HDL modification by secretory phospholipase A_2 promotes scavenger receptor class B type I interaction and accelerates HDL catabolism

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Abstract **During inflammatory states plasma levels of high density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I) are reduced. Secretory group IIa phospholipase A_2 ($sPLA_2$) is a cytokine-induced acute-phase enzyme associated with HDL. Transgenic mice overexpressing $sPLA_2$ have reduced HDL levels. Studies were performed to define the mechanism for the HDL reduction in these mice. HDL isolated from $sPLA_2$ transgenic mice have a significantly lower phospholipid content and greater triglyceride content. In autologous clearance studies, ^{125}I-labeled HDL from $sPLA_2$ transgenic mice was catabolized significantly faster than HDL from control mice (4.24 ± 1.16 vs. 2.84 ± 0.1 pools per day, $P < 0.008$). In both $sPLA_2$ transgenic and control mice, the cholesteryl ester component of HDL was more rapidly catabolized than the protein component, indicating a selective uptake mechanism. In vitro studies using CHO cells transfected with scavenger receptor class B type I (SR-BI) showed that $sPLA_2$-modified HDL was nearly twice as efficient as a substrate for cholesteryl ester transfer. These data were confirmed in in vivo selective uptake experiments using adenoviral vector overexpression of SR-BI. In these studies, increased hepatic selective uptake was associated with increased ^{125}I-labeled apolipoprotein uptake in the kidney. We conclude that during inflammation $sPLA_2$ hydrolysis of HDL phospholipids alters the lipid composition of the particle, allowing for more efficient SR-BI-mediated selective cholesteryl ester uptake. This enhanced SR-BI activity generates HDL remnants that are preferentially catabolized in the kidney.** —de Beer, F. C., P. M. Connell, J. Yu, M. C. de Beer, N. R. Webb, and D. R. van der Westhuyzen. **HDL modification by secretory phospholipase A_2 promotes scavenger receptor class B type I interaction and accelerates HDL catabolism.** ***J. Lipid Res.*** **2000.** 41: **1849–1857.**

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Epidemiological studies have indicated that high density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I) levels are inversely correlated to the risk for cardiovascular disease (1). HDL cholesterol and apoA-I levels decrease markedly during acute inflammatory processes and are also reduced in chronic inflammatory states such as rheumatoid arthritis (2, 3). Patients with rheumatoid arthritis reportedly have a cardiovascular mortality rate twice that of a matched population (4). In addition to decreased levels during inflammation, HDL is modified from an anti-inflammatory particle to a proinflammatory particle that is depleted of its protective enzyme systems such as paraoxonase (5). The factors responsible for influencing HDL cholesterol and apoA-I levels during inflammation are thus of importance.

Metabolic studies of HDL and apoA-I in humans have established that variations in HDL cholesterol and apoA-I concentrations are largely due to differences in the rate of apoA-I catabolism, not biosynthesis (6, 7). Factors known to alter HDL lipid composition are associated with changes in apoA-I catabolic rate. In particular, the accumulation of cholesteryl esters in HDL, such as occurs in human cholesteryl ester transfer protein deficiency, is associated with delayed apoA-I catabolism and increased HDL levels (8). Conversely, lecithin:cholesterol acyltransferase deficiency with diminished formation of HDL cholesteryl esters is associated with accelerated HDL catabolism (9). Hydrolysis of HDL phospholipids also holds potential to influence the metabolism of HDL and apoA-I (10, 11). Hydrolysis of HDL phospholipids by snake venom secretory group IIa phospholipase A_2 ($sPLA_2$) leads to alterations in HDL size and density and increased cholesterol uptake by rat hepatocytes (12). The focus of this article is on mammalian secretory group IIA $sPLA_2$. These enzymes are acute-phase proteins that are dramatically increased in the setting of acute and chronic inflammation and can alter

Abbreviations: apoA-I, apolipoprotein A-I; Adnull, control adenovirus containing no transgene; AdSR-BI, recombinant adenovirus expressing SR-BI; BSA, bovine serum albumin; CE, cholesteryl oleoyl ester; DLT, dilactitol tyramine; HDL, high density lipoprotein; LDL, low density lipoprotein; SAA, serum amyloid A protein; $sPLA_2$, group IIa phospholipase A_2; SR-BI, scavenger receptor class B type I.

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HDL metabolism (13–16). It has been shown that lipoproteins are substrates for $sPLA_2$ (17). In plasma from patients with sepsis (who have low levels of HDL cholesterol), $sPLA_2$ was almost entirely associated with HDL (18). HDL is likely a major substrate of $sPLA_2$, as evidenced by the human $sPLA_2$ overexpressing transgenic model that is characterized by markedly decreased HDL levels (11). The HDL in these transgenics was reduced in size and phospholipid content when compared with nontransgenic littermates (11).

Cytokines induce serum amyloid A (SAA) during inflammation (19). SAA in plasma associates with HDL, and during extreme inflammation SAA can constitute the major apolipoprotein of HDL. It has been proposed that SAA may be a major factor in the reduction of HDL cholesterol during the acute phase (20). However, we have demonstrated that adenoviral vector overexpression of SAA in the absence of a generalized acute-phase response did not reduce HDL cholesterol or apoA-I levels (21).

The scavenger receptor class B type I (SR-BI) is an HDL receptor that mediates the selective transfer of lipid from HDL to cells (22). SR-BI overexpression profoundly decreases HDL levels (23, 24). To examine a possible mechanism for the decrease in HDL levels during inflammation we studied the interaction of sPLA-modified HDL with SR-BI. Data indicate that enhanced SR-BI-specific selective cholesteryl ester uptake from $sPLA_2$-modified HDL leads to accelerated catabolism and decreased HDL levels.

EXPERIMENTAL PROCEDURES

Animals

$sPLA_2$ transgenic mice were obtained from Chrysalis DNX Transgenic Sciences (Princeton, NJ) and were produced as described previously (25). All $sPLA_2$ lines were backcrossed repeatedly to C57BL/6J mice. Nontransgenic littermates were used as controls. C57BL/6J mice have a natural null mutation for mouse $sPLA_2$ that facilitates studies of the effects of the human transgene (26). All animals were maintained in a pathogen-free facility under an equal light/dark cycle and with free access to water and food. Mice were fasted 6 h before either retro-orbital bleeding or organ collection under metophane anesthesia.

Analyses of plasma and HDL lipids

The concentrations of total cholesterol, triglycerides, and phospholipids in ultracentrifugally isolated HDL were enzymatically determined with kits (Biochemical Diagnostics, Edgewood, NY; Sigma, St. Louis, MO). Plasma HDL cholesterol was measured enzymatically after precipitation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) by heparin and manganese (Biochemical Diagnostics).

Apolipoprotein measurements

The concentration of apoA-I was determined by Western blot analyses, using purified apoA-I to generate a standard curve. ApoA-I was identified with a rabbit anti-mouse apoA-I antibody (gift from A. J. Lusis, Department of Medicine, University of California Los Angeles, Los Angeles, CA) and visualized by chemiluminescence followed by densitometric scanning (Molecular Dynamics, Sunnyvale, CA).

Isolation of lipoproteins

HDL was isolated from the plasma of $sPLA_2$ transgenic mice and control littermates as described previously (27). Briefly, plasma density was adjusted to 1.09 g/ml with solid potassium bromide and the tubes were centrifuged for 5.5 h at 55,000 rpm in a VTi 90 rotor (Beckman Instruments, Fullerton, CA), at 10°C. The density of the infranatants, containing HDL, was readjusted to 1.21 g/ml and the tubes were centrifuged for 9.5 h under the same conditions. HDL was collected from the top of each tube and extensively dialyzed against 150 mM NaCl, 0.01% ethylenediaminetetraacetic acid, pH 7.4. Protein concentrations were determined by the method of Lowry et al. (28).

Labeling of lipoproteins

HDL apolipoproteins were iodinated by the iodine monochloride method (29) to a specific activity of 400–700 cpm/ng protein. For in vivo analyses the HDL was double labeled. The apolipoprotein component of HDL was labeled with nondegradable, intracellularly trapped ^{125}I-labeled dilactitol tyramine (DLT) as described (30). Specific activities ranged from 50 to 90 cpm/ng protein. HDL was labeled in the lipid component with nonhydrolyzable, intracellularly trapped 1α,2α(*n*)-[^{3}H]cholesteryl oleoyl ester (CE) (Amersham Pharmacia Biotech, Piscataway, NJ) as described (31). Specific activities ranged from 8 to 11 dpm/ng protein.

HDL clearance studies

HDL was isolated from $sPLA_2$ transgenic mice and normal C57BL/6 mice (see above). Kinetic experiments were performed with the above-described mice, using autologous double-labeled HDL, as well as by administering $sPLA_2$ HDL to C57BL/6 mice. For in vivo studies, mice were injected via the jugular vein with HDL radiolabeled with ^{125}I-labeled DLT and [^{3}H]CE. Recipient animals were fasted throughout the study, but had free access to water. Blood samples (30 μl) were directly counted (β counter; Packard, Downers Grove, IL). Ten microliters of plasma was subjected to lipid extraction (32) and after adding 5 ml of scintillation fluid (Optifluor; Packard) was counted in a scintillation counter (Packard). Plasma decay curves were generated by expressing the radioactivity at each time point as a percentage of the radioactivity determined 3 min after tracer injection. A noncompartmental analysis was performed with WinNonlin (version 2.1; Pharsight, Palo Alto, CA). Estimates of the terminal slope were made on the basis of the last three observations. Statistical analyses were performed with the two-tailed Mann-Whitney test.

Cell culture

An LDL receptor-deficient CHO line, ldlA (clone 7) (provided by M. Krieger, Biology Department, Massachusetts Institute of Technology, Cambridge, MA), was cultured in Ham's F-12 medium containing 5% (v/v) fetal bovine serum, 2 mM glutamine, penicillin (50 units/ml), and streptomycin (50 μg/ml). For the production of stable transfectants, murine SR-BI cDNA coding sequence (24, 33) was cloned into the expression vector pCMV5 (34) and transfected into CHO ldlA cells, using a modified bovine serum transfection kit (Stratagene, La Jolla, CA). Expressing lines were isolated and maintained in medium containing G418 (0.5 mg/ml).

In vitro analysis of SR-BI-mediated HDL binding and selective uptake

Binding and uptake assays were carried out essentially as described (24). SR-BI-transfected CHO cells and control CHO ldlA cells were seeded at a density of 2.5×10^5 cells per well in six-well plates 48 h prior to assay. Cell association assays were carried out at 37°C in Ham's F-12 containing penicillin (50 units/ml),

streptomycin (50 μg/ml), 2 mM glutamine, and 0.5% essentially fatty acid-free bovine serum albumin (BSA) and ^{125}I-labeled HDL or [^{3}H]CE HDL. After incubating for the required time, unbound ligand was removed by washing the cells four times with 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl and essentially fatty acid-free BSA (2 mg/ml); the last wash remaining on the cells for 5 min. This was followed by two washes with 50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl. All washes were performed at 4°C. Cells were solubilized in 0.1 N NaOH for 30–60 min at room temperature prior to protein and radioactivity quantitation. The trichloroacetic acid-soluble degraded material in cell medium was assayed as described (35).

In vivo analysis of SR-BI-mediated binding, selective uptake, and organ uptake

These in vivo studies were conducted in C57BL/6 mice, using a recombinant adenovirus expressing SR-BI (AdSR-BI) and a control adenovirus containing no transgene (Adnull) (24). Initial titration experiments with the AdSR-BI were performed. A dose of 0.2×10^{11} particles resulted in an approximate 6-fold increase in hepatic SR-BI expression that was associated with an approximately 30% decrease in HDL levels. This dose was chosen and experiments were conducted 3 days after respective adenoviral vector injection, when a relative steady state expression was achieved. For in vivo studies, mice were injected via the jugular vein with $sPLA_2$-modified or control HDL double labeled with ^{125}I-labeled DLT and [^{3}H]CE. The $sPLA_2$-modified and control HDLs were, respectively, injected to generate five SR-BI-overexpressing mice (AdSR-BI) and five control mice (Adnull). Recipient animals were fasted throughout the study, but had free access to water. Blood samples (approximately 80 μl) were obtained from the retro-orbital sinus at 3, 60, 120, and 240 min. Aliquots of plasma were counted for ^{125}I or ^{3}H as described under HDL Clearance Studies (above). Plasma decay curves were generated by expressing the radioactivity at each time point as a percentage of the radioactivity determined 3 min after tracer injection. At the end of the time course, animals were anesthetized and perfused with 30 ml of saline, and livers and kidneys were collected and weighed. Radioactivity was determined by direct counting of tissue (^{125}I) or was quantified after tissue homogenization and lipid extraction (^{3}H) (32).

Statistical analyses

Results are expressed as means ± SD unless otherwise indicated. Differences between groups were evaluated by analysis of variance and Student's *t*-test. Significance was set at $P < 0.05$.

RESULTS

$sPLA_2$ expression results in decreased plasma HDL levels and altered HDL composition

Three separate pools of $sPLA_2$ and control plasma were utilized to prepare HDL and conduct these experiments. The HDL cholesterol levels in $sPLA_2$ transgenic mice (54 ± 4 mg/dl) were significantly lower than that in nontransgenic control mice (71 ± 3 mg/dl). ApoA-I levels in $sPLA_2$ transgenic and control mice, respectively, measured 105 ± 9 and 121 ± 11 mg/dl. This difference did not reach significance. Analysis of the lipid composition of the HDL particles showed a significant decrease in phospholipid content, from 29% to 24% of total HDL mass in $sPLA_2$ transgenic HDL, when compared with control mouse HDL (**Table 1**). Conversely, triglyceride content significantly increased from 2.2% to 4.3% of total HDL (Table 1). No significant difference in free cholesterol or cholesteryl ester content was detected.

TABLE 1. HDL composition in $sPLA_2$ transgenic mice and nontransgenic littermates

	Nontransgenic Littermates	$sPLA_2$ Transgenics
Protein	51.4 ± 3.1	57.4 ± 3.2
Phospholipid	29.2 ± 3.2	23.6 ± 2.0[a]
Cholesteryl ester	13.8 ± 4.2	11.2 ± 3.2
Free cholesterol	3.4 ± 0.8	3.5 ± 1.0
Triglycerides	2.2 ± 0.3	4.3 ± 0.6[a]

Values are means ± SD.
[a] Significantly different from control, $P < 0.05$.

Expression of $sPLA_2$ causes rapid catabolism of HDL

Kinetic experiments using autologous mouse HDL as a tracer in either $sPLA_2$ transgenic or control mice, or $sPLA_2$ HDL in control mice, were performed to investigate the metabolic mechanism responsible for the decreased HDL cholesterol and apoA-I levels in $sPLA_2$ transgenic mice. HDL was double labeled with ^{125}I-labeled DLT and [^{3}H]CE to trace both the protein and cholesteryl ester components of HDL, respectively. Plasma clearance of the ^{125}I-labeled DLT label of $sPLA_2$ transgenic HDL in $sPLA_2$ transgenic mice was significantly faster than clearance of normal ^{125}I-labeled HDL in normal mice (4.24 ± 1.16 vs. 2.84 ± 0.21 pools/day, respectively, $P < 0.008$) (**Fig. 1A** and **Table 2**). In both the $sPLA_2$ transgenic and control mice, [^{3}H]CE HDL was more rapidly cleared than the ^{125}I-labeled DLT HDL (Table 2), indicating whole-body selective lipid uptake in both strains of mice. $sPLA_2$ [^{3}H]CE HDL (5.43 ± 1.4 pools/day) cleared more rapidly than control [^{3}H]CE HDL (4.02 ± 0.78 pools/day), but this did not reach significance ($P < 0.08$) (Table 2). Confirmation that rapid catabolism of $sPLA_2$ HDL was not due to metabolic differences between $sPLA_2$ transgenic mice and controls was obtained in that HDL from $sPLA_2$ mice was also more rapidly catabolized in control mice (Table 2).

$sPLA_2$-modified HDL is a more efficient substrate for SR-BI-mediated selective uptake

The ability of SR-BI to mediate HDL association and subsequent selective lipid uptake was assessed in vitro by incubating SR-BI-transfected CHO cells with ^{125}I-labeled DLT- or [^{3}H]CE-labeled $sPLA_2$ transgenic or control HDL. For these in vitro experiments, SR-BI-specific cell association was defined as the difference between total cell association with transfected cells and control nontransfected CHO cells. SR-BI-expressing cells exhibited specific association of each of the ^{125}I-labeled ligands, with maximal association after 1–2 h of incubation (data not shown). When increasing concentrations of the respective HDLs were incubated in duplicate at 37°C, the specific association of control HDL was higher at each concentration point tested than that of $sPLA_2$ transgenic HDL (**Fig. 2** and **Table 3**). Inte-

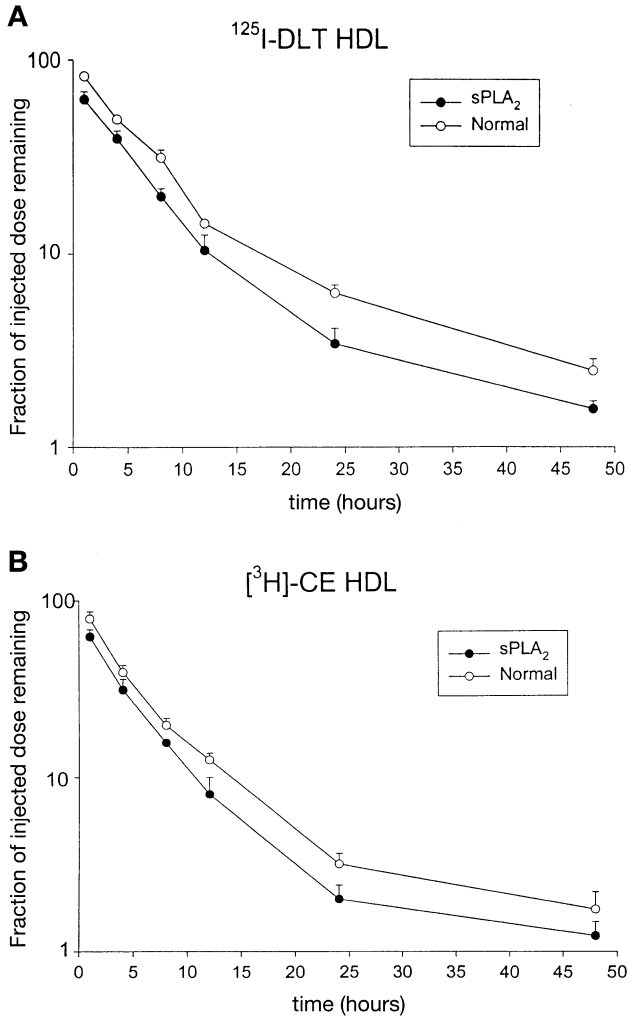

Fig. 1. Comparison of the kinetic properties of HDL isolated from $sPLA_2$ transgenic mice (solid circles) and control mice (open circles). HDLs were double labeled on the protein (^{125}I-DLT) and lipid [^{3}H]CE components. Respective HDLs were injected in the autologous strain via the jugular vein. Blood samples were collected at the indicated time points. Analyses were performed using WinNonlin (version 2.1; Pharsight) and FCR values were calculated. $sPLA_2$ transgenic mice had a significantly faster ^{125}I-DLT HDL catabolic rate than control mice ($P < 0.05$) (A). The [^{3}H]CE label tended to clear faster from the $sPLA_2$ transgenic mice but this did not reach significance ($P < 0.08$) (B). For both control and $sPLA_2$ transgenic mice, n = 5.

gration of the data from the three experiments did not reach significance because of interexperimental variability. Greater receptor-specific association of control HDL was observed in three separate experiments using three different preparations of $sPLA_2$-modified and control HDL (Table 3).

Incubation with [^{3}H]CE HDL (10 μg/ml) resulted in the association of increasing amounts of ^{3}H label during a 3-h incubation period (**Fig. 3**). For both HDL ligands, more [^{3}H]CE HDL was associated with the cells than can be accounted for by ^{125}I-labeled HDL association, demonstrating selective lipid uptake from control and $sPLA_2$-modified HDL. When expressed as the amount of apparent HDL protein associated with cells, greater amounts of the [^{3}H]CE label from $sPLA_2$-modified HDL specifically associated with the SR-BI-transfected cells when compared with the control HDL, indicating enhanced selective lipid uptake of cholesteryl ester from the $sPLA_2$-modified HDL. These findings were confirmed in three separate experiments (Table 3). In each of these experiments, the $sPLA_2$-modified HDL allowed for greater cholesteryl ester uptake when compared with control HDL. The ratio of SR-BI-specific [^{3}H]CE HDL uptake and SR-BI-specific ^{125}I-labeled HDL association provides an index of selective cholesteryl ester uptake efficiency. $sPLA_2$-modified HDL as a substrate allowed for 1.4- to 3.5-fold greater efficiency of SR-BI-mediated selective cholesteryl ester uptake than control HDL.

TABLE 2. HDL catabolic rates in $sPLA_2$ transgenic and control mice, using autologous double-labeled HDL as a tracer

	Control Mice (n = 5)	$sPLA_2$ Transgenic Mice (n = 5)	Control Mice (n = 5) ($sPLA_2$, HDL)
		pools/day	
^{125}I-labeled DLT HDL	2.84 ± 0.21	4.24 ± 1.16[a]	4.03 ± 0.62[a]
[^{3}H]CE HDL	4.02 ± 0.78	5.43 ± 1.4	4.91 ± 1.2

Values represent means ± SD.
[a] Significantly different from control HDL, $P < 0.05$.

$sPLA_2$ modification of HDL results in enhanced in vivo hepatic selective uptake and renal catabolism of HDL apolipoproteins

The more efficient cholesteryl ester uptake from $sPLA_2$-modified HDL in vitro was further evaluated in vivo using adenoviral vector-mediated SR-BI overexpression and HDL radiolabeled with nondegradable lipid and protein tracers. Selective uptake and catabolism were evaluated in vivo by correlating plasma decay with tissue accumulation

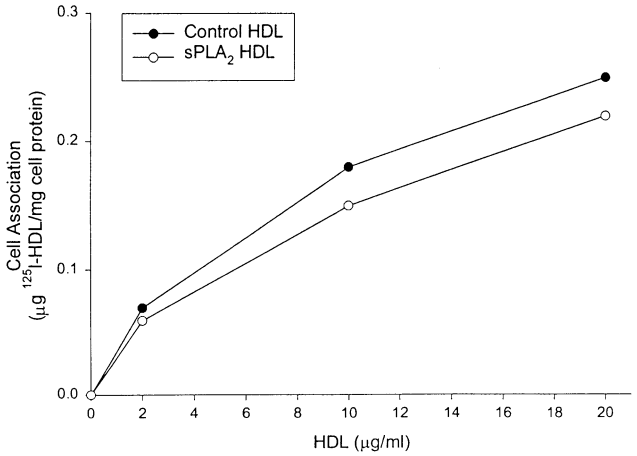

Fig. 2. SR-BI-specific cellular association of control and $sPLA_2$ transgenic ^{125}I-HDL. SR-BI-transfected ldlA cells were incubated at 37°C for 3 h with increasing concentrations of $sPLA_2$-modified and control HDL. SR-BI-specific values (defined as difference between ^{125}I-HDL association with transfected and nontransfected cells) shown are expressed on a cell protein basis. Each point is the mean of duplicate determinations.

TABLE 3. SR-BI selective uptake (ng HDL/mg cell protein) from $sPLA_2$ transgenic and control HDL

Experiment Number	HDL	Uptake		Selective Uptake	Uptake Efficiency
		^{125}I-HDL	[^{3}H]CE HDL		
		ng/mg protein		*ng/mg*	
1	Control	362	1,150	788	3.2
	$sPLA_2$-tg	159	1,800	1,641	11.3
2	Conrol	216	2,550	2,334	11.8
	$sPLA_2$-tg	177	2,920	2,743	16.5
3	Control	189	2,970	2,751	15.7
	$sPLA_2$-tg	160	3,500	3,340	21.9

Results of three experiments done in duplicate. SR-BI-specific cell association was defined as the difference between total cell association with SR-BI-transfected and nontransfected CHO ldlA cells. Cells were incubated with radiolabeled HDL (10 μg/ml) at 37°C for 3 h. Cell-associated radiolabel was measured as described in Experimental Procedures. Selective uptake is defined as the difference between SR-BI-specific [^{3}H]CE and ^{125}I-labeled HDL cell-associated labels. The efficiency of selective uptake is the ratio between [^{3}H]CE and ^{125}I-labeled HDL uptake.

of the double-labeled respective HDLs. We elected to perform these experiments in C57BL/6 mice rather than in autologous strains. The major reason was that $sPLA_2$ transgenic mice proved more sensitive to adenoviral vector administration than control mice, in that the already reduced HDL levels in these mice decreased more markedly than in control mice. When a dose of 0.2×10^{11} AdSR-BI or Adnull was administered, HDL cholesterol levels in C57BL/6 mice decreased by 35% from 77 ± 5 mg/dl (Adnull) to 49 ± 9 mg/dl (AdSR-BI). In $sPLA_2$ transgenic mice the same dose of AdSR-BI decreased HDL cholesterol levels by 70%, from 53 ± 7 to 16 ± 6 mg/dl. Such differences in HDL levels could influence HDL cholesteryl ester flux to the liver (36).

Plasma clearance of the ^{125}I-labeled DLT and [^{3}H]CE label from $sPLA_2$-modified and control HDL in AdSR-BI- or Adnull-administered mice was compared (**Fig. 4**). Reduction in the HDL pool size in the SR-BI-overexpressing mice was reduced by prior titration of the viral vector to levels that would not be expected to affect the fractional catabolism of HDL (36). Plasma HDL cholesterol in Adnull-injected animals was measured as 77 ± 5 mg/dl compared with 49 ± 9 mg/dl in AdSR-BI-injected animals. Experiments were terminated at 240 min as 90% of the [^{3}H]CE label had cleared from $sPLA_2$-modified HDL at that time (Fig. 4B). Data presented in Fig. 4A show that in Adnull-administered mice, ^{125}I-labeled DLT decayed more rapidly from $sPLA_2$-modified HDL than control HDL (60 ± 2.6% vs. 75 ± 3.7% of radiolabel remaining at 240 min, $P < 0.05$). Although SR-BI overexpression resulted in an accelerated clearance of ^{125}I-labeled DLT derived from both HDLs, significantly less ^{125}I-labeled DLT derived from $sPLA_2$-modified HDL remained at 240 min compared with control HDL (35 ± 1.7% vs. 63 ± 7.6%). For both double-labeled $sPLA_2$-modified HDL and con-

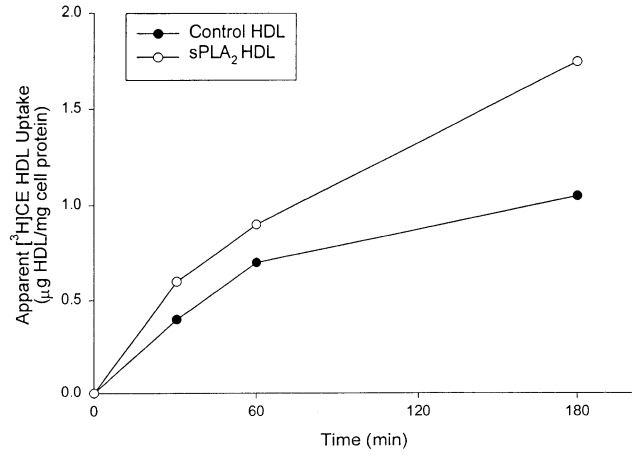

Fig. 3. SR-BI-mediated apparent [^{3}H]CE uptake from $sPLA_2$ transgenic and control HDL. [^{3}H]CE uptake from HDL was assessed by treating SR-BI-transfected ldlA cells and untransfected cells with the respective HDL preparations (10 μg/ml) at 37°C for the indicated time periods. Data on SR-BI-specific uptake of [^{3}H]CE HDL are expressed as apparent HDL protein uptake assuming whole particle uptake, and are normalized for total cell protein. Each point is the mean of duplicate determinations.

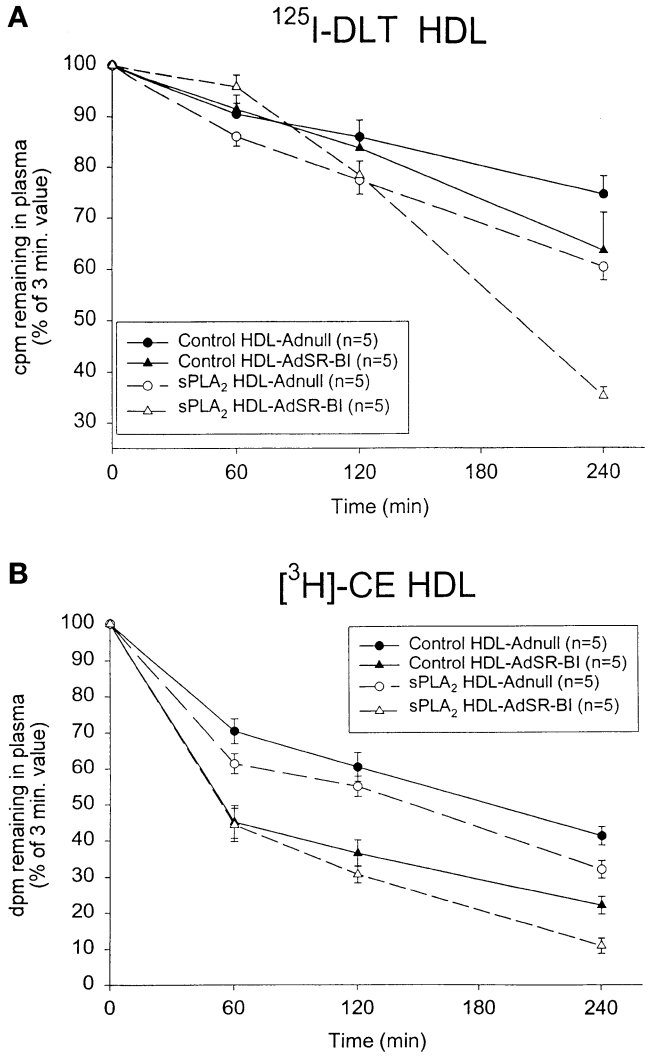

Fig. 4. Plasma decay of ^{125}I-DLT (A) and [^{3}H]CE (B) label from $sPLA_2$-modified and control HDL in SR-BI- or Adnull-administered C57BL/6 mice. Values are expressed as means ± SE.

trol HDL, fewer [^{3}H]CE disintegrations per minute remained in the plasma compared with the corresponding ^{125}I-labeled DLT. These data are compatible with the selective uptake of [^{3}H]CE. In Adnull-administered mice, significantly less [^{3}H]CE remained in the plasma at 240 min in the case of sPLA$_2$-modified HDL compared with control HDL (32 ± 4% vs. 41 ± 2.5%, $P < 0.05$). SR-BI overexpression resulted in a significant decrease in disintegrations per minute remaining in the plasma from both these HDLs, with [^{3}H]CE from sPLA$_2$ HDL again clearing more rapidly (11 ± 2% vs. 22 ± 4%, $P < 0.05$).

A second parameter monitored was the accumulation of ^{125}I-labeled DLT and [^{3}H]CE label from the sPLA$_2$ and control HDLs in the liver at 240 min. Radiolabel taken up into liver is expressed as the percentage of injected dose (**Fig. 5**). The results indicated that for both these HDLs more [^{3}H]CE label accumulated in the liver than ^{125}I-labeled DLT, indicative of the selective uptake mechanism. The finding that sPLA$_2$-modified HDL is a more efficient substrate for cholesteryl ester uptake in vitro is confirmed in the in vivo setting, where significantly more [^{3}H]CE label derived from the sPLA$_2$-modified HDL accumulated in the liver when compared with the control HDL (28 ± 4% vs. 39 ± 5% for Adnull and 40 ± 8% vs. 62 ± 8% for AdSR-BI-administered mice, $P < 0.05$) (Fig. 5). Overexpression of SR-BI significantly increased the amount of ^{125}I-labeled DLT label associated with the liver without significant differences between the HDLs. These data are compatible with the in vitro data presented in Table 3, indicating increased lipid uptake efficiency from sPLA$_2$-modified HDL but not increased association of the particles.

The third parameter investigated in the same animals

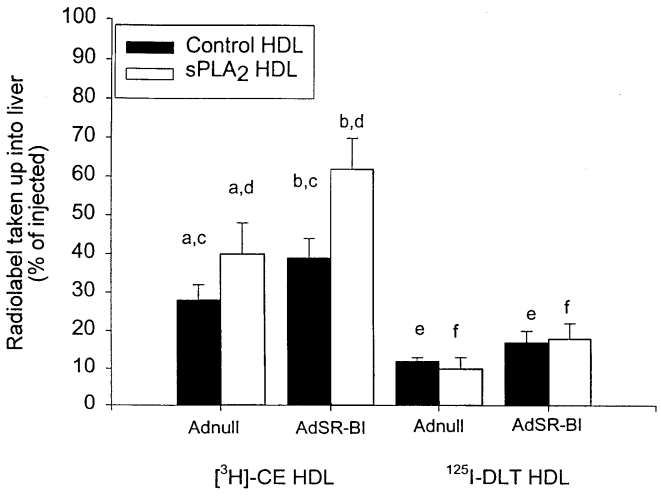

Fig. 5. Liver uptake of ^{125}I-DLT and [^{3}H]CE label from sPLA$_2$-modified and control HDL. Three days after treatment with AdSR-BI or Adnull, C57BL/6 mice were injected in the jugular vein with double-labeled sPLA$_2$-modified or control HDL. Four hours after tracer injection livers were collected after perfusion. Radioactivity was determined by direct counting of tissue (^{125}I) or quantified after tissue homogenization and lipid extraction (^{3}H). Values marked with the same letters are significantly different by analysis of variance ($P < 0.05$).

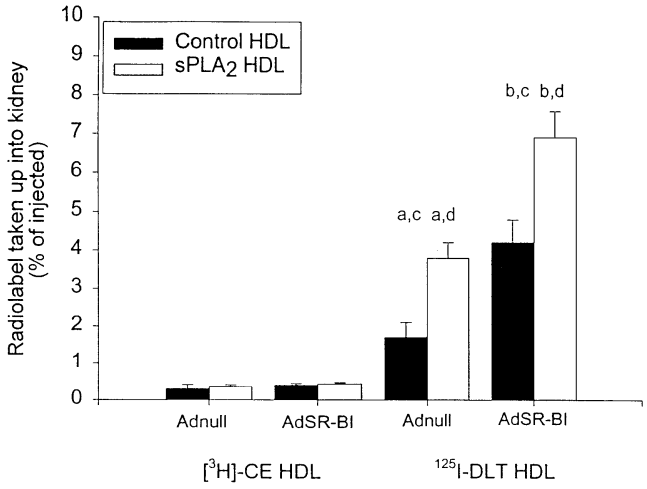

Fig. 6. Kidney uptake of ^{125}I-DLT and [^{3}H]CE label from sPLA$_2$-modified and control HDL. Three days after treatment with AdSR-BI or Adnull C57BL/6 mice were injected in the jugular vein with double-labeled sPLA$_2$-modified or control HDL. Four hours after tracer injection kidneys were collected after perfusion. Radioactivity was determined by direct counting of tissue (^{125}I) or quantified after tissue homogenization and lipid extraction (^{3}H). Values marked with the same letters are significantly different by analysis of variance ($P < 0.05$).

was the uptake of the respective radiolabels into the kidney. Data presented in **Fig. 6** indicate that in Adnull-treated mice, significantly more ^{125}I-labeled DLT label derived from sPLA$_2$-modified HDL accumulated in the kidney at 240 min compared with normal HDL (3.8 ± 0.4% vs. 1.7 ± 0.4%, $P < 0.05$). Expression of SR-BI (AdSR-BI) resulted in a significant increase in the ^{125}I-labeled DLT taken up into the kidney derived from both HDLs (6.9 ± 0.7% from sPLA$_2$ HDL vs. 4.2 ± 0.6% from control HDL, $P < 0.05$). In contrast to the liver, no significant differences in the [^{3}H]CE label in the kidney between sPLA$_2$-modified and control HDL was detected. SR-BI overexpression marginally increased the [^{3}H]CE label from both HDLs, but this did not reach significance.

DISCUSSION

The data presented in this article support the following contentions: *i*) Modification of HDL by sPLA$_2$ contributes to the reduction in HDL levels during inflammatory states; *ii*) sPLA$_2$ modification of HDL represents an example where remodeling of the lipid component of HDL results in enhanced SR-BI-mediated selective cholesteryl ester uptake; and *iii*) enhanced selective uptake in the liver is associated with increased HDL apolipoprotein catabolism, and at least some of this catabolism occurs in the kidney.

HDL isolated from the sPLA$_2$ transgenic mice was significantly depleted in phospholipids and enriched in triglycerides. This is in keeping with earlier reports that induction of the acute-phase response in mice resulted in a significant decrease in phospholipid composition of HDL (37), whereas triglyceride enrichment is a prominent fea-

ture of the acute-phase response (38). As triglyceride enrichment and phospholipid depletion are known determinants of HDL apoA-I catabolic rates, these findings were consistent with $sPLA_2$ modulating HDL metabolism in vivo.

It has been proposed that the reduction of HDL cholesterol and apoA-I levels during the acute-phase response is a direct result of SAA association with HDL (20). However, earlier studies of the effect of SAA on HDL metabolism were confounded by the fact that it involved the induction of the totality of the acute-phase response with a variety of metabolic effects that can affect HDL metabolism in ways beyond induction of SAA (39). Studies using adenoviral vector expression of SAA show, in fact, that neither high level expression of mouse nor human SAA resulted in decreased HDL cholesterol or apoA-I levels in wild-type or apoA-I transgenic mice (21). However, SAA might indirectly enhance the effect of $sPLA_2$ on HDL metabolism, as it was shown that its presence on HDL promotes the activity of $sPLA_2$ (17).

To define the mechanism whereby HDL is reduced in $sPLA_2$ transgenic mice, we performed initial autologous clearance studies in $sPLA_2$ and control mice using double-labeled nondegradable radiolabeled lipid and protein markers. Radioiodinated HDL apolipoproteins were catabolized nearly twice as fast in $sPLA_2$ transgenic mice when compared with control mice. The [^{3}H]CE clearance from $sPLA_2$-modified HDL was also more rapid than that of control mice, but did not reach significance ($P < 0.08$). In each case the [^{3}H]CE cleared more rapidly from the plasma than the apolipoprotein component radiolabeled with ^{125}I-labeled DLT, indicative of a selective uptake mechanism. These data and previous in vitro studies showing that snake venom $sPLA_2$ treatment of HDL resulted in increased uptake of [^{3}H]CE by hepatocytes (12) point to SR-BI involvement in this process. As SR-BI expression in the liver did not differ between $sPLA_2$ transgenic mice and control mice as assessed by Western blot analyses (data not shown), we investigated the possibility that $sPLA_2$ modification of the HDL ligand might influence HDL association and subsequent lipid transfer via SR-BI. In three separate experiments, three different batches of $sPLA_2$-modified HDL were significantly more efficient when compared with control HDL in promoting SR-BI-specific selective cholesteryl ester uptake. Increased selective uptake was apparent notwithstanding the fact that in each of these experiments $sPLA_2$-modified HDL associated somewhat less than control HDL with SR-BI. This dissociation between association and selective uptake is of interest. Evidence suggests an important role for apoA-I and its conformation/organization within HDL particles in mediating binding to SR-BI (40). By nuclear magnetic resonance spectroscopy of [^{13}C]lysine-apo-AI, the phospholipid content of recombinant HDL (rHDL particles) was found to be a major determinant of apo-AI conformation (41). In addition to influencing the phospholipid content of HDL, $sPLA_2$ thus also likely alters the conformation of apo-AI. This change in the HDL ligand results in a significant increase in selective cholesteryl ester uptake without a commensurate increase in HDL association (this is in fact marginally decreased, although it does not reach statistical significance). It is thus plausible that different regions of SR-BI might interact with different aspects of the HDL ligand to affect both association and efficient selective cholesteryl ester transfer. Changes in the HDL ligand could differentially influence these two aspects of SR-BI function. These data demonstrate that at constant SR-BI receptor levels, modification of the HDL ligand by $sPLA_2$ can influence lipid flow and subsequent HDL and apoA-I catabolism.

Adenoviral vector-mediated overexpression of SR-BI was used to study further the catabolism of $sPLA_2$-modified HDL in vivo. These experiments had two aims. The first was to confirm our in vitro data that $sPLA_2$-modified HDL allows for enhanced cholesteryl ester uptake in the in vivo setting. The second was to explore the relationship between selective uptake and subsequent catabolism of the apolipoprotein component of HDL. We evaluated the respective roles of the liver and kidney in the accelerated catabolism of $sPLA_2$-modified HDL. Measurements of organ uptake of HDL cholesteryl ester and apolipoprotein were performed with nondegradable radiolabeled lipid and protein tracers. The caveat in the execution of our in vivo selective uptake and catabolism evaluations was that drastically reduced HDL concentrations could influence the flux of HDL cholesteryl ester to the liver (36). Experiments were not conducted in autologous mice because $sPLA_2$ transgenics proved sensitive to adenoviral vector overexpression of SR-BI, with markedly decreased HDL levels (data not shown). It was established that HDL isolated from $sPLA_2$ mice was catabolized significantly faster than control HDL in control (C57BL/6) mice, indicating that the modification of HDL by $sPLA_2$ induced structural and compositional changes that were retained in normal mice lacking $sPLA_2$ (data now shown). The in vivo selective uptake evaluations were thus performed in control C57BL/6 mice and terminated at 4 h when nearly 90% of the [^{3}H]CE label was cleared from $sPLA_2$-modified HDL. The plasma decay curves corresponded to that obtained in the former kinetic studies in that both the protein and lipid label cleared more rapidly in the case of $sPLA_2$-modified HDL. Adenoviral vector-mediated SR-BI overexpression further enhanced this. Quantitative uptake by the liver showed that significantly more [^{3}H]CE accumulated in this organ when derived from $sPLA_2$-modified HDL and this increase was significantly higher with SR-BI overexpression. However, the liver accumulation of ^{125}I-labeled apolipoproteins did not differ significantly between the respective HDLs and increased similarly with SR-BI overexpression. Quantitative analyses of the accumulation of radiolabel in the kidney showed the reverse. Significantly more protein accumulated than lipid. No differences between the modest lipid accumulation from either HDL could be detected. SR-BI overexpression modestly increased the renal lipid accumulation, but this did not reach significance. More protein accumulated in the kidney derived from $sPLA_2$ HDL when compared with the control HDL. These accumulations were significantly enhanced with SR-BI overexpression. Taken together, these

data show that the enhanced selective ester uptake evident in the liver corresponds to enhanced protein accumulation in the kidney. It establishes a link between enhanced hepatic selective uptake and increases renal catabolism.

We suggest that under inflammatory conditions $sPLA_2$ hydrolyzes phospholipids on HDL, altering the composition of the particle. These modified particles are more efficient as substrates for SR-BI-mediated selective cholesteryl ester uptake. This enhanced SR-BI activity may lead to the entry of an SR-BI-generated HDL remnant into a distinct catabolic pathway that is particularly active in the kidney. The possibility needs to be explored that $sPLA_2$ inhibitors could ameliorate the low HDL state that exists in chronic inflammatory conditions such as rheumatoid arthritis. This holds the potential to reduce atherogenesis that complicates these diseases.

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