

# Dehydroepiandrosterone, an Adrenal Androgen, Increases Human Foam Cell Formation

## A Potentially Pro-Atherogenic Effect

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<b>OBJECTIVES</b>	We studied the effects of dehydroepiandrosterone (DHEA), an abundant adrenal androgen, on two key early events of atherogenesis: 1) human monocyte adhesion to vascular endothelium, and 2) human foam cell formation.
<b>BACKGROUND</b>	In the U.S., where DHEA is available without prescription, there has recently been a rapid increase in unsupervised self-administration of DHEA. The vascular biologic effects of DHEA are largely unknown, however.
<b>METHODS</b>	Regarding adhesion, human umbilical vein endothelial cells (HUVECs), exposed to either DHEA (42 or 420 nmol/l) or control, were incubated with human monocytes, and adhesion was measured by hemocytometry. Surface expression of endothelial cell adhesion molecules was measured by ELISA. Regarding foam cell formation, studies of lipid loading were performed on macrophages treated with DHEA or control and/or the androgen receptor antagonist hydroxyflutamide (HF) (4 $\mu$ mol/l). Intracellular cholesterol and cholesteryl esters (CE) were quantified by high-performance liquid chromatography. Expression of foam cell formation-related genes was measured by reverse-transcription polymerase chain reaction.
<b>RESULTS</b>	DHEA produced a dose-dependent receptor-mediated increase in the male macrophage CE content (up to $120 \pm 4\%$ of control values, $p = 0.015$ ). DHEA upregulated messenger ribonucleic acid expression of the lipoprotein-processing enzymes acyl coenzyme A:cholesterol acyltransferase I and lysosomal acid lipase by 3.4- and 5.3-fold, respectively ( $p < 0.05$ vs. control), but had no effect on scavenger receptor expression ( $p > 0.2$ ). There was no significant effect of DHEA on monocyte-endothelial adhesion ( $<10\%$ change in values, $p = 0.56$ ) or endothelial cell expression of cell adhesion molecules ( $p > 0.1$ ).
<b>CONCLUSIONS</b>	DHEA increases human macrophage foam cell formation, a potentially pro-atherogenic effect. This effect appears to be mediated via the androgen receptor and involves the upregulation of lipoprotein-processing enzymes. (J Am Coll Cardiol 2003;42:1967-74) © 2003 by the American College of Cardiology Foundation

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are sex hormone precursors of mainly adrenal origin, with weak androgenic action. To date, DHEA and DHEAS have been an endocrine paradox: they are the most abundant steroids in the circulation, yet their biologic significance is unknown. For over four decades, it has been hypothesized that DHEA may play a role in preventing the development of atherosclerosis and coronary artery disease (1). In recent years, there has been a surge of public interest in DHEA because of its reported anti-atherosclerotic and anti-aging effects. In the U.S., where DHEA is available without prescription, there has been a rapid increase in unsupervised self-administration, despite the fact that none of its purported benefits have been proven in the context of a large, randomized, placebo-controlled clinical trial (2).

The most persuasive evidence for an anti-atherogenic effect comes from a prospective cohort study of 242 men in whom a low baseline level of DHEAS ( $<140 \mu\text{g/dl}$ ) was associated with a threefold increase in cardiovascular mortality over a follow-up period of 12 years (3). However, subsequent prospective epidemiologic studies in women (4) and in other male cohorts (5-9) have failed to yield consistent results with respect to the relationship between DHEAS levels and cardiovascular outcomes. In animal experiments, DHEA administration has been reported to reduce aortic atherosclerosis in cholesterol-fed rabbits with or without aortic intimal injury (10,11). These anti-atherogenic effects were observed without significant changes in plasma lipids, suggesting a direct effect of DHEA on the arterial wall. It is difficult, however, to extrapolate these animal findings to humans, particularly as adrenal production of DHEA or DHEAS in most experimental animals is nonexistent or minute, and the experimental work is based on pharmacologic doses of exogenous hormones. To date, few mechanistic studies have examined the vascular effects of DHEA on humans.

There is increasing evidence that sex steroids play a role

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#### Abbreviations and Acronyms

ACAT	= acyl coenzyme A:cholesterol acyltransferase I
CE	= cholesteryl ester
DHEA	= dehydroepiandrosterone
DHEAS	= dehydroepiandrosterone sulfate
HCAEC	= human coronary artery endothelial cell
HF	= hydroxyflutamide
HUVEC	= human umbilical vein endothelial cell
ICAM-1	= intercellular adhesion molecule-1
IL-1-beta	= interleukin-1-beta
LAL	= lysosomal acid lipase
LDL	= low-density lipoprotein
MDM	= monocyte-derived macrophage
PBS	= phosphate-buffered saline
RT-PCR	= reverse-transcription polymerase chain reaction
VCAM-1	= vascular cell adhesion molecule-1

in the regulation of atherogenic processes. A prominent early event in atherogenesis is the adherence of monocytes to endothelial cells and their subsequent transmigration into the subendothelial space to differentiate into macrophages (12). The macrophages then accumulate very large amounts of cholesteryl ester (CE), giving them a foamy appearance (hence, "foam" cell) (13). In a series of human *in vitro* studies, we recently reported that androgens increase monocyte adhesion to endothelial cells and endothelial cell expression of vascular cell adhesion molecule-1 (VCAM-1, a mediator of monocyte-endothelial adhesion) (14). We also demonstrated that androgens, estrogen, and progesterone all exert gender-specific effects on human macrophage foam cell formation (15-17). As DHEA is both a weak androgen and a universal precursor for a number of androgenic and estrogenic products, we therefore sought to evaluate the effects of DHEA on two key early events of atherogenesis: human monocyte adhesion to vascular endothelium and foam cell formation.

## METHODS

### Human monocyte adhesion to vascular endothelial cells and endothelial cell adhesion molecule expression.

**ENDOTHELIAL CELL HARVESTING AND CULTURE.** Human umbilical vein endothelial cells (HUVECs) were harvested enzymatically from male infant umbilical cords under sterile conditions, as previously described by us (14). Human coronary artery endothelial cells (HCAECs) were purchased from Cell Applications, Inc. (San Diego, California). Endothelial cells (passages 2 to 4 for HUVECs and passages 5 to 10 for HCAECs) were grown to confluence before sex steroid hormone treatment and were used within 72 h. The purity of the endothelial cell monolayers was confirmed macroscopically by their cobblestone pattern and periodically by cell staining with a monoclonal antibody specific for von Willebrand factor. At the end of the 48-h treatments, cell viability was >95% (by trypan blue exclusion) for each condition.

**ISOLATION OF HUMAN MONOCYTES.** White cell concentrates (Red Cross Blood Bank) were obtained from the peripheral blood of healthy individual men. Monocytes were isolated by counterflow centrifugation elutriation, as previously described (15). Monocyte purity was >90% and viability was >95% by Trypan blue exclusion in all experiments. Monocytes were either: 1) resuspended in RPMI containing 2% human serum and used immediately for adhesion studies; or 2) resuspended in phenol red-free RPMI (Life Technologies, Carlsbad, California) for studies of lipid loading.

**MONOCYTE-ENDOTHELIAL CELL ADHESION ASSAY.** Confluent HUVEC monolayers were established in 24-mm-diameter wells before incubation for 48 h with the following treatments: 1) vehicle control (0.1% ethanol); 2) DHEA at a physiologic concentration (42 nmol/l); and 3) DHEA at a supraphysiologic concentration (420 nmol/l). Each treatment group was divided after 24 h of hormone treatment into basal and stimulated states, with the latter receiving interleukin-1-beta (IL-1-beta; Genzyme Corp., Cambridge, Massachusetts) at 50 U/ml for the final 24-h period. Each experiment used at least triplicate wells for each condition. Separate adhesion experiments were performed three times for all treatment groups. As described previously (15), the adhesion assay involved cell counting before and after a 1-h period of monocyte addition to the endothelial monolayer (Neubauer hemocytometer, Weber Scientific, London, UK). The initial suspensions and the suspension from each well were counted four times by an observer blinded to the treatment conditions. This method has been shown to have a low intra-observer error, with a coefficient of variation of <5%, and maximal basal adhesion after 1 h of incubation (14).

### ENDOTHELIAL CELL ADHESION MOLECULE EXPRESSION.

The cell-surface expression of adhesion molecules on the endothelial cell monolayers exposed to different treatments was assessed with an ELISA technique (15) using HUVECs and HCAECs. Confluent cell monolayers were established in 96-well plates and exposed for 48 h to the control or hormone treatments as outlined earlier, with or without IL-1-beta stimulation (50 U/ml) for the last 24 h of treatment. Monoclonal antibodies (obtained from Becton-Dickinson [Franklin Lakes, New Jersey], unless where indicated) to intercellular adhesion molecule-1 (ICAM-1), VCAM-1, E-selectin, or isotype mouse immunoglobulin G (ICN Immunobiologicals, Costa Mesa, California) (0.1 mg in 100 ml of Hank's Balanced Salt Solution with 10% heat-inactivated human serum) were added for 30 min before a 30-min incubation with sheep anti-mouse antibody/horseradish peroxidase conjugate (Amersham International) (1:500 in 100 ml of Hank's Balanced Salt Solution with 10% heat-inactivated human serum and 0.05% Tween-20). The ABTS substrate (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) was then added, and the results were expressed as units of optical density, measured

at 414 nm with an ELISA plate reader (Titretrek Multiscan, Flow Laboratories, Rickmansworth, UK).

**Human monocyte-derived macrophage (MDM) foam-cell formation. CULTURE AND LIPID LOADING OF HUMAN MDMS.** Monocytes isolated by elutriation were cultured in phenol red-free RPMI containing 10% postmenopausal human serum. From days 3 to 12, the following treatments were added with each media change: 1) vehicle control (0.1% ethanol); 2) DHEA at a variety of physiologic (4.2, 8.4, and 42 nmol/l) or supraphysiologic (420 nmol/l) concentrations; 3) DHEA at 42 nmol/l and hydroxyflutamide (HF), a nonsteroidal androgen-receptor antagonist at 4  $\mu$ mol/l; and 4) HF only at 4  $\mu$ mol/l. Each experiment used at least triplicate cultures for each condition.

After macrophage differentiation, lipid loading was achieved on days 9 through 12 during a 72-h incubation with lipid loading medium (50 mg/ml acetylated low-density lipoprotein [LDL] in phenol red-free RPMI containing 10% [vol./vol.] lipoprotein-deficient human serum [density >1.25 g/ml]). Cell viability for each treatment condition was established by trypan blue exclusion, with viability levels of 90% to 94%.

**PREPARATION AND ACETYLATION OF LDL.** Low density lipoprotein (1.05 > density > 1.02 g/ml) was isolated from the plasma of healthy, normolipidemic fasting subjects by two-step centrifugation at 10°C with a Beckman L8-M centrifuge and Ti70 rotor at 50,000 rpm (242,000 g) for 24 h. The LDL was dialyzed four times against 1 l of deoxygenated phosphate-buffered saline (PBS) (calcium and magnesium free; Flow Laboratories) containing 0.1 mg/ml chloramphenicol (Boehringer Mannheim, Mannheim, Germany) and 1.0 mg/ml EDTA. Soon after isolation, the LDL was acetylated at 4°C by a modification of a previous method (18) with 6 ml acetic anhydride per 1 mg LDL protein.

**PREPARATION OF MACROPHAGE CELL EXTRACTS.** After the human MDMs were washed three times with ice-cold PBS, cells were lysed with 0.6 ml of cold 0.2 mol/l NaOH at 4°C for 15 min. From the lysate, 0.2 ml was used for cell protein estimation, and the remaining 0.4 ml was added to 0.6 ml ice-cold PBS and immediately extracted into methanol (2.5 ml) and hexane (5 ml) in the presence of 20 mmol/l butylated hydroxytoluene (Sigma, St. Louis, Missouri) and 2 mmol/l EDTA. Samples were stored after extraction at -80°C until analysis for free cholesterol and CE was performed, usually within seven days.

**ENDOTHELIAL CELL AND MACROPHAGE LDL OXIDATION STUDIES.** Supernatant from control and DHEA-treated (42 nmol/l) endothelial cells and macrophages were collected after 24-h incubation with a lipid loading medium containing 50 mg/ml LDL for quantification of oxidation products. From the supernatant, 1 ml was added to 0.6 ml ice-cold PBS and then extracted into methanol and hexane, using the same protocol as for cell extracts.

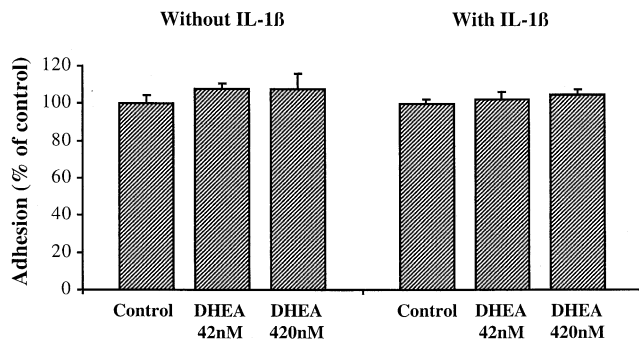
**ANALYSIS OF CHOLESTEROL, CES, AND CE OXIDATION PRODUCTS.** Cholesterol, CEs, cholesterol linoleate hydroperoxide, and alpha-tocopherol were separated and quantified by reverse-phase high-performance liquid chromatography at room temperature on a C-18 column (Supelco, Bellefonte, Pennsylvania), as described previously (19). Cholesterol, CEs, and alpha-tocopherol were quantified by the derivation of standard curves by use of commercially available standards (Sigma). The curves expressed a linear relationship between the chromatographic peak areas and the mass of the standard, which enabled us to quantify individual cholesterol compounds in nanomoles per milligram of cell protein.

**PROTEIN ESTIMATION.** All protein estimations were performed by the bicinchoninic acid method (Sigma) with bovine serum albumin used as a standard using 0.2 ml cell lysate. Samples were incubated for 60 min at 60°C before measurement of absorbance at 562 nm.

**RELATIVE REAL-TIME REVERSE-TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR).** The effect of DHEA on the macrophage expression of foam cell formation-related genes was undertaken by relative real-time RT-PCR with SYBR Green I monitoring. The genes assayed included acyl coenzyme A:cholesterol acyltransferase I (ACAT), lysosomal acid lipase (LAL), and scavenger receptors A1 and A2. Ribonucleic acid was isolated from control and DHEA-treated (42 nmol/l) macrophages at maturation (day 9). Reverse transcription of each RNA sample was performed in duplicate, according to standard protocols. Relative real-time PCR reactions were performed in duplicate for each cDNA sample for each gene of interest (Applied Biosystems, Inc. [ABI], model 7700 Sequence Detector, PE Biosystems) and analyzed using ABI Prism Sequence Detector software version 1.6.3 (PE Biosystems, Foster City, California). The housekeeping gene, 60S rRNA, was also measured for normalization of real-time PCR results.

The primer sequences were: ACAT forward 5'-AGTTGACAGCAGAGGCAGAG-3' and reverse 5'-GGATAAAGAGAATGAGGAGGG-3'; LAL forward 5'-GCAACAGCAGAGGAAATAC-3' and reverse 5'-GAGAATGACCCACATAATACAC-3'; scavenger receptor A1 forward 5'-CACAATCAACAGGAGGAC-3' and reverse 5'-CAATGAGAGGGATGAGAAC; and scavenger receptor A2 forward 5'-TACTCCACCA-TCACCTCTCTCC-3' and reverse 5'-TCGCTTT-GCTTGACTGAAC-3'.

**Statistical analysis.** Adhesion assay, ELISA, lipid loading, and gene expression data are expressed as the mean value  $\pm$  SEM of at least triplicate wells, per experiment per condition per donor. Results for the adhesion assays and ELISAs for cell adhesion molecule expression are expressed as a percentage of the control condition within each experiment, as each experiment involved endothelial cells and/or monocytes from different donors. In the case of lipid loading experiments, the results for each condition in each donor



**Figure 1.** Monocyte adhesion to endothelium (in the basal or interleukin-1-beta [IL-1-beta]-stimulated state) for each treatment condition, expressed as a percentage of control values. Dehydroepiandrosterone (DHEA) (in young adults and at supraphysiologic concentrations) had no significant effect on monocyte adhesion to endothelium ( $p = 0.56$  by analysis of variance [ANOVA] for adhesion in either the basal or IL-1-beta-stimulated state).

were calculated as a percentage of control values. Groups of three or more conditions were compared by one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test for multiple pairwise comparisons (for significant ANOVA results). Statistical comparisons between two treatment conditions were performed using an independent samples *t* test, with adjustment for multiple comparisons, where appropriate, in accordance with Hochberg's modification of the Bonferroni procedure. Statistical significance was inferred at a two-sided *p* value of  $<0.05$ . SPSS software (version 9.0) was used for statistical analyses.

## RESULTS

**Monocyte-endothelial cell adhesion and expression of endothelial cell adhesion molecules.** Exposure to DHEA, at physiologic (42 nmol/l) and supraphysiologic (420 nmol/l) concentrations, had no significant effect on monocyte adhesion to endothelial cell monolayers in the basal state ( $108 \pm 3\%$  and  $108 \pm 8\%$  for DHEA at 42 and 420 nmol/l, respectively, vs. control;  $p = 0.56$ ) (Fig. 1). Treatment of endothelial cell monolayers with IL-1-beta significantly increased monocyte-endothelial adhesion by  $66 \pm 10\%$  ( $p = 0.02$ ), as expected from previous work. However, DHEA, even at supraphysiologic concentrations, had no effect on monocyte-endothelial adhesion in the IL-1-beta-activated state ( $102 \pm 4\%$  and  $105 \pm 3\%$  for DHEA at 12 and 120 ng/ml, respectively, vs. vehicle control;  $p = 0.56$  by ANOVA) (Fig. 1). The effect of DHEA treatment on endothelial cell-surface adhesion molecule expression in both basal and IL-1-beta-stimulated states was also examined using an ELISA technique. Co-incubation of endothelial cells with IL-1-beta for 24 h increased cell-surface expression of all three molecules: ICAM-1, VCAM-1, and E-selectin. Administration of DHEA, at both physiologic and supraphysiologic concentrations, however, had no significant effect on expression of VCAM-1, ICAM-1, or E-selectin in the basal or IL-1-beta-stimulated states in HUVECs or HCAECs ( $p > 0.1$  by ANOVA for all cell adhesion molecules) (Fig. 2).

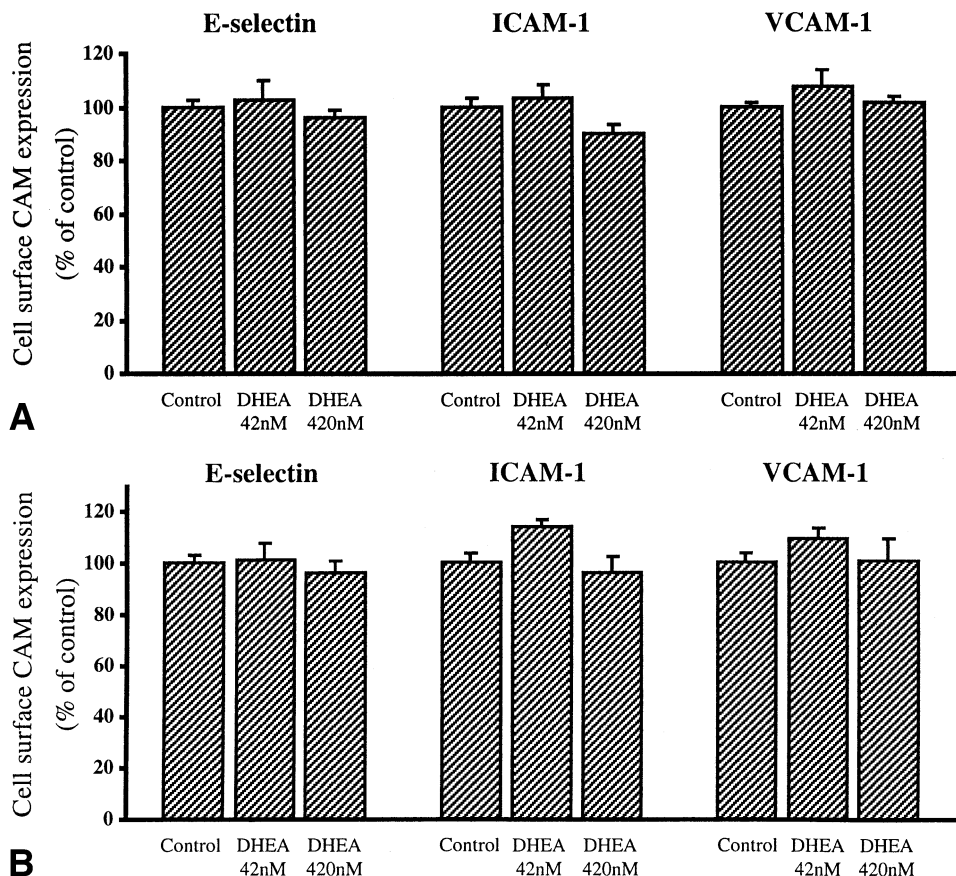
**Effects of DHEA on macrophage CE accumulation.** The effect of DHEA on MDM foam cell formation was studied over a range of physiologic (4.2 to 42 nmol/l) and supra-physiologic (420 nmol/l) concentrations. Exposure to DHEA produced a dose-dependent increase in the male macrophage CE content, which was maximal at a physiologic concentration of 42 nmol/l ( $104 \pm 5\%$ ,  $105 \pm 5\%$ ,  $120 \pm 4\%$ , and  $117 \pm 8\%$  for DHEA at 4.2, 8.4, 42, and 420 nmol/l, respectively, as a percentage of control values;  $p = 0.015$  by ANOVA) (Fig. 3). The DHEA-mediated effect on intracellular CE accumulation was abrogated by co-administration of the nonsteroidal androgen receptor antagonist HF (CE as a percentage of control value,  $91 \pm 5\%$  for DHEA at 42 nmol/l with HF,  $p > 0.1$  vs. control). Hydroxyflutamide alone did not significantly alter the CE content ( $101 \pm 6\%$ ,  $p > 0.8$  vs. control). Free cholesterol content was not significantly altered by DHEA exposure (free cholesterol as a percentage of control values:  $102 \pm 4\%$ ,  $104 \pm 1\%$ ,  $112 \pm 8\%$ , and  $90 \pm 8\%$  for DHEA at 4.2, 8.4, 42, and 420 nmol/l, respectively;  $p > 0.3$  by ANOVA).

**Mechanistic studies of the effect of DHEA on foam cell formation. EFFECT OF DHEA ON LDL OXIDATION BY ENDOTHELIAL CELLS AND MACROPHAGES.** To study the effect of DHEA on LDL oxidation, samples of media at the end of the loading period were extracted and analyzed for native and oxidized lipids and alpha-tocopherol content. No differences were found in any of the oxidation parameters (including alpha-tocopherol, cholesteryl linoleate, and cholesteryl linoleate hydroperoxide levels) measured between DHEA-treated and control cultures in both HCAECs and MDMs ( $p > 0.1$ ; data not shown). Consistent with previous studies on cell-mediated oxidation (19), only a very small proportion of cholesteryl linoleate, the major oxidizable lipid in LDL, was converted to cholesteryl linoleate hydroperoxide during the incubation ( $2.89 \pm 0.22\%$  for DHEA at 42 nmol/l and  $2.69 \pm 0.42\%$  for control in HCAECs;  $3.15 \pm 0.15\%$  for DHEA at 42 nmol/l and  $3.1 \pm 0.2\%$  for control in MDMs; cholesteryl linoleate hydroperoxide as a percentage of cholesteryl linoleate;  $p > 0.7$  vs. control).

**EFFECTS OF DHEA ON GENE EXPRESSION.** By relative RT-PCR, we examined the effect of physiologic concentrations of DHEA (42 nmol/l) on the expression of foam cell formation-related genes, including ACAT, LAL, and macrophage scavenger receptors A1 and A2. DHEA upregulated the expression of ACAT by 3.4-fold and LAL by 5.3-fold ( $p < 0.05$  vs. control for both genes) (Fig. 4A). By comparison, DHEA exposure had no effect on the expression of scavenger receptors A1 or A2 ( $p > 0.2$  vs. control for both genes) (Fig. 4B).

## DISCUSSION

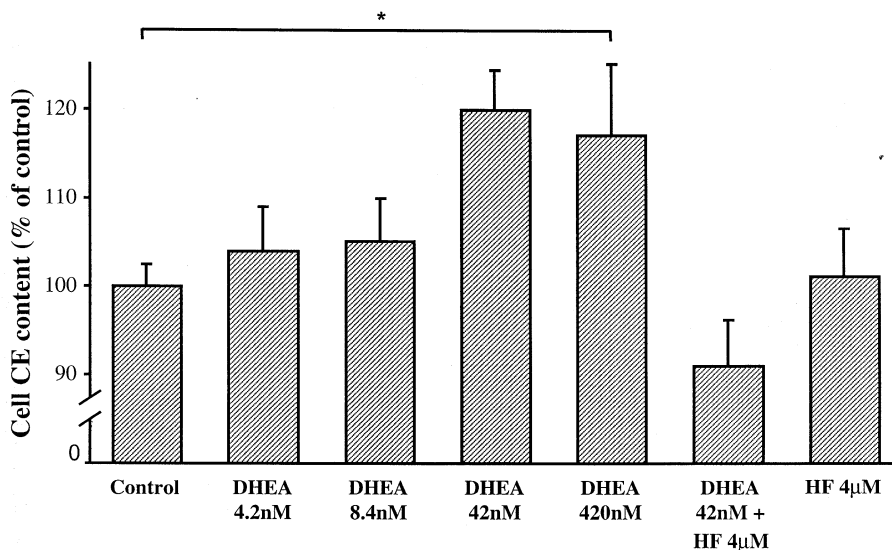
The effect of DHEA and its sulfated prohormone, DHEAS, on human atherogenic processes has received very little attention, despite longstanding conjecture that adrenal



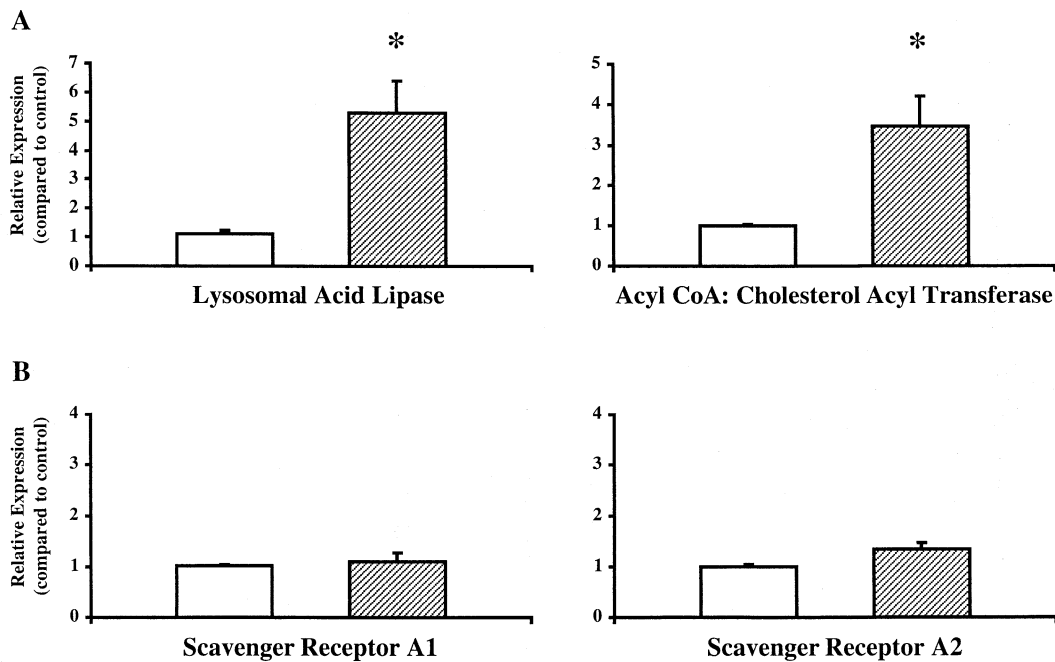
**Figure 2.** Endothelial cell-surface expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin for each treatment group in the basal (A) and IL-1-beta-stimulated (B) states. Results are expressed as a percentage of control values. DHEA had no significant effect on endothelial cell-surface expression of VCAM-1, ICAM-1, or E-selectin ( $p > 0.1$  by ANOVA for each cell adhesion molecule [CAM] in each treatment group). Abbreviations as in Figure 1.

androgens may be atheroprotective. Key early events in atherogenesis include monocyte adhesion to endothelium and macrophage lipid loading. In the present study, we

found that physiologic concentrations of DHEA increased CE formation in male donor human MDMs, an effect antagonized by androgen receptor blockade. By RT-PCR, we



**Figure 3.** Exposure to DHEA was associated with a dose-dependent increase in cholesteryl ester (CE) accumulation in human macrophages from male donors, over the physiologic range (4.2 to 42 nmol/l). This effect is abrogated by co-incubation with the androgen receptor antagonist HF. \* $p = 0.015$  by ANOVA. There was a polynomial relationship between the DHEA concentration and macrophage CE content ( $r = 0.997$ ). Abbreviations as in Figure 1.



**Figure 4.** Effects of dehydroepiandrosterone (DHEA) exposure on macrophage gene expression. **(A)** Gene expression examined by reverse-transcription polymerase chain reaction shows that DHEA at physiologic concentrations upregulates the lipoprotein-processing genes acyl coenzyme A:cholesterol acyltransferase I (ACAT) and lysosomal acid lipase (LAL). **(B)** In contrast, DHEA exposure had no significant effect on expression of the scavenger receptors A1 and A2. \* $p < 0.05$  versus control values for ACAT and LAL (vs.  $p > 0.2$  for scavenger receptors A1 and 2). **Open columns** = control; **shaded columns** = DHEA at 42 nmol/l.

have also shown that DHEA exposure increases the expression of two important lipoprotein-processing enzymes implicated in foam cell formation: LAL and ACAT. In other mechanistic studies, DHEA was found to have no significant effect on LDL oxidation or on scavenger receptor expression. In contrast to foam cell formation, DHEA had no direct effect on either human monocyte adhesion to endothelial cells or endothelial cell expression of the major cell adhesion molecules E-selectin, ICAM-1, and VCAM-1.

Although initially surprising, the finding of a potentially pro-atherogenic effect of DHEA on male human macrophage foam cell formation is consistent with our recent demonstration that androgen exposure (using the non-aromatizable physiologic androgen dihydrotestosterone) is associated with increased CE accumulation in male but not female MDMs (16). The effect of DHEA on MDM CE accumulation was maximal at a concentration consistent with that found in a young male adults (42 nmol/l). Like the effect observed with dihydrotestosterone (16), the effect of DHEA on foam cell formation could be abrogated by HF, suggesting a common androgen receptor-dependent effect.

In mechanistic experiments evaluating the role of DHEA in foam cell formation, we examined the effects of DHEA on: 1) the oxidation of LDL; and 2) the expression of key foam cell formation-related genes. Oxidation of LDL, postulated to be an important event in atherogenesis, has been shown to accelerate macrophage CE accumulation (19). We found that DHEA had no effect on LDL oxidation by endothelial cells or macrophages, as measured

by a number of parameters, including the accumulation of CE hydroperoxides. In contrast, we found that physiologic concentrations of DHEA upregulated macrophage genes involved in lysosomal lipoprotein processing (i.e., LAL) and intracytoplasmic cholesterol esterification (i.e., ACAT). Lysosomal acid lipase plays a major role in the delivery of atherogenic lipoproteins to the cell by hydrolyzing the CE moiety from lipoproteins delivered to the lysosome via endocytosis or phagocytosis into free cholesterol (20). This newly hydrolyzed free cholesterol is then made available to ACAT for intracytoplasmic synthesis of CEs. Increases in both LAL and ACAT activities have thus been implicated in promoting macrophage foam cell formation (13). In contrast, DHEA had no effect on the expression of scavenger receptors A1 or A2, receptors implicated in mediating the influx of atherogenic lipoproteins into MDMs (21). These gene expression findings are strongly consistent with our recent demonstration that androgen (dihydrotestosterone) exposure in male donor MDMs is associated with increased LAL and ACAT expression, but with no significant effects on scavenger receptor expression (22). The congruence between DHEA- and dihydrotestosterone-related effects on MDM gene expression is consistent with the observed DHEA effects being due to an androgenic mode of action.

In contrast to our current findings, Taniguchi et al. (23) have reported that short-term exposure to DHEA inhibits CE accumulation in the female-derived mouse macrophage cell line J774A.1. However, it is difficult to extrapolate the findings of this study to the human setting for a number of

reasons: 1) adrenal production of DHEA or DHEAS in mice is minimal, making the relevance of these observations to humans doubtful; and 2) a female-derived macrophage cell line was used (which may not have expressed the androgen receptor).

We have previously reported that dihydrotestosterone exposure is also associated with increased human monocyte-endothelial adhesion via enhanced endothelial cell expression of VCAM-1 (14). On the other hand, estrogens inhibit monocyte-endothelial adhesion and inhibit endothelial cell expression of E-selectin, ICAM-1, and VCAM-1 (24). Previous *in vitro* experiments have reported that human endothelial cells possess the capacity to convert DHEA to other biologically more potent sex steroids, particularly androgens such as testosterone and dihydrotestosterone (25). In our experiments, DHEA, even at supraphysiologic concentrations, had no significant effect on monocyte-endothelial cell adhesion or endothelial cell-surface expression of cell adhesion molecules, either at baseline or at IL-1- $\beta$ -stimulated states. This neutral finding suggests that DHEA does not simply exert androgen-like effects on endothelial cells, at least compared with the potent non-aromatizable androgen dihydrotestosterone.

**DHEA in atherogenesis: evaluation of the biologic plausibility of an effect.** To evaluate the biologic plausibility of a DHEA-mediated effect on atherogenesis, it is necessary to consider differences in DHEA levels that are observed with increasing age and variations in DHEA levels across different populations.

Multiple prospective studies of middle-aged and elderly subjects have investigated the relationship between DHEAS levels and atherosclerosis. The results of these studies have not been consistent with different studies reporting either neutral, positive, or inverse relationships between DHEAS levels and a variety of cardiovascular end points (1). Consideration of the steep age-related decline in DHEAS secretion may explain the apparent discrepancy between such studies. In both men and women, DHEAS levels reach a peak between the second and third decades of life (26). Thereafter, levels decrease markedly with age; by 70 years of age, DHEAS levels are down to only 20% of their peak values (26). In the context of the pathogenesis of atherosclerotic lesions, the timing of peak DHEAS concentrations coincide with the progression of arterial fatty streaks to a more advanced lesion morphology—the fibrous plaque. All published prospective studies investigating the relationship between DHEAS and atherosclerosis have been performed in late-middle-age or elderly groups, where DHEA and DHEAS concentrations are only a fraction of those in young adulthood, and at a stage where atherosclerosis is already well established. Therefore, prospective studies investigating the link between DHEAS and atherosclerosis have been undertaken in age groups where the question of a potential relationship between the hormone and the disease may be least biologically relevant. Consistent with this hypothesis, the most striking effects of DHEA on foam cell

formation in our study were observed at a DHEA concentration consistent with those used in young male adults (42 nmol/l), whereas concentrations in keeping with elderly subjects (4.2 to 8.4 nmol/l) yielded only very modest effects on CE accumulation.

As DHEAS concentrations decline in older age, it is possible that DHEA and DHEAS exert different effects at different stages in life. In the prospective study with the youngest cohort of patients thus far, Hautanen et al. (6), using data from the Helsinki Heart Study (a trial involving dyslipidemic men with a mean age of 48 years), found that after adjustment for smoking and age, men with high DHEAS levels had a twofold *increased* risk of myocardial infarction, as compared with men with lower levels. In contrast, three prospective studies (5,7,8) in much older men have reported neutral relationships between a variety of nonfatal cardiovascular end points in men, including nonfatal myocardial infarction and carotid atherosclerosis.

The picture is somewhat different when considering *prospective* data relating DHEAS levels and male cardiovascular mortality. Barrett-Connor et al. (3), using data from the Rancho Bernardo Study of men aged 50 to 79 years, and LaCroix et al. (5), studying men with a mean age of 57 years, have both reported an inverse relationship between low DHEAS levels and a future risk of coronary heart disease death. In comparison, two recent, large, prospective studies in older male populations by Trivedi et al. (9) (mean age 70 years) and Tilvis et al. (27) (age >75 years) failed to find a consistent relationship between DHEAS levels and cardiovascular mortality in the elderly male population. In an expanded and updated analysis of the Rancho Bernardo Study (28), which included 1,029 men (mean age 60 years) followed up for 19 years, DHEAS was reported to provide a far more modest protection against cardiovascular death in men than originally reported. In this re-analysis of the Rancho Bernardo Study data, it is notable that under 70 years of age, mean DHEAS levels tended to be *higher* in all five-year age groups of men dead from cardiovascular disease or from any cause compared with those still alive (28). When above 70 years, however, all-cause and cardiovascular mortality was associated with lower DHEAS levels.

In contrast to men, prospective data on DHEAS and female cardiovascular risk are more consistent. In four prospective studies conducted in middle-aged to elderly female study populations, no relationship was found between DHEAS levels and a variety of cardiovascular end points, including cardiovascular mortality and carotid atherosclerosis, as detected by ultrasonography (4,8,9,27).

Comparative data between populations also do not support an atheroprotective role of adrenal androgens. LaCroix et al. (5) and Khaw (1) have both noted that there is great variability in DHEAS levels between different populations. For example, Japanese men in Japan have the lowest age-specific mean DHEAS levels, and California Caucasian men have the highest, with Hawaiian Japanese men having levels in between. At 60 to 64 years of age, California men

have approximately threefold (4.1  $\mu\text{mol/l}$ ) and Japanese Hawaiian men twofold (2.7  $\mu\text{mol/l}$ ) the mean DHEAS levels of Japanese men in Japan (1.4  $\mu\text{mol/l}$ ). Corresponding rates of coronary heart disease in these populations follow the same pattern. Although such ecologic data can be criticized on account of potential confounding factors, they provide no support for the notion that low DHEAS levels increase coronary heart disease rates.

**Study limitations.** Our *in vitro* experiments evaluated the effects of *direct* DHEA administration to human MDMs and endothelial cells. It is well known that DHEA and DHEAS both serve as sex hormone precursors that are converted by 3- $\beta$ -hydroxysteroid dehydrogenase to androstenedione and then into potent androgens and/or estrogens in the peripheral tissues such as skin, adipose tissue, breast lung, liver, kidney, and brain. It is therefore possible that in the *in vivo* context, the "net" vascular biologic effect of DHEA may be different, being the summation of both direct effects (observed in this study) and indirect effects via androgenic and estrogenic metabolites. Furthermore, although using human serum and primary cells, these *in vitro* experiments may not necessarily represent the same conditions as in the arterial wall *in vivo*.

**Conclusions.** In physiologic concentrations, DHEA increases CE accumulation in male donor human MDMs. This effect is mediated, at least in part, via the androgen receptor and involves the upregulation of the lipoprotein-processing enzymes ACAT and LAL. In contrast, DHEA has no significant effect on human monocyte adhesion to endothelium or expression of the cell adhesion molecules ICAM-1, VCAM-1, and E-selectin. The potentially pro-atherogenic effect of DHEA on male macrophage foam cell formation is consistent with available interpopulation and age-specific epidemiologic data on DHEA concentrations, in the context of the natural history of atherosclerosis. Further basic and clinical studies are required to further elucidate the potentially adverse cardiovascular effects of DHEA.

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