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EXPEDITED REVIEW

Androgens Up-Regulate Atherosclerosis-Related Genes in Macrophages From Males But Not Females

Molecular Insights Into Gender Differences in Atherosclerosis

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OBJECTIVES This study investigated the effects of androgens on gene expression in male- and female-donor macrophages.

BACKGROUND Men have more severe coronary disease than women. Androgen exposure increases foam cell formation in male but not female macrophages, and male macrophages express >4-fold more androgen receptor messenger ribonucleic acid than female macrophages. Therefore, androgen exposure may have gender-specific and potentially pro-atherogenic effects in macrophages.

METHODS Utilizing complementary deoxyribonucleic acid arrays, we studied the effects of a pure androgen (dihydrotestosterone, 40 nmol/l) on human monocyte-derived macrophages from healthy male and female donors (n = 4 hybridizations; 2 men, 2 women). Differential expression of atherosclerosis-related genes was confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) in five male and five female donors. Functional corroboration of foam cell formation-related findings was undertaken by experiments using ^{125}I-acetylated low-density lipoprotein (AcLDL).

RESULTS In male macrophages, androgen treatment produced differential up-regulation of 27 genes concentrated in five functional classes: 1) lipoprotein processing; 2) cell-surface adhesion; 3) extracellular signaling; 4) coagulation and fibrinolysis; and 5) transport protein genes. By contrast, none of 588 genes were up-regulated in female macrophages. By RT-PCR, we confirmed the gender-specific up-regulation of six of these atherosclerosis-related genes: acyl coenzyme A:cholesterol acyl transferase I, lysosomal acid lipase (LAL), caveolin-2, CD40, vascular endothelial growth factor-165 receptor, and tissue factor pathway inhibitor. Functionally, androgen-treated male macrophages showed increased rates of lysosomal AcLDL degradation, by 45% to 75% after 15 to 20 h of ^{125}I-AcLDL incubation ($p = 0.001$), consistent with increased LAL activity.

CONCLUSIONS Androgens increase expression of atherosclerosis-related genes in male but not female macrophages, with functional consequences. These findings may contribute to the male predisposition to atherosclerosis. (J Am Coll Cardiol 2003;42:1306–13) © 2003 by the American College of Cardiology Foundation

Men have earlier and more severe atherosclerosis than women, independent of environmental risk factor exposure (1). Indeed, the incidence of coronary deaths in men age 35 to 64 years exceeds that in age-matched women by up to 500% (2). To date, the causes of this gender difference remain unclear. Such observations have produced considerable interest in the potential role of sex hormones in atherogenesis. The apparent atheroprotective effect of estrogen has been studied extensively, and, contrary to expectations, the first prospective randomized controlled trials to test this hypothesis have shown adverse effects (3,4). The role of androgens is now also being scrutinized.

Macrophages play a key role in both the early (via foam cell formation) and late (via inflammatory and other mediators) stages of atherosclerosis (5). Formation of the macrophage foam cell is due to the uptake of modified lipoproteins by monocyte-derived macrophages (MDMs) in the arterial wall (6). A growing body of evidence shows that sex steroid hormones regulate processes integral to human macrophage foam cell formation (7). Furthermore, sex steroids may act in a gender-specific manner, with greater effects of estrogens and/or androgens in male or female cells (7). This gender-related regulation might have important implications for understanding the basis of (and developing treatments for) the gender gap in atherosclerosis.

We recently demonstrated that androgen exposure increases foam cell formation in male- but not female-donor

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Abbreviations and Acronyms

ACAT	= acyl coenzyme A:cholesterol acyl transferase I
AcLDL	= acetylated low-density lipoprotein
AR	= androgen receptor
cDNA	= complementary deoxyribonucleic acid
DHT	= dihydrotestosterone
LAL	= lysosomal acid lipase
MDM	= monocyte-derived macrophage
RNA	= ribonucleic acid
RT-PCR	= reverse transcription-polymerase chain reaction
TFPI	= tissue factor pathway inhibitor
VEGF	= vascular endothelial growth factor

human MDMs, via an androgen receptor (AR)-mediated pathway (8). Furthermore, male-donor MDMs express at least four-fold more AR messenger ribonucleic acid (RNA) than MDMs from female donors (8). As the AR mediates the genomic effects of androgen action, androgens may have gender-specific and potentially pro-atherogenic effects on macrophage gene expression. Utilizing complementary deoxyribonucleic acid (cDNA) array analysis, we have systematically investigated the effects of androgen exposure on gene expression in male- and female-donor human MDMs in order to identify: 1) atherosclerosis-related genes potentially regulated by androgen exposure; and 2) cellular mechanisms involved in the androgen regulation of macrophage foam cell formation.

METHODS

Isolation, culture, and hormone treatment of human monocytes. Monocytes were isolated from whole blood of young healthy adult male and premenopausal female donors (inclusion criteria: Caucasian, <35 years of age, without pre-existing cardiovascular disease or cardiovascular risks, and no hormone therapy) by counterflow centrifugation elutriation (9) and cultured in phenol red-free RPMI (Life Technologies, Carlsbad, California) containing 10% postmenopausal human serum (17β-estradiol <75 pmol/l, progesterone <0.8 nmol/l, and testosterone <2 nmol/l), penicillin G (50 U/ml), and streptomycin (50 μg/ml). Over a nine-day maturation period, the following treatments were added with each media change from days 3 to 9: 1) dihydrotestosterone (DHT) at 40 nmol/l, or 2) vehicle control (0.1% ethanol). Monocyte purity was >95% and viability >95% (trypan blue) in all experiments.

RNA isolation and cDNA array experiments. To identify atherosclerosis-related genes potentially regulated by androgen exposure, cDNA arrays were used (ATLAS Human Cardiovascular Array containing 588 genes and 9 housekeeping genes, Clontech Laboratories, Palo Alto, California). Total RNA was isolated from both control- and androgen-treated macrophages at maturation (day 9). Four separate array analyses of gene expression in control- versus androgen-treated MDMs from four consecutive donors (two men and two women) were undertaken. Probe synthesis, array hybridization, and phosphorimager analysis were performed using a modification of published methods (10). Briefly, cDNA probes both control- and androgen-treated MDMs, synthesized by reverse transcription of 5 μg total RNA in the presence of α^{32}P-dATP, were hybridized for 16 h onto separate but identical array membranes, and the signal intensities were measured by phosphorimager analysis (Fujix Bas 1500 Phosphorimager, Fuji, Tokyo, Japan). Pairwise comparative gene expression between controls and androgen-treated MDMs from each donor was undertaken after signal intensities were converted to a ratio adjusted for background and housekeeping gene (60S rRNA) expression. Data for each gene were used only when the signals for that gene from both membranes were at least 50% above background. Hence, our experiments preferentially detected up-regulation rather than down-regulation.

The cDNA array reproducibility was evaluated by undertaking two consecutive array hybridizations using the same two samples of control- and androgen-treated RNA. These data demonstrated that 97% of differential expression values in each experiment were within two-fold of each other.

DIFFERENTIAL EXPRESSION CRITERIA. Individual genes that exhibited at least two-fold up-regulation in the androgen-treated cells relative to control in both membranes for each gender were defined as being differentially expressed.

SELECTION OF HOUSEKEEPER GENE. A non-linear power regression analysis of cDNA array signal intensities from both control- and androgen-treated arrays of each subject was used to identify the housekeeper gene. On all regression plots, 60S rRNA did not exhibit differential expression with androgen treatment and was selected as the housekeeper gene.

Relative real time reverse transcription-polymerase chain reaction (RT-PCR). Confirmation of array findings in six atherosclerosis-related genes was undertaken in MDMs from five male and five female donors by relative real-time RT-PCR with SYBR Green I (Molecular Probes, Eugene, Oregon) monitoring using a modification of published methods (11). The MDMs for these experiments were cultured using the same protocols and treated in the same hormonal conditions as for the cDNA array studies. The genes assayed included: acyl coenzyme A:cholesterol acyltransferase I (ACAT); lysosomal acid lipase (LAL); caveolin-2; CD40; vascular endothelial growth factor (VEGF)-165 receptor; and tissue factor pathway inhibitor (TFPI). 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 Incorporated [ABI] Model 7700 Sequence Detector, PE Biosystems, Foster City, California) and analyzed using ABI Prism Sequence Detector Software version 1.6.3 (PE

Biosystems). The housekeeping gene, 60S rRNA, was also measured for normalization of real-time PCR results.

The primer sequences were: ACAT forward 5′ AGTTGACAGCAGAGGCAGAG 3′, reverse 5′ GGATAAAGAGAATGAGGAGGG 3′; LAL forward 5′ GCAACAGCAGAGGAAATAC 3′, reverse 5′ GAGAATGACCCACATAATACAC 3′; caveolin-2 forward 5′ TGCAGACAATATGGAAGAG 3′, reverse 5′ GAAATGAACAGAACAGTGG 3′; CD40 forward 5′ GCACCTCAGAAACAGACAC 3′, reverse 5′ GGACCACAGACAACATCAG 3′; VEGF-165 receptor forward 5′ CTCATCACCATCATAGCC 3′, reverse 5′ CAACTTCACACCATCCAC 3′; TFPI forward 5′ GTGAATAACTCCCTGACTCC 3′, reverse 5′ TTTCACTCTCTGCTTCTTTC 3′; and 60S rRNA forward 5′ GGAGAAGAGGAAAGAGAAAG 3′, reverse 5′ CAACGCATGAGGAATTAAC 3′.

Functional studies on lipoprotein metabolism. Lipoprotein uptake and lysosomal degradation studies were conducted in both control- and androgen-treated (DHT 400 nmol/l) male-donor MDMs after incubation with ^{125}I-acetylated low-density lipoprotein (AcLDL). Cell-surface–bound AcLDL was determined after a 4-h incubation at 4°C with ^{125}I-AcLDL with and without a 30-fold excess of unlabeled AcLDL. Specific binding was calculated by subtracting the results in the presence of unlabeled AcLDL from that in its absence (12). Binding parameters were analyzed using the LIGAND computer program (BIOSOFT, Cambridge, United Kingdom). Lysosomal degradation of AcLDL was determined as trichloroacetic acid-soluble, non-iodide ^{125}I in the post-incubation medium (12) following incubation at 37°C with 20 μg/ml ^{125}I-AcLDL (with or without a 30-fold excess of unlabeled Ac LDL) for 6, 16, and 20 h. Retroendocytosis studies were conducted to using a modification of published methods (13). Briefly, MDMs were incubated with 20 μg/ml ^{125}I-AcLDL at 37°C for up to 120 min. Following incubation, cells were cooled to 4°C and washed five times with phosphate buffered saline containing 2 mg/ml bovine serum albumin, once with an acid wash (0.2 mol/l acetic acid, 0.5 mol/l sodium chloride, pH 2.4), followed by three times with phosphate buffered saline. Retroendocytosis of AcLDL was determined by measuring release of trichloroacetic acid-precipitable ^{125}I-material during a 1-h chase period at 37°C (13).

Statistical analysis. All results are expressed as mean ± SEM. Statistical comparisons were performed using paired or unpaired *t* tests, as appropriate. In the RT-PCR experiments, a repeated measures design was adopted in that both control- and DHT-treated macrophages are derived from the same subject. For these experiments, gene expression in control- versus DHT-treated cells in each gender were compared one gene at a time using a paired *t* test. Where multiple comparisons were performed—for example, when comparing gene expression by real-time RT-PCR, p values were adjusted according to Hochberg's modification of the Bonferroni procedure (14). Statistical analysis was performed using GraphPad Prism version 3.0a (GraphPad Software, San Diego, California), and regression plots were generated using Kaleidagraph 3.0 (Synergy Software, Reading, Pennsylvania).

RESULTS

Effects of androgen exposure on male and female donor macrophage gene expression: cDNA array findings. In cDNA array analyses of MDMs from two consecutive subjects for each gender, androgen exposure in male-donor MDMs up-regulated the expression of 27 genes. The genes encoded for proteins that were categorizable into five functional clusters: 1) lipoprotein and other metabolic pathways; 2) adhesion, inflammation, and cell-surface antigens; 3) coagulation and fibrinolysis; 4) extracellular signaling/receptor-associated proteins; and 5) transport proteins (Table 1, Figs. 1A and 1B). By contrast, in female-donor MDMs, no gene met our criteria of being up-regulated at least two-fold in two consecutive individuals by cDNA array analysis.

Real time relative RT-PCR. The androgen-dependent, gender-specific up-regulation of six atherosclerosis-related genes was confirmed by real time RT-PCR, by comparing the expression of each gene in androgen- versus control-treated MDMs from five consecutive male and five consecutive female donors. Gene expression for each subject was quantified four times—twice on each of two separate cDNA syntheses). In male-donor MDMs, androgen exposure up-regulated the expression of ACAT by 5-fold, LAL by 3.8-fold, caveolin-2 by 3.4-fold, CD40 by 5.5-fold, VEGF-165 receptor-1 by 3.7-fold, and TFPI by 4.8-fold, respectively ($p < 0.005$ vs. control for all genes). By comparison, androgen exposure had no significant effect on the expression of the same genes in female donor MDMs when compared with control ($p > 0.1$ vs. control for all genes) (Fig. 1C).

Functional studies: the effect of androgen exposure and macrophage lipoprotein metabolism. Because DHT increases cholesteryl ester accumulation in male-donor MDMs (8), we used the cDNA array findings to identify cellular mechanisms for androgen action. Functional analysis of cDNA array findings indicated that androgen effects on foam-cell-formation–related processes involved up-regulation of genes involved in lysosomal degradation (LAL) and post-lysosomal processing (ACAT and hormone sensitive lipase). In contrast, the expression of genes involved in pre-lysosomal processes, such as modified low-density lipoprotein binding and uptake (macrophage scavenger receptor types A1 and A2), were unaffected by androgen exposure. Therefore, in vitro experiments were undertaken to evaluate the effects of androgen exposure on the uptake and lysosomal processing of modified low-density lipoprotein by male-donor MDMs and to corroborate the functional implications of our array findings.

Table 1. Genes Up-Regulated by Androgen Exposure in Male- and Female-Donor Human Macrophages*

Gender	Gene Functional Class	Accession No.	Gene Name
Male	Lipoprotein and other metabolic pathways	L21934	Acyl coenzyme A:cholesterol acyltransferase
		U40002	Hormone-sensitive lipase
		M74775	Lysosomal acid lipase
		AF035752	Caveolin-2
		X17025	Isopentenyl diphosphate delta isomerase
		M34788	Adrenodoxin/ferredoxin 1
		L19501	Cystathionine beta-synthase
		M58342	Iduronate 2-sulfatase
		M36693	Superoxide dismutase 2
	Adhesion, inflammation, and cell surface antigens	X60592	CD40
		AF047826	Cadherin 19, type 2
		U41070	Leukotriene B4 receptor
		X05908	Annexin I
		U34802	Gap junction protein alpha-8
		U49240	Symplekin
	Coagulation and fibrinolysis	D00017	Annexin II
		J03225	Tissue factor pathway inhibitor
		D29992	Tissue factor pathway inhibitor 2
		V00595	Prothrombin precursor
	Extracellular signaling/receptor-associated genes	L25615	Arginine vasopressin receptor 1A
		X15357	Atrial natriuretic peptide receptor A
		L13436	Atrial natriuretic peptide receptor B
		AF016050	Vascular endothelial cell growth factor 165 receptor
		AF016098	Vascular endothelial cell growth factor 165 receptor 2
	Transport proteins	Y10255	Fatty acid binding protein 3
		M94856	Fatty acid binding protein 5
		M20747	Insulin-responsive glucose transporter type 4
Female	Nil	Nil	Nil

*Differential up-regulation was defined as an at least two-fold up-regulation in androgen-treated cells versus control in both arrays for each gender.

LYSOSOMAL DEGRADATION OF MODIFIED LOW-DENSITY LIPOPROTEIN. To evaluate the functional significance of increased LAL expression, we studied the effects of androgen exposure on lysosomal lipoprotein degradation in male-donor macrophages by incubation of macrophages with radiolabelled AcLDL. Androgen-treated male donor macrophages showed increased rates of lysosomal degradation of AcLDL at 37°C compared with controls (Fig. 2A). This difference was highly significant by 20 h of incubation with radiolabelled AcLDL ($p = 0.001$).

SCAVENGER RECEPTOR BINDING AND RETROENDOCYTOSIS STUDIES. No difference existed between control- and DHT-treated male MDMs in specific or non-specific cell-surface binding of AcLDL with increasing concentrations of radiolabelled ligand (Fig. 2B). This was confirmed by computational analysis of binding parameters (LIGAND program, BIOSOFT), which showed no difference in binding affinity (Kd 1.77×10^{-8} mol/l and Kd 1.91×10^{8} mol/l in controls and DHT-treated cells, respectively; $p > 0.9$) or receptor site concentrations (1.23×10^{-10} mol/l and 1.35×10^{-10} mol/l in controls and DHT-treated cells, respectively; $p > 0.3$) between androgen and control treatments. In addition, there was no difference in retroendocytosis of undegraded AcLDL between control- and DHT-treated cells during pulse-chase experiments where the only source of labeled lipoprotein was from an intracellular site of previously endocytosed I^{125}-AcLDL (100 ± 5 % and 103 ± 4% for control- and DHT-treated cells, respectively; $p > 0.1$).

In the absence of androgen effects on scavenger receptor binding and retroendocytosis, the effect of DHT on lysosomal lipoprotein degradation is consistent with a primary effect on increasing the activity of LAL. Overall, these functional data are consistent with the gene expression findings noted above.

DISCUSSION

Male gender is a strong risk factor for atherosclerosis, independent of environmental risk factor exposure (1). Therefore, gender differences in sex hormones and genetics may contribute to the male predisposition to atherosclerosis. To date, few studies have investigated gender-dependent differences in the regulation of atherosclerosis-related genes. Using cDNA array analysis, we report that androgens regulate the macrophage expression of a large number of atherosclerosis-related genes in a strikingly gender-dependent manner. In male-donor human MDMs, androgen exposure up-regulated the expression of 27 genes with a range of atherosclerosis-related functions, including lipoprotein metabolism, adhesion, inflammation, coagula-

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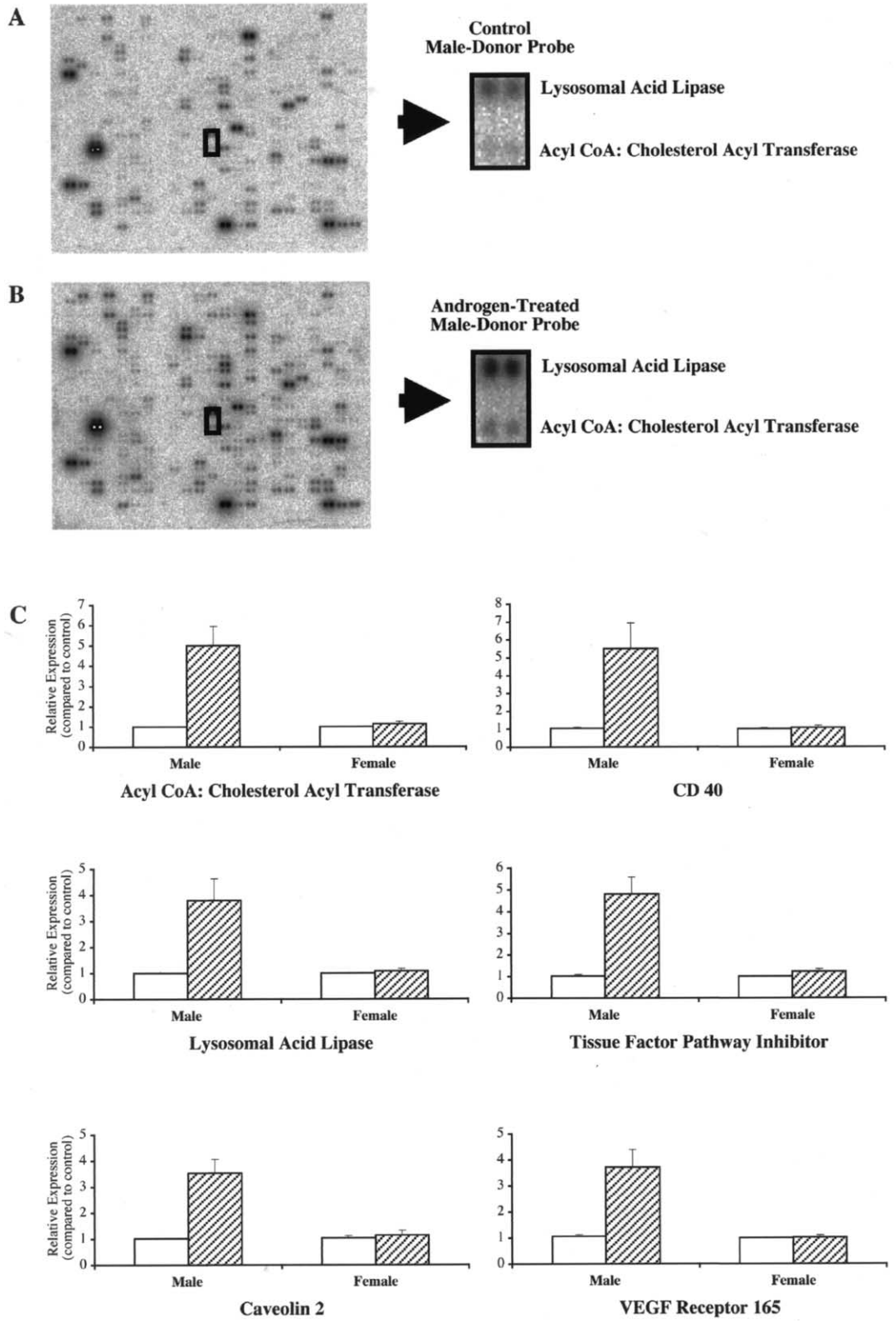

Figure 1. Effects of androgen exposure on macrophage gene expression. **(A, B)** Gene expression profiles in control- and androgen-treated male macrophages, respectively. There is enhanced expression of a large number of atherosclerosis-related genes (see Table 1). For example, the **boxed areas** containing lysosomal acid lipase (LAL) and acyl coA:cholesterol acyl transferase (ACAT) are enlarged and shown on the **right**. **(C)** Differential gene expression examined by reverse transcription-polymerase chain reaction (RT-PCR) shows the androgen-dependent gender-specific up-regulation of six atherosclerosis-associated genes: 1) ACAT; 2) LAL; 3) caveolin-2; 4) CD40; 5) tissue factor pathway inhibitor; and 6) vascular endothelial growth factor (VEGF)-165 receptor. In all genes studied by RT-PCR, androgen exposure produced up-regulation in gene expression in male-donor but not female-donor macrophages ($p < 0.005$ vs. control for all genes in male-donor cells as compared with $p > 0.1$ vs. control for all genes in female-donor cells). **Open bars** = controls; **striped bars** = 40 nmol/l dihydrotestosterone.

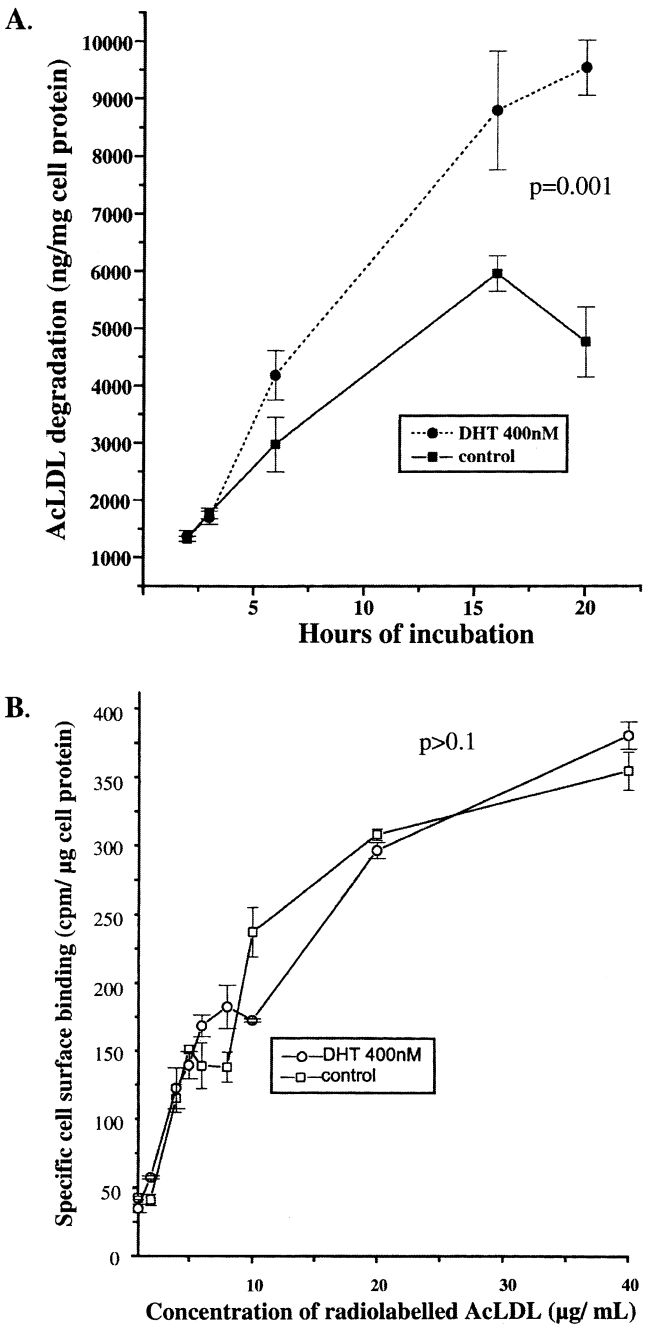

Figure 2. Effects of androgen exposure on macrophage lipoprotein metabolism. **(A)** Androgen exposure increased the rate of lysosomal degradation of acetylated low-density lipoprotein (AcLDL) by male-donor monocyte-derived macrophages—a finding consistent with increased lysosomal acid lipase activity ($p = 0.001$ for dihydrotestosterone [DHT]-treated vs. control cells, at 20 h). **(B)** Androgen exposure had no effect on cell surface binding of ^{125}I-AcLDL ($p > 0.1$ for androgen vs. control macrophages).

tion, and angiogenesis (Table 1). By contrast, *no* genes were affected in female-donor MDMs. These array findings were confirmed by real-time RT-PCR findings, as well as by the demonstration of functional effects of androgen exposure on macrophage lipoprotein metabolism.

We previously reported that androgen exposure increases human macrophage foam cell formation in male-donor but not in female-donor MDMs (8). The present study extends these findings by demonstrating that androgens exert complex and gender-specific effects on the expression of lipoprotein metabolism genes in male-donor MDMs. This involves the up-regulation of genes involved in: 1) lysosomal lipoprotein processing (LAL), and 2) post-lysosomal processes including intracytoplasmic cholesterol esterification (ACAT) and cholesteryl ester hydrolysis (hormone-sensitive lipase). By contrast, the expression of genes involved in modified low-density lipoprotein binding and uptake (scavenger receptors class A1 and A2) were unaffected by androgen exposure. As androgens exert both genomic and non-genomic effects and as changes in gene expression do not always reflect functional changes, we undertook a series of lipoprotein metabolism studies to examine androgen regulation of modified low-density lipoprotein cell surface binding, retroendocytosis, and lysosomal degradation of lipoproteins. Our gene expression findings are corroborated by in vitro functional studies demonstrating that androgen exposure increases the rate of lysosomal hydrolysis of lipoproteins in male-donor MDMs, in the absence of androgen effects on scavenger receptor binding, or retroendocytosis. These findings are in keeping with an androgen-dependent up-regulation of LAL activity in male-donor MDMs. The LAL plays a major role in the delivery of atherogenic lipoproteins to the cell, by hydrolyzing the cholesteryl ester moiety from lipoproteins delivered to the lysosome via endocytosis or phagocytosis into free cholesterol, thereby making free cholesterol available for intracytoplasmic re-esterification by ACAT (15). Increase in LAL activity has thus been implicated in promoting macrophage foam cell formation (15,16). Our current findings also support a previous observation of positive correlation between serum testosterone levels during development and hormone-sensitive lipase protein levels and enzymatic activity in the guinea pig testis (17). In male MDMs, therefore, androgen exposure exerts widespread effects on the expression of genes involved in lipoprotein metabolism, with a net functional effect of increasing the accumulation of cholesteryl esters.

The adherence of monocytes to vascular endothelium and their subsequent transmigration into the vessel wall are key early events in atherogenesis (5). We have previously reported that androgen treatment of endothelial cells increases human monocyte adhesion to endothelium in male-donor cells via up-regulation of endothelial cell expression of vascular cell adhesion molecule-1 (18). The present study extends these observations from endothelial cells to monocytes. In the current study, we found that androgen exposure in macrophages produced a gender-specific up-regulation of genes implicated in promoting the adherence of monocytes to vascular endothelium (including CD40, leukotriene B4 receptor, and cadherin 19). CD40 ligation in human macrophages has been shown to trigger the expression of adhesion molecules including lymphocyte function-associated antigen-1 and intercellular adhesion molecule-1 (19). Leukotriene B4, a potent chemoattractant and pro-

inflammatory mediator, has also been implicated in monocyte recruitment, activation, and adhesion. Leukotriene B4 receptor antagonism has been shown to reduce monocyte infiltration and lipid accumulation in apoE-deficient mice (20).

A number of the array findings from this study are also consistent with available observational data. In clinical studies evaluating the effect of androgens on coagulation, exogenous testosterone has been reported to increase plasma levels of prothrombin fragment F1.2 in healthy men (21), but not in women with severe premenstrual syndrome (22), which is consistent with a gender-dependent effect. In animal models, androgen exposure up-regulates arginine vasopressin receptor 1A messenger RNA in the male Syrian hamster brain (23) and increases fatty acid binding protein content in rat heart and skeletal muscle (24). Increasing evidence shows the role of androgens in the control of angiogenesis. Dihydrotestosterone up-regulates the expression of VEGF messenger RNA and VEGF biological activity in an adult rat prostate (25), and testosterone increases VEGF expression via an AR-dependent manner, in an immortalized cell line S115 (26).

We and others have previously shown that male human macrophages express higher levels of AR message than female-donor cells (8,27). Moreover, in rat aortic smooth muscle cells, AR protein levels are higher in male than in female animals (28). Therefore, the marked gender specificity of androgen effects on macrophage gene expression is most likely related to gender differences in MDM AR content. Hence, gender differences in AR content may be a key mediator of gender differences in vascular biology. This hypothesis is supported by a recent study using male testicular feminized rats, which express a non-functional AR. In rats, the contractile response of the thoracic aorta to vasopressin is three-fold higher in females than in males (29). Stallone et al. (29) found that this gender difference in vascular reactivity in rat aortas was abolished in AR-deficient male testicular feminized rats, where responses to vasopressin were similar to normal female rather than male animals.

Regarding limitations, we used arrays with 588 cDNAs with a high concentration of genes with established relevance to atherosclerosis and vascular biology. A larger, genome-wide screen might identify more androgen-regulated genes within macrophages.

Dihydrotestosterone, a potent physiologic androgen specific for AR, was used at a concentration of 40 nmol/l on the basis of previous foam cell and cell adhesion experiments (8,18). As DHT is not aromatizable to estrogenic metabolites, unlike testosterone (30), its use avoids the potentially confounding effects of estrogens, as they have also been shown to influence both gene expression and macrophage lipid loading (31). Whereas DHT at 40 nmol/l is higher than normal circulating levels (being ten-fold above the normal serum concentration for male adults), this concentration is physiologically relevant in our experiments as: 1) testosterone, the major circulating androgen (usual serum concentrations 20 to 32 nmol/l in men), is absent from our culture milieu; and 2) plasma levels of DHT do not reflect tissue formation (32), particularly in tissues possessing 5α-reductase activity, such as macrophages (33,34). The 5α-reductase enzyme converts testosterone to DHT, accounting for 75% of tissue DHT, which is the final androgenic messenger in many target tissues. This enzyme is also present in the other major cell types in the atherosclerotic lesion: the endothelial cell (35) and the vascular smooth muscle cell (36). Therefore, in the absence of testosterone, the use of 40 nmol/l DHT for in vitro treatment of MDMs is likely to be physiologically relevant because DHT concentrations in tissues possessing 5α-reductase activity can approach concentrations in the order of 100 nmol/l (34,37). Moreover, the functional effects of gender-dependent, AR-mediated up-regulation of genes are evident at DHT concentrations as low as 4 nmol/l (8). In our previous dose-ranging studies, DHT produced a dose-dependent, AR-dependent increase in male-donor MDM cholesteryl ester accumulation which was demonstrable between 4 to 400 nmol/l (inclusive), but had no effect on cholesteryl ester accumulation in female-donor MDMs even at concentrations as high as 400 nmol/l (8).

In summary, using differential gene-expression techniques, we report for the first time that androgens regulate the macrophage expression of a large number of atherosclerosis-related genes in a highly gender-dependent manner. Androgen exposure up-regulated the expression of 27 genes, many with known atherosclerosis-related functions, in male-donor MDMs but had no significant effect on gene expression in female-donor MDMs. These findings provide novel insights into the effects of androgens on gene expression in the macrophage and suggest that androgens may be involved in the complex and gender-dependent regulation of a range of atherosclerosis-related processes including foam cell formation, adhesion, inflammation, coagulation, and angiogenesis. These gene expression findings are corroborated by functional data confirming the androgen-dependent up-regulation of lysosomal degradation of modified lipoproteins in male-donor MDMs—a process integral to the delivery of atherogenic lipoproteins to the macrophage. These effects may contribute to the male predisposition to atherosclerosis.

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