Increases in Bone Mineral Density in Response to Oral Dehydroepiandrosterone Replacement in Older Adults Appear to Be Mediated by Serum Estrogens

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Context: The mechanisms by which dehydroepiandrosterone (DHEA) replacement increases bone mineral density (BMD) in older adults are not known.

Objective: The aims were to determine the effects of DHEA therapy on changes in sex hormones and IGF-I and their associations with changes in BMD.

Design, Setting, and Participants: A randomized, double-blinded, placebo-controlled trial was conducted at an academic research institution. Participants were 58 women and 61 men, aged 60–88 yr, with low serum DHEA sulfate (DHEAS) levels.

Intervention: The intervention was oral DHEA 50 mg/d or placebo for 12 months.

Main Outcome Measures: BMD and serum DHEAS, testosterone, estradiol (E_2), estrone (E_1), SHBG, IGF-I, and IGF binding protein 3 were measured before and after intervention. Free testosterone and estrogen (FEI) indices were calculated.

Results: The average changes in hip and spine BMD (DHEA *vs.* placebo) ranged from 1.1 to 1.6%. Compared with placebo, DHEA replacement increased serum DHEAS, testosterone, free testosterone index, E_1, E_2, FEI, and IGF-I (all $P < 0.001$) and decreased SHBG ($P = 0.02$) in women and, in men, increased DHEAS, E_1, FEI (all $P < 0.001$), and E_2 ($P = 0.02$) and decreased SHBG ($P = 0.037$). The changes in total and regional hip BMD were associated with 12-month E_2 (all $P \leq 0.001$) and FEI (all $P \leq 0.013$). The effects of DHEA treatment were eliminated by adjustment for 12-month E_2.

Conclusions: The significant increases in hip BMD in older adults undergoing DHEA replacement were mediated primarily by increases in serum E_2 rather than direct effects of DHEAS. (***J Clin Endocrinol Metab*** **93: 4767–4773, 2008)**

The adrenal hormone dehydroepiandrosterone (DHEA) is a major source of androgens and estrogens in postmenopausal women and older men (1). The age-related decline in DHEA and its sulfate, DHEAS, may predispose older adults to loss of bone mass secondary to a changing sex hormone milieu. Previously we reported that, when compared with placebo, DHEA replacement therapy (50 mg/d) for 1 yr improved hip (total, trochanter, and shaft regions) bone mineral density (BMD) in older women and men and lumbar spine BMD in women (2). The mechanisms by which DHEA replacement promotes an increase in BMD are not known. DHEAS may impart direct effects on bone metabolism (3) or may act through its conversion to testosterone and/or subsequent aromatization to estrogens (4). Another possibility is that the increase in serum IGF-I that is commonly observed in response to DHEA therapy exerts anabolic effects on bone (5, 6). The purpose of this study was to determine the effects of DHEA therapy on changes in serum testosterone, estrogens, IGF-I, and IGF binding protein

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Abbreviations: BAP, Bone-specific alkaline phosphatase; BMD, bone mineral density; CTX, C-terminal telopeptide of type 1 collagen; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DXA, dual-energy x-ray absorptiometry; E_1, estrone; E_2, estradiol; FEI, free estrogen index; FTI, free testosterone index; IGFBP, IGF binding protein.

(IGFBP)-3, and evaluate the independent associations of serum DHEAS, testosterone, estradiol (E_2), and IGF-I with the changes in BMD that occurred in response to DHEA replacement therapy in older women and men.

Subjects and Methods

Study participants

Participant characteristics and intent-to-treat and secondary compliance analyses of the changes in BMD and body composition in response to DHEA replacement therapy have been reported (2). Briefly, participants were women and men aged 60+ years, with low serum DHEAS (<3.8 μmol/liter, 140 μg/dl), and no use of prescribed or over-the-counter hormone therapies or oral glucocorticoids in the previous 6 months. Volunteers were excluded for unstable health and contraindications for sex hormone therapy. The study was approved by the Colorado Multiple Institutional Review Board. Written informed consent was obtained from all volunteers.

Intervention

Participants were randomly assigned with stratification by sex to receive oral DHEA 50 mg/d (Belmar Pharmacy, Lakewood, CO) or placebo for 1 yr. The intervention was administered in a double-blinded manner. The dose was selected because it raises serum DHEAS levels of older adults to the normal range for young adults (6, 7). Compliance with the intervention was assessed by measuring serum DHEAS levels at 3-month intervals. The secondary analyses reported herein included only participants compliant to the intervention (119 of 140), as previously described (2).

Procedures

Dual-energy x-ray absorptiometry (DXA)

BMD of the proximal femur (total hip, neck, trochanter, and shaft regions) and lumbar spine (L_2-L_4) was measured by DXA at baseline and after 12 months of intervention as described previously (2). Body mass, fat mass, and fat-free mass were measured by DXA.

DHEAS, sex hormones, and bone markers

Serum DHEAS was measured as described previously (2). Serum total testosterone (Beckman Coulter, Fullerton, CA), E_2, estrone (E_1), and SHBG (all Diagnostic Systems Laboratory, Webster, TX), were measured at baseline and 12 months and stored at −80 C for subsequent batched analyses by RIA. Intra- and interassay coefficients of variation (CVs) were: 1) testosterone, 2.1 and 5.1%; 2) E_2, 9.0 and 7.7%; 3) E_1, 8.7 and 11.7%; and 4) SHBG, 5.1 and 12.0%. Serum albumin was measured using the bromcresol purple method at the time of collection at baseline and 12 months (interassay CV 1.3–2.0%).

Free testosterone index (FTI) and free estradiol index (FEI) were calculated using the equations of van den Beld *et al.* (8). Testosterone binding constants for albumin and SHBG were 4.06×10^4 and 5.97×10^8 liters/mol, respectively; E_2 binding constants for albumin and SHBG were 4.21×10^4 and 3.15×10^8 liters/mol, respectively (9).

Serum C-terminal telopeptide of type 1 collagen (CTX) was measured by ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark) and bone-specific alkaline phosphatase (BAP; Quidel Corp., San Diego, CA) by enzyme immunoassay at baseline and 12 months. The intra- and interassay CVs were 6.6 and 17.6% for CTX and 3.0 and 13.1% for BAP.

Statistical analyses

The primary outcome variables were specified *a priori* as the 12-month changes in BMD (expressed as percent of baseline) at the lumbar spine and hip regions. The primary explanatory variables were E_1, E_2, FEI, testosterone, FTI, and IGF-I. The 12-month concentrations, as opposed to the 12-month changes, were used because baseline testosterone levels were undetectable in some women. Separate analyses were also conducted using the 12-month changes in serum sex hormone and IGF-I concentrations to determine whether findings were consistent across approaches. Serum testosterone was assumed to be 0.58 nmol/liter (16.9 ng/dl) when concentrations were below the detection limit of 0.59 nmol/liter (17.0 ng/dl). Based on preliminary descriptive analyses, a logarithmic transformation of the hormone and IGF-I data was used to reduce skew and reduce influence from high-leverage cases. No transformation of percent BMD change was necessary.

The primary analyses used linear regression methods with percent BMD change as the outcome and 12-month serum hormone or IGF-I concentration as explanatory variables. These models were adjusted for baseline BMD to increase the precision of the inference. A sequence of multivariate regression models was used to evaluate potential mediators of DHEA effects on BMD. Specifically, the role of sex hormones as mediators of the DHEA effect on BMD was assessed by evaluating the difference between the crude (unadjusted) DHEA coefficient and the DHEA coefficient after adjustment for changes in the sex hormone(s). A difference between the crude and adjusted coefficients was evidence of mediation by the hormone. All analyses were performed in S-plus version 7 (Insightful Corporation, Seattle, WA); results are reported as estimates, 95% confidence intervals, and two-sided *P* values unless otherwise indicated.

Results

Baseline characteristics

There were no significant differences between the placebo and DHEA groups in baseline characteristics (Table 1). Body weight

TABLE 1. Baseline characteristics (mean ± SD)

	Placebo	DHEA	*P* value
Women, n	33	25	
Age, yr	69.1 (6.4)	69.3 (7.5)	0.88
Weight, kg	68.7 (14.7)	69.1 (13.4)	0.93
Height, m	1.62 (0.06)	1.61 (0.06)	0.76
Fat-free mass, kg	39.6 (4.8)	38.2 (5.5)	0.45
Fat mass, kg	27.5 (11.2)	28.8 (8.6)	0.62
Fat mass, percent of body mass	38.7 (8.2)	41.0 (5.8)	0.23
BMD, g/cm^2			
Total hip	0.836 (0.136)	0.837 (0.113)	0.98
Femoral neck	0.758 (0.154)	0.753 (0.113)	0.90
Trochanter	0.667 (0.117)	0.689 (0.096)	0.46
Femoral shaft	0.983 (0.171)	0.957 (0.148)	0.56
Lumbar spine	0.996 (0.162)	1.040 (0.177)	0.33
Men, n	31	30	
Age, yr	69.1 (6.5)	69.4 (6.7)	0.88
Weight, kg	86.8 (13.0)	81.0 (11.5)	0.07
Height, m	1.76 (0.05)	1.74 (0.06)	0.27
Fat-free mass, kg	57.3 (5.7)	54.9 (4.6)	0.08
Fat mass, kg	26.5 (9.4)	23.1 (8.4)	0.15
Fat mass, percent of body mass	29.7 (6.7)	27.8 (6.6)	0.28
BMD, g/cm^2			
Total hip	1.035 (0.148)	1.027 (0.138)	0.83
Femoral neck	0.955 (0.152)	0.927 (0.144)	0.47
Trochanter	0.865 (0.159)	0.896 (0.141)	0.43
Femoral shaft	1.201 (0.170)	1.170 (0.160)	0.48
Lumbar spine	1.241 (0.263)	1.252 (0.249)	0.87

TABLE 2. Serum sex hormones, IGF-I, and IGFBP-3

	Placebo		DHEA		
	Baseline	12 months	Baseline	12 months	*P* value[a]
Women					
DHEAS (μmol/liter)	1.2 (0.7)	1.1 (0.7)	1.5 (1.0)	7.1 (5.0)	<0.001
Total testosterone (nmol/liter)[b]	1.0 (0.3)	0.9 (0.4)	1.2 (0.8)	2.2 (1.3)	<0.001
SHBG (nmol/liter)	182.5 (83.7)	183.2 (88.4)	149.1 (67.1)	121.9 (61.7)	0.005
FTI (pmol/liter)[c]	7.0 (4.0)	8.0 (4.0)	9.0 (6.0)	25.0 (15.0)	<0.001
E_2 (pmol/liter)	116.7 (43.0)	118.9 (45.9)	113.8 (30.8)	183.2 (71.6)	<0.001
E_1 (pmol/liter)	94.7 (43.3)	94.0 (37.4)	105.0 (42.9)	187.2 (99.1)	<0.001
FEI (pmol/liter)[c]	2.2 (1.2)	2.3 (1.0)	2.3 (1.4)	5.1 (2.7)	<0.001
IGF-I (nmol/liter)	14.8 (6.6)	13.8 (5.2)	14.0 (5.3)	17.2 (6.3)	0.028
IGFBP-3 (nmol/liter)	114.8 (31.2)	117.6 (34.3)	119.7 (36.4)	118.3 (36.4)	0.941
Men					
DHEAS (μmol/liter)	1.7 (0.7)	2.9 (0.6)	1.7 (0.7)	8.8 (4.9)	<0.001
Total testosterone (nmol/liter)	13.9 (4.6)	14.1 (4.7)	13.6 (3.9)	12.4 (3.2)	0.114
SHBG (nmol/liter)	126.0 (64.3)	126.7 (66.5)	107.8 (41.4)	96.7 (39.3)	0.037
FTI (pmol/liter)	156.0 (48.0)	161.0 (42.0)	169.0 (52.0)	172.0 (56.0)	0.417
E_2 (pmol/liter)	171.4 (45.2)	175.8 (34.1)	174.7 (42.6)	235.3 (83.3)	0.001
E_1 (pmol/liter)	127.2 (58.1)	128.0 (53.3)	119.5 (53.6)	176.8 (102.1)	0.022
FEI (pmol/liter)	5.9 (1.6)	6.0 (1.4)	6.6 (1.4)	8.7 (2.9)	0.001
IGF-I (nmol/liter)	15.5 (6.5)	14.1 (5.6)	15.5 (5.6)	16.0 (6.1)	0.207
IGFBP-3 (nmol/liter)	98.7 (34.0)	96.2 (36.0)	104.6 (34.3)	104.0 (36.0)	0.413

Values are mean (SD).

[a] Between-group comparisons of 12-month values; differences between groups at baseline were not significant ($P > 0.05$).

[b] Total testosterone was below the limit of detection (0.59 nmol/liter) in 26 women (11 placebo, 14 DHEA) at baseline and 16 women (11 placebo, five DHEA) at 12 months.

[c] Cases with undetectable total testosterone concentrations were assumed to be 0.58 nmol/liter for the calculations of the FTI and FEI.

and fat-free mass tended ($P = 0.07$ and $P = 0.08$, respectively) to be greater in the men in the placebo arm compared with men in the DHEA arm. Relative body fat was not significantly different between the placebo and DHEA groups in the women or men.

There were no significant differences between the placebo and DHEA groups in baseline serum hormone concentrations, IGF-I, IGFBP-3, CTX, or BAP (Table 2 and Fig. 1). Total testosterone concentration at baseline was below the limit of detection in 25 women (11 placebo, 14 DHEA).

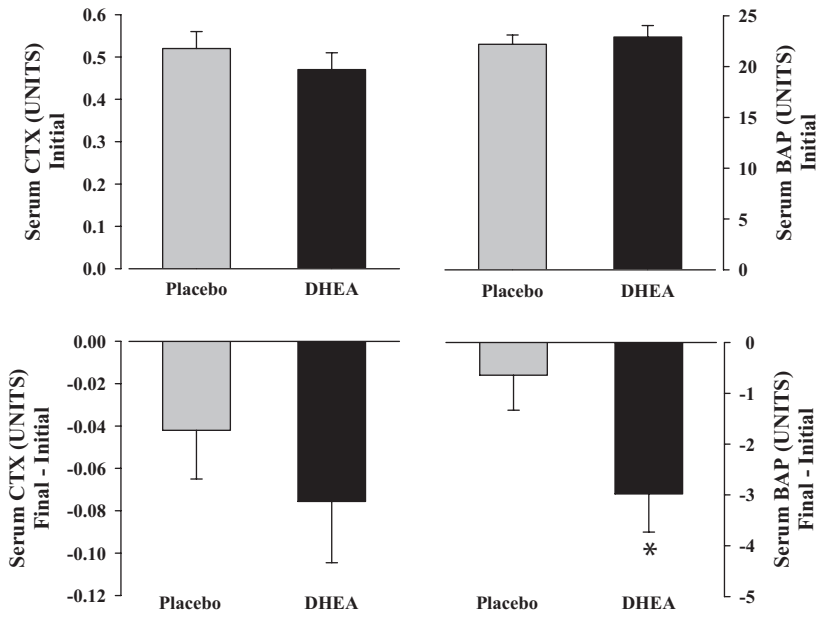

FIG. 1. Serum markers of bone resorption (CTX) and formation (BAP) before intervention (*top panels*) and changes in response to 12 months of DHEA or placebo therapy (*bottom panels*). Values are mean ± SE. *, Different from change in placebo group, $P < 0.05$.

Changes in BMD and bone markers

As previously reported (2), women and men who were compliant to DHEA replacement therapy for 1 yr had significant increases in hip BMD (total, trochanter, and shaft regions). Additionally, in exploratory sex-specific analyses, women on DHEA had a significant increase in lumbar spine BMD. Average changes in BMD (DHEA *vs.* placebo; adjusted for baseline BMD) in the cohort presented herein were: total hip, 1.14% (95% confidence interval: 0.19–2.10; $P = 0.02$); femoral shaft, 1.56% (0.26–2.86; $P = 0.02$); trochanter, 1.46% (0.17–2.75; $P = 0.03$); and lumbar spine, 1.09% (−0.24 to 2.43; $P = 0.10$). Although both CTX and BAP tended to decrease in response to DHEA therapy, only the decrease in BAP was significantly ($P = 0.02$) different from the change in the placebo group (Fig. 1).

Sex-specific changes in sex hormones and IGF-I (Table 2)

DHEA replacement therapy resulted in increases in serum DHEAS ($P < 0.001$) in women and men. In women, there were increases (all $P < 0.001$) in serum total testosterone, FTI, E_1, E_2, FEI, and IGF-I and a

decrease ($P = 0.02$) in SHBG in the DHEA group when compared with the placebo group. Total testosterone remained below the limit of detection at the end of the study in the 11 placebo-treated women in whom levels were undetectable at baseline and in five DHEA-treated women. Men in the DHEA group also had increases in FEI, E_1 (both $P < 0.001$), and E_2 ($P = 0.02$) and a decrease in SHBG ($P = 0.037$) when compared with controls. There was a trend for a reduction in total testosterone in men in the DHEA group ($P = 0.11$). IGFBP-3 concentration did not change significantly in women or men in response to DHEA replacement.

Univariate associations of sex hormones and IGF-I with changes in BMD (Table 3)

The changes in hip (total and regional) and spine BMD were not significantly associated with total testosterone, FTI, or IGF-I. In contrast, the changes in total hip, femoral shaft, and trochanter BMD were associated with E_2 (all $P \leq 0.001$) and FEI (all $P \leq 0.013$). Increases in total hip and trochanter BMD were also associated with E_1 concentrations (both $P \leq 0.02$). Because of the discordant effects of DHEA replacement on total testosterone and IGF-I by sex, we repeated these analyses in women only, but neither testosterone nor IGF-I was significantly associated with changes in BMD at any site.

TABLE 3. Associations of the 12-month serum sex hormone and IGF-I values with changes in BMD[a]

	Coefficient[b]	95% CI	*P* value
Total testosterone[c]			
Total hip BMD	0.02	(−0.29, 0.33)	0.904
Femoral shaft BMD	0.03	(−0.38, 0.43)	0.903
Trochanter BMD	−0.02	(−0.43, 0.40)	0.940
Lumbar spine BMD	0.15	(−0.26, 0.55)	0.471
FTI[c]			
Total hip BMD	0.13	(−0.15, 0.41)	0.372
Femoral shaft BMD	0.13	(−0.24, 0.50)	0.498
Trochanter BMD	0.10	(−0.28, 0.49)	0.595
Lumbar spine BMD	0.18	(−0.19, 0.55)	0.342
E_2			
Total hip BMD	2.08	(1.15, 3.00)	<0.001
Femoral shaft BMD	2.07	(0.85, 3.30)	0.001
Trochanter BMD	2.62	(1.25, 3.99)	<0.001
Lumbar spine BMD	0.93	(−0.29, 2.14)	0.137
FEI[c]			
Total hip BMD	1.01	(0.42, 1.61)	0.001
Femoral shaft BMD	1.15	(0.37, 1.93)	0.005
Trochanter BMD	1.08	(0.24, 1.92)	0.013
Lumbar spine BMD	0.57	(−0.18, 1.33)	0.142
E_1			
Total hip BMD	0.77	(0.13, 1.41)	0.020
Femoral shaft BMD	0.56	(−0.31, 1.44)	0.209
Trochanter BMD	1.35	(0.48, 2.22)	0.003
Lumbar spine BMD	0.82	(−0.04, 1.68)	0.066
IGF-I			
Total hip BMD	−0.05	(−0.88, 0.77)	0.897
Femoral shaft BMD	−0.25	(−1.37, 0.86)	0.656
Trochanter BMD	0.28	(−0.88, 1.45)	0.635
Lumbar spine BMD	−1.10	(−2.22, 0.01)	0.056

CI, Confidence interval.

[a] Adjusted for baseline BMD.

[b] Slope coefficient for the regression of the relative change in BMD on $\log_2$ of the 12-month hormone or IGF-I levels. The slope coefficient is interpreted as the percent change in BMD if the 12-month serum analyte concentration differed by 2-fold.

[c] Cases with undetectable total testosterone concentrations were assumed to be 0.58 nmol/liter for the calculations of the FTI and ETI.

TABLE 4. Associations of DHEA treatment and 12-month serum sex hormone concentrations with changes in BMD[a]

	Coefficient (*P* value)		
Model	DHEA	Testosterone[b]	E_2[b]
Total hip BMD			
DHEA	1.14 (0.019)		
T		0.00 (0.991)	
E_2			2.03 (<0.001)
$T + E_2$		−0.31 (0.056)	2.44 (<0.001)
DHEA + T	1.18 (0.018)	−0.07 (0.666)	
DHEA + E_2	0.22 (0.686)		1.91 (<0.001)
DHEA + T + E_2	0.17 (0.742)	−0.31 (0.059)	2.34 (<0.001)
Femoral shaft BMD			
DHEA	1.56 (0.019)		
T		0.01 (0.971)	
E_2			2.15 (0.001)
$T + E_2$		−0.37 (0.099)	2.68 (<0.001)
DHEA + T	1.63 (0.017)	−0.10 (0.634)	
DHEA + E_2	0.60 (0.432)		1.84 (0.016)
DHEA + T + E_2	0.56 (0.460)	−0.36 (0.105)	2.38 (0.004)
Trochanter BMD			
DHEA	1.46 (0.027)		
T		−0.03 (0.878)	
E_2			2.31 (0.001)
$T + E_2$		−0.34 (0.120)	2.75 (<0.001)
DHEA + T	1.49 (0.025)	−0.08 (0.688)	
DHEA + E_2	0.62 (0.386)		2.00 (0.011)
DHEA + T + E_2	0.54 (0.452)	−0.33 (0.137)	2.47 (0.004)
Lumbar spine BMD			
DHEA	1.09 (0.108)		
T		0.12 (0.545)	
E_2			0.93 (0.145)
$T + E_2$		−0.04 (0.875)	0.99 (0.183)
DHEA + T	1.06 (0.125)	0.08 (0.696)	
DHEA + E_2	0.82 (0.281)		0.59 (0.401)
DHEA + T + E_2	0.81 (0.288)	−0.01 (0.969)	0.61 (0.458)

DHEA, DHEA replacement group; T, serum total testosterone.

[a] Adjusted for baseline BMD.

[b] $\log_2$.

Mediating effects of sex hormones on BMD (Table 4)

The potential mediating effects of sex hormones on BMD were evaluated by determining whether there was a substantial change in significance of the unadjusted compared with hormone-adjusted DHEA effects. For example, adjusting for E_2 concentrations abolished the significant association of DHEA treatment with change in hip BMD (unadjusted $P = 0.019$, adjusted $P = 0.686$). Using total hip BMD as an example, Table 4 illustrates the following: 1) in the T+E_2 model, the association of E_2 remained as strong or stronger than in the univariate model (E_2 alone), and there was a trend ($P = 0.056$) for testosterone to become inversely associated with change in hip BMD; 2) in the DHEA+T model, the association of DHEA treatment with BMD remained as strong as in the univariate model (DHEA alone) after

adjusting for testosterone; 3) in the DHEA+E_2 model, adjusting for E_2 concentrations abolished the significant univariate association of DHEA treatment with change in BMD; and 4) in the DHEA+T+E_2 model, only E_2 was significantly associated with change in BMD; as in the T+E_2 model, there was a trend ($P = 0.059$) for testosterone to become inversely associated with change in BMD. Similarly, for the femoral shaft and trochanter, the increases in BMD in response to DHEA therapy were mediated by serum E_2 and not testosterone. Although changes in lumbar spine BMD were not significantly associated with DHEA treatment, adjustment for E_2 (and not testosterone) resulted in a major shift in the DHEA coefficient (unadjusted: 1.09; adjusted: 0.82), which was consistent with the E_2 mediation effects observed for other skeletal sites. Similar results were obtained in sex-specific models and when DHEA treatment group was replaced by 12-month serum DHEAS as a continuous variable in the multivariate models (data not shown). Note that although Tables 3 and 4 have certain (*i.e.* univariate) models in common, the coefficients within these models differ because cases with missing data were excluded from the multivariate analyses.

Discussion

The primary aims of the study were to determine the effects of DHEA therapy on changes in circulating sex hormones and IGF-I concentrations and to evaluate whether the changes in these factors mediated the increases in BMD that occurred in response to DHEA replacement therapy. The major findings were that DHEA therapy resulted in significant increases in serum DHEAS and estrogens in women and men and significant increases in serum testosterone and IGF-I in women and that the effects of DHEA treatment to increase BMD of the total hip and trochanteric and shaft subregions were mediated by increases in serum estradiol but not testosterone or IGF-I.

Changes in serum sex hormones, IGF-I, and bone markers

Although the biological effects of DHEA and DHEAS in humans are not well understood, it is likely that effects are mediated through conversion to sex hormones (4). This conversion occurs in peripheral target tissues via the actions of steroidogenic enzymes (3). In both women and men, the increase in serum DHEAS concentration in response to DHEA replacement therapy was about 600%, which brought levels into the normal range for young women and men (1). This robust increase in prohormone resulted in modest changes in serum sex hormone levels. Serum E_1, E_2, and testosterone levels increased in women on DHEA by 60, 78, and 157%, respectively, and changes in men were 36, 50, and −5%, respectively.

The relatively small changes in serum sex hormone levels in response to the large increase in DHEAS were likely related to two factors. First, the capacity for synthesis of androgens and estrogens from DHEAS in target tissues is limited by steroidogenic enzyme activity. It was reported recently that saturation of androgen synthesis occurs at a serum DHEA concentration of 27.4 nmol/liter (7.9 ng/ml) (10). Although we did not measure serum DHEA in the current study, this would be roughly equivalent to a DHEAS concentration of 2.74 μmol/liter (102 μg/dl; based on 1:100 ratio of DHEA to DHEAS). Thus, serum DHEAS in the women and men on treatment in the current study exceeded levels for saturation of androgen synthesis by 2- to 3-fold. This suggests that a lower dose of DHEA than that used in the current study could optimize the conversion to sex hormones. Second, it has been suggested that only a fraction of the androgens that are synthesized locally diffuse into the circulation; it is not clear whether the same is true for estrogens (11). Support for this comes from observations that serum levels of glucuronide metabolites of androgens increase to a relatively greater extent than do androgens in response to DHEA therapy (11). As a result of the local synthesis and metabolism of androgens and estrogens in target tissues in response to DHEA therapy, it is possible that changes in serum levels of sex hormones provide only crude estimates of the potential biological effects of DHEA in bone. The enzymatic machinery for hormone production in bone is evidenced by the expression in human osteoblast-like cells of steroid-metabolizing enzymes, including some 17β-hydroxysteroid dehydrogenases (12) and aromatase (13).

The relative increases in serum testosterone, E_2, and E_1 and decrease in SHBG in response to DHEA in women were generally consistent with magnitudes of change observed in previous studies of DHEA replacement therapy (5, 7, 14–18). One notable exception was the study of Genazzani *et al.* (14), in which both early and late postmenopausal women had 5-fold increases in serum E_2 levels, from about 18 to about 90 pg/ml, in response to 12 months of oral DHEA (25 mg/d). The reason for the robust increase in E_2 in that study was not apparent.

The increase in serum E_2 and decrease in SHBG in men in the current study were consistent with previous studies of DHEA replacement therapy (7, 17, 19). However, changes in serum E_2 in response to DHEA replacement vary widely in men (5, 6, 15, 18). The lack of increase in serum testosterone in men was also consistent with previous reports (5, 7, 15, 18–21) Interestingly, both we and Nair *et al.* (17) observed trends for total serum testosterone to decrease in men in response to oral DHEA therapy. In young men treated with an aromatase inhibitor and varying doses of E_2, there was a dose-dependent effect of serum E_2 to inhibit gonadotropin secretion and a strong inverse association ($r = -0.80$) between serum E_2 and testosterone concentrations (22). This suggests that the increase in E_2 in response to DHEA therapy in men could have suppressed endogenous testosterone production.

The biological effects of DHEA and DHEAS in humans may also be mediated through the GH-IGF-I axis. Serum IGF-I responses to DHEA replacement have been varied across studies but overall support an increase in IGF-I (5–7, 14, 20, 23). We found an approximately 23% increase in serum IGF-I concentrations in women but a nonsignificant 3% increase in men in the DHEA treatment groups. As also reported by von Muhlen *et al.* (18), the increase in serum IGF-I in women was not due to an increase in IGFBP-3, suggesting greater bioavailable IGF-I. It is not clear whether DHEA replacement increases serum IGF-1 by direct action on the GH-IGF-I axis, by modulating hepatic release of IGFs or indirectly by raising circulating estrogens and

androgens. DHEA replacement did not increase the GH response to a GHRH challenge in postmenopausal women despite increased basal serum IGF-I (23). These responses argue against a direct effect of oral DHEA replacement on GH production but favor stimulation of hepatic IGF-I release. Whether the estrogenic and/or androgenic effects of DHEA replacement alter the IGF-I/IGFBP-3 system is difficult to elucidate. Short-term transdermal testosterone administration did not alter serum IGF-I or IGFBP-3 in postmenopausal women (24). These findings suggest that the effects of oral DHEA replacement on IGF-I likely result from hepatic metabolism.

The finding that DHEA therapy resulted in a decrease in serum BAP and a trend for a decrease in CTX suggests that there was an attenuation of bone turnover in the DHEA group. This is consistent with the notion that the effects of DHEA on bone were mediated through estrogen, rather than through anabolic actions of testosterone or IGF-I.

Associations of DHEA treatment and sex hormones with changes in BMD

The changes in circulating sex hormones and IGF-I that we found in response to oral DHEA replacement support the plausibility that these factors could have mediated increases in BMD. The multivariate regression analyses underscored the dominant role of serum E_2 as mediator of the increases in BMD of the total hip, femoral shaft, and greater trochanter in response to DHEA therapy. When E_2 was in the model, DHEA therapy was no longer an independent determinant of changes in BMD.

The importance of estrogens for maintaining BMD in women is well established. In men, the importance of E_2 has been demonstrated in cases of estrogen receptor (25) or aromatase anomalies (26–28) and observational studies (29, 30). Men with estrogen receptor-α deficiency or mutations of the CYP19 (P450 aromatase) gene have been reported to have osteopenia and an increased rate of bone turnover. In prospective observational studies, serum E_2 concentration was directly correlated with changes in BMD in older men (29, 30). Gennari *et al.* (29) found that the greatest losses of hip and spine BMD over 4 yr occurred in older men in the lowest quartile of free E_2 and that neither free nor total testosterone was associated with the change in BMD. Slemenda *et al.* (30) found that serum E_2 was a significant, positive correlate of radial, lumbar spine, femoral neck, and trochanteric BMD in older men who were followed up for approximately 2 yr, even after controlling for age, body weight, and serum testosterone. Interestingly, Slemenda *et al.* (30) found that testosterone was negatively associated with lumbar spine and trochanteric BMD after controlling for E_2. This latter finding is intriguing because we also found inverse, albeit not significant, associations of serum testosterone with total and regional hip BMD when E_2 was included in the multivariate models. It seems unlikely that testosterone has detrimental effects on BMD because testosterone is known to increase bone formation and inhibit bone resorption (31). This finding could be an artifact of statistical analyses based on linear models. It may also have a biological basis in that the inverse association of testosterone with BMD, after accounting for E_2, may be an indicator of low aromatase activity.

A limitation of this study was that serum measures of sex hormones and IGF-I in response to DHEA replacement are only surrogates of their bone-specific activity. It was beyond the scope of the study to differentiate the potential paracrine and/or autocrine actions of DHEAS and IGF-I.

In summary, DHEA replacement for 12 months increased circulating estrogens in women and men and testosterone and IGF-I in women. The significant increases in BMD in older women and men in response to DHEA replacement were mediated primarily by increases in serum E_2, rather than by testosterone or direct effects of DHEAS.

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