppression test; *cort/DHEA-S*, baseline cortisol to DHEA-S ratio; *24-h UFC*, 24-h urinary free cortisol; *BMD*, bone mineral density; *LS*, lumbar spine; *FN*, femoral neck; *TF*, total femur

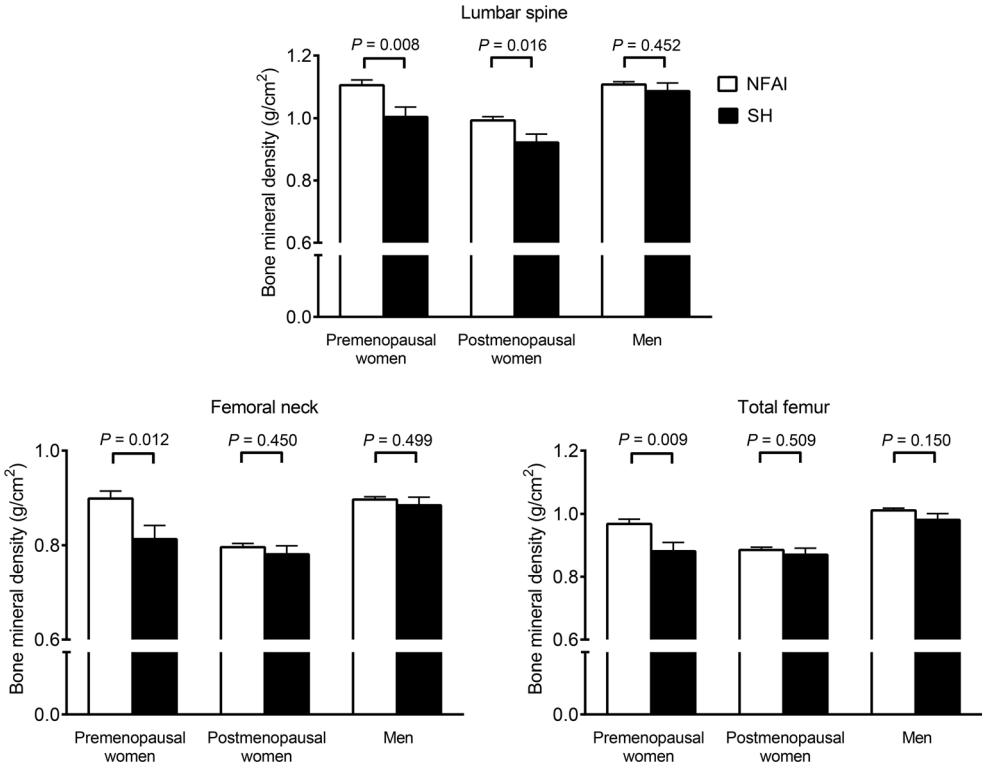

Fig. 1 Bone mineral density (BMD) at the lumbar spine, femoral neck, and total femur in subjects with non-functional adrenal incidentaloma (NFAI) and subclinical hypercortisolism (SH). We adjusted for the following confounders: centers for BMD measurements, age, body mass index, smoking, drinking, exercise. *P* values were generated using analysis of covariance

Table 2 Multivariate analyses to determine the independent associations of cortisol levels after 1-mg dexamethasone suppression test (1-mg DST), dehydroepiandrestrone-sulfate (DHEA-S), or baseline cortisol to DHEA-S ratio (cort/DHEA-S) with bone mineral density (BMD) and bone turnover markers

	Cortisol levels after 1-mg DST			DHEA-S			cort/DHEA-S		
	β	SE	*P*	*β*	SE	*P*	*β*	SE	*P*
Premenopausal women (*N* = 77)									
LS BMD	*− 0.042*	*0.017*	*0.019*	0.056	0.039	0.155	− 0.052	0.038	0.170
FN BMD	*− 0.033*	*0.016*	*0.040*	0.050	0.035	0.156	− 0.048	0.034	0.165
TF BMD	*− 0.035*	*0.015*	*0.024*	0.032	0.034	0.344	− 0.043	0.033	0.192
CTX	0.037	0.025	0.151	−0.021	0.056	0.709	0.024	0.054	0.660
BALP	0.805	1.033	0.438	−0.615	2.245	0.785	1.981	2.172	0.365
Postmenopausal women (*N* = 237)									
LS BMD	− 0.025	0.017	0.138	*0.096*	*0.030*	*0.001*	*− 0.081*	*0.028*	*0.004*
FN BMD	− 0.009	0.013	0.394	0.005	0.021	0.819	− 0.001	0.019	0.963
TF BMD	− 0.010	0.013	0.441	0.021	0.024	0.385	− 0.012	0.023	0.603
CTX	− 0.028	0.024	0.250	0.010	0.044	0.812	0.044	0.040	0.279
BALP	− 0.244	1.315	0.853	− 2.616	2.407	0.278	1.771	2.233	0.429
Male (*N* = 481)									
LS BMD	− 0.022	0.015	0.145	*0.029*	*0.014*	*0.038*	*− 0.029*	*0.011*	*0.011*
FN BMD	− 0.012	0.010	0.210	0.016	0.009	0.079	− 0.014	0.008	0.075
TF BMD	− 0.020	0.011	0.079	0.020	0.011	0.059	− 0.013	0.009	0.136
CTX	0.023	0.015	0.121	− 0.016	0.014	0.251	0.019	0.012	0.095
BALP	1.028	0.865	0.236	− 2.084	0.830	0.121	0.804	0.685	0.241

Significant results (P<0.05) are in italics

P values were determined by multiple linear regression analyses with respect to the log-transformed cortisol levels after 1-mg DST, log-transformed DHEA-S, and log-transformed cort/DHEA-S after adjusting for the following: centers for BMD measurements, age, body mass index, current smoking, drinking (≥ 3 U/day), and regular outdoor exercise (≥ 30 min/day)

LS, lumbar spine; *FN*, femoral neck; *TF*, total femur; *CTX*, C-terminal telopeptide of type I collagen; *BSALP*, bone-specific alkaline phosphatase

Table 3 Multivariate analyses to determine the associations of dehydroepiandrosterone-sulfate (DHEA-S) or baseline cortisol to DHEA-S ratio (cort/DHEA-S) with bone mineral density (BMD) independent of cortisol levels after 1-mg dexamethasone suppression test (1-mg DST)

	DHEA-S and cortisol levels after 1-mg DST						cort/DHEA-S and cortisol levels after 1-mg DST					
	DHEA-S			Cortisol levels after 1-mg DST			cort/DHEA-S			Cortisol levels after 1-mg DST		
	β	SE	*P*	*β*	SE	*P*	*β*	SE	*P*	*β*	SE	*P*
Premenopausal women (*N* = 77)												
LS BMD	− 0.019	0.051	0.707	*− 0.052*	*0.024*	*0.036*	0.036	0.054	0.502	*− 0.058*	*0.026*	*0.028*
FN BMD	0.007	0.048	0.891	− 0.030	0.022	0.182	0.002	0.050	0.962	− 0.033	0.024	0.174
TF BMD	− 0.038	0.045	0.405	*− 0.049*	*0.021*	*0.025*	0.025	0.047	0.593	− 0.045	0.023	0.052
Postmenopausal women (*N* = 237)												
LS BMD	*0.094*	*0.030*	*0.002*	− 0.005	0.016	0.777	*− 0.079*	*0.029*	*0.006*	− 0.003	0.016	0.859
FN BMD	0.002	0.021	0.933	− 0.008	0.011	0.465	0.002	0.020	0.906	− 0.008	0.012	0.495
TF BMD	0.018	0.025	0.465	− 0.008	0.013	0.533	− 0.008	0.023	0.718	− 0.008	0.013	0.549
Male (*N* = 481)												
LS BMD	0.025	0.014	0.079	− 0.015	0.015	0.321	*− 0.026*	*0.012*	*0.029*	− 0.012	0.016	0.432
FN BMD	0.014	0.010	0.139	− 0.009	0.010	0.387	− 0.012	0.008	0.145	− 0.008	0.010	0.429
TF BMD	0.016	0.011	0.137	− 0.016	0.012	0.181	− 0.009	0.009	0.323	− 0.017	0.012	0.150

Significant results (P<0.05) are in italics

P values were determined by multiple linear regression analyses after adjusting for the following: centers for BMD measurements, age, body mass index, current smoking, drinking (≥ 3 U/day), and regular outdoor exercise (≥ 30 min/day)

LS, lumbar spine; *FN*, femoral neck; *TF*, total femur

Odds ratios of lower BMD according to cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S

The ORs for lower BMD according to cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S (after adjusting for all potential confounders) are shown in Table 4. In premenopausal women, the risk for lower BMD was not associated with cortisol levels after 1-mg DST, DHEA-S, or cort/DHEA-S probably due to low incidence of lower BMD (4 of 77, 5.2%). In postmenopausal women, the ORs for lower BMD were 0.26 (95% CI, 0.08–0.82) for DHEA-S and 3.40 (95% CI, 1.12–10.33) for cort/DHEA-S after adjusting for confounders. In premenopausal women, there was no statistically significant difference in the AUCs of cortisol levels after 1-mg DST (0.726, 95% CI, 0.611–0.822) for lower BMDs compared with those of DHEA-S (0.701, 95% CI, 0.585–0.801, $P = 0.479$) and cort/DHEA-S (0.729, 95% CI, 0.615–0.825; $P = 0.888$). In postmenopausal women, the AUCs of the DHEA-S (0.755, 95% CI, 0.690–0.811) for lower BMD were similar to those of cort/DHEA-S (0.751, 95% CI, 0.686–0.808; $P = 0.877$) and higher than those of cortisol levels after 1-mg DST (0.680, 95% CI, 0.612–0.742; $P = 0.042$). In men, cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S all had no significant association with the risk of lower BMD after adjusting for potential confounders. In men, there was no statistically significant difference in the AUCs of cortisol levels after 1-mg DST (0.796, 95% CI, 0.754–0.833) for lower BMDs compared with those of DHEA-S (0.783, 95% CI, 0.741–0.821; $P = 0.281$) and cort/DHEA-S (0.786, 95% CI, 0.744–0.824; $P = 0.317$).

Table 4 Multiple logistic regression analyses of the odds ratios for lower bone mineral density (BMD) according to cortisol levels after 1-mg dexamethasone suppression test (1-mg DST), dehydroepiandrosterone-sulfate (DHEA-S), or baseline cortisol to DHEA-S ratio (cort/DHEA-S)

Variable	OR	95% CI	*P*
Postmenopausal women (*N* = 237)			
Cortisol levels after 1-mg DST	0.99	0.50–1.95	0.476
DHEA-S	*0.26*	*0.08–0.82*	*0.022*
Cort/DHEA-S	*3.40*	*1.12–10.33*	*0.031*
Men (*N* = 481)			
Cortisol levels after 1-mg DST	1.51	0.67–3.40	0.318
DHEA-S	0.70	0.36–1.27	0.299
Cort/DHEA-S	1.08	0.55–2.11	0.825

Significant results (P<0.05) are in italics

The multivariable adjustment factors in these analyses were centers for BMD measurements, age, body mass index, current smoking, drinking (≥ 3 U/day), and regular outdoor exercise (≥ 30 min/day). Lower BMD was defined by Z-score ≤ − 2.0 for men aged < 50 years or T-score ≤ − 2.5 for postmenopausal women and men aged ≥ 50 years

OR, odd ratio; *CI*, confidence interval

Table 5 Baseline characteristics of subjects participating in two large studies (Korean and Italian) for the relationship between subclinical hypercortisolism and bone mineral density

Group	Italian group ($N = 85$)	Present study ($N = 109$)	P
Age (years)	*62.9 ± 9.9 (34–79)*	*54.8 ± 11.2 (27–77)*	*< 0.001*
BMI (kg/m^2)	*29.2 ± 4.7 (21.3–40.9)*	*24.7 ± 3.7 (17.0–30.6)*	*< 0.001*
Females/males	53 (62.4%) /32 (37.6%)	56 (51.4%) /53 (48.6%)	0.189
LS BMD (Z-score)	*− 0.7 ± 1.4 (− 4.5 to 3.1)*	*− 0.0 ± 1.2 (− 2.5 to 3.3)*	*< 0.001*
FN BMD (Z-score)	*− 0.4 ± 1.1 (− 2.5 to 2.2)*	*0.2 ± 0.9 (− 1.7 to 2.3)*	*< 0.001*
No. of fractured patients (%)	*60 (70.6%)*	*0 (0.0%)*	*< 0.001*
Diameter (cm)	*2.9 ± 1.2 (0.9–5.5)*	*2.2 ± 0.7 (1.0–4.3)*	*< 0.001*
ACTH (pmol/L)	*1.6 ± 0.6 (0.2–3.6)*	*3.8 ± 1.0 (0.3–4.6)*	*< 0.001*
24-h UFC (nmol/day)	179.7 ± 86.9 (27.6–449.9)	160.6 ± 112.1 (33.1–819.7)	0.198
Cortisol levels after 1-mg DST (nmol/L)	*118.6 ± 77.3 (13.8–331.1)*	*149.0 ± 88.3 (63.5–471.8)*	*0.013*

Significant results (P<0.05) are in italics

Values are expressed as means ± standard deviations, medians with interquartile ranges, or numbers with percentages, unless otherwise specified. Data of Italian group were modified from Chiodini et al. [15]

P values were generated by Student's *t* test and chi-square tests

BMI, body mass index; *LS*, lumbar spine; *FN*, femoral neck; *BMD*, bone mineral density; *ACTH*, adrenocorticotrophic hormone; *24-h UFC*, 24-h urinary free cortisol; *1-mg DST*, 1-mg dexamethasone suppression test

Comparing the baseline characteristics with Caucasian patients in a previous study for the relationship between SH and BMD

We compared the baseline characteristics of patients with SH ($n = 109$) in our study to those of Caucasians ($n = 85$) from a previous study [15] (Table 5). The age ($P < 0.001$) and BMI ($P = 0.001$) were significantly lower, while Z-scores of the LS ($P < 0.001$) and FN ($P < 0.001$) were significantly higher in our Asian cohort than they were in the Caucasian population. The prevalence of fractures was significantly higher in the Caucasians (60 of 85, 70.6%) than in the Asians (none of 109, 0.0%) ($P < 0.001$). In addition, adenomas were significantly smaller ($P < 0.001$) and ACTH ($P < 0.001$) and cortisol levels after 1-mg DST ($P = 0.013$) were significantly higher in our Asian cohort than they were in the Caucasian population.

Discussion

In this study, Korean pre- and postmenopausal women with SH had lower LS BMD than did those with NFAI. In premenopausal women, patients with SH also had lower FN and TF BMD than did those with NFAI. However, in Korean men, there was no significant difference in the BMDs between patients with SH and NFAI. In Korean premenopausal women, cortisol levels after 1-mg DST was inversely associated with BMDs at all skeletal sites. In Korean postmenopausal women and men, DHEA-S and cort/DHEA-S had positive and negative associations with BMDs only at LS, respectively. These positive and negative associations of DHEA-S and cort/DHEA-S with LS BMDs were significant regardless of cortisol levels after 1-mg DST in postmenopausal women. The negative association of cort/DHEA-S with LS BMD in men was also significant regardless of cortisol levels after 1-mg DST. The risk of lower BMD increased according to a decrease in DHEA-S and an increase in cort/DHEA-S only in postmenopausal women.

Based on the known detrimental effects of cortisol excess on trabecular bone [16], most previous studies found a reduction in trabecular BMD at LS by DXA in patients with SH. The data regarding cortical bone were discordant due to the small sample size and diverse criteria for diagnosing SH among previous studies [15, 17]. Since these prior studies have been conducted mostly in Western countries, the bone phenotype of Asian patients with SH remains controversial. In this context, our results were similar to those from studies performed in Caucasian populations in that we found lower LS BMD in both pre- and postmenopausal women with SH and lower BMDs at the hip in premenopausal women. We also found inverse associations of cortisol levels after 1-mg DST with BMDs at all skeletal sites in premenopausal women [15, 18, 19]. Therefore, subtle cortisol excess is an important determinant for reduced BMD in patients with SH.

One interesting finding of our study was that DHEA-S, independent of subtle cortical excess, may be another important determinant of BMD reduction in postmenopausal women and men with SH (especially at LS). DHEA-S is the most abundant circulating androgen precursor secreted by adrenal glands under the regulation of ACTH [20]. Suppression of ACTH, through the increased autonomous cortisol secretion in ACTH-independent hypercortisolism, can reduce adrenal

DHEA-S secretion. Therefore, one might hypothesize that patients with SH would have a low DHEA-S level [10, 20]. Moreover, DHEA-S has been previously reported having a positive relationship with BMDs in healthy subjects [21, 22]. Also, there was more severe bone involvement in patients with adrenal CS (ACTH-independent hypercortisolism), who might have lower DHEA-S levels, than in those with CD (ACTH-dependent hypercortisolism) [23, 24]. These findings suggested that reduced DHEA-S secretion contributes to lower BMD in patients with SH [2, 9, 23]. Moreover, one study in Caucasian women with SH ($n = 35$) or overt hypercortisolism ($n = 36$) had previously shown that higher cort/DHEA-S was even a predictor of VFs [9]. However, less is known regarding the association between DHEA-S and bone metabolism in Asian patients with SH. In this study, DHEA-S and cort/DHEA-S had positive and inverse association with BMD at the LS (largely trabecular) in postmenopausal women and men, respectively. Moreover, the inverse associations of cort/DHEA-S with LS BMDs in postmenopausal women and men persisted after adjusting for cortisol levels after 1-mg DST. To our knowledge, this study is the first to show that reduced DHEA-S in SH may contribute to the reduced LS BMD, independently of subtle cortisol excess.

We also identified some differences in the association of cortisol and DHEA-S with BMD according to sex, menopausal status, and site. We found that there was a decrease in BMDs at all skeletal sites only in premenopausal women with SH. There were also inverse associations between cortisol levels after 1-mg DST and BMDs at all skeletal sites only in premenopausal women. In postmenopausal women and men, both DHEA-S and cort/DHEA-S showed positive and negative associations with BMD at LS, respectively, but not at the hip. We could not determine the exact reason for this finding here. However, these findings suggest that subtle cortisol excess may strongly contribute to the reduced BMD at both LS and hip in premenopausal women and reduced adrenal androgen may mainly contribute to the reduced LS BMD in postmenopausal women and men. Actually, premenopausal women of this study had significantly higher cortisol levels after 1-mg DST, which reflects more excess of cortisol, compared to those in postmenopausal women. This might cause more deleterious impact of subtle cortisol excess on BMDs of premenopausal women. In postmenopausal women, the loss of estrogen's protective effects on LS BMD might lead them to be more sensitive to DHEA-S, the only source of androgen in women. We also could not exactly elucidate the reason for the associations of cortisol levels after 1-mg DST, DHEA-S, and cort/DHEA-S mostly with LS BMD in our study. The increased metabolic activity of the LS (largely trabecular), compared to that at the hip [25], possibly explains these findings. Our findings also agree with those from a clinical study that showed that the difference in BMDs due to different levels of DHEA-S between adrenal CS and CD was only evident at the LS, but not at the femur [23].

Another interesting difference between the present study and prior studies in Caucasians was the frequency of fracture in patients with SH. Despite its large sample size, none of the 109 SH patients in this study experienced fractures, compared with 60 of 85 SH patients who had fractures (70.6%) in the previous study [15]. The reason to explain this discrepancy is not clear. However, our SH patients were younger and had higher BMD Z-scores at LS and FN compared to those of previous Caucasian study, which means that subjects in our study were in generally lower risk of fractures. Moreover, our SH patients showed smaller adenomas and higher ACTH compared to those of previous Caucasian study. These might reflect that our SH patients had relatively shorter duration of disease and less suppression of adrenal androgen production, which might cause in decreased BMD, but not yet increase in fractures [26]. It may also reflect ethnic differences in the skeletal effect of cortisol excess through individual sensitivity to cortisol based on polymorphisms in GR [6] and 11βHSD1 [7, 8]. Alternatively, the increased risk of fracture in SH patients may be explained by a deteriorated bone quality [15, 27, 28]. In this context, our group recently reported trabecular bone score (TBS) changes in patients with various degree of endogenous hypercortisolism using a slightly different subset of AI patients ($n = 435$; 355 NFAI, 61 SH, and 19 adrenal CS) from those of present study [29]. Although there was a little inconsistency in the relationship between parameters of endogenous hypercortisolism and LS BMD from those of our present study due to the different characteristics of study subjects, TBS was lower in female patients with endogenous hypercortisolism than those in NFAI patients. However, there was also no increase in vertebral fractures in patients with endogenous hypercortisolism in that study.

This study has several strengths. First, this is the first multicenter study performed in an Asian population that evaluated the relationship of SH with BMD and BTMs according to sex and menopausal status. In addition, we enrolled patients with newly diagnosed AI in order to minimize selection bias. Despite its strengths, this study also has several limitations. First, there was no consensus regarding the diagnosis of SH, given the lack of specific signs and symptoms of subclinical cortisol excess and the possible fluctuation between cortisol secretion over time in patients with AI [1, 2, 4]. Therefore, we used the diagnostic criteria for SH to predict the occurrence of postsurgical hypocortisolism, pre-surgical cortisol excess, and chronic complications of SH (including metabolic syndrome and low bone mass) [10]. Moreover, despite the different DXA equipment by each medical center participating in this study, we could not standardize the BMD results. Instead, we adjusted for centers for BMD measurements in all statistical analyses for excluding confounding effects

of different DXA equipment. In addition, our data were obtained from Koreans; there is a need for studies of independent cohorts.

In conclusion, this study is the first to demonstrate that subtle cortisol excess in premenopausal women and reduced levels of DHEA-S in postmenopausal women and men may contribute to the reduced BMD, especially in trabecular bone. These findings suggest that the detection of a high cortisol level after 1-mg DST and/or a low or suppressed DHEA-S level in patients with AI should trigger more detailed assessments of bone health according to sex and menopausal status.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest None.

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