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Effects of Testosterone Replacement in Androgen-Deficient Women with Hypopituitarism: A Randomized, Double-Blind, Placebo-Controlled Study

K. K. Miller, B. M. K. Biller, C. Beauregard, J. G. Lipman, J. Jones, D. Schoenfeld, J. C. Sherman, B. Swearingen, J. Loeffler, and A. Klibanski

Neuroendocrine Unit (K.K.M., B.M.K.B., C.B., J.G.L., J.J., A.K.) and Departments of Neurosurgery (B.S.) and Radiation Oncology (J.L.), Massachusetts General Hospital Biostatistics Center (D.S.), and Psychology Assessment Center (J.C.S.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Context: Hypopituitarism in women is characterized by profound androgen deficiency due to a loss of adrenal and/or ovarian function. The effects of testosterone replacement in this population have not been reported.

Objective: The objective of the study was to determine whether physiologic testosterone replacement improves bone density, body composition, and/or neurobehavioral function in women with severe androgen deficiency secondary to hypopituitarism.

Design: This was a 12-month randomized, placebo-controlled study.

Setting: The study was conducted at a general clinical research center.

Study Participants: Fifty-one women of reproductive age with androgen deficiency due to hypopituitarism participated.

Intervention: Physiologic testosterone administration using a patch that delivers 300 μg daily or placebo was administered.

Main Outcome Measures: Bone density, fat-free mass, and fat mass were measured by dual x-ray absorptiometry. Thigh muscle and abdominal cross-sectional area were measured by computed tomography scan. Mood, sexual function, quality of life, and cognitive function were assessed using self-administered questionnaires.

Results: Mean free testosterone increased into the normal range during testosterone administration. Mean hip ($P = 0.023$) and radius ($P = 0.007$), but not posteroanterior spine, bone mineral density increased in the group receiving testosterone, compared with placebo, as did mean fat-free mass ($P = 0.040$) and thigh muscle area ($P = 0.038$), but there was no change in fat mass. Mood ($P = 0.029$) and sexual function ($P = 0.044$) improved, as did some aspects of quality of life, but not cognitive function. Testosterone at physiologic replacement levels was well tolerated, with few side effects.

Conclusions: This is the first randomized, double-blind, placebo-controlled study to show a positive effect of testosterone on bone density, body composition, and neurobehavioral function in women with severe androgen deficiency due to hypopituitarism. **(*J Clin Endocrinol Metab* 91: 1683–1690, 2006)**

TESTOSTERONE REPLACEMENT IN androgen-deficient men improves bone density, muscle mass, mood, and libido (1–3), but little is known about the effects of testosterone replacement in women with androgen deficiency. Anterior pituitary dysfunction, or hypopituitarism, is a potential complication of neoplastic or infiltrative disease, surgery, and/or radiation to the pituitary or hypothalamus. This syndrome is characterized by loss of many or all anterior pituitary hormones leading to endogenous thyroid, glucocorticoid, estrogen, and/or GH deficiency. Hypopituitarism in women results in profound androgen deficiency due to dysfunction of the adrenal glands and/or ovaries, the primary sources of androgens in women (4). Therefore, hypopituitarism provides a model of severe acquired androgen deficiency in women of reproductive age.

Data regarding the effects of testosterone administration in women are primarily derived from a menopausal population and focus on neurobehavioral effects, particularly libido and sexual function. This includes a report by Shifren *et al.* (5), who administered low-dose testosterone by patch to a group of surgically menopausal women and demonstrated an improvement in libido, sexual function, and mood in those who received testosterone, compared with placebo. The data with regard to libido and sexual function in surgically menopausal women were recently confirmed by three larger additional studies (6–8). There are few published data on the effects of low-dose testosterone replacement on bone density and body composition in women and none in women with hypopituitarism.

We report the results of a randomized, placebo-controlled study of the effects of testosterone replacement on bone density, body composition, and neurobehavioral function in a group of women with profound androgen deficiency due to hypopituitarism.

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Abbreviations: ALT, Alanine aminotransferase; ANCOVA, analysis of covariance; BDI, Beck Depression Inventory; BMD, bone mineral density; CEE, conjugated equine estrogen; CT, computed tomography; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DXA, dual x-ray absorptiometry; HDL, high-density lipoprotein; HRT, hormone replacement therapy; LDL, low-density lipoprotein; NHP, Nottingham Health Profile; PA, posteroanterior; PGWB, Psychologic General Well-Being; WASI, Wechsler Abbreviated Scale of Intelligence.

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Subjects and Methods

Study participants

Fifty-three women, aged 19–50 yr, with hypopituitarism leading to adrenal insufficiency and/or hypogonadism, participated in the study, and 51 were included in the analysis. A serum free testosterone level less than the median of the reference range for premenopausal women, 3.1 pg/ml, as determined by Esoterix Endocrinology (Calabasas Hills, CA), was required at the time of screening. All patients were estrogen replete, whether with regular menstrual periods (n = 7) or receiving exogenous hormone administration in the form of oral contraceptives or hormone replacement therapy (n = 41), for at least 1 yr before study participation. Subjects were offered Ortho-Cyclen 28 (Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ); 15 women therefore received this preparation, whereas the remainder of the subjects continued to take the preparation prescribed by their physicians before study participation. Patients were excluded from participation if receiving androgens, dehydroepiandrosterone (DHEA), supraphysiologic glucocorticoid therapy, anabolic agents, bisphosphonates, or any other medications known to affect bone density within the year before study enrollment. In addition, smokers older than 35 yr who were receiving oral contraceptives were not allowed to participate. Because of the known effects of GH on bone density, body composition, and quality of life, potential subjects were required to be GH naïve or to have been on a stable dose of GH for at least 2 yr to participate in the study. Study participants fell into four groups regarding GH status: 1) not evaluated for GH deficiency (n = 10), 2) diagnosed with GH deficiency and receiving stable replacement doses for at least 2 yr (n = 19), 3) diagnosed with GH deficiency and not receiving GH replacement (n = 18), and 4) not GH deficient (n = 4). Serum creatinine or alanine aminotransferase (ALT) more than three times the upper limit of normal were additional exclusion criteria. The study was approved by the Massachusetts General Hospital Institutional Review Board, and all subjects gave informed, written consent before study participation.

Protocol

Study subjects were recruited through referrals from physicians and through advertisements. At the screening visit, a complete medical history and physical examination were performed. Blood was drawn for measurement of free testosterone, total testosterone, ALT, and creatinine.

Each eligible subject returned for a baseline visit during which the following tests were performed. Blood was drawn for determination of free testosterone, total testosterone, SHBG, ALT, and a lipid profile [total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides], which were all measured in real time. Serum was frozen at −80 C for measurement of other hormones, including estradiol and IGF-I, which were assayed at the end of the study. Urine pregnancy tests were performed on all patients. Bone mineral density and body composition were measured by dual x-ray absorptiometry (DXA), using a Hologic QDR-4500 (Hologic Inc., Waltham, MA), with an accuracy error for bone of less than 1% (9), body fat mass of 1.7%, and fat-free mass of 2.4% (10). Cross-sectional muscle area was determined using single-slice quantitative computed tomography (CT) scans of the midthigh using 10-mm-thick images (General Electric RP High Speed Helical CT scanner, Milwaukee, WI) and graphical analysis software (General Electric Advantage Windows Workstation version 2.0; General Electric). All measurements were made in duplicate. Technical factors for the scan were as follows: 80 kVp, 70 mA, 2-sec scan time. Abdominal adipose deposition, including cross-sectional areas of total fat, sc fat, and intraabdominal fat were determined in duplicate using single-slice quantitative CT scans at the level of L4 using 10-mm-thick axial images with the same scanner and software as described above. The Beck Depression Inventory (BDI) was administered to measure mood (11). Sexual function was measured using the Derogatis Interview for Sexual Function-Female Version (12). Quality of life was measured using the RAND 36-Item Health Survey 1.0 (13, 14), the Nottingham Health Profile (NHP) (15) and the Psychologic General Well-Being (PGWB) Index questionnaire (16). Cognitive function was measured using the Wechsler Abbreviated Scale of Intelligence (WASI) (17), the Vanderberg and Kuse Mental Rotations Test (18), the Hopkins Verbal Learning Test-Revised (19), the Woodcock Johnson Cross-Out Test (20), and the Grooved Pegboard Test (21).

After completion of all baseline testing, participants were randomly assigned to receive transdermal testosterone (300 μg, Intrinsa; Procter & Gamble Pharmaceuticals, Cincinnati, OH), in the form of two 150-μg patches, placed on the abdomen and changed twice weekly, or two placebo patches, also changed twice weekly. The 150-μg patch delivers a mean 150 μg of testosterone daily, resulting in a mean time-averaged increment in serum-free testosterone concentration of 2.7 $\pm$ 0.9 pg/ml, and the free testosterone level remains relatively constant for 96 h after patch application (22). The 300-μg dose used in this study is approximately 6% of a typical 5-mg male dose. A health care professional not involved with the study monitored free testosterone levels and implemented dose reductions in subjects with serum-free testosterone levels above the upper limit of normal for women of reproductive age. Dose reductions were from 300 μg (two patches) to 150 μg (one patch). A study subject receiving placebo patches was sham dose reduced concurrently with each such subject receiving testosterone to maintain blinding of study subjects and investigators. Dose increases were not made. This resulted in nine participants being dose reduced from 300 to 150 μg testosterone and another nine, who were receiving placebo, being sham dose reduced. Randomization was stratified based on whether subjects were receiving GH, and assignments were blinded to the investigators and subjects.

Each subject returned for study visits at 1, 3, 6, 9, and 12 months after the baseline visit. Safety evaluation, including pregnancy testing, blood testing for free testosterone, ALT and lipid profile, hirsutism evaluation using the Lorenzo scale (23), and patch site evaluation, was performed at each visit, as was testing for mood, quality of life, and cognitive function end points, except for the WASI, which was administered at baseline and 12 months only. Total testosterone and SHBG were also measured at all study time points. In addition, blood was collected, and serum frozen at −80 C for measurement of estradiol and IGF-I after study completion. Bone density and body composition were measured at baseline and 6- and 12-month visits only.

Laboratory methods

Total testosterone was measured by column chromatography (Esoterix Endocrinology). The sensitivity of this assay is 3 ng/dl (to convert total testosterone to nanomoles per liter, multiply nanograms per deciliter by 0.0347) and the intraassay coefficient of variation (CV) less than 8.1%. The normal range for women of reproductive age is 10–55 ng/dl, as determined by Esoterix Endocrinology. Free testosterone concentration was calculated as the product of percent free testosterone, measured by equilibrium dialysis (Esoterix Endocrinology), and total testosterone concentration. The sensitivity of the determination of percent free testosterone by this method is 0.1%, with an intraassay CV of 6.9%. The normal range of free testosterone for women of reproductive age is 1.1–6.3 pg/ml (to convert free testosterone to picomoles per liter, multiply picograms per milliliter by 3.467), as determined by Esoterix Endocrinology. SHBG was measured by an in-house immunoradiometric assay (Esoterix Endocrinology), with a lower limit of detection of 10 nM, intraassay CV of 2.4–3.9% and a normal female range of 40–120 nM. Estradiol was determined using a RIA kit (Diagnostic Systems Laboratories, Webster, TX), with a sensitivity of 2.2 pg/ml (to convert estradiol to picomoles per liter, multiply picograms per milliliter by 3.671), an intraassay CV of 6.5–8.9%, and a normal range in women of reproductive age of 35–375 pg/ml. IGF-I was measured using a RIA kit (Nichols Institute Diagnostics, San Clemente, CA), with an intraassay CV of 2.4–3.0%, a sensitivity of 14 ng/ml (to convert IGF-I to nanomoles per liter, multiply nanograms per milliliter by 0.131), and a normal range of 182–780 ng/ml (ages 16–24 yr), 114–492 ng/ml (ages 25–39 yr), and 90–360 ng/ml (ages 40–54 yr). Total cholesterol, HDL, triglycerides, ALT, and creatinine were measured using previously described methods (24).

Statistical analysis

JMP Statistical Discoveries (version 4.0.2; SAS Institute, Inc., Cary, NC) was used for statistical analysis of bone density and body composition data and baseline clinical variables. Clinical characteristics were compared by ANOVA. All variables were tested for normality by the

Shapiro-Wilk test. For all variables not normally distributed, the Wilcoxon rank-sums test was used to determine significance. For bone density and body composition, analysis of covariance (ANCOVA) was used to determine whether there was an effect of testosterone *vs.* placebo on 12-month values, controlling for baseline values. Undetectable hormone levels were assigned values just below the lower limit of detection.

Effects of testosterone on all variables measured at more than three time points, including hormone levels, safety end points, mood, sexual function, quality of life, and cognitive function were determined using a mixed model. We examined graphs of a representative variable (BDI) without regard to treatment group to determine the best transformation to normality, independence from baseline, and homoscedasticity. We considered the following transformations: untransformed, square root, and log (y+1). Because the log (y+1) transformation was superior, we then used this transformation on all positive variables. Variables involving T or Z scores were left untransformed. Then the change from baseline was analyzed using a random intercepts model, with a fixed treatment effect and a random intercept with baseline value as a single covariate. This analysis averages the difference between the on-study value and the baseline and uses this as a measure of response to therapy. Side effect frequencies were compared using χ^2.

Statistical significance was defined as a two-tailed $P < 0.05$. Baseline clinical characteristics are mean ± SD. All other results are reported as mean ± SEM.

Results

Participant characteristics

Clinical characteristics of the study subjects are shown in Table 1, including the etiology of pituitary dysfunction. Table 1 also compares baseline clinical characteristics in those patients randomized to testosterone with those randomized to placebo patches. There were no significant differences between the two groups in the parameters tested. Fifty-nine percent of participants were depressed at baseline, as measured by the BDI. Forty-six percent had total Derogatis T scores more than 2 SD and 68% more than 1 SD below the normal mean.

One potential study subject was excluded from participation because she did not meet inclusion criteria; her free testosterone level was above the median of the reference range for premenopausal women (Fig. 1.) One participant did not receive placebo as assigned because it was learned after randomization that she was no longer eligible for study participation due to recent discontinuation of GH replacement therapy (Fig. 1.) One participant in the placebo group was excluded from analysis when it was learned that she had not met inclusion criteria (data were obtained that demonstrated primary, not secondary, adrenal insufficiency); she had discontinued study participation after the 1-month visit because of an unrelated illness (Fig. 1.)

Hormone levels

Mean free testosterone was below normal for women of reproductive age (Esoterix Endocrinology), with undetectable levels in 28 of 51 women (55%) at baseline, and increased into the normal range during testosterone administration (Fig. 2). Nine of 24 (37.8%) of subjects randomized to receive 300 μg of testosterone were dose reduced to 150 μg testosterone daily because of free testosterone levels above the upper limit of normal for women of reproductive age. Estradiol, IGF-I, and SHBG did not change with testosterone administration *vs.* placebo.

TABLE 1. Baseline clinical characteristics

	Testosterone group	Placebo group
No. of patients	24	27
Type of pituitary disease		
Pituitary adenomas	11	17
Craniopharyngiomas	4	6
Miscellaneous pituitary abnormalities[a]	9	4
Treatment		
Radiation	0	1
Surgery	8	10
Radiation and surgery	10	11
Neither surgery nor radiation	6	5
Age, yr	41.9 ± 6.2	40.0 ± 8.3
Weight, kg	74.4 ± 20.2	78.2 ± 24.1
Body mass index, kg/m^2	28.4 ± 7.2	28.8 ± 7.7
Percent IBW	132.1 ± 10.6	135.0 ± 14.7
Percent body fat (DXA)	37.6 ± 4.9	36.6 ± 7.6
Fat free mass, kg	39.7 ± 5.4	45.1 ± 8.0
BMD (DXA)		
Total hip BMD	0.92 ± .15	0.91 ± 0.13
Total hip Z score	0.0 ± 1.1	0.1 ± 1.1
Radius BMD	0.60 ± 0.07	0.60 ± 0.05
Radius Z score	0.9 ± 1.2	0.8 ± 0.7
PA spine BMD	0.97 ± 0.13	0.96 ± 0.12
PA spine Z score	−0.1 ± 1.3	−0.2 ± 1.1
Total testosterone, ng/dl[b]	8.6 ± 8.2	7.1 ± 6.1
Free testosterone, pg/ml[b]	0.6 ± 0.9	0.5 ± 0.6
SHBG, nmol/liter[b]	139 ± 67	191 ± 120
BDI score[c]	11.0 ± 8.3	12.1 ± 7.7
Derogatis total (area T score)[d]	30.9 ± 13.4	36.0 ± 17.6

Mean ± SD. SI conversion factors: To convert free testosterone to picomoles per liter, multiply by 3.467; to convert total testosterone to nanomoles per liter, multiply by 0.0347. IBW, Ideal body weight.

[a] Includes apoplexy, lymphocytic hypophysitis, Sheehan's syndrome, empty sella, brain tumors, Rathke's cleft cysts, congenital hypopituitarism, and idiopathic hypopituitarism.

[b] Normal range for women of reproductive age: free testosterone, 1.1–6.3 pg/ml; total testosterone, 10–55 ng/dl; SHBG, 40–120 nmol/liter.

[c] BDI: less than 10, no or minimal depression; 10–18, mild or moderate depression; 19–29, moderate to severe depression; more than 30, severe depression.

[d] A T score of 50 is the mean normal, with SD of 10.

Bone density

Bone density increased significantly at the hip and radius [$P = 0.023$ and $P = 0.007$, respectively, by ANCOVA). The mean percent changes at the hip in the testosterone and placebo groups were 0.9 ± 0.5 and −1.2 ± 0.6%, respectively, and at the radius 0.8 ± 0.2 and −0.5 ± 0.4%, respectively (Fig. 3). There was no significant change in PA spine bone density in patients receiving testosterone *vs.* placebo (Fig. 3). There was a trend toward an association between percent increase bone mineral density (BMD) and both percent increase fat-free mass ($R = 0.37$, $P = 0.073$) and percent increase thigh muscle area ($R = 0.35$, $P = 0.091$) at the hip but not at the spine or radius.

Body composition

Fat-free mass, as measured by DXA, increased significantly in patients receiving testosterone *vs.* placebo ($P = 0.040$ by ANCOVA), with a mean increase of 3.4 ± 0.9 and 0.6 ± 0.9% in the testosterone and placebo groups, respectively (Fig. 4). Cross-sectional CT of the thigh demonstrated

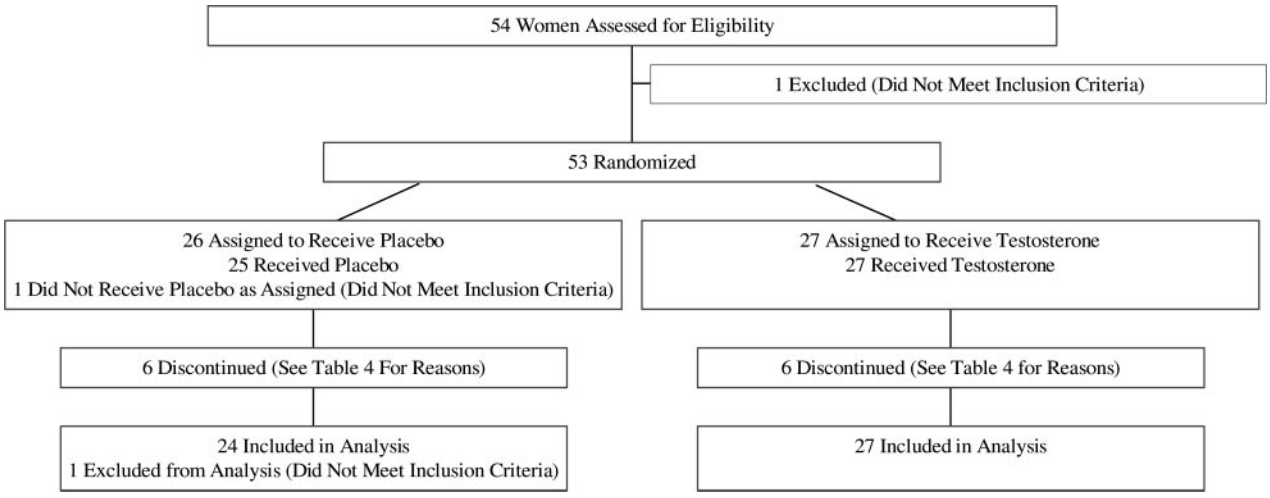

FIG. 1. Flow of participants through the study.

a significant increase in muscle area, compared with placebo ($P = 0.038$ by ANCOVA), with a mean increase in the testosterone group of 6.6 ± 1.4%, compared with 1.5 ± 1.3% in the placebo group (Fig. 4). There was no change in fat mass, as measured by DXA [1.4 ± 3.4% (placebo) *vs.* 4.8 ± 2.2% (testosterone), $P = 0.83$]. Total, intraabdominal and sc fat cross-sectional area, as measured by CT, did not change significantly with testosterone compared with placebo, nor did body weight.

Mood, sexual function, well-being, and cognitive function

Mood, as measured by BDI, improved in study subjects receiving testosterone, compared with those receiving placebo ($P = 0.029$) (Fig. 5). Sexual function improved in subjects receiving testosterone, compared with those receiving placebo ($P = 0.044$) (Fig. 6). The following subscales also improved in patients receiving testosterone, compared with placebo: arousal ($P = 0.047$) and behavior/experience ($P = 0.025$). There was no change in the following subscales: orgasm, cognition/fantasy, or drive/relationship (Fig. 6).

Women receiving testosterone demonstrated significant improvement, compared with placebo, in the following quality-of-life scales: PGWB self-control ($P = 0.005$), RAND energy/fatigue ($P = 0.017$), RAND general health ($P = 0.026$), and NHP sleep ($P = 0.038$). There was a trend toward improvement in the PGWB composite score ($P = 0.056$), the PGWB positive well-being scale ($P = 0.051$), and the NHP social isolation scale ($P = 0.060$) in subjects receiving testosterone, compared with placebo, whereas there was no improvement in other scales (Table 2).

There was a trend toward improved spatial function as measured by the WASI block design test ($P = 0.078$) but no improvement in spatial function as measured by the Vanderberg and Kuse Mental Rotations Test nor any other changes in cognitive function as measured by the other tests performed when compared with placebo (data not shown).

Side effects

Side effects in the testosterone and placebo groups are compared in Table 3. One third of patients receiving testosterone reported increased acne, compared with only one participant (4%) who received placebo ($P = 0.004$). There were no other differences in side effects between the groups. Sixty-five percent of subjects (33 of 51) experienced mild local irritation at patch sites, and three of these subjects (6% of the total number) dropped out of the study because of severe patch reactions (Table 3); patch reactions were distributed equally between the testosterone and placebo groups. All

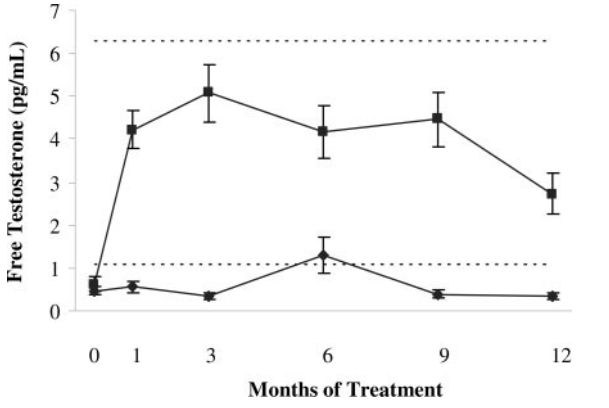

FIG. 2. Free testosterone, measured using equilibrium dialysis, in subjects with hypopituitarism receiving testosterone (■) or placebo (◆). *Horizontal dotted lines* delineate the normal range for women of reproductive age.

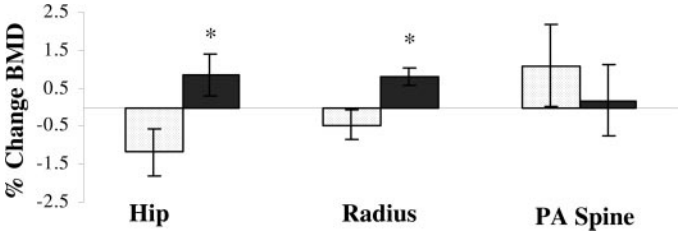

FIG. 3. Percent change in BMD over 12 months in women with hypopituitarism receiving testosterone replacement (*black bars*) or placebo (*hatched bars*). There was a significant increase in mean hip ($P = 0.023$) and radius ($P = 0.007$), but not PA spine, BMD in the group receiving testosterone, compared with the group receiving placebo. *, $P < 0.05$.

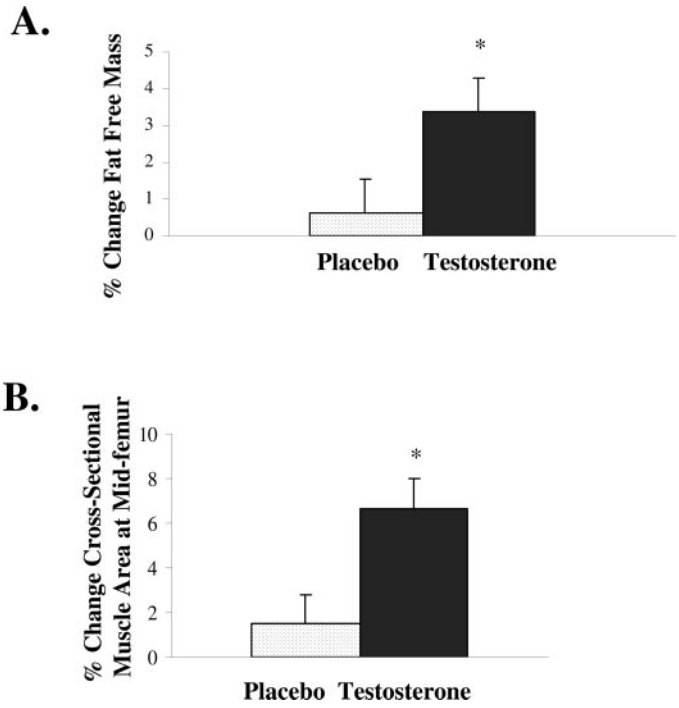

FIG. 4. A, Mean fat-free mass, as measured by DXA, increased significantly in the group of women receiving testosterone (*black bars*), compared with placebo (*hatched bars*) ($P = 0.040$). B, Cross-sectional muscle area of the thigh, as measured by CT, increased significantly in the group of women receiving testosterone (*black bars*), compared with placebo (*hatched bars*) ($P = 0.038$). *, $P < 0.05$.

rashes were local. Reasons for discontinuation of nine additional patients are delineated in Table 3.

There was a mean 6% increase in total cholesterol in women receiving testosterone, compared with placebo ($P = 0.020$). Two women in the testosterone group and one woman in the placebo group experienced increases in total cholesterol from baselines in the normal range (<200 mg/dl) to levels greater than 200 mg/dl [208 and 212 mg/dl (testosterone); 228 mg/dl (placebo) at 12 months]. There were no

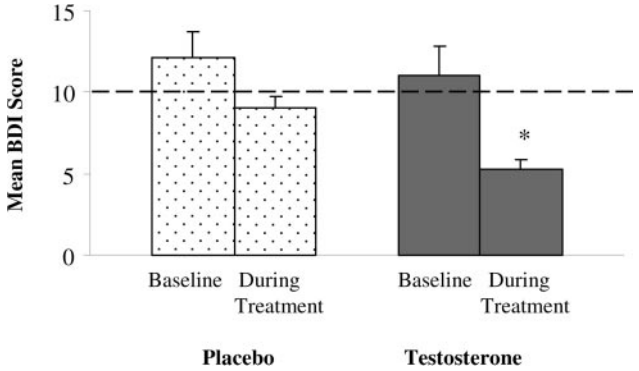

FIG. 5. Mood improved significantly in the group of women receiving testosterone replacement (*black bars*), compared with placebo (*hatched bars*) ($P = 0.029$). During treatment, values are the mean of BDI scores for all visits after randomization to testosterone (*black bars*) or placebo (*hatched bars*). Lower BDI values indicate that subjects are less depressed. BDI score less than 10: no or minimal depression (area below *horizontal dotted line*); 10–18: mild to moderate depression; 19–29: moderate to severe depression; more than 30: severe depression. *, $P < 0.05$.

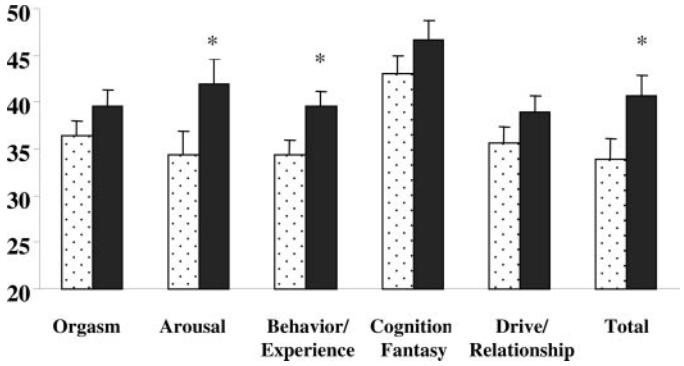

FIG. 6. Area T scores for sexual function, as measured by the Derogatis Interview for Sexual Function-Female Version. Improvements were observed in the total score ($P = 0.044$) and the arousal ($P = 0.047$) and behavior/experience ($P = 0.025$) subscales in the group of women receiving testosterone replacement (*black bars*), compared with placebo (*hatched bars*). All *bar graphs* depict 12-month values, controlled for baseline values. Area T scores are normalized percentile rankings. A value of 50 designates the normal mean, with SD of 10. *, $P < 0.05$.

significant changes in other lipids or lipoproteins, including HDL or LDL, in patients receiving testosterone, compared with placebo.

There was a trend toward a decrease in mean ALT in women receiving testosterone ($P = 0.083$), compared with placebo.

Discussion

Although the effects of testosterone replacement in hypogonadal men are well known (1–3), the effects of such replacement in androgen-deficient women are not well understood. Physiologic testosterone doses for women are a fraction of those for men, and the physiologic roles for low levels of endogenous androgens in women have not been well characterized. This randomized, placebo-controlled protocol is the first to demonstrate increases in bone density and changes in body composition due to physiologic testosterone replacement in a group of women with severe androgen deficiency. Moreover, this is the first study to show improvements in mood, sexual function, and quality of life in women with hypopituitarism receiving testosterone replacement therapy.

To our knowledge, there are two previously published randomized, controlled trials investigating the effects of testosterone on bone density in women. Both demonstrated improvements in bone density in women receiving testosterone. They differ from the current study in that both investigated postmenopausal women, an older and less severely androgen-deficient population, and they both used modes of testosterone delivery that resulted in transient supraphysiologic free testosterone levels. Davis *et al.* (25) randomized 30 postmenopausal women to testosterone plus estradiol implants *vs.* estradiol alone in a single-blind study and reported an increase in hip and spine bone density at 24 months in women receiving the testosterone, compared with those receiving estradiol alone. Miller *et al.* (26) demonstrated increases in hip, but not spine, bone density in postmenopausal women receiving sublingual testosterone in addition

TABLE 2. Quality of life questionnaires

	Testosterone group	Placebo group	Significance
PGWB			
Composite score	77.49 ± 2.66	70.53 ± 2.31	0.056
Anxiety	17.28 ± 0.80	16.58 ± 0.73	NS
Depressed mood	12.63 ± 0.43	11.68 ± 0.39	NS
General health	9.40 ± 0.44	9.81 ± 0.44	NS
Positive well-being	12.56 ± 0.57	11.05 ± 0.48	0.051
Self-control	12.63 ± 0.47	10.82 ± 0.38	0.005
Vitality	10.86 ± 0.85	9.09 ± 0.68	NS
RAND			
Energy/fatigue	44.79 ± 4.76	30.88 ± 3.28	0.017
Emotional well-being	73.69 ± 3.21	67.96 ± 2.98	NS
General health	49.19 ± 3.85	38.12 ± 2.91	0.026
Pain	74.42 ± 4.03	73.16 ± 3.82	NS
Physical functioning	72.48 ± 5.35	64.38 ± 4.63	NS
Role limitation due to emotional problems	68.42 ± 8.78	60.24 ± 7.68	NS
Role limitation due to physical health	44.33 ± 11.02	32.98 ± 7.91	NS
Social functioning	69.07 ± 4.21	71.26 ± 4.28	NS
NHP			
Emotional reaction	2.98 ± 0.85	4.10 ± 1.03	NS
Energy level	3.62 ± 1.58	7.66 ± 2.87	NS
Pain	1.29 ± 0.49	2.13 ± 0.66	NS
Physical abilities	1.07 ± 0.36	1.59 ± 0.43	NS
Sleep	2.91 ± 0.92	6.94 ± 1.84	0.038
Social isolation	1.51 ± 0.56	3.62 ± 1.00	0.060

Scores reported are the means of scores for months 1, 3, 6, 9, and 12 after randomization to testosterone or placebo, controlled for baseline. PGWB ranges: composite score 0–110; anxiety, 0–25; depressed mood, 0–15; general health, 0–15; positive well-being, 0–20; self-control, 0–15; vitality, 0–20. Higher score is indicative of better quality of life. RAND scores vary from 0 to 100. Higher scores are indicative of better quality of life. NHP scores vary from 0 to 100. Lower scores are indicative of better quality of life. NS, Not significant.

to micronized hormone replacement therapy (HRT), compared with micronized HRT alone. In the current study, we demonstrate increases in hip bone density in young women with severe androgen deficiency with testosterone replacement to physiologic levels for healthy women of reproductive age.

There are also few published studies investigating the effects of other androgens on bone density in women. Two double-blind, controlled studies of the effects of methyltestosterone, a synthetic oral compound active at androgen receptors, plus conjugated equine estrogen (CEE), compared with CEE alone in women with bilateral oophorectomy, yielded conflicting results. Barrett-Connor *et al.* (27) demonstrated increases in bone density in women with bilateral oophorectomy receiving methyltestosterone at the higher of the two doses used. However, Watts *et al.* (28) failed to detect a significant increase in bone density in the group that received this same higher dose of methyltestosterone. Methyltestosterone is not converted to testosterone but binds to androgen receptors (29) and may increase bioavailable testosterone by suppressing SHBG levels (30). Because the compound is not endogenously produced, it cannot be readily assessed whether such therapy approximates normal physiology, nor can the results be compared directly with those of our study.

TABLE 3. Related adverse events and reasons for study discontinuation

	Testosterone group (n = 24)	Placebo group (n = 27)	Significance
Related adverse events			
Skin reaction to patch	16	17	NS
Oily skin	3	1	NS
Acne	8	1	0.004
Hirsutism	7	3	NS
Alopecia	0	2	NS
Reasons for discontinuation			
Skin reaction to patch	2	1	NS
Unrelated illness	2	2	NS
Started taking DHEA	0	1	NS
Study subject preference	2	2	NS

NS, Not significant.

In the current study, hip bone density increased with 1 yr of physiologic testosterone replacement, compared with placebo, as did radial bone density, whereas spine bone density did not. These findings suggest that testosterone replacement, at the low doses that are appropriately physiologic for women, may be more effective in increasing cortical than trabecular bone density. This is consistent with our previously reported strong cross-sectional correlations of androgen levels with hip, but not spine, bone density in women with hypopituitarism (31). This is also consistent with the results of the study by Miller *et al.* (26) that showed an increase in hip, but not spine, bone density in postmenopausal women randomized to sublingual estradiol with or without progesterone plus micronized testosterone, compared with micronized HRT alone. Additional studies are necessary to investigate this hypothesis further.

We demonstrate increased fat-free mass with physiologic testosterone replacement in women with severe androgen deficiency due to hypopituitarism. Although testosterone replacement at male replacement doses, *i.e.* much higher than used in the current study of women, has been shown to increase lean body mass in men with hypogonadism (1, 3), there are few studies investigating these end points in women. In a previous report from our group, body composition did not change in women with AIDS wasting treated

with the same testosterone preparation as in our current report (32). Likewise, Davis *et al.* (33) were not able to demonstrate changes in body composition in postmenopausal women receiving testosterone plus estradiol implants, compared with estradiol alone, although they did report increases in fat-free mass, as measured by DXA, within the testosterone group when compared with baseline. Moreover, there are scant published data regarding the effects of administration of other compounds with androgenic action on body composition in women. Methyltestosterone administration has been investigated in postmenopausal women by Dobs *et al.* (34) in a randomized, placebo-controlled study that demonstrated an increase in fat-free mass, a decrease in percent body fat, and an increase in lower body strength in women receiving methyltestosterone plus CEE *vs.* CEE alone. In another study, the addition of nandrolone decanoate, an anabolic steroid with weak androgenic activity, to caloric restriction resulted in an increase in lean body mass and decrease in total body fat, compared with diet alone (35). Although these two synthetic compounds have androgenic activity, they are not equivalent to testosterone replacement. Nevertheless, their effects on body composition are consistent with our results.

Our study was not powered or designed to determine whether the combination of testosterone and GH replacement might have an additive or synergistic effect on BMD or muscle. Patients receiving GH were accepted for participation only if they had been receiving stable GH replacement for at least 2 yr. The effect of combination therapy is an important area of investigation for future trials.

This is the first study to demonstrate improvements in mood and sexual function in women with severe androgen deficiency due to hypopituitarism, which is consistent with effects of testosterone in other female populations. Shifren *et al.* (5) demonstrated improved libido, sexual function, mood and quality of life in women with androgen deficiency due to bilateral oophorectomy. Only the group that received the higher dose of testosterone, 300 μg daily, experienced improvement, compared with placebo, and subjects with supraphysiologic testosterone levels were not dose reduced. This resulted in a mean free testosterone level for the group near the upper limit of normal, raising the question of whether strict physiologic dosing would be efficacious in women (5). Three subsequent recent randomized, placebo-controlled reports (6–8) confirmed those of Shifren *et al.* (5) with respect to the effectiveness of testosterone, 300 μg daily by patch, to improve sexual function and libido in women with bilateral oophorectomy. Other, less profoundly hypoandrogenic populations have also been demonstrated to experience improvements in libido and/or sexual function with testosterone administration, including postmenopausal women (25) and premenopausal women with decreased libido (36). In the latter study, Goldstat *et al.* (36) also reported improvements in mood and quality of life with testosterone therapy. Lobo *et al.* (30) showed improved sexual function with methyltestosterone plus oral esterified estrogens, compared with esterified estrogens alone in postmenopausal women with hypoactive sexual desire.

Other studies have investigated the effects of DHEA in women with androgen deficiency due to adrenal insufficiency (37–42) or hypopituitarism (43, 44). Of note, DHEA, a compound classified by the U.S. Food and Drug Administration as a food supplement, is converted endogenously to testosterone, estradiol, and other hormones (45). Arlt *et al.* (37) studied women with adrenal insufficiency, primary or secondary, and demonstrated an improvement in libido, sexual function, and mood in subjects receiving DHEA, 50 mg daily, compared with placebo. Subsequent studies (38–44) showed less dramatic results. Some, but not all, of these studies used lower doses or combined men and women in their data analysis, which may explain some of the variance with the results of Arlt *et al.*. Trials comparing DHEA and testosterone directly will be required to determine whether one is more effective than the other.

Testosterone was well tolerated in this study, with a significant increase in acne, but not hirsutism or alopecia, in the group of women receiving testosterone, compared with placebo. A small increase in mean total cholesterol was detected, but only two subjects in the testosterone group increased from normal to slightly elevated, compared with one subject in the placebo group. There were no changes in HDL, which has been shown to decrease with oral preparations of androgens and preandrogens (37, 46), and no increase in LDL or triglycerides to explain an increase in total cholesterol. The particular preparation used in this study caused local irritation in many subjects, severe enough in some women (6% of the total) to prompt discontinuation from the study. Most of the rashes were mild, all were local, and the majority of subjects who experienced them chose to remain in the study. The irritation was not likely caused by the testosterone itself because it was experienced by similar numbers of women in both groups.

Our data demonstrate that physiologic testosterone replacement increased hip and radial bone density, fat-free mass, and thigh muscle area and improved mood, sexual function, and some aspects of quality of life in women with severe androgen deficiency due to hypopituitarism in response to physiologic testosterone replacement. In addition, testosterone at physiologic replacement levels was well tolerated with few side effects. Further studies will be needed to determine long-term efficacy and safety of such a replacement strategy.

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Address all correspondence and requests for reprints to: Karen K. Miller, Neuroendocrine Unit, Bulfinch 457B, Massachusetts General Hospital, Boston, Massachusetts 02114. E-mail: kkmiller@partners.org.

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References

1. **Katznelson L, Finkelstein J, Schoenfeld D, Rosenthal D, Anderson E, Klibanski A** 1996 Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. J Clin Endocrinol Metab 81:4358–4365
2. **Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto A, Snyder P, Weber T, Berman N, Hull L, Swerdloff R** 2004 Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. J Clin Endocrinol Metab 89:2085–2098
3. **Woodhouse L, Gupta N, Bhasin M, Singh A, Ross R, Phillips J, Bhasin S** 2004 Dose-dependent effects of testosterone on regional adipose tissue distribution in healthy young men. J Clin Endocrinol Metab 89:718–726
4. **Miller K, Sesmilo G, Schiller A, Schoenfeld D, Burton S, Klibanski A** 2001 Androgen deficiency in women with hypopituitarism. J Clin Endocrinol Metab 86:561–567
5. **Shifren J, Braunstein G, Simon J, Casson P, Buster J, Redmond G, Burki R, Ginsburg E, Rosen R, Leiblum S, Caramelli K, Mazer N** 2000 Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 343:682–688
6. **Braunstein G, Sundwall D, Katz M, Shifren J, Buster J, Simon J, Bachman G, Aguirre O, Lucas J, Rodenberg C, Buch A, Watts N** 2005 Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. Arch Intern Med 165:1582–1589
7. **Buster J, Kingsberg S, Aguirre O, Brown C, Breaux J, Buch A, Rodenberg C, Wekselman K, Casson P** 2005 Testosterone patch for low sexual desire in surgically menopausal women: a randomized trial. Obstet Gynecol 105:938–940
8. **Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, Waldbaum A, Bouchard C, Derzko C, Buch A, Rodenberg C, Lucas J, Davis S** 2005 Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 90:5226–5233
9. **Barthe N, Braillon P, Ducassou D, Basse-Cathalinat B** 1997 Comparison of two Hologic DXA systems (QDR 1000 and QDR 4500/A). Br J Radiol 70:728–739
10. **Koutkia P, Canavan B, Breu J, Torriani M, Kissko J, Grinspoon S** 2004 Growth hormone-releasing hormone in HIV-infected men with lipodystrophy. JAMA 292:210–218
11. **Beck A, Ward C, Mendelson M** 1961 An inventory for measuring depression. Arch Gen Psychiatry 4:561–571
12. **Derogatis L** 1997 The Derogatis Interview for Sexual Functioning (DISF/DISF-SR): an introductory report. J Sex Mar Ther 231:291–304
13. 1992 Rand 36-Item Health Survey 1.0. Santa Monica, CA: Rand Health Sciences Program
14. **Hays R, Sherbourne C, Mazel R** 1993 The RAND 36-Item Health Survey 1.0. Health Econ 2:217–227
15. **Hunt S, McKenna S, McKewen S, Williams J, Papp E** 1981 The Nottingham Health Profile: subjective health status and medical consultations. Soc Sci Med 15:221–229
16. **Dupuy H** 1984 The Psychological General Well-Being (PGWB) Index. In: Wenger N, Mattson M, Furberg C, Elinson J, eds. Assessment of quality of life. New York: Le Jacq Publishing, Inc.
17. 1999 Wechsler abbreviated scale of intelligence manual. San Antonio, TX: The Psychological Corp.
18. **Vandenberg S, Kuse A** 1978 Mental rotations, a group test of three-dimensional spatial visualization. Percept Mot Skills 47:599–604
19. **Shapiro A, Benedict R, Schretlen D, Brandt J** 1999 Construct and concurrent validity of the Hopkins Verbal Learning Test-revised. Clin Neuropsychol 13:348–358
20. **Woodcock R, Bonner Johnson M** 1989 Tests of cognitive ability: standard and supplemental batteries. Allen, TX: DLM Teaching Resources
21. **Mitrushina M, Boone K, D'Elia L** 1999 Handbook of normative data for neuropsychological assessment. New York: Oxford University Press
22. **Mazer N, Shifren J** 2003 Transdermal testosterone for women: a new physiological approach for androgen therapy. Obstet Gynecol Surv 58:489–500
23. **Moncada E** 1970 Familial study of hirsutism. J Clin Endocrinol Metab 31:556–564
24. **Kratz A, Ferraro M, Sluss P, Lewandrowski K** 2004 Laboratory reference values. N Engl J Med 351:1548–1563
25. **Davis SR, McCloud P, Strauss BJ, Burger H** 1995 Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. Maturitas 21:227–236
26. **Miller B, De Souza M, Slade K, Luciano A** 2000 Sublingual administration of micronized estradiol and progesterone, with and without micronized testosterone: effect on biochemical markers of bone metabolism and bone mineral density. Menopause 7:318–326
27. **Barrett-Connor E, Young R, Notelovitz M, Sullivan J, Wiita B, Yang H, Nolan J** 1999 A two-year, double-blind comparison of estrogen-androgen and conjugated estrogens in surgically menopausal women. Effects on bone mineral density, symptoms and lipid profiles. J Reprod Med 44:1012–1020
28. **Watts NB, Notelovitz M, Timmons MC, Addison WA, Wiita B, Downey LJ** 1995 Comparison of oral estrogens and estrogens plus androgen on bone mineral density, menopausal symptoms, and lipid-lipoprotein profiles in surgical menopause. Obstet Gynecol 85:529–537
29. **Wiita B, Artis A, Ackerman DM, Longcope C** 1995 Binding of 17-α-methyltestosterone in vitro to human sex hormone binding globulin and rat ventral prostate androgen receptors. Ther Drug Monit 17:377–380
30. **Lobo RA, Rosen RC, Yang HM, Block B, Van Der Hoop RG** 2003 Comparative effects of oral esterified estrogens with and without methyltestosterone on endocrine profiles and dimensions of sexual function in postmenopausal women with hypoactive sexual desire. Fertil Steril 79:1341–1352
31. **Miller K, Biller B, Hier J, Arens E, Klibanski A** 2002 Androgens and bone density in women with hypopituitarism. J Clin Endocrinol Metab 87:2770–2776
32. **Miller K, Corcoran C, Armstrong C, Caramelli K, Anderson E, Cotton D, Basgoz N, Hirschhorn L, Tuomala R, Schoenfeld D, Daugherty C, Mazer N, Grinspoon S** 1998 Transdermal testosterone administration in women with acquired immunodeficiency syndrome wasting: a pilot study. J Clin Endocrinol Metab 83:2717–2725
33. **Davis S, Walker K, Strauss B** 2000 Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. Menopause 7:395–401
34. **Dobs A, Nguyen T, Pace C, Roberts C** 2002 Differential effects of oral estrogen versus oral estrogen-androgen replacement therapy on body composition in postmenopausal women. J Clin Endocrinol Metab 87:1509–1516
35. **Lovejoy J, Bray G, Bourgeois MO, Macchiavelli R, Rood JC, Greeson C, Partington C** 1996 Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women—a clinical research center study. J Clin Endocrinol Metab 81:2198–2203
36. **Goldstat R, Briganti E, Tran J, Wolfe R, Davis S** 2003 Transdermal testosterone therapy improves well-being, mood, and sexual function in premenopausal women. Menopause 10:390–398
37. **Arlt W, Callies F, van Vlijmen J, Koehler I, Reincke M, Bidlingmaier M, Huebler D, Oettel M, Ernst M, Schulte H, Allolio B** 1999 Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 341:1013–1020
38. **Hunt P, Gurnell E, Huppert F, Richards C, Prevost A, Wass J, Herbert J, Chatterjee V** 2000 Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. J Clin Endocrinol Metab 85:4650–4656
39. **Lovas K, Gebre-Medhin G, Trovik T, Fougner K, Uhlving S, Nedrebo B, Myking O, Kampe O, Husebye E** 2003 Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. J Clin Endocrinol Metab 88:1112–1118
40. **Callies F, Fassnacht M, Christoph van Vlijmen J, Koehler I, Huebler D, Seibel M, Arlt W, Allolio B** 2001 Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. J Clin Endocrinol Metab 86:1968–1972
41. **van Thiel SW, Romijn JA, Pereira AM, Biermasz NR, Roelfsema F, van Hemert A, Ballieux B, Smit JW** 2005 Effects of DHEA, superimposed on growth hormone substitution, on quality of life and IGF-I in patients with secondary adrenal insufficiency: a randomized, placebo controlled, crossover trial. J Clin Endocrinol Metab 90:3295–3303
42. **Libe R, Barbetta L, Dall'Asta C, Salvaggio F, Gala C, Beck-Peccoz P, Ambrosi B** 2004 Effects of dehydroepiandrosterone (DHEA) supplementation on hormonal, metabolic and behavioral status in patients with hypoadrenalism. J Endocrinol Invest 27:736–741
43. **Johannsson G, Burman P, Wiren L, Engstrom BE, Nilsson AG, Ottosson M, Jonsson B, Bengtsson BA, Karlsson FA** 2002 Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. J Clin Endocrinol Metab 87:2046–2052
44. **Bilger M, Speraw S, LaFranchi SH, Hanna CE** 2005 Androgen replacement in adolescents and young women with hypopituitarism. J Pediatr Endocrinol Metab 18:355–362
45. **Young J, Couzinet B, Nahoul K, Brailly S, Chanson P, Baulieu E, Schaison G** 1997 Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. J Clin Endocrinol Metab 82:2578–2585
46. **Raisz LG, Wiita B, Artis A, Bowen A, Schwartz S, Trahiotis M, Shoukri K, Smith J** 1996 Comparison of the effects of estrogen alone and estrogen plus androgen on biochemical markers of bone formation and resorption in postmenopausal women. J Clin Endocrinol Metab 81:37–43

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