Induction of Adrenal Scavenger Receptor BI and Increased High Density Lipoprotein-Cholesteryl Ether Uptake by *in Vivo* Inhibition of Hepatic Lipase*

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Hepatic lipase (HL) and scavenger receptor type B class I (SR-BI) have both been implicated in high density lipoprotein (HDL)-cholesteryl ester uptake in cholesterol-utilizing tissues. Inactivation of HL by gene-directed targeting in mice results in up-regulation of SR-BI expression in adrenal gland (Wang, N., Weng, W., Breslow, J. L., and Tall, A. R. (1996) *J. Biol. Chem.* 271, 21001–21004). The net effect on HDL-cholesteryl ester uptake is not known.

We determined the impact of acute *in vivo* inhibition of rat adrenal HL activity by antibodies on SR-BI expression and on human and rat HDL-[³H]cholesteryl ether (CEth) uptake in the adrenal gland. Rat HDL was isolated from rats in which HL activity had been inhibited for 1 h. The rats were studied under basal conditions (not ACTH-treated) and after previous treatment with ACTH for 6 days (ACTH-treated). Intravenous injection of anti-HL resulted in 70% lowering of adrenal HL activity in both conditions which were maintained for at least 8 h. In not ACTH-treated rats, inhibition of adrenal HL increased adrenal SR-BI mRNA (5.2-fold) and mass (1.6-fold) within 4 h. HL inhibition resulted in 41% and 14% more adrenal accumulation of human HDL-[³H]CEth during 4 and 24 h, respectively. The adrenal uptake of rat HDL-[³H]CEth increased by 68%, 4 h after the antibody injection. ACTH treatment increased total adrenal HL activity from 3.7 ± 0.5 milliunits to 34.0 ± 17.2 milliunits, as well as adrenal SR-BI mRNA from 2.9 ± 0.7 arbitrary units (A.U.) to 86.8 ± 41.1 A.U. and SR-BI mass from 7.7 ± 1.8 A.U. to 63.16 ± 46.7 A.U. The human HDL-[³H]CEth uptake by adrenals was also significantly increased from 0.58 ± 0.11% of injected dose to 7.24 ± 1.58% of injected dose. Inhibition of adrenal HL activity did not result in further induction of SR-BI expression and did not affect human HDL-[³H]CEth uptake.

These findings indicate that SR-BI expression may be influenced by changes in HL activity. HL activity is not needed for the SR-BI-mediated HDL-cholesteryl ester uptake by rat adrenal glands.

In the rat, hepatic lipase (HL[1]; E.C. 3.1.1.34) is extracellu-

larly localized at the parenchymal cell microvilli of the liver (1–3). A related enzyme, also indicated as liver (L)-type lipase, is present in the zona fasciculata of the adrenal gland and in the corpora lutea of the ovary (2, 4–6). We proposed a role of HL in the uptake of HDL-unesterified cholesterol and cholesteryl esters in the lipase-containing tissues (7, 8). *In vitro* studies, with either isolated cell systems or perfused rat liver, showed that HL activity may stimulate the uptake of HDL-cholesteryl esters as well as unesterified cholesterol (9–11). However, *in vivo* only indirect support for a role of HL in HDL-cholesterol and cholesteryl ester uptake has been obtained. Jansen *et al.* (12) showed that plasma HDL-cholesterol increased by *in vivo* inhibition of HL. At the same time *de novo* cholesterol synthesis in liver (13) and in superovulated rat ovaries (14) is induced. These findings are compatible with the involvement of HL in the uptake of extracellular cholesterol. HDL-cholesteryl ester uptake has been studied in a wide range of tissues under different metabolic conditions and may be taken up via several mechanisms. Besides the classical endocytotic pathway (see Ref. 15 as review) a selective uptake mechanism, in which HDL-cholesteryl esters are taken up without concomitant internalization of the protein part, has been proposed (8, 16, 17). The scavenger receptor class B type I (SR-BI), exclusively present in liver and non-placental steroidogenic tissues, is involved in this process (18–20). In endocrine tissues the SR-BI expression is regulated by trophic hormones (21). Additionally, cellular cholesterol levels may modulate SR-BI expression (22, 23). Investigations in HL-deficient (knock-out) mice suggest a link between HL and SR-BI expression (22). In female HL knock-out mice, SR-BI expression in adrenal gland was strongly enhanced. The induction of SR-BI was suggested to result from a lowering of intracellular cholesterol stores because of HL deficiency. An alternative mechanism may be that SR-BI expression is stimulated compensatory to changes in plasma lipoprotein metabolism because of the long-term HL deficiency. Remarkably, despite the greatly enhanced SR-BI levels (3.5-fold), adrenal cholesterol (ester) stores were largely depleted suggesting that the increase in SR-BI did not result in adequate cholesterol supply to support steroidogenesis. This may indicate that HL activity is required for the optimal activation of selective HDL-cholesteryl ester uptake.

In the present investigation we studied the impact of acute *in vivo* inhibition of HL activity on adrenal SR-BI expression. In addition, we measured the consequences for HDL-cholesteryl ester uptake under these conditions.

lipoprotein; CEth, cholesteryl ether; SR-BI, scavenger receptor class B type I; IgG, immunoglobulin G; PCR, polymerase chain reaction; ACTH, adrenocorticotrophic hormone; A.U., arbitrary unit(s).

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (200–300 g) were kept under controlled conditions of humidity, light, and temperature with free access to tap water and chow diet. The animals were fasted overnight before use. ACTH treatment consisted of daily subcutaneous administration of 0.2 mg of Synacthen (a synthetic ACTH analogue, Ciba) per kg body weight for 6 days. Control rats were injected daily with saline for the same period.

In Vivo Inhibition of Hepatic Lipase Activity—The IgG fraction of goat anti-rat HL and non-immune goat serum was isolated by protein G affinity chromatography. The IgGs were dialyzed against 5 mM (NH$_4$)HCO$_3$ and lyophilized. The obtained pellets were resuspended in 0.15 M NaCl to a concentration of 30 mg of protein/ml. The antibody preparation was tested for its ability to inhibit HL activity of heparin-containing rat liver perfusate. To inhibit adrenal lipase activity, the rats were treated with an amount of antibodies that inhibited the enzyme activity equivalent to all heparin-releasable HL activity in rats of the same weight. Control animals were injected with the same amount of control IgG. Four, eight, or twenty-four hours after antibody injection, adrenals were homogenized in 10 volumes of ice-cold phosphate-buffered saline (pH 7.4) containing 10 IU/ml heparin and 1 mM benzamidine. After centrifugation (10,000 × g, 2 min, 4 °C), the postnuclear fraction was assayed for hepatic lipase activity as described elsewhere (24). Enzyme activity was determined as triacylglycerol hydrolase and expressed as milliunits (nmol of free fatty acids released per min).

Northern Blot and Immunoblot Analysis of SR-BI—Total tissue RNA was isolated from adrenal glands as described (25). Adrenal RNA (7.5 µg/lane) was electrophoresed on a formaldehyde-agarose gel and transferred to a nylon membrane (Highbond-N+, Amersham). The SR-BI cDNA probe for *in situ* hybridization was prepared by reverse transcriptase-PCR using 1 µg of adrenal RNA. The reverse transcriptase-PCR was performed as described previously (26), using the primers SR-BI(1)-(5′-CGG AAT TCA GGG GTG TTT GAA GGC-3′) and SR-BI(2)-(5′-CGG GAT CCT GAA TGG CCT CCT TAT CC-3′) according to the human cDNA sequence (27). This primer combination yields a PCR product of 550 base pairs, which is ~98% homologous with that of the rat (data not shown). The RT-PCR product (550 base pairs) was extracted from agarose gel and resuspended in sterile water. A glyceraldehyde-3-phosphate dehydrogenase probe (570 base pairs) was also prepared by reverse transcriptase-PCR from rat heart RNA and used as reference. Both probes were labeled using 1 mCi of [^{32}P]ATP. Membranes were hybridized following standard methods (28). Radioactive bands were analyzed using a GS363 Molecular Imager System from Bio-Rad. Values of the SR-BI mRNA were normalized for glyceraldehyde-3-phosphate dehydrogenase contents in the same samples. Crude membranes of adrenal glands were isolated as described elsewhere (29). Twenty µg of membrane protein were separated by 10% SDS-polyacrylamide gel electrophoresis under reducing conditions (30) and transferred to a nitrocellulose membrane (Schleicher & Schuell). The membranes were incubated for 2 h at room temperature with a rabbit polyclonal anti-rat SR-BI antibody followed by incubation for 1 h with alkaline phosphatase-conjugated goat anti-rabbit IgGs as secondary antibody. SR-BI protein bands were detected and scanned using a Hewlett Packard ScanJet 4C and quantified.

HDL Isolation and Labeling—Human HDL was isolated from blood of healthy volunteers at a density between 1.063 and 1.21 g/ml by sequential ultracentrifugation using standard techniques (31). HDL was passed over a Sepharose-heparin column to remove apoE-containing lipoproteins (32). After dialysis against 0.15 M NaCl, containing 1 mM EDTA, pH 7.4, the lipoprotein was labeled in its lipid moiety with [1α,2α-^{3}H]cholesteryl oleyl ether (Amersham Pharmacia Biotech) as described previously for LDL (33). The labeled HDL was reisolated by gradient ultracentrifugation (33) and dialyzed against 0.15 M NaCl. Before use the preparation was filtered through a 0.45-µm Millipore filter. Rat HDL was isolated from blood of control animals injected with polyclonal anti-HL antibody and sacrificed 1 h later. During this period HL activity in the liver is inhibited 90–98%. The HDL fraction was isolated and handled as described above for human HDL, except that the lipoprotein fraction was collected at density between 1.050 and 1.21 g/ml. The labeling of rat HDL occurred in the presence of human lipoprotein-deficient serum as a source of cholesteryl ester transfer protein (33).

HDL-[^{3}H]CEth Uptake in Vivo—To study adrenal HDL[^{3}H]CEth uptake *in vivo*, two different procedures were used. In the first procedure rats were intravenously injected with 0.1 ml of concentrated anti-HL or non-immune IgG preparation. Four hours later 0.2 ml of human HDL-

TABLE I
In vivo effect of anti-HL antibody administration on adrenal HL activity and SR-BI expression in control rats

Animals were injected with goat non-immune IgG (control IgG) or with a goat polyclonal anti-hepatic lipase antibody (anti-HL) and sacrificed after 4 h or 24 h. Adrenal glands were removed, homogenized, and assayed for HL activity. HL activity was assayed as triacylglycerol hydrolase and expressed as milliunits (nanomoles of free fatty acids released per min) per 2 adrenals. SR-BI mRNA and mass were determined as described under "Experimental Procedures." Values are mean ± S.D. ($n = 7$) except for HL activity at 24 h ($n = 3$) and for SR-BI expression ($n = 3$). All values are statistically significant if compared with control IgG, except when NS is indicated using one way analysis of variance with the Student-Newman-Keuls test.

|	HL activity	SR-BI mRNA	SR-BI mass
	(total milliunits/organ)	*(A.U./organ)*	*(A.U./organ)*
Control IgG (4 h)	3.68 ± 0.46	2.9 ± 0.7	7.7 ± 1.8
Anti-HL (4 h)	1.18 ± 0.48[a]	15.1 ± 4.2[a]	12.8 ± 0.4[a]
Anti-HL (24 h)	8.38 ± 1.08[a]	2.4 ± 0.6[NS]	13.7 ± 3.5[a]

[a] $p < 0.0001$.

[^{3}H]cholesteryl ether solution, corresponding to 124 nmol of total cholesterol and 1×10^6 dpm, was injected intravenously. Animals were sacrificed 4 or 24 h after the injection of labeled HDL. In the second procedure, animals were intravenously injected with 0.1 ml of concentrated anti-HL or non-immune IgG preparation. Two hours later 0.2 ml of rat HDL[^{3}H]CEth corresponding to 100 nmol of total cholesterol and 1×10^6 dpm was intravenously injected. Two hours after the injection of the labeled HDL, 0.1 ml of concentrated anti-HL or control IgGs were administrated again. Animals were sacrificed 4 h after the labeled lipoprotein injection. The adrenals were excised, cleaned from adherent fat tissue, and weighed. Tissue samples were dissolved in Soluene-350 (Packard Instrument) for 4 h at 55 °C and analyzed for radioactivity. The radioactivity in the adrenals was corrected for contamination of plasma radioactivity and used 9.9% (v/w) plasma per organ (34). In the experiments with rat HDL the radioactivity in the adrenals was also corrected for the increase in HDL-cholesteryl esters between 2 and 4 h after the antibody injection (12.3%) (12).

RESULTS

HL activity was lowered *in vivo* by administration of HL activity inhibiting antibodies. Four hours after anti-HL administration to control (not ACTH-treated) rats, the adrenal lipase activity was inhibited by 68% (Table I). The adrenal HL activity remained inhibited for at least 4 h. After 8 h the adrenal HL activity was still 40% lower than in controls (non-immune) (2.21 ± 0.36 *versus* 3.68 ± 0.46 milliunits/organ). After 24 h, the adrenal lipase activity had increased to 2- to 3-fold over the basal activity. Four hours after the injection of anti-HL antibody into control (not ACTH-treated) animals the adrenal SR-BI mRNA content was greatly increased (5.2-fold) (Table I). SR-BI mass increased during the same period by 66%. Twenty-four h after the injection of anti-HL, when HL activity had increased above the basal activity, SR-BI mRNA was about 18% below control values (n.s.). SR-BI mass, however, remained increased at the level already reached 4 h after inhibition of HL activity.

In the following experiments we studied the consequences of the changes in HL activity and SR-BI expression for the adrenal uptake of HDL-cholesteryl ester. To this end, the rats were injected with HDL labeled with a non-degradable cholesteryl ester analogue, [^{3}H]cholesteryl ether (HDL-[^{3}H]CEth). The accumulation of radioactivity represents the uptake of HDL-[^{3}H]cholesteryl ether (HDL-[^{3}H]CEth) as an estimation of HDL-cholesteryl ester. In the first 4 h after administration of labeled human HDL, 72.0 ± 2.4% of the injected HDL-[^{3}H]CEth was cleared from the plasma compartment in control rats, compared with 66.0 ± 2.7% in antibody-treated rats (n.s). Most of this label is removed by the liver (not shown). The adrenals took up 0.58 ± 0.11% of the total injected dose during this time period (Fig. 1). In rats treated with anti-HL, the

FIG. 1. **Effect of HL activity inhibition on HDL-[³H]CEth uptake by adrenal glands of control rats.** The rats were injected with control IgGs or with a polyclonal anti-rat HL antibody preparation. Labeled rat (*r*) or human (*h*) HDL was intravenously injected 2 or 4 h later, respectively, as described under "Experimental Procedures." The animals were sacrificed 4 h or 24 h later. HDL-[³H]CEth uptake by adrenal glands was expressed as percentage of the injected dose per organ. Values are mean ± S.D. (*n* = 4). The effect of anti-HL was tested using one-way analysis of variance with the Student-Newman-Keuls test.
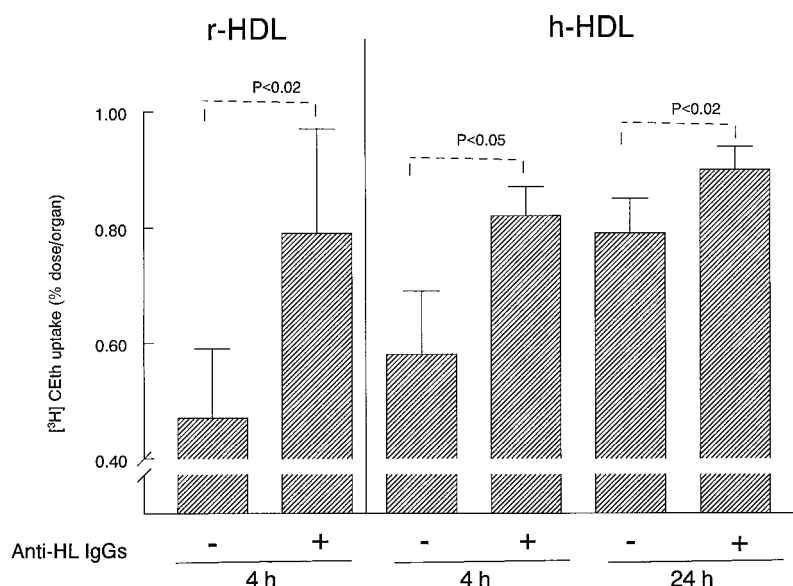
adrenal uptake of HDL-[³H]CEth was 41% higher than in the controls. This effect on adrenal HDL-[³H]CEth uptake was also present when the rats were studied 24 h after HDL-[³H]CEth injection, although it tended to be smaller (Fig. 1). Additional experiments were carried out using HDL isolated from rats in which HL activity had been functionally inactivated by anti-HL antibody for 1 h. In the first 4 h after the administration of rat HDL-[³H]CEth about 50% (controls, 53.0 ± 5.2%; anti-HL-treated rats, 47.6 ± 3.0%, n.s.) of the label was cleared from the plasma compartment. During this period control adrenals took up 0.47 ± 0.12% of the injected dose per organ. In rats treated with anti-HL, the adrenal uptake was increased by 68% (Fig. 1) (0.47 ± 0.12, *n* = 4 *versus* 0.79 ± 0.18, *n* = 5, *p* < 0.02). From these experiments, we concluded that SR-BI rather than HL activity corresponds with the uptake of HDL-cholesteryl ester in the adrenal gland. On the other hand, SR-BI expression may be modulated by changes in HL activity.

Next we studied whether HL activity may affect HDL-cholesteryl ester uptake when the adrenal gland is stimulated by ACTH treatment and HL activity is greatly enhanced. Rats were treated with ACTH for 6 days, leading to about a 2-fold increase in HL activity (107 ± 13 *versus* 213 ± 50 milliunits/g wet weight). Because the adrenal weight increased during ACTH treatment (35 ± 5 *versus* 174 ± 61 mg/2 adrenals), the total lipase activity in the adrenals increased even more (9.2-fold) (Fig. 2). Under these conditions SR-BI expression is also greatly enhanced (Fig. 2). Total SR-BI mRNA in stimulated adrenals was 30-fold higher than in the control (2.9 ± 0.7 *versus* 86.8 ± 41.1 A.U./2 adrenals). SR-BI mass was less increased (4.9 ± 0.6 *versus* 19.5 ± 14.8 A.U./mg of protein) (Fig. 2), but total SR-BI mass in the adrenals was 8.1-fold higher than in the controls (7.7 ± 1.8 *versus* 63.2 ± 46.7 A.U./2 adrenals). Under these conditions, the stimulated adrenals took up 7.2% of the injected dose of (HDL-[³H]CEth) in 4 h, which is about 12 times more than in the unstimulated adrenals (Fig. 2). Inhibition of HL activity under these conditions had no effect on SR-BI expression either in total mRNA (86.8 ± 41.1 *versus* 67.1 ± 9.9 A.U./2 adrenals) or in total SR-BI mass (63.2 ± 46.7 *versus* 60.9 ± 33.6 A.U./2 adrenals). In addition, inhibition of HL activity did not influence [³H]CEth uptake (7.24 ± 1.58 *versus* 6.67 ± 1.40% of injected dose/2 adrenals) in ACTH-treated rats for 4 h.
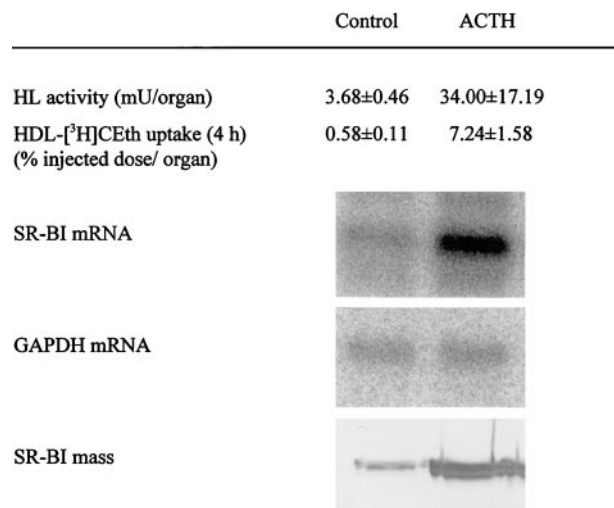
FIG. 2. **Effect of ACTH treatment on rat adrenal HDL-[³H]CEth uptake and SR-BI expression.** Control and ACTH animals were treated with control IgG and sacrificed 4 h later. Both adrenals were removed subsequently and frozen. HL activity and SR-BI mRNA and mass were analyzed by enzymatic assay, Northern blot, and immunoblot, respectively, as described under "Experimental Procedures." The figure shows a representative experiment of Northern blot and immunoblot. HDL-[³H]CEth was injected 4 h after the IgG and the animals were sacrificed 4 h later in order to measure the uptake of radioactivity in the adrenal glands. Values are mean ± S.D. (*n* = 4) except for HL activity (*n* = 7).

DISCUSSION

Several mechanisms have been proposed for the (selective) uptake of HDL-cholesteryl ester in the adrenal gland. Both L-type lipase, the adrenal form of HL, and SR-BI may play a role in adrenal cholesterol homeostasis. *In vitro*, several studies on the effect of HL on HDL-cholesterol (ester) uptake in cultured cells have been reported, but no *in vivo* data are available. HL and SR-BI expression may be coordinately regulated. Gene-targeted inactivation of HL in mice was found to be associated with increased expression of SR-BI. Despite the increase in SR-BI expression, adrenal cholesteryl ester stores were partly depleted (22). This suggested that the induction of SR-BI could not fully compensate for the loss of HL activity in cholesterol homeostasis. The effect of the increased SR-BI on HDL-cholesteryl ester uptake was not evaluated. In the pres-

ent study, we determined the effect of an acute inhibition of adrenal HL activity on SR-BI expression and on HDL-cholesteryl ether uptake *in vivo*. Administration of anti-HL to rats leads to a rapid inactivation of HL activity in adrenals and liver. The adrenal HL turnover is relatively slow. Once adrenal lipase activity is inhibited it remains lowered during at least 8 h, while the HL activity in the liver is restored to control values in 4 h. Twenty-four hours after the injection of antibody the adrenal lipase activity is increased above the control values. Acute inhibition of HL in the adrenal gland led to a greatly increased expression of SR-BI within 4 h, which was accompanied by a significant increase in HDL-[^{3}H]CEth uptake. Twenty-four hours after antibody administration, SR-BI expression had returned to control values. The actual rate of adrenal uptake of HDL-cholesteryl esters at this time point cannot be determined as the major part of HDL-[^{3}H]CEth uptake is by the liver (35) and takes place within the first 4 h after injection. Between 4 and 24 h after HDL-[^{3}H]CEth administration the increase in uptake of label in the adrenal gland was much smaller in the antibody-treated animals than in the controls. In this time period SR-BI mRNA decreased to control values in the antibody-treated animals. This may partly explain the lower rate of uptake of HDL-[^{3}H]CEth in the adrenals. We also used rat HDL isolated from animals in which HL activity had been functionally inactivated for 1 h. Therefore, this HDL had hardly been processed by HL *in vivo* prior to intravenous injection and is enriched in phospholipids and cholesterol (12). Uptake of [^{3}H]CEth from these "unprocessed" homologous rat HDL was similar to that from human HDL. Our data are compatible with a model in which adrenal HL activity is a determinant of SR-BI expression and SR-BI is the most important determinant of HDL-[^{3}H]CEth uptake. The latter is further supported by findings in ACTH pretreated rats. ACTH pretreatment led to a considerable increase in SR-BI expression, adrenal HL activity, and HDL-[^{3}H]CEth uptake. The increase in SR-BI mass was in line with the increased HDL-[^{3}H]CEth uptake. In stimulated rats the inhibition of HL did not affect either SR-BI expression or HDL-[^{3}H]CEth uptake. This clearly rules out adrenal HL activity as a major determinant of HDL-cholesteryl ester uptake under these conditions. The mechanism of the interaction between adrenal HL activity and SR-BI expression in the control rats can only be speculated about. HL is an enzyme with high phospholipase activity. Its preferred substrates are HDL-phospholipids. HL has been shown to be able to modulate HDL-unesterified cholesterol fluxes between HDL and cells and specifically to diminish the efflux of cholesterol from cells to HDL (36, 37). SR-BI expression is likely to be regulated by the cellular cholesterol content (22). Therefore, it could be that *in vivo* inhibition of HL leads to an increased efflux (or diminished influx) of non-esterified cholesterol in the adrenal gland which in turn gives rise to induction of SR-BI expression. Subsequently, SR-BI may stimulate HDL-cholesteryl ester uptake. In this model the primary role of HL would be in the modulation of fluxes of unesterified HDL-cholesterol and that of SR-BI in the mediation of HDL-cho-

lesteryl ester uptake. Taken together, HL and SR-BI may be part of mechanisms ensuring an optimal cholesterol supply for steroid hormone synthesis under a variety of conditions.

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