Effects on Lipoprotein Particles of Long-Term Dehydroepiandrosterone in Elderly Men and Women and Testosterone in Elderly Men

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Context: Although age-related declines in dehydroepiandrosterone sulfate (DHEAS) and testosterone are associated with cardiovascular risk, it remains to be determined whether replacement of these hormones improves cardiovascular risk factors.

Objective: This study sought to determine the effect of long-term replacement of dehydroepiandrosterone (DHEA) in elderly men and women and testosterone in elderly men on lipid and lipoprotein concentrations and particle sizes.

Methods: A 2-yr randomized, placebo-controlled, double-blind study was conducted in 87 elderly men with low levels of DHEAS and bioavailable testosterone and 57 elderly women with low levels of DHEAS. Among elderly men, 29 received DHEA (75 mg/d), 27 received testosterone (5 mg/d), and 31 received placebo. Among the elderly women, 27 received DHEA (50 mg/d), and 30 received placebo. Baseline lipoprotein profiles in the elderly were compared to healthy younger participants. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particle sizes and concentrations were quantified using nuclear magnetic resonance spectroscopy.

Results: The elderly had higher concentrations of total cholesterol, triglycerides, LDL cholesterol, total LDL particles, and small, dense LDL particles than the young. In men, neither DHEA nor testosterone affected LDL or HDL particle concentrations. In women, DHEA reduced HDL cholesterol [median difference (95% confidence intervals), −5.0 (−8.0, −2.0) mg/dl; $P = 0.002$] and the number of large HDL particles [−1.0 (−1.8, −0.2) μmol/liter; $P = 0.003$].

Conclusions: Long-term DHEA and testosterone had no significant effect on plasma lipoproteins in elderly men, but elderly women showed a lowering of the large HDL particles that may have potential adverse clinical implications. **(*J Clin Endocrinol Metab* 95: 1617–1625, 2010)**

Worldwide, the segment of the adult population 60 yr of age and older is rapidly expanding due in part to a prolonged life expectancy. This rapid expansion of the aging population is associated with concomitant increases in the incidence of many age-related chronic diseases, including cardiovascular diseases (CVDs), which places an enormous burden on health care delivery and cost. Hence, it is vital to develop strategies to prevent or delay the onset of these age-related chronic diseases. One such strategy may be to replace hormones that decline with age.

There are many observational studies in humans (1, 2) and experimental studies in animals (3, 4) that link dehydroepiandrosterone (DHEA) and cardiovascular risk. DHEA and its sulfated ester (DHEAS) are the most abundant circulating steroid hormones in the human body (5). There is a progressive decline in DHEA concentrations in

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Abbreviations: BMI, Body mass index; CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; DHEAS, sulfated form of DHEA; HDL, high-density lipoprotein cholesterol; IQR, interquartile range; LDL, low-density lipoprotein cholesterol; NMR, nuclear magnetic resonance.

both men and women with advancing age (5). Based mainly on data from observational studies in humans (1, 2) and experiments in animals (3, 4), DHEA has been marketed as an "antiaging" supplement claimed to protect against CVD and other chronic illness. Being a sex steroid precursor hormone, DHEA is thought to influence lipid metabolism and thereby CVD risk (6). Similar to DHEA, testosterone also declines with age in men (7). Cross-sectional studies have also suggested that low levels of bioavailable testosterone may lead to increased risk of coronary artery disease (CAD) in men (8, 9). In particular, testosterone deficiency has been shown to increase the atherogenic potential of lipoprotein particles (7). Therefore, we hypothesized that DHEA replacement in healthy elderly men and women, as well as testosterone replacement in healthy elderly men, would result in improvements in the lipid and lipoprotein profile.

There is growing evidence indicating that lipoprotein particle size plays a role in mitigating CVD risk, with large low-density lipoprotein (LDL) and large high-density lipoprotein (HDL) particles associated with reductions in CVD risk (10, 11). For example, families with exceptional longevity have been shown to have larger LDL and HDL particle sizes, irrespective of their total concentrations of lipids and apolipoproteins (12). Moreover, there is also an inverse relationship between CVD risk factors and LDL and HDL particle sizes (12, 13). Although the clinical utility is not fully established, lipoprotein particle sizes and concentrations assessed by nuclear magnetic resonance (NMR) have been shown to be superior to traditional lipid measures in predicting CVD risk (14).

Lipoprotein particles have been shown to exhibit age- and sex-related characteristics (15). It is known that females have less atherogenic lipoprotein profile compared with males (*e.g.* higher HDL concentrations) (15, 16). Irrespective of sex, lipoprotein particle sizes undergo undesired changes with age (17), specifically increasing their atherogenic potential. Whether replacement of DHEA or testosterone has any effect on reversing these age-related changes in lipoprotein particle profile is unknown. Due to the widespread use of these hormones, it is important to address the effectiveness and safety of DHEA or testosterone replacement in improving cardiovascular health on a long-term basis (18).

Hence, the aim of the current study was: 1) to assess the impact of age and sex on lipoprotein particle concentration and size as assessed by NMR; and 2) to evaluate the role of DHEA or low-dose testosterone replacement for 2 yr in elderly men with low DHEAS and bioavailable testosterone levels and DHEA in elderly women with low DHEAS on the lipoprotein profiles, especially on LDL and HDL particle sizes and concentrations as measured by NMR.

Subjects and Methods

Subjects

The study was approved by the Mayo Clinic Institutional Review Board (Rochester, MN). A detailed description of the study design and methodology has already been described as part of another publication (19). Briefly, this is a 2-yr randomized, double-blind, placebo-controlled study done in the absence of industry support. Potential research volunteers were recruited from the greater Rochester, Minnesota, and Olmstead county area by local radio and newspaper advertisements. Volunteers were eligible to participate in this study if they were 60 yr of age or greater and had both a DHEAS concentration less than 1.57 μg/ml (4.3 μmol/liter) and a bioavailable testosterone concentration of less than 103 ng/dl (3.6 nmol/liter) for elderly men and a DHEAS concentration of less than 0.95 μg/ml (2.6 μmol/liter) for elderly women. These cutoff values, which represented the 15th percentile of levels for normal young men and women (20), were chosen to ensure that a sufficient number of healthy elderly people could participate in the study, as previously reported (19). Exclusion criteria included evidence of clinically important coexisting illness or conditions. A total of 92 elderly men and 62 elderly women were randomly assigned to the study groups. Also included were 37 healthy young men and 38 healthy young women between the ages of 18 and 31 yr to obtain a baseline comparison of outcome measures. For the present subanalysis, only the participants with a complete set (baseline and 24-month) of unfrozen EDTA plasma samples were included, which was necessary for the NMR lipoprotein profiling. Baseline characteristics are presented in Table 1 and have been reported previously (19).

Experimental design

Elderly men received either a DHEA tablet (75 mg/d) plus a transdermal placebo patch; a placebo tablet plus a transdermal testosterone patch (5 mg/d, D-TRANS; Alza Corp., Mountain View, CA); or a placebo tablet plus a placebo transdermal patch. Elderly women received either DHEA (50 mg/d) or placebo. Every 3 months, participants received a new supply of tablets and patches from the Mayo Clinic's Research pharmacy and were asked to return any unused tablets or patches to monitor study compliance. Fasting blood samples were collected at baseline and every 3 months for the measurement of liver enzymes, hematocrit, testosterone, and DHEAS. In addition, fasting blood samples were collected at baseline and at 24 months for measurement of lipid and lipoprotein concentrations. For young participants only baseline blood samples were collected.

Analytic procedures

Plasma concentrations of DHEAS and total and bioavailable testosterone were measured by competitive chemiluminescence immunoassay. SHBG was measured by solid-phase, two-site chemiluminescence immunometric assay (Immulite; Diagnostic Products Corp., Los Angeles, CA). Bioavailable testosterone was measured on the basis of differential precipitation of SHBG by ammonium sulfate after the equilibration of serum samples with

TABLE 1. Baseline characteristics of participants

Variable label	Elderly females		Elderly males		
	Placebo	DHEA	Placebo	DHEA	Testosterone
n	30	19	29	25	23
Age (yr)	70 (66, 75)	68 (65, 69)	66 (64, 72)	68 (66, 72)	66 (62, 72)
BMI (kg/m^2)	27.9 (25.7, 30.4)	26.0 (24.7, 27.1)[b]	27.0 (25.9, 30.0)	27 (24.4, 28.7)	28.4 (25.7, 30.5)
Body fat (%)	42.4 (39.3, 45.5)	41.9 (36.9, 43.5)	29.1 (23.8, 32.0)	25.1 (21.7, 28.9)	27.5 (24.3, 29.7)
Visceral fat/total fat ratio	0.13 (0.11, 0.16)	0.11 (0.08, 0.15)	0.20 (0.14, 0.21)	0.21 (0.17, 0.23)	0.21 (0.18, 0.27)
FFM (kg)	39.7 (37.5, 42.5)	38.1 (36.2, 40.5)	62.3 (58.9, 64.6)	59.9 (57.7, 63.7)	59.7 (56.3, 64.3)
Fasting glucose (mg/dl)	94.2 (89.0, 97.1)	89.6 (86.1, 93.1)[b]	93.6 (89.2, 99.1)	93.5 (90.9, 95.8)	92.9 (89.9, 98.1)
Fasting insulin (μU/ml)	3.9 (3.1, 4.8)	3.2 (2.7, 5.4)	4.3 (3.2, 5.4)	3.7 (2.9, 5.3)	4.0 (3.2, 5.4)
Insulin sensitivity[a]	9.3 (6.4, 16.3)	12.2 (5.5, 19.6)	11.4 (6.0, 14.1)	12.7 (7.7, 16.7)	9.6 (6.7, 16.8)
C-peptide (nmol/liter)	1.2 (0.9, 1.4)	1.1 (1.0, 1.5)	1.2 (1.1, 1.5)	1.2 (0.9, 1.5)	1.1 (1.0, 1.4)
DHEAS levels (μg/ml)	0.32 (0.30, 0.43)	0.40 (0.30, 0.53)	0.73 (0.53, 1.20)	0.63 (0.50, 0.97)	0.77 (0.40, 0.90)
Total testosterone (ng/dl)	30 (27, 35)	28 (27, 33)	398 (296, 465)	390 (251, 442)	357 (282, 465)
Bioavailable testosterone (ng/dl)	NA	NA	52.8 (46.7, 62.3)	62.3 (52.4, 69.0)	56.3 (50.9, 65.3)
Estradiol (pg/ml)	8.5 (6.0, 12.7)	7.1 (5.6, 10.5)	23.5 (19.6, 27.7)	19.9 (15.4, 24.0)	20.0 (16.3, 24.2)
Bioavailable estradiol (pg/ml)	2.8 (1.6, 5.2)	2.1 (1.6, 3.2)	8.9 (7.0, 10.9)	8.3 (6.9, 10.1)	7.8 (6.3, 11.3)

Data are expressed as median (interquartile range). Normal ranges: DHEAS—women 18–30 yr old, 0.44–3.32 μg/ml; and men 18–30 yr old, 0.89–4.57 μg/ml; total testosterone—women 20–40 yr old, 10–55 ng/dl; and men 20–40 yr old, 350-1230 ng/dl; bioavailable testosterone—men 20–40 yr old, 128–430 ng/dl; estradiol—premenopausal women, 30–100 pg/ml; and men, 8.0–35.0 pg/ml; bioavailable estradiol—premenopausal women, 0.6–7.1 pg/ml; and men, 0.2–1.5 pg/ml. FFM, Fat free mass; NA, not available.

[a] Insulin sensitivity index calculated from an oral glucose minimal model as described previously (40).

[b] Wilcoxon rank sum $P < 0.05$ (elderly females: placebo *vs.* DHEA).

tracer amounts of tritium-labeled testosterone. The intraassay coefficients of variation for DHEA, total and bioavailable testosterone, and SHBG were below 7%, below 8%, below 6%, and below 5%, respectively. The interassay coefficients of variation for DHEA, total and bioavailable testosterone, and SHBG were below 12%, below 10%, below 8%, and below 6%, respectively.

The lipoprotein particle size and concentrations were measured in plasma in the Cardiovascular Medicine Laboratory at the Mayo Clinic using a 400-MHz NMR lipoprotein analyzer system developed by LipoScience Inc. (Raleigh, NC), as described previously (21). Samples were run one or two times per week in batches of 60–64 samples. The first few analysis batches contained a mixture of elderly male, elderly female, young male, and young female samples.

Statistical analysis

Baseline demographic characteristics are presented as the median with the interquartile range (IQR). Changes in the outcome measures were calculated by comparing the value at baseline with that taken at the last measurement. Baseline comparisons between treatment groups were conducted using a nonparametric Wilcoxon rank sum test. Nonparametric analyses were chosen in the present study because the lipoprotein particle data were not normally distributed. Multiple regression analyses were conducted to examine the treatment effects on the change in each of the outcome variables; the dependent variable was the change from baseline to 24 months (with the use of a rank transformation), and the independent variables included were the treatment group, the sex of the participant, the age of the participant at enrollment, length of follow-up, and the baseline value. Because approximately 25% of the elderly participants were taking statins (including atorvastatin, lovastatin, pravastatin, and simvastatin), statin therapy was considered as a potential covariate. However, because there was no effect of statin therapy on the change in any of the clinical outcomes in response to DHEA or testosterone, statin therapy was not included in the final model. Data maintenance, editing, and analysis were carried out in the Division of Biostatistics at the Mayo Clinic.

Results

The median duration of treatment within the DHEA, testosterone, and placebo groups in elderly men was 23.2 (22.6 to 23.5), 23.2 (22.2 to 23.8), and 23.1 (22.7 to 24.0) months, respectively. For elderly women, the median duration of treatment with DHEA and placebo was 23.0 (22.7 to 23.7) and 23.3 (22.4 to 23.5) months, respectively. In the group of elderly men, 31 of 32 subjects completed the placebo arm, 29 of 30 subjects completed the DHEA arm, and 27 of 30 subjects completed the testosterone arm in the parent study (19). In the group of elderly women, 30 of 30 subjects completed the placebo arm, and 27 of 30 subjects completed the DHEA arm in the parent study (19). Overall study compliance on average was above 95% for all treatments.

Baseline demographics

Table 1 summarizes the baseline characteristics for the participants included in the present analysis. There were no differences noted between groups in terms of age, weight, body mass index (BMI), total body fat, ratio of visceral fat to total body fat, visceral fat, or fat free mass. However, in elderly women there were significant differences in BMI and

fasting glucose at baseline between treatment groups. In addition, there were no significant differences observed between groups for DHEAS, total testosterone, bioavailable testosterone (in men), fasting insulin, fasting glucose concentrations, or insulin sensitivity index.

Effect of DHEA or testosterone in elderly men and DHEA in elderly women on DHEA and testosterone concentrations

The changes in DHEAS, bioavailable testosterone, and total testosterone have been previously reported (19). In brief, DHEA therapy increased the plasma levels of DHEAS by a median of 3.4 μg/ml (9.2 μmol/liter) in elderly men and by 3.8 μg/ml (10.3 μmol/liter) in elderly women. DHEAS values on therapy reached levels equivalent to that of high normal levels seen in younger adults. Treatment with DHEA increased the level of total testosterone by a median of 19.8 ng/dl (0.7 nmol/liter) in elderly women relative to placebo. In contrast, treatment with DHEA did not significantly affect total testosterone in elderly men relative to placebo. Importantly, Fig. 2 in Ref. 19 of the original publication of the results from this study demonstrates that the DHEA treatment resulted in elevations in DHEAS concentrations after 3 months of treatment and that these elevations were maintained for the duration of the study in both elderly men and elderly women. Treatment with testosterone increased the level of total testosterone by a median of 104.5 ng/dl (3.6 nmol/liter) and bioavailable testosterone by a median of 30.4 ng/dl (1.1 nmol/liter) in elderly men relative to placebo. As previously reported (22), treatment with testosterone raised total testosterone concentrations to levels seen in young adults and also raised bioavailable testosterone by approximately 50%, which would represent a partial restoration toward levels seen in young adults. The incomplete restoration of bioavailable testosterone may be due in part to a testosterone-induced suppression of endogenous testosterone production as indicated by declines in the concentrations of both LH and FSH relative to placebo (−1.8 IU/liter and 3.0 IU/liter, respectively) (19). Figure 2 from the original publication of the results from this study (19) also demonstrates that testosterone treatment resulted in elevations in bioavailable testosterone after approximately 6–9 months of treatment and that these elevations were maintained for the duration of the study in elderly men.

Baseline lipoprotein particle sizes and concentrations

The baseline values for all LDL and HDL lipoprotein particle concentrations and sizes are summarized in Tables 2 and 3, respectively. No major differences in lipoprotein

TABLE 2. Baseline and 24-month (final) LDL cholesterol profiles by sex and treatment

	Elderly females		Elderly males		
Variable label	**Placebo**	**DHEA**	**Placebo**	**DHEA**	**Testosterone**
n	30	19	29	25	23
LDL cholesterol (mg/dl)					
Baseline	108 (97, 127)	93 (85, 119)	93 (83, 112)	101 (88, 110)	105 (91, 112)
Final	108 (98, 122)	97 (77, 123)	94 (82, 108)	98 (92, 115)	93 (82, 110)
Total LDL particles (nmol/liter)					
Baseline	1195 (984, 1303)	958 (827, 1309)	1042 (834, 1323)	1167 (876, 1325)	1325 (928, 1498)
Final	1126 (988, 1386)	1057 (828, 1403)	1014 (821, 1339)	1044 (869, 1328)	1205 (1039, 1445)
Large LDL particles (nmol/liter)					
Baseline	452 (340, 582)	501 (336, 613)	401 (283, 480)	374 (264, 555)	304 (207, 445)
Final	484 (349, 627)	436 (368, 627)	376 (269, 472)	337 (205, 437)	288 (192, 528)
Medium-small LDL particles (nmol/liter)					
Baseline	129 (87, 168)	104 (61, 176)	147 (80, 195)	140 (82, 197)	201 (110, 221)
Final	128 (85, 171)	100 (64, 174)	121 (82, 202)	156 (94, 196)	177 (104, 224)
Very small LDL particles (nmol/liter)					
Baseline	511 (318, 683)	411 (253, 664)	527 (336, 841)	541 (317, 746)	822 (300, 890)
Final	532 (364, 678)	421 (232, 749)	490 (267, 767)	700 (421, 897)	586 (350, 780)
Total small LDL particles (nmol/liter)					
Baseline	648 (408, 821)	508 (321, 821)	668 (422, 1020)	679 (398, 923)	1029 (413, 1097)
Final	662 (426, 848)	522 (295, 939)	609 (334, 960)	744 (444, 965)	885 (516, 1125)
LDL mean particle size (nm)					
Baseline	21.3 (20.6, 21.8)	21.5 (20.6, 21.9)	21.1 (20.4, 21.4)	20.8 (20.3, 21.4)	20.4 (20.2, 21.6)
Final	21.3 (20.8, 21.7)	21.4 (20.5, 22.0)	20.7 (20.5, 21.5)	20.7 (20.4, 21.4)	20.6 (20.0, 21.4)

Data are expressed as median (interquartile range). No significant differences were observed between treatment groups at baseline.

TABLE 3. Baseline and 24-month (final) LDL cholesterol profiles by sex and treatment

Variable label	Elderly females		Elderly males		
	Placebo	DHEA	Placebo	DHEA	Testosterone
n	30	19	29	25	23
HDL cholesterol (mg/dl)					
Baseline	46 (40, 52)	48 (42, 55)	37 (34, 43)	38 (34, 44)	36 (30, 41)
Final	47 (40, 55)	46 (34, 54)	39 (34, 43)	36 (34, 46)	39 (35, 45)
Total HDL particles (μmol/liter)					
Baseline	31.3 (30.3, 34.7)	32.8 (28.4, 34.6)	29.1 (26.0, 31.4)	28.7 (27.5, 31.4)	29.1 (25.2, 32.7)
Final	32.3 (29.7, 35.2)	29.7 (27.0, 33.1)	29.0 (27.3, 31.4)	28.7 (26.5, 30.4)	29.7 (26.9, 31.9)
Large HDL particles (μmol/liter)					
Baseline	5.4 (4.4, 6.9)	6.0 (3.4, 8.7)	2.8 (2.5, 5.8)	3.5 (2.4, 5.4)	3.4 (2.2, 4.5)
Final	6.1 (4.1, 8.0)	5.6 (2.4, 7.8)	4.1 (2.5, 6.2)	3.7 (2.3, 6.0)	3.4 (2.5, 6.2)
Medium HDL particles (μmol/liter)					
Baseline	1.8 (0.9, 4.0)	0.9 (0.4, 3.0)	1.1 (0.5, 1.8)	0.8 (0.0, 1.5)	0.7 (0.0, 2.7)
Final	2.5 (0.0, 4.0)	4.0 (1.1, 5.0)	1.2 (0.4, 3.0)	1.5 (0.3, 2.8)	2.2 (0.4, 5.0)
Small HDL particles (μmol/liter)					
Baseline	23.7 (21.7, 26.5)	23.3 (19.3, 27.6)	23.2 (21.4, 25.5)	24.3 (19.8, 26.1)	23.4 (20.6, 27.3)
Final	22.4 (19.8, 26.1)	19.3 (17.3, 23.5)	21.9 (19.1, 24.3)	22.1 (18.8, 25.5)	22.3 (19.7, 24.8)
HDL mean particle size (nm)					
Baseline	9.0 (8.7, 9.2)	9.0 (8.6, 9.4)	8.6 (8.4, 9.1)	8.6 (8.4, 9.1)	8.6 (8.4, 8.9)
Final	9.0 (8.8, 9.2)	9.1 (8.5, 9.5)	8.7 (8.5, 9.0)	8.6 (8.5, 9.0)	8.6 (8.5, 9.0)

Data are expressed as median (interquartile range). No significant differences were observed between treatment groups at baseline.

size or concentration were noted ($P > 0.05$) between treatment groups at baseline within the elderly men. However, at baseline the elderly women in the placebo treatment group had higher triglyceride concentrations than the DHEA group [median (IQR), 107 mg/dl (87,135) *vs.* 87 mg/dl (67,113); $P = 0.026$].

Effect of age and sex on lipoprotein particle sizes and concentrations

Figure 1 shows the effect of age and sex on total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, and LDL and HDL particle concentrations. Both elderly men and women had higher total cholesterol ($P < 0.001$) and triglyceride ($P < 0.05$) concentrations compared with their younger counterparts. With regard to LDL, the elderly group had elevated LDL cholesterol ($P = 0.001$), total LDL particle ($P < 0.001$), and small, dense LDL particle concentrations ($P < 0.001$). Total HDL cholesterol, total HDL particle, and large HDL particle concentrations were higher in women compared with men ($P < 0.001$). The large HDL particles were lower in both elderly men ($P = 0.04$) and elderly women ($P = 0.03$) than their younger counterparts.

Effect of DHEA or testosterone in elderly men and DHEA in elderly women on lipoprotein particle sizes and concentrations

Table 3 summarizes the treatment effect with DHEA compared with placebo in elderly women and DHEA or testosterone compared with placebo in elderly men on lipoprotein particle size and numbers. Two years of treatment with DHEA in elderly women significantly reduced total HDL cholesterol concentrations ($P = 0.002$), total HDL particle concentrations ($P = 0.004$), and large HDL particle concentrations ($P = 0.003$) relative to placebo. In contrast, DHEA replacement did not alter the LDL particle profile in elderly women. Two years of treatment with either DHEA or testosterone in elderly men did not significantly affect the LDL or HDL particle profiles ($P > 0.05$).

Discussion

The main findings from the current study are: 1) elderly men and women had higher atherogenic potential of their lipoprotein particle profiles than their younger counterparts; 2) 2 yr of DHEA replacement resulted in reductions of HDL cholesterol, total HDL particle, and large HDL particle concentrations in elderly women; 3) 2 yr of either DHEA or testosterone replacement had no measurable affect on the lipoprotein particle profiles in elderly men, despite significantly increasing DHEAS and testosterone concentrations, respectively, to levels found in young men.

The current study supports previous data suggesting that an individual's sex influences his/her lipoprotein profile. Specifically, the present data demonstrated that women had significantly higher concentrations compared with men of total HDL cholesterol, total HDL particles, and large HDL particles (Fig. 1). This may be related to differences in sex hormone profiles (15, 16). In addition, there were also measurable effects of age on the lipoprotein particle profiles in both sexes as shown previously (15). Both elderly men and women had higher total cho-

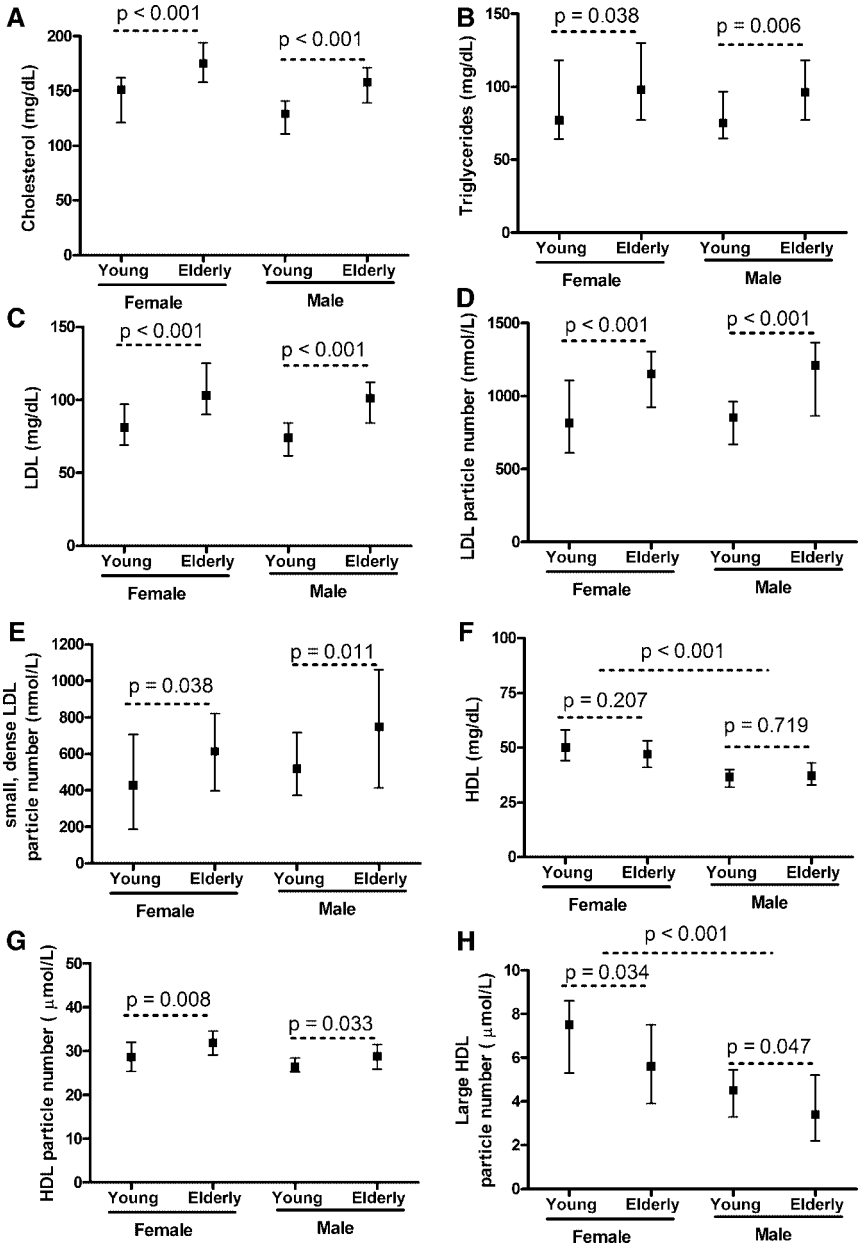

FIG. 1. Effect of age and sex on various lipoprotein particles. Data are presented as median (interquartile range). All *P* values are based on nonparametric Wilcoxon rank sum tests.

lesterol and triglycerides compared with their younger counterparts. With advancing age, the concentrations of atherogenic small, dense LDL particles increased substantially in men, thus indicating a higher risk for coronary artery disease (15).

We observed that DHEA replacement in elderly men had no measurable affect on the lipoprotein profiles, whereas DHEA replacement significantly altered the HDL lipoprotein profiles in elderly women. Specifically, DHEA significantly reduced HDL cholesterol, total HDL particle, and large HDL particle concentrations in elderly women. These results are consistent with previous reports that indicate that exogenous DHEA administration results in reductions in HDL cholesterol in women (23–25). Similarly, we also recently reported that short-term (3 months) DHEA supplementation (50 mg/d) in hypoadrenal women (characterized by absolute deficiency of DHEA) results in reductions in the large HDL particle concentration (26). It remains uncertain why the lowering of HDL cholesterol, total HDL particle, and large HDL particle concentrations occurs in response to DHEA replacement in elderly women, but not elderly men. However, these lipid alterations may have been mediated by the DHEA-induced elevation in total testosterone that was observed among the elderly women receiving DHEA. The potential clinical implications that the DHEA-induced lowering of HDL cholesterol, total HDL particle, and large HDL particle concentrations have on CVD outcomes among elderly women remain to be fully understood.

CVD, in particular CAD, is the leading cause of morbidity and mortality among adults in the United States, and its incidence is increasing in women and with age (27). Lipoprotein particle sizes as estimated by NMR spectroscopy have recently emerged as an important tool in the risk stratification for CAD (14). Females generally have a less atherogenic lipoprotein profile compared with males (15, 16). However, independent of sex, lipoprotein particles undergo several distinct modifications with age that make them more susceptible to oxidation (28, 29), thereby increasing CAD risk in both sexes. Moreover, substantial evidence is emerging about the relationship between lipoprotein particle sizes and longevity. In a study involving Ashkenazi Jewish centenarians and their offspring, it has been reported that lower levels of small LDL particles were associated with better cardiovascular health and longevity (12). Concentrations of large HDL and large LDL particles were significantly higher in individuals with exceptional longevity (13) and had an inverse relationship with CVD risk factors. It therefore is a concern that DHEA replacement resulted in an unfavorable effect on lipoprotein particles in women.

In randomized trials, short-term (3–6 months) replacement of DHEA has been shown to enhance cardiometa-

bolic parameters by improving insulin sensitivity and reducing visceral adiposity (30, 31). Therefore, it could be argued that these beneficial effects of DHEA replacement on insulin sensitivity and visceral adiposity outweigh the negative effects of DHEA on the lipoprotein particle profile. However, these beneficial effects on insulin sensitivity and visceral adiposity have not been shown to be sustained over longer periods (2 yr) of treatment (19). Specifically, we have previously reported from this same cohort that 2 yr of DHEA replacement had no measurable effects on either insulin sensitivity or visceral adiposity (19).

It is known that serum concentrations of testosterone decline with age in elderly men (7), and testosterone deficiency has been shown to increase the predisposition to insulin resistance, visceral adiposity, dyslipidemia, and the metabolic syndrome (32, 33), thereby increasing CVD risk. There is conflicting evidence regarding testosterone replacement on cardiometabolic parameters in randomized studies as indicated by a recent meta-analysis (34). This is probably due to a difference in doses, duration, and importantly the route of administration (oral *vs.* im *vs.* transdermal) employed in different studies. Although there are number of studies that have examined the impact of testosterone replacement on lipid profile (7, 35), data regarding the impact of testosterone on lipoprotein particle sizes and concentrations is still limited. In the current study, 2 yr of testosterone replacement had no measurable affect on the lipid and lipoprotein profile. The present findings are in contrast to previous reports that indicate that testosterone administration at supraphysiological doses decreases HDL cholesterol in healthy young and elderly adults (36–38). It is noteworthy that in the present study low-dose testosterone (5 mg/d) was used to minimize the risk of adverse effects like prostate enlargement and cancer, as previously reported (19). Although a higher replacement dose (200 mg/every 2 wk) for shorter duration (3 months) has been shown to improve cardiometabolic parameters (39), safety and efficacy for longer duration of its use remains uncertain. The route of administration (transdermal patch *vs.* im injection) may also play a role in mitigating the effects of testosterone on cardiometabolic parameters.

As stated previously, the present study is a subanalysis of a larger clinical trial that was initially powered to detect significant effects of long-term supplementation with DHEA in elderly men and women and testosterone in elderly men on body composition, bone mineral density, physical performance, and glucose tolerance in elderly adults with low androgen levels (19). Therefore, an *a priori* power analysis was not feasible for the present outcomes. However, on the basis of the 95% confidence intervals (CIs) shown in Table 4, it is not likely that our

TABLE 4. The impact of DHEA and testosterone on LDL and HDL cholesterol profiles

Variable label	Elderly females		Elderly males		
	DHEA *vs.* placebo	*P* value	DHEA *vs.* placebo	*P* value	Testosterone *vs.* placebo	*P* value
LDL cholesterol						
LDL cholesterol (mg/dl)	1.0 (−10.0, 12.0)	0.691	−4.0 (−12.0, 4.0)	0.347	−1.0 (−9.0, 8.0)	0.984
Total LDL particles (nmol/liter)	48.0 (−98.0, 186.0)	0.844	−19.0 (−114.0, 106.0)	0.774	19.0 (−114.0, 143.0)	0.443
Large LDL particles (nmol/liter)	−29.0 (−100.0, 60.0)	0.422	−49.0 (−115.0, 29.0)	0.243	−5.0 (−68.0, 68.0)	0.632
Medium-small LDL particles (nmol/liter)	9.0 (−20.0, 38.0)	0.732	8.0 (−17.0, 42.0)	0.603	2.0 (−30.0, 34.0)	0.502
Very small LDL particles (nmol/liter)	43.5 (−65.0, 167.0)	0.5732	13.0 (−77.0, 126.0)	0.758	38.0 (−91.0, 142.0)	0.248
Total small LDL particles (nmol/liter)	48.5 (−96.0, 205.0)	0.670	27.0 (−89.0, 160.0)	0.687	36.0 (−121.0, 180.0)	0.261
LDL mean particle size (nm)	−0.05 (−0.30, 0.20)	0.737	−0.10 (−0.40, 0.10)	0.358	−0.10 (−0.40, 0.20)	0.237
HDL cholesterol						
HDL cholesterol (mg/dl)	−5.0 (−8.0, −2.0)	0.002	0.0 (−3.0, 3.0)	0.961	2.0 (−1.0, 5.0)	0.279
Total HDL particles (μmol/liter)	−2.5 (−4.3, −0.6)	0.004	0.3 (−1.6, 1.9)	0.862	0.5 (−1.0, 3.0)	0.591
Large HDL particles (μmol/liter)	−1.0 (−1.8, −0.2)	0.003	−0.2 (−0.8, 0.5)	0.611	0.0 (−0.7, 0.8)	0.844
Medium HDL particles (μmol/liter)	−0.7 (−2.1, 0.7)	0.099	0.0 (−1.3, 0.9)	0.988	0.7 (−0.6, 1.9)	0.290
Small HDL particles (μmol/liter)	−0.3 (−3.2, 2.1)	0.966	1.2 (−0.9, 2.5)	0.339	0.2 (−2.0, 2.5)	0.927
HDL mean particle size (nm)	−0.1 (−0.2, 0.0)	0.118	0.0 (−0.1, 0.1)	0.971	0.1 (−0.1, 0.2)	0.799

Data are expressed as median difference (95% CI). All *P* values are two-sided and are based on multiple regression analysis in which the dependent variable was the change from baseline (with the use of a rank transformation) and the independent variables were the study group, sex, age at the time of randomization, length of follow-up, and baseline values.

negative findings can be attributed to the small number of subjects in the study. As we have previously reported (19), these 95% CIs indicate that the number of participants was sufficient to establish any clinically meaningful treatment effects. For example, the 95% CI in the testosterone group for the changes in total LDL particle concentrations in elderly men ranged from −114 to 143 nmol/liter, thus ruling out reductions in the LDL particle concentrations greater than 114 nmol/liter (~8% reduction) or increases in LDL particle concentrations greater than 143 nmol/liter (~10% increase). If the study had enrolled more subjects, the CI would have been even narrower. Similarly, the 95% CI in the DHEA group for the changes in total LDL particle concentrations in elderly men ranged from −114 to 106 μmol/liter (~10% reduction to ~10% increase), and in elderly women ranged from −98 to 186 μmol/liter (~10% reduction to ~20% increase). Moreover, the 95% CI in the testosterone group for the changes in total HDL particle concentrations in elderly men ranged from −1 to 3 μmol/liter (~3% reduction to ~10% increase). Finally, the 95% CI in the DHEA group for the changes in total HDL particle concentrations in elderly men ranged from −3 to 3 μmol/liter (~8% reduction to ~8% increase).

It should be recognized that approximately 25% of the elderly participants in the present study were taking statins at baseline. Among the elderly population in the greater Rochester, Minnesota, and Olmstead county areas, it is difficult to find people who are not treated with diet and/or therapeutic agents when their LDL cholesterol concentrations are above 100 mg/dl. However, the participants in the present study were randomly assigned to receive either of the treatments or a placebo. Importantly, adding statin therapy as a covariate had no affect on change in the individual lipoprotein particles.

In summary, both in elderly men and women, DHEA replacement increased the plasma levels of DHEAS to levels typically observed in younger adults. Long-term (2 yr) DHEA replacement in elderly women resulted in a lowering of the HDL cholesterol, total HDL particle, and large HDL particle concentrations. In contrast, DHEA replacement had no measurable effects on the lipoprotein particle profile in elderly men. Similarly, long-term physiological replacement of testosterone had no measurable effects on the lipoprotein particle profile in elderly men.

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References

1. **Feldman HA, Johannes CB, Araujo AB, Mohr BA, Longcope C, McKinlay JB** 2001 Low dehydroepiandrosterone and ischemic heart disease in middle-aged men: prospective results from the Massachusetts Male Aging Study. Am J Epidemiol 153:79–89
2. **Barrett-Connor E, Khaw KT, Yen SS** 1986 A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. N Engl J Med 315:1519–1524
3. **Sato K, Iemitsu M, Aizawa K, Ajisaka R** 2008 Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle. Am J Physiol Endocrinol Metab 294:E961–E968
4. **Gordon GB, Bush DE, Weisman HF** 1988 Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand white rabbit with aortic intimal injury. J Clin Invest 82:712–720
5. **Orentreich N, Brind JL, Rizer RL, Vogelman JH** 1984 Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551–555
6. **Porsova-Dutoit I, Sulcova J, Starka L** 2000 Do DHEA/DHEAS play a protective role in coronary heart disease? Physiol Res 49(Suppl 1):S43–S56
7. **Alexandersen P, Christiansen C** 2004 The aging male: testosterone deficiency and testosterone replacement. An up-date. Atherosclerosis 173:157–169
8. **English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS** 2000 Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. Eur Heart J 21:890–894
9. **Yarnell JW, Beswick AD, Sweetnam PM, Riad-Fahmy D** 1993 Endogenous sex hormones and ischemic heart disease in men. The Caerphilly Prospective Study. Arterioscler Thromb 13:517–520
10. **Colhoun HM, Otvos JD, Rubens MB, Taskinen MR, Underwood SR, Fuller JH** 2002 Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. Diabetes 51:1949–1956
11. 2002 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 106:3143–3421
12. **Heijmans BT, Beekman M, Houwing-Duistermaat JJ, Cobain MR,**

Powell J, Blauw GJ, van der Ouderaa F, Westendorp RG, Slagboom PE 2006 Lipoprotein particle profiles mark familial and sporadic human longevity. PLoS Med 3:e495

13. **Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R, Cheng S, Shuldiner AR** 2003 Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA 290: 2030–2040
14. **Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, McNamara JR, Bloomfield HE, Robins SJ** 2006 Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 113:1556–1563
15. **Freedman DS, Otvos JD, Jeyarajah EJ, Shalaurova I, Cupples LA, Parise H, D'Agostino RB, Wilson PW, Schaefer EJ** 2004 Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. Clin Chem 50:1189–1200
16. **Pascot A, Lemieux I, Bergeron J, Tremblay A, Nadeau A, Prud'homme D, Couillard C, Lamarche B, Després JP** 2002 HDL particle size: a marker of the gender difference in the metabolic risk profile. Atherosclerosis 160:399–406
17. **Khalil A, Fortin JP, LeHoux JG, Fülöp T** 2000 Age-related decrease of dehydroepiandrosterone concentrations in low density lipoproteins and its role in the susceptibility of low density lipoproteins to lipid peroxidation. J Lipid Res 41:1552–1561
18. **Liverman C, Blazer D** 2004 Committee on Assessing the Need for Clinical Trials of Testosterone Replacement Therapy. Executive summary. Washington DC: National Academy Press; 1–10
19. **Nair KS, Rizza RA, O'Brien P, Dhatariya K, Short KR, Nehra A, Vittone JL, Klee GG, Basu A, Basu R, Cobelli C, Toffolo G, Dalla Man C, Tindall DJ, Melton 3rd JL, Smith GE, Khosla S, Jensen MD** 2006 DHEA in elderly women and DHEA or testosterone in elderly men. N Engl J Med 355:1647–1659
20. **Khosla S, Melton 3rd LJ, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL** 1998 Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 83: 2266–2274
21. **Jeyarajah EJ, Cromwell WC, Otvos JD** 2006 Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med 26:847–870
22. **Henderson GC, Dhatariya K, Ford GC, Klaus KA, Basu R, Rizza RA, Jensen MD, Khosla S, O'Brien P, Nair KS** 2009 Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements. FASEB J 23:631–641
23. **Morales AJ, Nolan JJ, Nelson JC, Yen SS** 1994 Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. J Clin Endocrinol Metab 78:1360–1367
24. **Mortola JF, Yen SS** 1990 The effects of oral dehydroepiandrosterone on endocrine-metabolic parameters in postmenopausal women. J Clin Endocrinol Metab 71:696–704
25. **Mattson LA, Cullberg G, Tangkeo P, Zador G, Samsioe G** 1980 Administration of dehydroepiandrosterone enanthate to oophorectomized women–effects on sex hormones and lipid metabolism. Maturitas 2:301–309
26. **Srinivasan M, Irving BA, Dhatariya K, Klaus KA, Hartman SJ, McConnell JP, Nair KS** 2009 Effect of dehydroepiandrosterone replacement on lipoprotein profile in hypoadrenal women. J Clin Endocrinol Metab 94:761–764
27. **Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, Ford E, Furie K, Go A, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott M, Meigs J, Mozaffarian D, Nichol G, O'Donnell C, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Steinberger J, Thom T, Wasserthiel-Smoller S, Wong N, Wylie-Rosett J, Hong Y** 2009 Heart disease and stroke statistics—2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 119: 480–486
28. **Khalil A, Jay-Gerin JP, Fülöp Jr T** 1998 Age-related increased susceptibility of high-density lipoproteins (HDL) to in vitro oxidation induced by γ-radiolysis of water. FEBS Lett 435:153–158
29. **Khalil A, Wagner JR, Lacombe G, Dangoisse V, Fülöp Jr T** 1996 Increased susceptibility of low-density lipoprotein (LDL) to oxidation by γ-radiolysis with age. FEBS Lett 392:45–48
30. **Dhatariya K, Bigelow ML, Nair KS** 2005 Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. Diabetes 54:765–769
31. **Villareal DT, Holloszy JO** 2004 Effect of DHEA on abdominal fat and insulin action in elderly women and men: a randomized controlled trial. JAMA 292:2243–2248
32. **Oh JY, Barrett-Connor E, Wedick NM, Wingard DL** 2002 Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. Diabetes Care 25:55–60
33. **Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB** 2000 Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts Male Aging Study. Diabetes Care 23:490–494
34. **Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, Lenzi A, Fabbri A** 2005 Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol (Oxf) 63:280–293
35. **Snyder PJ, Peachey H, Berlin JA, Rader D, Usher D, Loh L, Hannoush P, Dlewati A, Holmes JH, Santanna J, Strom BL** 2001 Effect of transdermal testosterone treatment on serum lipid and apolipoprotein levels in men more than 65 years of age. Am J Med 111:255–260
36. **Meriggiola MC, Marcovina S, Paulsen CA, Bremner WJ** 1995 Testosterone enanthate at a dose of 200 mg/week decreases HDL-cholesterol levels in healthy men. Int J Androl 18:237–242
37. **Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R** 1996 The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 335:1–7
38. **Bagatell CJ, Knopp RH, Vale WW, Rivier JE, Bremner WJ** 1992 Physiologic testosterone levels in normal men suppress high-density lipoprotein cholesterol levels. Ann Intern Med 116:967–973
39. **Kapoor D, Goodwin E, Channer KS, Jones TH** 2006 Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol 154:899–906
40. **Dalla Man C, Caumo A, Cobelli C** 2002 The oral glucose minimal model: estimation of insulin sensitivity from a meal test. IEEE Trans Biomed Eng 49:419–429