Cellular SR-BI and ABCA1-mediated cholesterol efflux are gender-specific in healthy subjects[S]

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Abstract We evaluated the impact of gender differences in both the quantitative and qualitative features of HDL subspecies on cellular free cholesterol efflux through the scavenger receptor class B type I (SR-BI), ABCA1, and ABCG1 pathways. For that purpose, healthy subjects (30 men and 26 women) matched for age, body mass index, triglyceride, apolipoprotein A-I, and high density lipoprotein-cholesterol (HDL-C) levels were recruited. We observed a significant increase (+14%; $P < 0.03$) in the capacity of whole sera from women to mediate cellular free cholesterol efflux via the SR-BI-dependent pathway compared with sera from men. Such enhanced efflux capacity resulted from a significant increase in plasma levels of large cholesteryl ester-rich HDL_2 particles (+20%; $P < 0.04$) as well as from an enhanced capacity (+14%; $P < 0.03$) of these particles to mediate cellular free cholesterol efflux via SR-BI. By contrast, plasma from men displayed an enhanced free cholesterol efflux capacity (+31%; $P < 0.001$) via the ABCA1 transporter pathway compared with that from women, which resulted from a 2.4-fold increase in the plasma level of preβ particles ($P < 0.008$). Moreover, in women, SR-BI-mediated cellular free cholesterol efflux was significantly correlated with plasma HDL-C ($r = 0.72$, $P < 0.0001$), whereas this relationship was not observed in men. In conclusion, HDL-C level may not represent the absolute indicator of the efficiency of the initial step of the reverse cholesterol transport.—Catalano, G., E. Duchene, Z. Julia, W. Le Goff, E. Bruckert, M. J. Chapman, and M. Guerin. **Cellular SR-BI and ABCA1-mediated cholesterol efflux are gender-specific in healthy subjects. *J. Lipid Res.* 2008.** 49: **635–643.**

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Plasma HDL particles are highly heterogeneous in their structure, metabolism, and biological functions (1). HDL particles display a spectrum of antiatherogenic properties, including cellular cholesterol efflux, a key feature of the initial phase of the reverse cholesterol transport pathway, protection of LDL particles from oxidative stress, and anti-inflammatory, antiapoptotic, vasodilatory, antithrombotic, and anti-infectious activities (2). Several mechanisms of cellular cholesterol efflux have been proposed (3). One is the nonspecific release of cholesterol from the cell surface membrane to extracellular acceptors mediated by passive aqueous diffusion. This efflux pathway occurs in all cell types, but it has been considered inefficient compared with receptor- or transporter-mediated efflux, although recent studies (4, 5) suggest that aqueous transfer mechanism may be quantitatively as important as cholesterol efflux mediated by specific transporters or receptors. ABCA1, a key membrane cholesterol transporter and a member of the large ATP binding cassette family, has been reported to play a major role in cholesterol efflux from macrophages and foam cells (6). Indeed, ABCA1 promotes phospholipid and free cholesterol efflux to lipid-poor or lipid-free apolipoprotein A-I (apoA-I), thereby allowing the formation of spherical HDL particles after the esterification of free cholesterol by LCAT. Scavenger receptor class B type I (SR-BI), a member of the CD36 family, mediates cellular cholesterol efflux to large, cholesteryl ester (CE)-rich HDL particles (7). Indeed SR-BI interacts with a broad range of cholesterol acceptors but preferentially mediates the efflux of free cholesterol to large phospholipid- and CE-rich HDL particles (8). Similarly, ABCG1 facilitates cellular cholesterol export to phospholipid-rich acceptors such as HDL (9, 10).

Atherogenic dyslipidemias, which are associated with an increased risk of premature atherosclerosis, are characterized by increased plasma levels of proatherogenic apoB-containing lipoproteins relative to subnormal con-

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centrations of antiatherogenic apoA-I-containing HDL particles. Indeed, low circulating levels of high density lipoprotein-cholesterol (HDL-C) in men (<40 mg/dl) represent a strong and independent risk factor for premature atherosclerosis and coronary heart disease (11). Thus, it has been suggested that increasing plasma HDL-C levels might afford clinical benefits. Indeed, in epidemiological studies, an increment of 1 mg/dl in plasma HDL-C is associated with a 2–4% reduction in the risk of cardiovascular events (12). High levels of HDL-C, apoA-I, and apoA-I-containing lipoprotein subspecies have been reported to be negatively associated with the risk of coronary heart disease. It is well established that women have consistently higher HDL-C levels, especially the HDL_2 subfraction (13), as well as higher concentrations of HDL-apoA-I compared with men (14) and that premenopausal women display a lower risk of developing cardiovascular diseases than similarly aged men. In addition, estrogen administration is associated with increases in HDL-C and apoA-I levels in both young premenopausal and postmenopausal women (15, 16). These data suggest that sex hormones influence the metabolism of both the lipid and protein components of HDL particles and protection from cardiovascular disease. In this context, many studies have been undertaken to evaluate the role of estradiol in this protection. It appears that the atheroprotective effect of estradiol is mainly related to a direct effect on the cells of the arterial wall (17). However, the mechanisms involved in the protection from cardiovascular diseases observed in women are not fully understood.

The potential relevance of gender differences to the capacity of HDL to mediate cholesterol efflux is indeterminate. Therefore, the objective of this study was to evaluate whether potential differences in the quantitative and qualitative features of plasma HDL particles between healthy nondyslipidemic men and women might affect cellular free cholesterol efflux through the SR-BI, ABCA1, and ABCG1 pathways. Our data clearly demonstrate that plasma from women displayed a higher capacity to mediate cellular free cholesterol efflux via the SR-BI pathway compared with that from men. Such enhanced efflux capacity resulted from significant increases in plasma levels of large CE-rich HDL_2 particles as well as from an enhanced capacity of these particles to mediate cellular free cholesterol efflux via the SR-BI pathway. By contrast, plasma from healthy nondyslipidemic men displayed an enhanced free cholesterol efflux capacity via the ABCA1 transporter pathway compared with that from women, which resulted from an increased level of poorly lipidated apoA-I particles (preβ-HDL).

METHODS

Subjects

Plasma was obtained from healthy volunteers (30 men and 26 women) selected specifically for their healthy nondyslipidemic status. Selection criteria for subjects were as follows: total cholesterol < 250 mg/dl, triglyceride < 150 mg/dl, LDL-C < 150 mg/dl, apoB < 140 mg/dl, and body mass index < 25 kg/m^2 (**Table 1**). They were nonobese and free of cardiovascular disease, hypertension, diabetes, hyperlipidemia, medication use, including lipid-lowering drugs, and thyroid, renal, or liver dysfunction. Eight of the women were under contraceptive treatment; none of the postmenopausal women (n = 11) was currently receiving estrogen replacement therapy. After an overnight fast, blood samples were collected into sterile EDTA-containing tubes for the isolation of plasma or into anticoagulant-free tubes for the isolation of serum. Plasma was immediately separated from blood cells by low-speed centrifugation at 2,500 rpm for 20 min at 4°C and frozen at −80°C until used.

This study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Isolation of lipoprotein subfractions

Lipoproteins were isolated from plasma by density gradient ultracentrifugation in a Beckman SW41 Ti rotor at 40,000 rpm for 48 h in a Beckman XL70 at 15°C and by a slight modification of the method of Chapman et al. (18) as described previously (19). After centrifugation, gradients were collected from the top of the tubes with an Eppendorf precision pipette in aliquots of 0.4 ml. Fractions corresponding to HDL subspecies, HDL_{2b} (d = 1.063–1.091 g/ml), HDL_{2a} (d = 1.091–1.110 g/ml), HDL_{3a} (d = 1.110–1.133 g/ml), HDL_{3b} (d = 1.133–1.156 g/ml), and HDL_{3c} (d = 1.156–1.179 g/ml), were analyzed for their lipid and protein contents. In addition, plasma lipoproteins were also fractionated by gel filtration on two Superose 6 (Amersham Biosciences) columns connected in series using the Biologic DuoFlow Chromatography System (Bio-Rad).

Lipid and protein analysis

The lipid contents of plasma and isolated lipoprotein fractions, total protein, and apoA-I, apoA-II, and apoB were quantified with an Autoanalyzer (Konelab 20). Reagent kits from Roche Diagnostics and ThermoElectron were used for the determination of total cholesterol and triglyceride levels, respectively. The levels of unesterified cholesterol and phospholipids were determined with reagent kits (Wako Diagnostics). CE mass was calculated as total cholesterol minus free cholesterol × 1.67 and thus represents the sum of the esterified cholesterol and fatty acids moieties (18). Bicinchoninic acid assay reagent (Pierce) was used for protein quantification. Fasting plasma LDL-C was calculated using the Friedewald formula. HDL-C levels were determined after dextran sulfate-magnesium precipitation of

TABLE 1. Clinical characteristics and plasma parameters in healthy nondyslipidemic subjects

Characteristics	Men (n = 30)	Women (n = 26)
Age (years)	46 ± 13	45 ± 18
Body mass index (kg/m^2)	24 ± 2	22 ± 2
Cholesterol (mg/dl)		
Total	217 ± 26	219 ± 22
LDL	141 ± 28	140 ± 17
HDL	58 ± 13	60 ± 13
Triglycerides (mg/dl)	90 ± 26	96 ± 19
Apolipoproteins (mg/dl)		
apoA-I	144 ± 26	161 ± 27[a]
apoA-II	44 ± 7	44 ± 7
apoB	109 ± 13	116 ± 26
Cholesteryl ester transfer protein activity (%)	26 ± 9	32 ± 8[a]

ApoA-I, apolipoprotein A-I. Values are means ± SD.
[a] $P < 0.05$ versus men.

apolipoprotein B-containing lipoproteins. Plasma apoA-I, apoB, and apoA-II concentrations were determined using immunoturbidimetric assays (ThermoElectron reagents and calibrators; Wako Diagnostics reagents and calibrators). Lipoprotein mass was calculated as the sum of the mass of the individual lipid and protein components for each lipoprotein fraction. Molecular weights of HDL subfractions were calculated by transforming concentration data (mg/dl) into absolute molar units using molecular weights of CE, free cholesterol, phospholipid, and triglyceride of 650, 387, 750, and 850, respectively (20). The protein moiety was considered to consist of two apolipoproteins, apoA-I and apoA-II, and the molecular weight of the protein moiety in each HDL subfraction was calculated using the total protein content (mg/dl) converted to molarity on the basis of the relative mass contents of apoA-I and apoA-II (21).

Quantification of preβ-HDL

The quantification of preβ-HDL in serum was performed as described previously (22). Serum from healthy nondyslipidemic men and women (5 μl) was electrophoresed (2 h at 200 V) at 7°C on a 0.75% agarose gel in 50 mM barbital buffer (pH 8.6) on Gelbond using the Multiphor system (Amersham Pharmacia Biotech, Inc., Orsay, France) and transferred to a nitrocellulose membrane. After blocking in 5% skim milk powder buffer, the bound serum proteins were immunoreacted with a monoclonal mouse antiserum raised against human apoA-I (AbCys, Paris, France). Immunodetection of apoA-I was subsequently localized with horseradish peroxidase-conjugated goat anti-mouse IgG (Bio-Rad, Ivry-sur-Seine, France) and visualized by staining with a 4-chloro-1-naphthol solution kit (Bio-Rad). The relative abundance of the human apoA-I among the α- or preβ-HDL species was determined by scanning reflectance densitometry (Quantity One software; Bio-Rad). The amount of preβ-HDL was expressed as the percentage of total apoA-I (relative concentration) and as absolute concentration (mg/l apoA-I) by multiplying its percentage by the serum apoA-I levels. A normolipidemic serum and a hypertriglyceridemic serum were systematically included in each experiment as controls.

Determination of endogenous CE transfer from HDL to apoB-containing lipoproteins

Determination of endogenous CE transfer from HDL to apoB-containing lipoproteins was assayed by a modification of the method of Guerin et al. (23), which estimates net physiological CE transfer between lipoprotein donor and acceptor particles in the plasma of individual patients. To obtain radiolabeled HDL, a single plasma from a normolipidemic healthy man was depleted of apoB-containing lipoproteins by ultracentrifugation. The $d > 1.063$ g/ml plasma fraction was then labeled with [^{3}H]cholesterol overnight (4 μCi/ml). Radiolabeled [^{3}H]HDL was then isolated from the $d > 1.063$ g/ml plasma fraction as described previously (23). CE transfer was determined after incubation of whole plasma (200 μl) from individual subjects at 37°C or 0°C for 3 h in the presence of radiolabeled HDL (2 μg) and iodoacetate (final concentration, 1.5 mmol/l) for the inhibition of LCAT. After incubation, apoB-containing lipoproteins were precipitated using the dextran sulfate-magnesium procedure. The radioactive content of the supernatant was quantified by liquid scintillation spectrometry with a Trilux 1450 (Perkin-Elmer). Cholesteryl ester transfer protein (CETP) activity (expressed as percentage) was calculated as the amount of the label recovered in the supernatant after incubation and divided by the label present in the supernatant before incubation. The CETP-dependent CE transfer was calculated from the difference between the radioactivity transferred at 37°C and 0°C.

Cell culture and lipid efflux assays

Fu5AH cells were grown at 37°C in Eagle's MEM and 5% newborn calf serum, HepG2 and RAW267.4 cells in DMEM plus 10% fetal bovine serum, and CHO-K1 cells (wild-type and human ABCG1-transfected cells), kindly provided by Dr. W. Jessup, in Ham's F-12 medium (Gibco) supplemented with 10% fetal bovine serum. Lipid efflux assays were performed as described previously for the different cellular models (10, 24, 25) in cultured rat hepatoma Fu5AH cells (24) expressing high levels of SR-BI, in mouse macrophage RAW264.7 cells expressing ABCA1 after stimulation with 8-Br cAMP (25), and in CHO cells overexpressing the human ABCG1 gene (10).

Fu5AH and HepG2 cells. After plating, cells were labeled by incubation with [^{3}H]cholesterol (1 μCi/ml) for 48 h in Eagle's MEM for Fu5AH or in DMEM for HepG2. Subsequently, cells were incubated for 24 h in the presence of BSA (0.5%) and newborn calf serum (25%) for Fu5AH or in fetal bovine serum (25%) for HepG2 to allow equilibration of the label. After equilibration, cholesterol acceptors (2.5% diluted serum, 2.5% diluted apoB-depleted serum, or 10 μg phospholipid/ml isolated HDL subfractions) were added in serum-free medium and incubated with cells for 4 h at 37°C. For cholesterol efflux studies performed in the presence of rabbit antiserum directed to human SR-BI (Cla-1) (26), the assay was carried out as described above but the cells were preincubated with a 1:50 dilution of whole rabbit antiserum for 30 min before the efflux period.

RAW264.7 cells. The day after cell plating, cells were loaded and labeled with acetylated LDL (50 μg/ml) and 0.5 μCi/ml [^{3}H]cholesterol for 24 h in serum-free DMEM containing glucose (4.5 g/l) and BSA (0.2%) (DGGB). After incubation, RAW264.7 cells were incubated with DGGB in the absence or presence of 8-Br cAMP (0.3 mM) for 16 h to induce ABCA1 expression. Cholesterol acceptors (2.5% diluted serum or 30 μg apoA-I/ml isolated HDL subfractions) were added to RAW264.7 cells in serum-free DMEM for 4 h at 37°C in the presence or absence of 0.3 mM 8-Br cAMP.

CHO-K1 cells (wild type and human ABCG1). Two days after plating, cellular cholesterol was labeled by incubation of cells with serum-free Ham's F-12 medium and 1 μCi/ml [^{3}H]cholesterol. Equilibration of the label was performed for 90 min in serum-free medium and BSA (0.1%). After equilibration of labeling, acceptors (2.5% diluted serum or 5 μg phospholipid/ml isolated HDL subfractions) were added to the cells in serum-free medium containing BSA (0.1%) for 4 h at 37°C. Net efflux was expressed as the difference between efflux to human ABCG1-transfected CHO-K1 cells and wild-type CHO-K1 cells.

All efflux experiments were performed in triplicate for each sample. Fractional cholesterol efflux (expressed as a percentage) was calculated as the amount of the label recovered in the medium divided by the total label in each well (radioactivity in the medium + radioactivity in the cells) obtained after lipid extraction from cells in a mixture of 3:2 hexane-isopropanol (v/v). The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux values obtained with the test samples. Plasma coming from a single normolipidemic subject not included in the study was prepared in aliquots, frozen, and used as an internal standard for each experiment. All efflux values were normalized with the fractional efflux obtained with the 2.5% diluted standard plasma. The capacity of HDL subfractions, HDL_2 and HDL_3, to mediate free cholesterol efflux is expressed as percentage of cholesterol efflux per mole of acceptor particle.

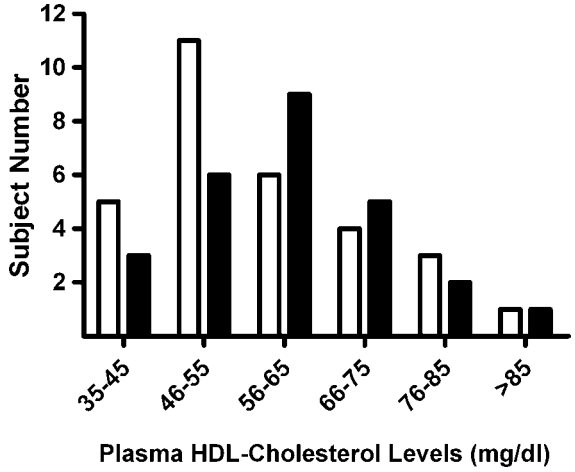

Fig. 1. Distribution of individuals as a function of high density lipoprotein-cholesterol (HDL-C) in a population of healthy nondyslipidemic men and women. The bar graph shows the number of men (open bars) and women (closed bars) as a function of HDL-C levels.

Statistical analyses

Differences in cellular cholesterol efflux to the various acceptors from healthy nondyslipidemic men or women were tested for significance by ANOVA. Results were considered statistically significant at $P < 0.05$.

RESULTS

Plasma lipid and clinical characteristics of healthy subjects

As shown in Table 1, no significant differences in mean age, body mass index, plasma lipid profile, and apoB and apoA-II levels were observed between men and women. By contrast, women displayed significant increases in both plasma apoA-I levels (+12%; $P < 0.02$) and endogenous CETP activity (+23%; $P < 0.05$) compared with men. Based on those parameters, the population is representative of a general population of healthy nondyslipidemic subjects. As expected, analysis of the distribution of HDL-C levels showed (**Fig. 1**) that even if the arithmetic mean value for HDL-C level is not significantly different between genders, men and women exhibit distinct distributions around this value, with the HDL-C peak value being shifted toward higher values in women compared with men.

Gender-specific effects of sera on cellular free cholesterol efflux via SR-BI

We measured the efflux capacity of whole serum from healthy nondyslipidemic women and men in different cellular models, each representative of a specific cholesterol efflux pathway (10, 24, 25). Using 40-fold diluted serum, we observed a significant increase (+14%; $P < 0.03$) in the capacity of whole sera from women to mediate cellular cholesterol efflux via the SR-BI-dependent pathway compared with sera from men (**Table 2**). Because apoB-containing lipoproteins have been shown to represent potential cellular cholesterol acceptors for the SR-BI-mediated cholesterol efflux (26), we confirmed our present observations in efflux experiments using apoB-depleted sera (Table 2), suggesting that apoB-containing lipoproteins did not account for this observed gender difference. Interestingly, we observed that SR-BI-mediated cellular free cholesterol efflux was significantly correlated with plasma HDL-C ($r = 0.72$, $P < 0.0001$) in women, whereas this relationship was not observed in men ($r = 0.11$, $P = 0.56$) (**Fig. 2**). To better define how HDL parameters could induce this gender difference in the efflux capacity of total serum, the men and women groups were divided into subgroups according to the mean of fractional efflux values observed in the whole nondyslipidemic population (Table 2). This observation led us to identify two subgroups of men and women with HDL-C levels above and below 55 mg/dl, respectively, with potentially distinct capacity in terms of cholesterol efflux (Fig. 2). Interestingly, when the capacity of serum to mediate cholesterol efflux via the SR-BI pathway was analyzed according to plasma HDL-C levels, only women with HDL-C > 55 mg/dl displayed a significantly higher efflux capacity (+18%; $P < 0.05$) compared with women with HDL-C < 55 mg/dl or compared with men irrespective of their HDL-C levels (**Fig. 3A**). Similar observations were reached using the human hepatocarcinoma cell line HepG2 (Fig. 3B). In addition, when efflux experiments in HepG2 cells were performed in the presence of an anti-Cla-1 serum, gender-specific differences in serum efflux capacity were not observed, indicating that SR-BI-mediated cholesterol efflux is enhanced in plasma from women displaying increased HDL-C levels.

TABLE 2. Serum cellular free cholesterol efflux capacities in nondyslipidemic men and women

Efflux Pathway	Cellular Model	Total Population (n = 56)	Men (n = 30)	Women (n = 26)	*P* (men vs. women)
SR-BI	Fu5AH cells (total serum)	23.6 ± 2.3	22.1 ± 3.7	25.3 ± 5.1	<0.03
	Fu5AH cells (apoB-depleted serum)	10.7 ± 2.6	9.7 ± 1.9	11.7 ± 3.0	<0.03
ABCA1	RAW264.7 cells	9.6 ± 2.7	9.8 ± 2.7	9.3 ± 2.8	NS
	cAMP-pretreated RAW264.7 cells	23.7 ± 7.1	27.9 ± 4.9	19.3 ± 6.5	<0.001
	ABCA1-dependent efflux	14.1 ± 5.9	18.1 ± 4.6	10.0 ± 3.9	<0.001
ABCG1	CHO cells	22.3 ± 4.6	22.5 ± 3.3	22.0 ± 5.6	NS
	CHO-human ABCG1 cells	48.5 ± 5.7	47.6 ± 4.8	49.4 ± 6.6	NS
	ABCG1-dependent efflux	26.4 ± 4.0	25.6 ± 3.6	27.3 ± 4.2	NS

SR-BI, scavenger receptor class B type I. Values are means ± SD and are expressed as fractional efflux (%). Efflux to 2.5% apoB-depleted serum was measured after dextran-sulfate precipitation of apoB-containing lipoproteins.

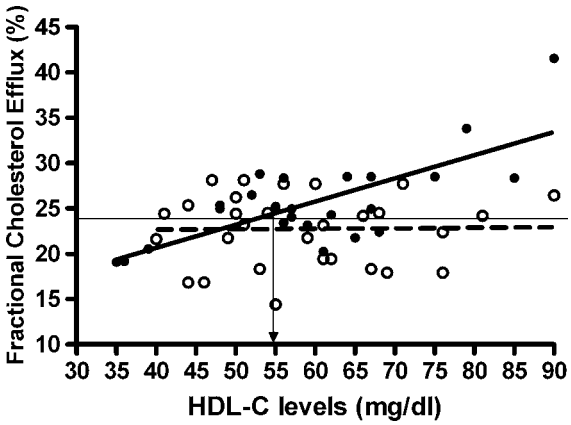

Fig. 2. Correlation between HDL-C levels and the capacity of serum to mediate scavenger receptor class B type I (SR-BI)-dependent cholesterol efflux. Cholesterol efflux capacity of sera through SR-BI was significantly correlated with plasma HDL-C ($r = 0.72$, $P < 0.0001$) in healthy nondyslipidemic women (closed circles, continuous line), whereas this relationship was not observed in men (open circles, dotted line) ($r = 0.11$, $P = 0.56$).

Gender-specific features of HDL subfraction levels

To determine whether the observed gender differences in serum efflux capacity might be induced by variations in quantitative and/or qualitative features of HDL, plasma apoA-I-containing lipoprotein particles were separated by density gradient ultracentrifugation to yield multiple HDL subfractions. The distribution of HDL lipoprotein mass among density gradient subfractions from plasma of men and women is shown in **Fig. 4**. Within the hydrated density range of HDL, a significant increase (+11%; $P < 0.05$) in the mean total HDL mass (expressed as total chemical mass of lipids and protein) was observed in women compared with men (347 ± 13 and 386 ± 15 mg/dl in men and women, respectively), consistent with higher concentrations of plasma apoA-I in women (Fig. 4A). The increase in plasma HDL concentration resulted primarily from a significant and specific increase in plasma HDL_2 subfraction mass levels (+20%; $P < 0.04$) (Fig. 4A), and more precisely from an increase in large HDL_{2b} levels (+35%; $P < 0.02$) (Fig. 4B), in women compared with men. Equally, the molar concentration of HDL_2 subfractions was increased significantly (+24%; $P < 0.03$) in nondyslipidemic women compared with men, reflecting an increased number of circulating HDL_2 particles in the women's plasma (6.6 ± 0.4 and 5.3 ± 0.4 μmol/l in women and men, respectively). As shown in Fig. 4C, a large increase (+60%; $P < 0.0001$) in plasma total HDL_2 mass was observed in women with HDL-C > 55 mg/dl compared with women with HDL-C < 55 mg/dl. Specifically, in women displaying HDL-C > 55 mg/dl, the increase in total HDL_2 mass resulted from significantly higher HDL_{2b} subfraction levels (+76%; $P < 0.003$). By contrast, no significant variation was detected in circulating levels of HDL_3 subfractions between men and women (Fig. 4A, B).

Gender-specific effects of HDL subfractions on cellular free cholesterol efflux

The capacity of isolated HDL_2 and HDL_3 subfractions to mediate cellular free cholesterol efflux via SR-BI, ABCA1, and ABCG1 pathways is presented in **Table 3**. HDL_2 isolated from the plasma of women displayed a significantly higher capacity to mediate free cholesterol efflux via the SR-BI pathway (+14%; $P < 0.03$) compared with the corresponding subfractions from men. The increased efflux capacity of HDL_2 from women compared with men arose mainly from a high capacity of HDL_2 from women with HDL-C > 55 mg/dl compared with HDL_2 from women with lower HDL-C, or from men irrespective of plasma HDL-C concentration (**Fig. 5**). In addition, HDL_2 and HDL_3 subfractions isolated from both healthy

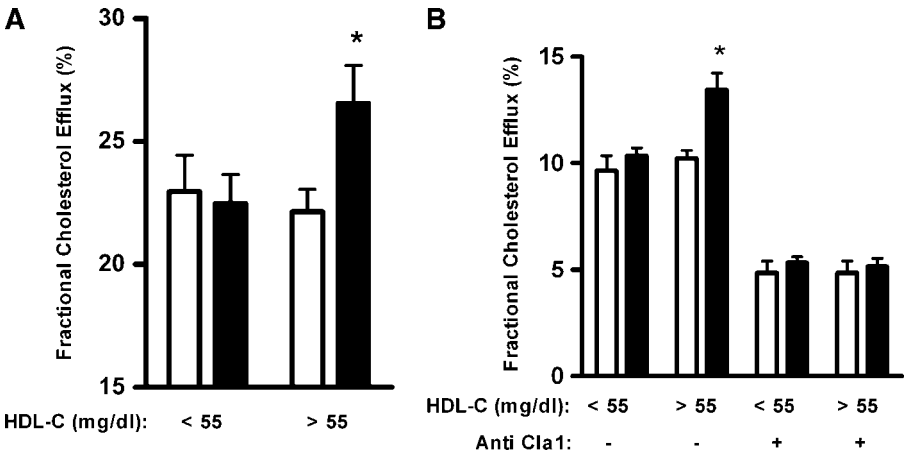

Fig. 3. Fractional cholesterol efflux to total serum in nondyslipidemic men (open bars) and women (closed bars) as a function of plasma HDL-C levels determined using rat hepatoma Fu5AH cells (A) or the human hepatocarcinoma cell line HepG2 (B). Cholesterol efflux studies in HepG2 cells were performed in the absence or presence of rabbit antiserum directed to human SR-BI (Cla-1). Values are means ± SEM. * $P < 0.04$ versus men.

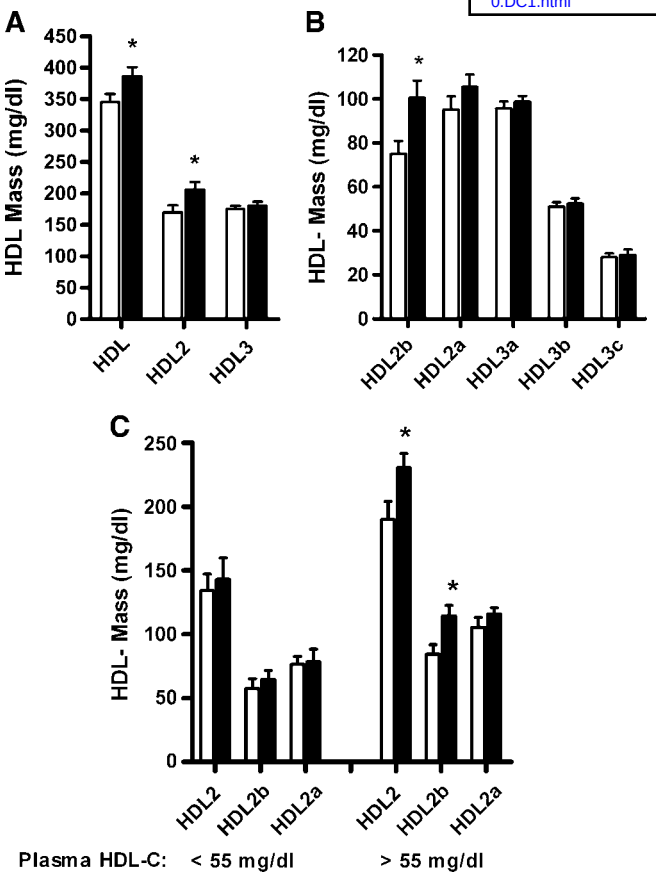

Fig. 4. Distribution of HDL lipoprotein mass in nondyslipidemic men (open bars) and women (closed bars). A: Plasma lipoprotein mass of total HDL, HDL_2, and HDL_3. B: Plasma lipoprotein mass of individual HDL subspecies. C: Plasma lipoprotein mass of HDL_2 subfractions in nondyslipidemic men and women as a function of plasma HDL-C levels. Values are means ± SEM. * $P < 0.05$, women versus men.

dyslipidemic men and women displayed similar capacities to mediate cellular cholesterol efflux via ABCA1 or ABCG1.

Gender-specific differences in HDL particle size as a function of HDL-C levels

To further evaluate HDL particle profiles in terms of lipoprotein size as a function of HDL-C level, plasma from healthy nondyslipidemic men and women was separated using gel filtration chromatography. HDL from women displaying HDL-C < 55 mg/dl coeluted with HDL from men with HDL-C above or below the cutoff value, whereas HDL from women displaying HDL-C > 55 mg/dl eluted earlier (elution volume, 30 ml), indicating a larger mean size of HDL particles in this subgroup of subjects (see supplementary figure).

Gender-specific effects of sera on cellular free cholesterol efflux via ABC transporters

No significant gender effect in the capacity of serum from nondyslipidemic subjects to mediate free cholesterol efflux via the ABCG1 transporter pathway was detected in the present study (Table 2). By contrast, using 40-fold diluted serum, free cholesterol efflux from ABCA1-expressing RAW264.7 mouse macrophage cells was increased significantly by 31% ($P < 0.001$) in nondyslipidemic men compared with women. Because it was recently demonstrated that preβ-HDL levels predict the variance of the ABCA1-dependent efflux (22), it is likely that the present increased capacity of plasma from nondyslipidemic men, compared with those from women, to mediate cellular ABCA1-dependent free cholesterol efflux was attributable to the increase of preβ-HDL levels in the plasma from men compared with those from women subjects.

Gender-specific differences in preβ-HDL concentration in healthy nondyslipidemic subjects

As shown in **Fig. 6**, quantification of preβ-HDL in plasma from nondyslipidemic subjects revealed that the relative concentration of preβ-HDL particles, expressed in percentage of apoA-I, was increased significantly (+40%; $P < 0.003$) in men compared with women (3.5 ± 0.2 and 1.4 ± 0.1 in men and women, respectively). Equally, absolute plasma concentrations of preβ-HDL were increased significantly (+42%; $P < 0.008$) in men compared with women (54.9 ± 4.0 and 22.9 ± 0.7 mg/l apoA-I in men and women, respectively).

DISCUSSION

In the present study, we demonstrate for the first time that serum from healthy nondyslipidemic men and women present distinct cholesterol efflux capacities through both SR-BI and ABCA1 pathways. HDL_2 from

TABLE 3. HDL subfraction cellular free cholesterol efflux capacities in nondyslipidemic men and women

		HDL_2		HDL_3	
Efflux Pathway	Cellular Model	Men	Women	Men	Women
SR-BI	Fu5AH cells	7.7 ± 1.0	9.4 ± 2.3[a]	5.2 ± 1.0	5.2 ± 1.0
ABCA1	RAW264.7 cells	1.3 ± 0.4	1.4 ± 0.3	1.2 ± 0.2	1.3 ± 0.2
	cAMP-pretreated RAW264.7 cells	2.2 ± 0.9	2.7 ± 0.8	3.4 ± 0.8	3.7 ± 0.8
	ABCA1-dependent efflux	1.0 ± 0.5	1.2 ± 0.7	2.2 ± 0.6	2.4 ± 0.7
ABCG1	CHO cells	14.3 ± 4.0	18.3 ± 5.7	10.5 ± 4.7	12.8 ± 5.5
	CHO-human ABCG1 cells	36.6 ± 5.9	43.2 ± 9.8	26.2 ± 4.7	31.1 ± 7.7
	ABCG1-dependent efflux	22.3 ± 2.5	24.8 ± 5.4	15.7 ± 1.3	18.3 ± 5.5

Values are means ± SD and are expressed as fractional efflux per mole of HDL particle (%).
[a] $P = 0.001$ versus men.

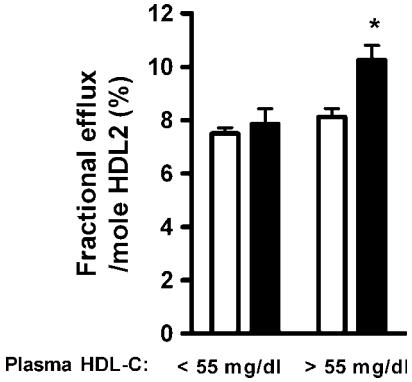

Fig. 5. Capacity of the HDL_2 subfraction isolated from men (open bars) and women (closed bars) to mediate free cholesterol efflux through the SR-BI pathway in Fu5AH cells as a function of plasma HDL-C levels. Values are means ± SEM. * $P < 0.02$ versus men.

women with increased HDL-C levels (>55 mg/dl) showed an increased capacity to mediate cholesterol efflux through the SR-BI pathway compared with those from men, as a result of a larger HDL_2 particle size. In addition, sera from healthy nondyslipidemic men showed an increased capacity to mediate cellular free cholesterol efflux via the ABCA1 pathway compared with women, as a result of increased plasma levels of preβ-HDL.

It is likely that the gender-specific mechanisms of HDL-mediated cholesterol efflux result mainly from gender differences in the intravascular metabolism of HDL particles and thus in HDL particle profile. Indeed, it is well established that apoA-I, lipoprotein lipase, hepatic lipase, CETP, phospholipid transfer protein (PLTP) activities, and SR-BI expression, which are intimately involved in intravascular HDL formation and remodeling, are regulated by sex hormones (14). We observed here an increase in plasma apoA-I concentrations in healthy nondyslipidemic women relative to men. Because apoA-I represents the major apolipoprotein moiety of HDL particles, this observation is consistent with the increased number of

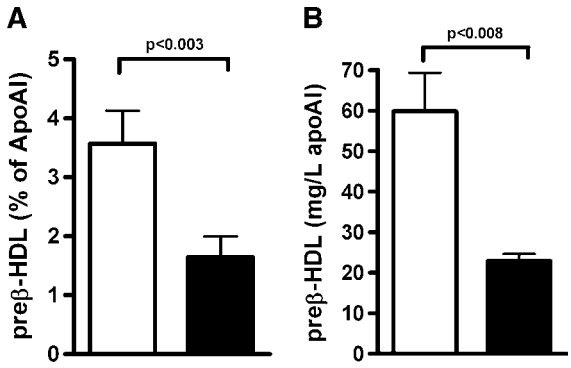

Fig. 6. Quantification of preβ-HDL in plasma from nondyslipidemic men (open bars) and women (closed bars) expressed as a percentage of total plasma apolipoprotein A-I (apoAI; A) and as absolute plasma concentrations of preβ-HDL (B). Values are means ± SEM.

circulating HDL_2 particles observed in women's plasma compared with men's plasma. Equally, these findings are in good agreement with earlier studies showing that estrogens modulate hepatic apolipoprotein expression and secretion, in particular those of apoA-I, thus enhancing both HDL formation and plasma HDL-C levels (14, 27).

Hepatic lipase is a major determinant for HDL level and influences both HDL-C and HDL_2 levels, high HL activity being associated with low HDL_2 concentrations. Sex differences in plasma HDL_2 levels, in particular in the HDL_{2b} subfraction, are entirely consistent with the downregulation of HL by estradiol (28). Indeed, HL activity is twice as high in men as in women, and the hepatic expression and activity of HL has been shown to be upregulated by testosterone (27, 29); by contrast, levels of lipoprotein lipase activity do not appear to be significantly different between men and women (14). In good agreement with data previously reported by O'Connor et al. (28), we observed a gender difference in preβ-HDL levels, with men displaying significant increases in plasma levels of preβ-HDL compared with women. It is well established that PLTP is involved in the intravascular remodeling of HDL particles and in the formation of preβ-HDL particles (30, 31). The interaction of PLTP with HDL results in the release of small, lipid-poor preβ-HDL particles and at the same time produces large α-migrating HDL by a process that involves particle fusion (32, 33). In agreement with our present findings showing an increase in plasma preβ-HDL levels in men compared with women, a sex difference in PLTP activity has been reported in a normolipidemic population of 200 healthy subjects, with women displaying lower PLTP activity than men (34). Thus, it is likely that increased PLTP activity results in increased levels of lipid-poor preβ-HDL acceptor particles for the ABCA1 pathway in men.

The observed differences in preβ-HDL levels between men and women also can be explained by gender differences in intravascular HDL remodeling. Indeed, apoA-I recycles extensively between large lipid-rich HDL and small lipid poor HDL particles as a result of various mechanisms, such as CETP-mediated neutral lipid transfer, PLTP-mediated phospholipid transfer, hepatic lipase-mediated triglyceride hydrolysis, and SR-BI-mediated HDL-CE selective liver uptake. Finally, it is well established that both plasma CETP concentration and activity are equally influenced by sex hormones. Indeed, endogenous levels of CETP activity are increased to a greater degree (+25%) in women compared with men (35), and a positive correlation has been demonstrated between serum CETP concentration and estradiol levels in women (36). In agreement with these earlier studies, we observed an increase in endogenous plasma CETP activity in healthy nondyslipidemic women compared with men. Interestingly, variation in plasma CETP activity was associated with variations in plasma HDL_2 levels, specifically those of the HDL_{2b} subfraction. Intravascular remodeling of HDL particles involves CE accumulation in the hydrophobic core of small, dense, lipid-poor HDL_{3c} particles through the action of lecithin:cholesterol acyltransferase. In consequence, HDL_{3c} particles are progressively transformed

into the larger phospholipid- and CE-rich lipoprotein subspecies, namely HDL_{2a} and HDL_{2b}. Because CETP mediates the heterotransfer of triglycerides and CEs primarily between HDL_{2a} and apoB-containing lipoprotein particles (37), such neutral lipid exchange results in the formation of HDL_{2b} subspecies. HDL_{2b} is then transformed back to HDL_{3c} by the hydrolysis of triglycerides and phospholipids, involving the simultaneous action of HL, CETP, and PLTP. Thus, HDL_{2b} subfraction particles accumulate in the plasma of women subjects as a result of increased plasma CETP activity and reduced PLTP and HL activities.

Using the mouse macrophage cell line RAW264.7, in which the expression of ABCA1 was stimulated with 8-Br cAMP, we observed a significant increase in the capacity of whole serum from men (+31%; $P < 0.0001$) to remove cellular free cholesterol compared with serum from women. In this context, it is relevant that apoE expression by RAW264.7 cells is negligible (38). Because ABCA1 mediates the export of cellular cholesterol and phospholipid to lipid-poor apolipoproteins, mainly apoA-I, to form nascent HDL, it is likely that the observed gender effect on the efflux capacity of sera is intimately related to gender differences observed on preβ-HDL levels. The role of preβ-HDL particles in mediating ABCA1-dependent efflux is reinforced by the lack of relationship between ABCA1 efflux and efflux from control RAW264.7 cells expressing low ABCA1 levels. In addition, our present findings are consistent with an earlier study showing that plasma from human PLTP transgenic mice displayed an increased potential to form preβ particles and an increased plasma capacity to prevent cholesterol accumulation in macrophages (30).

Together, our findings clearly demonstrate that the initial step in the reverse cholesterol transport pathway may be subject to gender-specific constraints that are related to gender-specific HDL remodeling. Indeed, increased circulating levels of large HDL_2 particles enhance cholesterol efflux through the SR-BI pathway in healthy nondyslipidemic women. By contrast, an increased cellular free cholesterol efflux capacity of sera through the ABCA1 pathway occurs in healthy nondyslipidemic men compared with women as a result of the increased circulating levels of lipid-poor preβ-HDL particles. Moreover, the capacity of serum to mediate cellular free cholesterol efflux via SR-BI, ABCA1, or ABCG1 in healthy nondyslipidemic men is not dependent on plasma HDL or HDL-C levels. In conclusion, the steady-state level of HDL-C may not represent the appropriate index to evaluate the efficiency of the initial step of the reverse cholesterol transport system in all circumstances. Thus, the present study strongly suggests that the therapeutic impact of HDL-increasing drugs on the reverse cholesterol transport pathway and on the development of atherosclerosis needs to be addressed separately in men and women.

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