Selective Uptake from LDL Is Stimulated by Unsaturated Fatty Acids and Modulated by Cholesterol Content in the Plasma Membrane: Role of Plasma Membrane Composition in Regulating Non-SR-BI-Mediated Selective Lipid Transfer†

Toru Seo,‡ Wanda Velez-Carrasco,§ Kemin Qi,‡ Marni Hall,‡ Tilla S. Worgall,‡ Rebecca A. Johnson,‡ and Richard J. Deckelbaum*,‡

Institute of Human Nutrition and Department of Pediatrics, Columbia University, New York, New York 10032, and Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, New York 10021

Received October 18, 2001; Revised Manuscript Received April 2, 2002

ABSTRACT: We previously reported that unsaturated fatty acids stimulated low-density lipoprotein (LDL) particle uptake in J774 macrophages by increasing LDL receptor activity. Since free fatty acids (FFA) also change plasma membrane properties, a putative cholesteryl ester (CE) acceptor for selective uptake (SU), we questioned the ability of FFA to modulate SU from LDL. Using [^{3}H]cholesteryl ether/^{125}I-LDL to trace CE core and whole particle uptake, we found that oleic acid and eicosapentaenoic acid, but not saturated stearic acid, increased SU by 30% over control levels. An ACAT inhibitor, Dup128, abolished FFA effects on SU, indicating that increased SU by FFA was secondary to changes in cell-free cholesterol (FC). Consistent with these observations, ACAT inhibition increased cell FC and reduced LDL SU by half. The important role of plasma membrane composition was further demonstrated in that β-cyclodextrin-(β-CD-) mediated FC removal from the plasma membrane increased SU from LDL and was further stimulated by U18666A, a compound that inhibits FC transport between lysosomes and the plasma membrane. In contrast, cholesterol-saturated β-CD markedly reduced LDL SU. In contrast to LDL SU, oleic acid, ACAT inhibition, U18666A, or β-CD had no effects on HDL SU. Moreover, HDL SU was inhibited by antimouse SR-BI antibody by more than 50% but had little effect on LDL SU. In C57BL/6 mice fed a high fat diet, plasma FFA levels increased, and SU accounted for an almost 4-fold increased proportion of total cholesterol delivery to the arterial wall. Taken together, these data suggest that LDL SU is mediated by pathways independent of SR-BI and is influenced by plasma membrane FC content. Moreover, in conditions where elevated plasma FFA occur, SU from LDL can be an important mechanism for cholesterol delivery in vivo.

Low-density lipoprotein (LDL) is the major lipoprotein carrier for cholesterol in human plasma. LDL metabolism in humans is well characterized (*1*). Although LDL receptors are the primary pathway for plasma LDL clearance via the liver in human and many animal models, other uptake mechanisms exist in peripheral tissues (*2*, *3*) where few LDL receptors are expressed, such as the arterial wall (*4*). Moreover, these processes, which involve non-LDL receptor pathways, contribute to development of atherosclerosis (*5*). These include scavenger class A receptors, or classical scavenger receptors, and CD36 that mediates uptake of modified lipoproteins by macrophages and smooth muscle cells (*6*−*8*). In addition, low-affinity, high-capacity pathways such as cell surface proteoglycans (*9*−*11*) have also been suggested to play an important role in atherosclerotic lesion development (*12*).

Selective lipid transfer from lipoproteins, or selective uptake, is another pathway for cholesterol delivery to cells, whereby CE[1] from the lipoprotein core is taken up by cells without concomitant uptake of whole lipoprotein particles. Selective uptake from HDL is mediated by scavenger receptor type BI, or SR-BI, and is important in steroidogenesis and reverse cholesterol transport (*13*, *14*). Although less information is available, selective uptake also occurs with other lipoproteins such as VLDL, LDL, and IDL (*15*−*17*). SR-BI can also contribute to selective uptake from these lipoproteins (*18*), and in some cells, SR-BI can almost exclusively mediate selective uptake from LDL (*19*). Nevertheless, selective uptake from LDL occurs in tissues where little or no SR-BI is present (*20*). This indicates that, in

† This study was supported by NIH Grant HL40404. T.S. was supported by NIH Training Grant in Atherosclerosis Research HL07343-22 and is currently supported by NIH Grant DK60497.

* Address correspondence to this author at the Institute of Human Nutrition. Tel: (212) 305-4808. Fax: (212) 305-3079. E-mail: rjd20@columbia.edu.

‡ Columbia University.

§ Rockefeller University.

[1] Abbreviations: SU, selective uptake; FFA, free fatty acids; OA, oleic acid; EPA, eicosapentaenoic acid; ACAT, acyl-coenzyme A cholesterol acyltransferase; FC, free cholesterol; CE, cholesteryl ester; β-CD, β-hydroxypropylcyclodextrin; SR-BI, scavenger receptor class B-1; PL, phospholipid; FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum.

10.1021/bi011949g CCC: $22.00
Published on Web 06/01/2002

addition to SR-BI, alternative mechanisms for selective uptake are likely present in these cells. We have reported that cell surface proteoglycans mediate selective uptake from LDL and that this pathway is enhanced by lipoprotein lipase (*21*). The physiological significance of selective uptake from non-HDL lipoproteins is not fully delineated. However, with the relatively high content of CE in LDL, it is possible that selective uptake from LDL can contribute substantially to CE delivery to cells and to the development of atherosclerosis.

The plasma membrane is a putative CE acceptor for selective uptake (*22*). Still, little information is available as to how properties of the plasma membrane might affect selective uptake. We previously found that oleic acid (OA) stimulated LDL particle uptake via increased LDL receptor activity in J774 macrophages (*23*). We hypothesized that this was due to a decrease in cholesterol content in the plasma membrane as a result of fatty acid induction of increased ACAT activity and a concomitant need for the plasma membrane to supply FC required for cholesterol esterification (*23*). Potential effects of FFA on altering cholesterol in the plasma membrane are consistent with studies showing that FFA changed the membrane fluidity in various cell types (*24*, *25*) and can alter the rate of cholesterol efflux in vascular smooth muscle cells (*26*). Since the ability of plasma membrane lipids to accommodate CE is influenced by changes in plasma membrane cholesterol content (*27*, *28*), the presence of FFA and subsequent changes in membrane cholesterol might influence selective uptake from LDL.

In the current studies, we examined selective uptake from LDL in J774 macrophages and the potential of FFA to modulate selective uptake from LDL and HDL. To examine potential roles of LDL selective uptake in vivo, studies were also performed to determine whether elevated FFA levels in plasma influence LDL particle uptake and selective uptake in the arterial wall. Our results show that unsaturated FFA stimulate selective uptake for LDL but not HDL. OA decreases cholesterol content in the plasma membrane, and other factors that change cholesterol content in the plasma membrane markedly affect selective uptake from LDL but not HDL. Studies described herein suggest different mechanisms for selective uptake from LDL as compared to HDL. HDL selective uptake was primarily mediated by SR-BI, while pathways that were influenced by the cholesterol content in the plasma membrane largely contributed to selective uptake from LDL. Moreover, mice fed a high fat diet showed a 2-fold increase in plasma FFA levels, and selective uptake from LDL was enhanced in the arterial wall.

EXPERIMENTAL PROCEDURES

Materials. Sodium [^{125}I]iodide (NEZ033) and [1,2,6,7-^{3}H(n)]cholesteryl oleate were purchased from Dupont NEM, Wilmington, DE. [1α,2α(n)-^{3}H]Cholesteryl oleoyl ether (TRK888) was purchased from Amersham Life Science, Arlington Heights, IL. Fatty acids, bovine serum albumin (BSA), (+)-4-cholesten-3-one, β-hydroxypropylcyclodextrin (β-CD), cholesterol oxidase, and filipin were purchased from Sigma Chemical Co. (St. Louis, MO). β-CD was solubilized in deionized H_2O (46%, w/v) and kept sterile. Cholesterol-saturated β-CD was prepared as described (*29*). U18666A was purchased from BIOMOL Research Laboratories, Inc. (Philadelphia, PA). Rabbit antimouse SR-BI antibodies (*30*) were kindly provided by Drs. David Silver and Alan Tall (Columbia University). Fetal bovine serum (FBS) was purchased from Hyclone Laboratories (Logan, VT). Lipoprotein-deficient serum (LPDS) was prepared from FBS as described (*21*). Dulbecco's modified Eagle's medium (DMEM), RPMI, glutamine (200 mM), penicillin (10000 units/mL), and streptomycin (10000 μg/mL) were purchased from Gibco laboratories (Grand Island, NY). The ACAT inhibitor, Dup128 (*31*), was kindly provided by Dr. Jefferey Billheimer, Dupont Pharmaceutical Co., Wilmington, DE. Rabbit antirat cytochrome P450 reductase antibody (OSA-300C) was purchased from StressGen Biotechnologies Corp. (Victoria, BC, Canada).

Cells. J774 (A2) murine macrophages (*32*) were grown in culture in RPMI medium containing 10% FBS, vitamins, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 95% atmosphere with 5% CO_2. Two days before the experiments, cells were plated to either 16 or 35 mm dishes at a density of 10^4 or 10^5 cells/dish, respectively, and grown to 80% confluency. For the plasma membrane isolation, cells were plated on 100 mm dishes and maintained in the above medium until 100% confluency.

Lipoprotein Preparation and Labeling. LDL and HDL were isolated from normolipidimic subjects and labeled with [^{3}H]cholesteryl oleoyl ether ([^{3}H]CEt) and ^{125}I and assayed for CE content using gas−liquid chromatography as described (*21*). For in vivo studies, LDL was labeled with nondegradable [^{125}I]tyramine cellobiose (^{125}I-TC) instead of conventional ^{125}I labeling (*21*). The calculated cholesterol to protein ratios of LDL and HDL ranged from 1.6 to 1.8 ($n = 20$) and 0.2 to 0.5 ($n = 6$), respectively. In both HDL and LDL, more than 85% of total cholesterol was accounted for by CE.

Determination of LDL and HDL Metabolism in Cultured Cells. J774 macrophages were incubated with double-labeled LDL or HDL in RPMI containing 1% BSA in the presence or absence of 0.3 mM FFA (2:1 mol ratio FFA:BSA). At the end of the incubation, medium was removed to determine LDL degradation, and cells were washed twice with PBS and solubilized in 0.1 N NaOH to determine cell-associated [^{3}H]CEt and ^{125}I by a Wallac 4090 scintillation counter as described (*21*). In separate experiments, LDL was incubated with FFA or cells for extended periods and was characterized for structural and potential oxidation effects (*33*, *34*). Under the described experimental conditions, there was no evidence of changes in LDL structure, and oxidized LDL was not detected during incubation with cells. Cell-associated and degraded ^{125}I-apo B were summed to show total cell apo B metabolism, and values were used to calculate cholesterol delivery to cells using cholesterol to protein ratios of the experimental LDL. This value was compared to CE uptake using [^{3}H]CEt, and the difference between CE uptake from [^{3}H]CEt and ^{125}I uptake was defined as selective uptake.

To promote FC accumulation in the plasma membrane, LDL incubation was performed in the presence of 10 μM Dup128, an ACAT inhibitor (*31*). Also, cells were incubated with RPMI containing 5 mM β-CD or cholesterol-saturated β-CD for 1 h at 37 °C before being incubated with lipoproteins. β-CD rapidly removes cholesterol from the membrane, while treatment of cells with cholesterol-saturated β-CD leads to a cholesterol-rich plasma membrane (*35*).

Similar experiments were also performed in the presence of 2.5 μM U18666A, an amphiphile that inhibits the transport of hydrolyzed cholesterol from lysosomes to the plasma membrane (*36*) or vice versa (*37*) to prevent the redistribution of cell cholesterol between the plasma membrane and intracellular compartments (*38*, *39*).

To examine the roles of cell surface proteins, cells were pretreated with Pronase (3 μg/mL) for 30 min at 37 °C prior to experiments to digest cell surface proteins. Cells were then incubated with labeled LDL for 4 h in the presence or absence of cyclohexamide (0.89 mM) to inhibit protein synthesis. A 4 h incubation was chosen for the latter studies since longer incubations of cells with cyclohexamide leads to substantial cell death.

Filipin Staining of Cell-Free Cholesterol. Cells were plated on the slide chamber and incubated with 100 μg/mL LDL in the presence or absence of 10 μM ACAT inhibitor or 2.5 μM U18666A for 30 min to 18 h at 37 °C. After incubation, cells were incubated with DMEM containing heparin (1400 IU/mL) for 45 min at 4 °C to remove cell surface bound LDL and fixed with 3% paraformaldehyde for 1 h at room temperature. After extensive washing with PBS, cells were incubated with PBS containing 10% FBS and filipin (0.05 mg/mL) for 1 h at room temperature and washed with PBS to remove unbound filipin (*40*). Studies were repeated twice and showed very similar results. The stained cells were visualized with a Leica Orthlux II florescent microscope and were photographed using a Leica Vario-Orthomat at a magnification of 400×. Equal exposure times were used for all photographs. Thus, the 30 min incubation shows only minimal staining. This was necessary to show the variations of filipin intensity under the treatment conditions (C−F) that were much higher than after 30 min incubation.

Determination of Cholesterol Content in the Plasma Membrane Using Cholesterol Oxidase. After incubation of cells with 100 μg/mL [^{3}H]CE-labeled LDL for 30 min to 18 h, cells were washed with PBS and incubated with DMEM containing heparin (1400 IU/mL) for 45 min at 4 °C to remove surface-bound LDL. After the cells were quickly washed with PBS, the cells were fixed with 1% glutaldehyde at room temperature for 10 min and were washed with PBS to remove the fixative. Cells were then incubated with RPMI containing cholesterol oxidase (1 units/mL) for 1 h to allow the conversion of cholesterol to cholestenone in the plasma membrane (*41*). After the cells were washed with PBS, cellular lipids were extracted with 2-propanol containing 60 μg/mL cholestenone as an internal standard. [^{3}H]Cholesterol, [^{3}H]cholestenone, and [^{3}H]CE were separated on thin-layer chromatography as described (*23*) and measured for radioactivity.

Plasma Membrane Isolation and Characterization. DEAE-Sephadex beads were washed, coated with 0.1% nitrocellulose, and stored in PBS containing 100 units/mL penicillin and 100 μg/mL streptomycin at 4 °C as described by Gotlib and Searls (*42*). Six 100 mm confluent dishes per condition were used to isolate the plasma membrane using cationized beads as described by DeGrella et al. (*43*). There was very little endoplasmic reticulum membrane contamination as determined by western blot with antirat cytochrome P450 reductase antibody. Isolated plasma membranes were extracted with 2-propanol and measured for cholesterol and PL content using Waco enzymatic assays (Richmond, VA), and the plasma membrane cholesterol content was normalized per 100 μg of phospholipid (PL).

Cell Phospholipid Measurement. After incubation of cells with FFA, cell lipids were extracted with hexane−2-propanol (3:2) overnight. Isolated lipids were assayed for PLs by the method of Bartlett (*44*), and cell FC and CE were determined by GLC as described previously (*21*).

LDL Uptake in the Arterial Wall in Vivo. Male C57BL/6 mice (4 weeks old) weighing approximately 15−18 g were fed a normal chow or a high fat diet (45% calories come from fats that are primarily saturated coconut oil; TD97108, Harlan Tekland, Indianapolis, IN) in the clean room at Rockefeller University. At the end of a 12 week feeding period, mice were injected with double-labeled human LDL (200 μg) through the tail vein, and mice were continued on their respective diets for 24 h. Previous studies indicated that 99% of injected LDL was cleared after 24 h (*21*). At the end of the experiments, the vascular system was thoroughly perfused with 20 mL of phosphate-buffered saline (PBS) containing 1 mg/mL EDTA and 100 IU/mL heparin. Aortas from these mice were isolated and quickly rinsed with saline containing EDTA (1 mg/mL) followed by separation of lipid and protein phases for determination of ^{125}I-TC-apoB and [^{3}H]CEt uptake as described in previous studies (*21*). In separate sets of mice fed the same diets, blood was obtained by retro-orbital sampling, and plasma lipid profiles were determined by enzymatic kits.

Statistics. Two-tailed *t*-tests were performed to determine the statistical significance of experiments ($p < 0.01$).

RESULTS

Effects of Oleic Acid, ACAT Inhibition, and U18666A on Plasma Membrane Cholesterol. As previously reported (*23*), incubation of OA increased LDL particle uptake by increasing LDL receptor activity. We have hypothesized that this is likely due to decreases in cholesterol content in the plasma membrane cholesterol secondary to increased ACAT activity (*23*). Since the plasma membrane is important for selective uptake (*14*, *22*, *45*), we hypothesized that changes in the plasma membrane cholesterol would influence selective uptake from LDL. To test this, we first examined whether OA would modulate cholesterol content in the plasma membrane in cells incubated with LDL by using the cholesterol binding resin, filipin (Figure 1). Effects of ACAT inhibitor and U18666A on filipin staining were also examined since these compounds are reported to alter the plasma membrane cholesterol content (*46*). Compared to cells incubated with LDL alone for 30 min (Figure 1B), LDL incubation increased filipin staining at both 4 and 18 h (Figure 1C,D), an effect associated with increasing membrane cholesterol. Filipin staining was more pronounced in the presence of ACAT inhibitor, particularly at the cell periphery, and at 18 h, entire cells were stained, indicating substantial cholesterol accumulation within cells (Figure 1E). In contrast, the presence of U18666A during LDL incubation led to less filipin staining of the plasma membrane at 4 h (Figure 1G) and punctated staining patterns in lysosomes at 18 h, indicative of intracellular but not plasma membrane cholesterol accumulation (Figure 1H). There were no apparent differences in filipin staining intensity between cells incubated with or without OA, presumably due to the lower

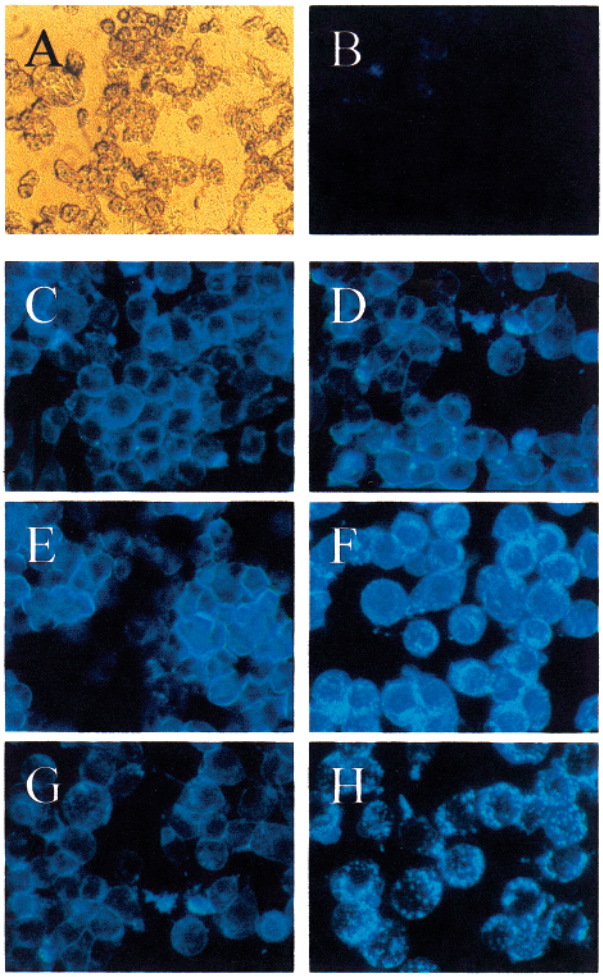

FIGURE 1: Changes in free cholesterol content in the plasma membrane of J774 cells by Dup128 ACAT inhibitor and U18666A as determined by filipin staining. Cells were incubated with 100 μg/mL LDL in the presence or absence of Dup128 ACAT inhibitor (10 μM) or U18666A (2.5 μM) for 30 min to 18 h. Cells were stained with filipin and visualized using a florescent microscope (400× magnification) to determine changes in cholesterol content in the plasma membrane as described in Experimental Procedures. Cells incubated with LDL for 30 min (light microscope image) (A), filipin staining (B), LDL incubation for 4 h (C) and 18 h (D), LDL + Dup128 for 4 h (E) and 18 h (F), and LDL + U18666A for 4 h (G) and 18 h (H).

sensitivity of filipin assays to changes in cell membrane cholesterol in the presence of FFA (data not shown).

Thus, we quantitatively assessed the LDL-derived cholesterol content in the plasma membrane utilizing cholesterol oxidase assays. [^{3}H]CE-labeled LDL (100 μg/mL) was incubated with cells for 4 h in the presence or absence of OA, Dup128 ACAT inhibitor, or U18666A, and changes in LDL-derived cholesterol in the plasma membrane were determined (Figure 2). Incubation of LDL with OA caused a significant reduction (~25%) in plasma membrane FC in cells compared to control cells. In contrast, ACAT inhibition caused a 1.5-fold increase in cholesterol content in the plasma membrane. LDL-derived cholesterol in the plasma membrane was consistently lower (50% of control) in cells incubated with U18666A despite continuous LDL uptake, consistent with an effect of inhibiting cholesterol transport from lysosomes.

Effects of FFA and ACAT inhibition on membrane FC content were further assessed by direct measurement of FC

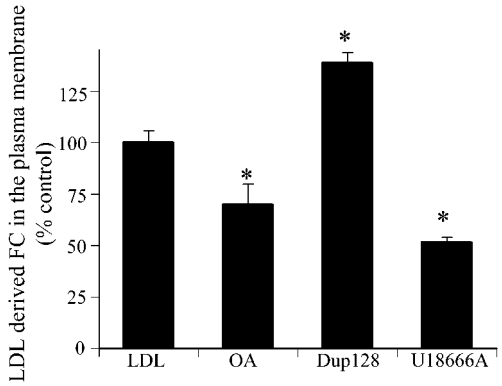

FIGURE 2: Changes in LDL-derived free cholesterol in the plasma membrane by OA, Dup128 ACAT inhibitor, and U18666A as determined by cholesterol oxidase. J774 cells were incubated with 100 μg/mL [^{3}H]cholesteryl ester labeled LDL in the presence or absence of 0.3 mM OA, 10 μM Dup128, or 2.5 μM U18666A for 4 h at 37 °C. At the end of the experiments, cell surface cholesterol was determined by cholesterol oxidase as described in Experimental Procedures. Percent changes in LDL-derived cholesterol content in the plasma membrane are compared to control cells incubated with LDL alone set to 100%. Results are the mean of triplicate determinations ± SD of a representative experiment. An asterisk indicates significant differences in cholestenone content in the plasma membrane of control cells ($p < 0.01$).

Table 1: Effect of Oleic Acid and Dup128 ACAT Inhibitor on FC Content in the Plasma Membrane[a]

	FC (μg/100 μg of PL)	FC/PL ratio (mol/mol)
LDL	26.9 ± 1.10	0.52 ± 0.02
LDL + FFA	20.5 ± 0.84[b]	0.40 ± 0.02[b]
LDL + Dup128	44.0 ± 1.58[b]	0.87 ± 0.03[b]

[a] J774 cells were incubated with 100 μg/mL LDL in the presence or absence of 0.3 mM oleic acid or ACAT inhibitor Dup128 (10 μM) for 8 h at 37 °C, and the plasma membrane was isolated as described in Experimental Procedures. Free cholesterol (FC), phospholipid (PL), and protein content were determined by enzymatic assays and expressed as the mean of quadruple determinations ± SD of a representative experiment. [b]Significant difference from LDL alone ($p < 0.05$).

and PL in isolated plasma membrane from J774 cells. Compared to cells incubated with LDL alone, addition of OA caused a 20% reduction in FC content and a corresponding 20% decrease in FC to PL molar ratios in isolated plasma membrane after 8 h (Table 1). Similar reductions in FC content with FFA were also observed at 4 h (data not shown). In contrast, ACAT inhibition led to substantial increases in FC content in the plasma membrane, in keeping with results from the filipin and cholesterol oxidase studies. Incubation of FFA alone or with LDL did not increase cell phospholipid content, indicating that changes in FC to PL ratios induced by FFA are due to modulation of FC in the plasma membrane. Collectively, these studies demonstrated that incubation of fatty acids reduced cholesterol in the plasma membrane. In contrast, ACAT inhibition increased plasma membrane FC.

Selective Uptake from LDL Is Dose and Time Dependent and Stimulated by Oleic Acid. To determine whether selective uptake from LDL was stimulated by OA, J774 murine macrophages were incubated with double-labeled LDL in the presence or absence of OA for up to 18 h, and cellular CE uptake was calculated on the basis of ^{125}I and ^{3}H measurements. LDL concentration was 100 μg/mL, a level

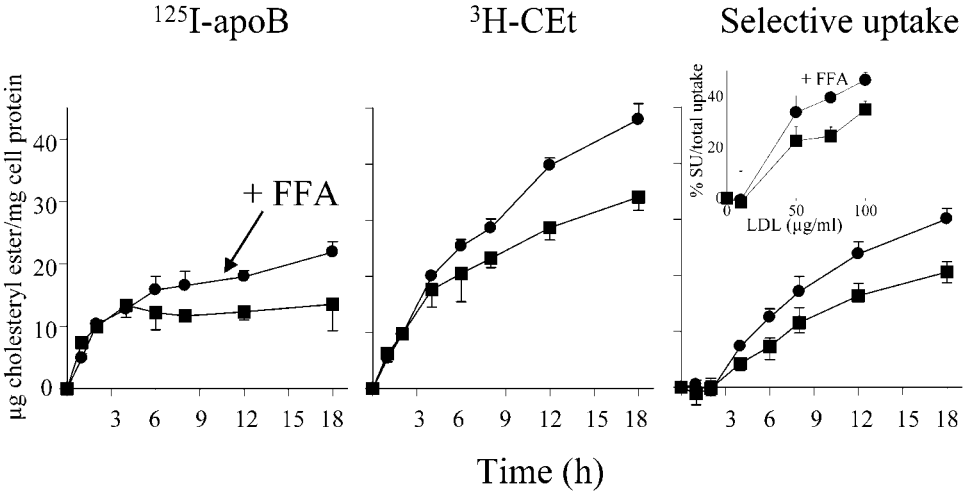

FIGURE 3: Effects of oleic acid on selective uptake from LDL in J774 macrophages. Cells were incubated with 100 μg/mL LDL, labeled with [^{3}H]CEt and ^{125}I as described in Experimental Procedures, in the presence (circles) or absence (squares) of 0.3 mM OA for 1−18 h at 37 °C. At the end of the experiments, LDL CE uptake was determined on the basis of uptake of ^{125}I-apo B (^{125}I-apoB) and [^{3}H]CEt as described in Experimental Procedures. Selective uptake is shown as the difference in [^{3}H]CEt and ^{125}I-apo B. Inset: Cells were incubated with varying concentrations of LDL for 8 h in the presence or absence of 0.3 mM OA. Results are shown as the mean of triplicate determinations ± SD of a representative experiment. After 4 h incubation, all differences in selective uptake between OA-treated and control cells are significant at $p < 0.01$.

Table 2: Effects of Different Fatty Acids and ACAT Inhibition on Selective Uptake from LDL in J774 Cells[a]

	selective uptake (μg of CE/mg of cell protein)	
FFA	LDL	LDL + Dup128
none	13.3 ± 0.4	10.4 ± 1.1
stearic acid (18:0)	12.4 ± 0.6	8.4 ± 0.2
oleic acid (18:1)	18.2 ± 2.4[b]	11.5 ± 2.2
EPA (20:5)	16.7 ± 1.2[b]	12.6 ± 1.0

[a] Cells were incubated with 100 μg/mL double-labeled LDL in the presence or absence of 0.3 mM stearic acid, oleic acid, and eicosapentaenoic acid (EPA) with or without the ACAT inhibitor Dup128 (10 μM) for 8 h at 37 °C. Results are expressed as the mean of triplicate determinations ± SD of a representative experiment. [b]Significant difference from LDL alone ($p < 0.05$).

which saturates cell uptake via the LDL receptor (*11*). Selective uptake was calculated from the differences between ^{125}I and ^{3}H uptakes (Figure 3). At early incubation times (1−2 h), calculated CE delivery from ^{125}I or ^{3}H was similar, suggesting that LDL and CE uptake was initially mediated primarily by receptor-mediated endocytosis. However, selective uptake was noted at 4 h, and at 18 h selective uptake accounted for nearly 50% of cell CE uptake. Selective uptake from LDL was also concentration dependent (inset). In the presence of 0.3 mM OA, selective uptake increased by an additional 30%. Taken together, these studies indicated that selective uptake from LDL in J774 macrophages was both time and dose dependent and showed that OA promoted selective uptake from LDL.

Fatty Acid Specificity in Stimulating LDL Selective Uptake. Previous studies showed that certain unsaturated FFA such as OA and EPA were more effective in stimulating ACAT activity in J774 cells compared to saturated fatty acids (*23*). Since increased ACAT activity by fatty acids would lead to reduced cholesterol in the plasma membrane, different fatty acids with varying effects on ACAT activity were tested for their abilities to stimulate selective uptake from LDL (Table 2). Dup128 ACAT inhibitor was used to prevent fatty acid induced cholesterol esterification. Stearic acid, a poor substrate for ACAT (*23*, *47*), failed to increase selective uptake compared to control cells. However, OA and EPA, which both efficiently enhance ACAT activity in J774 cells (*23*, *48*), led to a substantial increase in LDL selective uptake, and this effect was abolished by ACAT inhibition. Since stearic acid and OA are incorporated into cell membranes at very similar rates (*49*, *50*), this effect on stimulating selective uptake was not due to differences in plasma membrane uptakes between these fatty acids. Rather, increased selective uptake by OA and EPA was likely due to their ability to stimulate ACAT activity, an effect abolished by ACAT inhibition.

Effects of Increased Cholesterol in the Plasma Membrane on LDL Selective Uptake. FFA-stimulated selective uptake from LDL was inhibited by an ACAT inhibitor, suggesting that changes in cell cholesterol partitioning affected selective uptake from LDL. Thus, we next examined whether changes of cholesterol in the plasma membrane in the absence of added FFA would modulate selective uptake from LDL and whether this would be affected by ACAT inhibition. Selective uptake from LDL was compared in cells that were relatively cholesterol enriched as compared to cholesterol depleted by growing them in culture with fetal bovine serum (FBS) and cholesterol-poor lipoprotein-deficient serum (LPDS), respectively. Consistent with different effects of FBS or LPDS on modulating cell cholesterol, LPDS incubation for 24 h resulted in a significantly reduced FC content in isolated plasma membranes compared to cells incubated with FBS (19.9 vs 30.12 μg per 100 μg of phospholipid, with a reduction in FC/PL molar ratios of over 25%). When selective uptake from LDL was determined in FBS- vs LPDS-treated cells, cells grown in FBS showed less selective uptake (Figure 4). Selective uptake from LDL was much reduced by ACAT inhibition under both growing conditions. These studies indicated that increasing cholesterol mass in the plasma membrane decreased selective uptake from LDL.

Effect of β-Cyclodextrin and U18666A on LDL Selective Uptake. Since FFA and ACAT inhibition might have effects on other cell pathways, we directly tested the role of

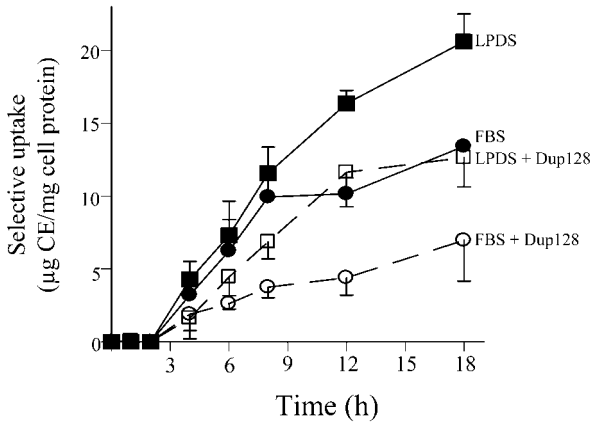

FIGURE 4: Effect of ACAT inhibition on selective cholesteryl ester uptake from LDL in cholesterol-enriched (FBS) and -depleted (LPDS) J774 macrophages. Cells were incubated in RPMI containing 10% LPDS or 10% FBS, conditions to deplete or enrich cellular cholesterol, respectively, for 24 h at 37 °C. On the day of the experiments, cells were incubated with 100 μg/mL double-labeled LDL with or without Dup128 ACAT inhibitor (10 μM) for the indicated time periods, and CE uptake from LDL was determined on the basis of ^{125}I-apo B and [^{3}H]CEt counts. The resulting selective uptake is shown as the mean of triplicate determinations ± SD of a representative experiment. After 9 h incubation, all differences in selective uptake in the presence or absence of Dup128 ACAT inhibitor are significant at $p < 0.05$.

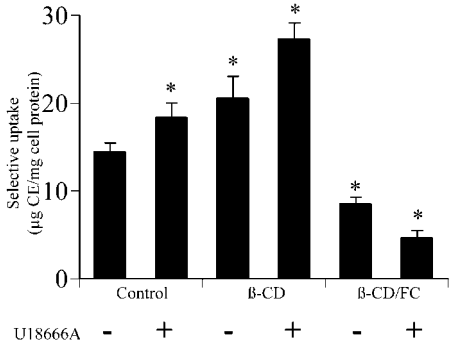

FIGURE 5: Modulation of plasma membrane free cholesterol with β-cyclodextrin and resulting effects on selective uptake from LDL. Cells were preincubated with 2.5 mM β-CD for 1 h at 37 °C to rapidly remove cholesterol from the plasma membrane. To inhibit replenishment of cholesterol to the plasma membrane and the movement of newly synthesized cholesterol from endoplasmic reticulum, U18666A was also incubated in some dishes. To saturate the plasma membrane with cholesterol, cholesterol- saturated β-CD (β-CD/FC) was incubated in the presence or absence of 2.5 mM U18666A to prevent redistribution of membrane cholesterol. After β-CD treatment, cells were incubated with 100 μg/mL double-labeled LDL for 8 h to assess selective uptake from LDL. Results are shown as the mean of triplicate determinations ± SD of a representative experiment. An asterisk indicates significant differences in LDL uptake from control cells ($p < 0.01$).

cholesterol in the plasma membrane on LDL selective uptake by using β-CD. We used β-CD to rapidly extract cholesterol from the plasma membrane or cholesterol-saturated β-CD to enrich cholesterol in the plasma membrane (*35*) (Figure 5). U18666A was also incubated with β-CD or cholesterol-saturated β-CD. Rationales for inclusion of U18666A in these studies were that, in the case of β-CD, the plasma membrane would remain cholesterol depleted since U18666A prevents transport of intracellular cholesterol to the plasma membrane. In contrast, when the plasma membrane is saturated with cholesterol by cholesterol-saturated β-CD, U18666A would also inhibit redistribution of plasma membrane cholesterol within cells, leaving the plasma membrane cholesterol enriched. Incubations of U18666A together with LDL led to a 20% increase in selective uptake from LDL compared to nontreated cells. This indicates blockage of LDL-derived cholesterol delivery to the plasma membrane by U18666A-promoted selective uptake. When cells were treated with β-CD, selective uptake from LDL increased by 30%, and the presence of U18666A caused an additional 20% increase in selective uptake. In contrast, when FC-saturated β-CD was used to saturate the plasma membrane with cholesterol, we observed a 50% reduction in selective uptake from LDL, reduced further when cell redistribution of the newly added plasma membrane cholesterol was inhibited by U18666A. These results are consistent with selective uptake from LDL being directly influenced by changes in the plasma membrane cholesterol content and not by other changes in cell cholesterol distribution that might be induced by ACAT inhibition or changes in intracellular cholesterol trafficking.

Effect of OA, ACAT Inhibition, and U18666A on LDL vs HDL Selective Uptake. To test whether selective uptake from LDL and HDL is similarly affected by cholesterol content in the plasma membrane, cells were incubated with double-labeled LDL or HDL in parallel studies in the presence or absence of OA, Dup128 ACAT inhibitor, or U18666A (Figure 6). Similar to results described above, selective uptake from LDL was increased by both OA and U18666A, while ACAT inhibition reduced selective uptake from LDL (Figure 6A). In contrast, HDL selective uptake was not affected by incubation with OA or ACAT inhibitor. U18666A consistently caused a small reduction in HDL selective uptake, contrasting the effect on LDL (Figure 6B). Differing effects of cholesterol content in the plasma membrane on HDL vs LDL selective uptake were further observed in studies using β-CD. In contrast to effects with LDL shown in Figure 4, there was little effect on HDL selective uptake after treatment with β-CD in the presence or absence of U18666A (Figure 6C). Cholesterol-saturated β-CD had a small effect on reducing HDL selective uptake, but effects were much less than that with LDL. Thus, changes in the plasma membrane cholesterol had little or no effects on HDL selective uptake, suggesting that different processes mediated LDL and HDL selective uptake.

Role of SR-BI on LDL and HDL Selective Uptake. HDL selective uptake is mediated by SR-BI (*13*, *14*). We next examined the role of SR-BI on HDL as compared to LDL selective uptake. Cells were incubated with LDL or HDL in the presence or absence of antimouse SR-BI antibodies for 8 h at 37 °C (Table 3). When LDL was incubated with antimouse SR-BI antibody, there was a reduction in both ^{3}H and ^{125}I uptake, consistent with previous data that LDL also binds to SR-BI (*21*, *51*). Nonetheless, this antibody caused only a small nonsignificant reduction in selective uptake from LDL, suggesting that most LDL selective uptake was mediated by non-SR-BI pathways in J774 cells. The major effect of antimouse SR-BI antibodies on LDL was on decreasing whole particle uptake but not selective uptake. Antimouse SR-BI antibodies had very different effects on HDL. While HDL ^{125}I uptake remained unchanged, HDL lipid uptake, as determined by ^{3}H uptake, was reduced by ~65%. As a result, selective uptake from HDL was markedly

Table 3: Effect of Antimouse SR-BI Antibodies on Selective Uptake from LDL and HDL in J774 Macrophages[a]

	control (μg of CE/mg of cell protein)			αSR-BI (μg of CE/mg of cell protein)		
	[^{125}I]apolipoprotein	[^{3}H]CEt	SU	[^{125}I]apolipoprotein	[^{3}H]CEt	SU
LDL	18.6 ± 2.3	33.2 ± 1.1	14.6 ± 1.0	13.8 ± 1.3[b]	25.6 ± 1.7[b]	11.9 ± 2.7
HDL	1.2 ± 0.2	6.7 ± 0.7	5.4 ± 0.5	1.1 ± 0.2	3.1 ± 0.6[b]	2.0 ± 0.4[b]

[a] Cells were incubated with 100 μg/mL double-labeled LDL or HDL in the presence or absence of irrelevant antimouse IgG (control) or antimouse SR-BI antibodies (1:20 dilution, 2 μg/mL) (αSR-BI) for 8 h at 37 °C. At the end of the experiments, CE uptake from LDL or HDL was determined as described in Experimental Procedures. Results are shown as the mean of triplicate determinations ± SD of a representative experiment. [b]Significant differences from control ($p < 0.01$).

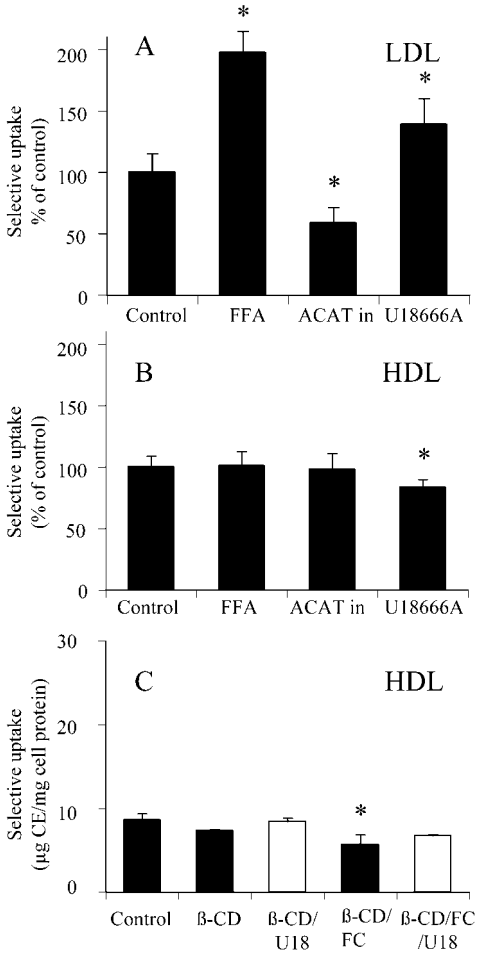

FIGURE 6: Selective uptake from LDL and HDL in J774 macrophages after treatment with oleic acid, Dup128, U18666A, and β-cyclodextrin. Cells were incubated for 8 h at 37 °C with 100 μg/mL double-labeled LDL (A) or HDL (B) in the presence or absence of 0.3 mM OA, 10 μM ACAT inhibitor, or 2.5 mM U18666A or with (C) HDL in the presence of β-CD. At the end of the experiments, selective uptake was determined as described in Experimental Procedures. Selective uptake is shown and expressed as percent changes over control where cells were incubated with only HDL or LDL. Actual amounts of selective uptake in control cells were 13.54 ± 1.75 μg/mg of cell protein and 8.24 ± 0.70 μg/mg of cell protein for LDL and HDL, respectively. Alternatively, cells were treated with β-CD or cholesterol-saturated β-CD with (open bars) or without (filled bars) U18666A as described in the legend of Figure 5 (C). The resulting selective uptake is shown as the mean of triplicate determinations ± SD of a representative experiment. An asterisk indicates significant differences in selective uptake from control cells ($p < 0.01$).

reduced by antimouse SR-BI antibody, in keeping with HDL selective uptake being primarily mediated by SR-BI.

To determine further whether selective uptake from LDL was mediated by cell surface proteins such as SR-BI, cells were incubated with or without a proteolytic enzyme, Pronase, followed by incubation with double-labeled LDL or HDL for 4 h. To inhibit new cellular protein synthesis, LDL and HDL were also incubated in the presence or absence of cyclohexamide (Figure 7). In cells incubated with LDL, there was no apparent difference in selective uptake with Pronase or Pronase with cyclohexamide (Pronase + cyclo). Although selective uptake from LDL was not altered by proteolytic digestion of cell surface proteins, whole particle uptake of LDL was reduced; e.g., with cells treated with Pronase + cyclohexamide LDL protein uptake was 2.2 μg/mg of cell protein compared to 6.0 μg/mg of cell protein in control cells. This was consistent with reduced LDL particle uptake via cell receptors such as LDL receptor. In contrast to LDL selective uptake, HDL selective uptake was markedly reduced in cells treated with Pronase and/or cyclohexamide. These studies strengthen our conclusion from previous experiments that SR-BI, and likely other cell surface proteins, are not essential for LDL selective uptake, but SR-BI is the predominant pathway for HDL selective uptake.

Contribution of Selective Uptake on Cholesterol Delivery to the Arterial Wall. To determine a potential role for LDL selective uptake in CE delivery to the arterial wall and whether increased FFA levels could modify this process, C57BL/6 mice were fed a normal chow or a high fat diet for 12 weeks. In experiments performed in parallel, mice fed a high fat diet demonstrated elevated plasma FFA levels (0.89 ± 0.20 vs 0.38 ± 0.11 mmol/L, respectively, $n = 8$ in each group) but had no atherosclerotic lesions as determined by Oil Red O staining (data not shown). Mice were injected with double-labeled LDL, and isolated aortas were measured for [^{3}H]CEt and ^{125}I-TC radioactivity to determine LDL-CE and whole particle uptakes, respectively (Table 4). When arterial LDL was assessed in these mice, there was a slight reduction in radiolabeled LDL particle uptake in mice fed a high fat diet (^{125}I-TC-apoB). This was likely due to dilution of labeled LDL with endogenous mouse lipoproteins, as total plasma cholesterol was almost 2-fold higher in mice fed a high fat diet compared to control (130.5 ± 21.1 vs 69.3 ± 11.5 mg/dL, respectively). There was no difference in plasma triglycerides in control vs mice fed a high fat diet (60.5 ± 15.0 vs 73.2 ± 22.0 mg/dL). Despite reduced total LDL particle uptake, relative ratios of selective uptake to total LDL uptake (SU/total) were increased substantially by 4-fold (20% as compared to 5% in control and normal chow-fed mice). These experiments indicated that cholesterol delivery via LDL selective uptake may be physiologically important and is enhanced by a high fat diet.

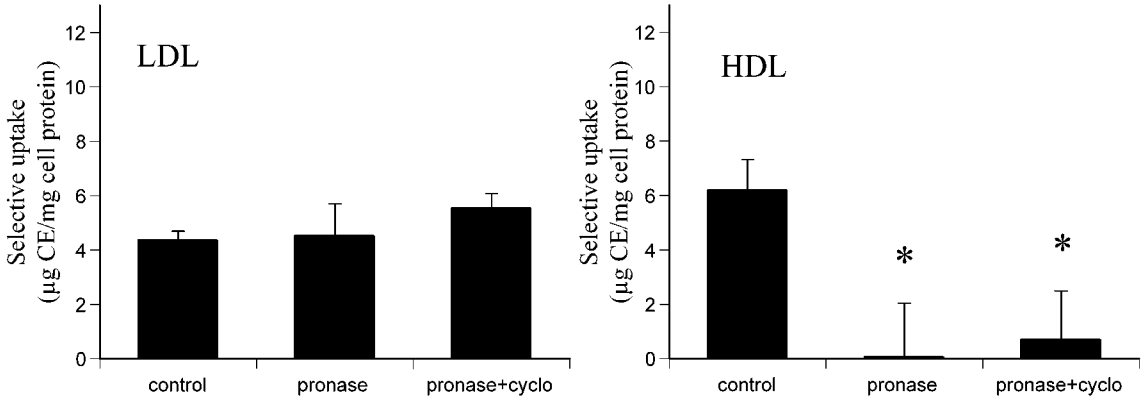

FIGURE 7: Effect of Pronase and cyclohexamide on selective uptake from LDL and HDL in J774 macrophages. Cells were incubated with serum-free RPMI containing Pronase (3 μg/mL) for 30 min at 37 °C. Treated and untreated control cells were incubated with 100 μg/mL double-labeled LDL or HDL for 4 h at 37 °C. To inhibit new cell protein synthesis, cyclohexamide (0.89 mM) was also added to the incubation medium. At the end of the experiments, CE uptake from LDL or HDL was determined as described in Experimental Procedures. Results are expressed as the amount of selective uptake from triplicate dishes ± SD of a representative experiment. An asterisk indicates significant differences from control (no treatment) ($p < 0.01$).

Table 4: Effects of a High Fat Diet on Aortic LDL Uptake and Selective Uptake in C57BL/6 Mice[a]

	CE uptake (ng/mg of artery)			% total CE uptake from LDL SU
	^{125}I-TC-apoB	[^{3}H]CEt	SU	
normal chow ($n = 4$)	46.2 ± 8.1	49.1 ± 9.9	2.7 ± 2.4	5.6 ± 3.1
high fat diet ($n = 6$)	34.1 ± 5.6	43.8 ± 6.8	9.3 ± 2.7	21.6 ± 6.2
	$p = 0.06$	$p = 0.4$	$p = 0.007$	$p = 0.002$

[a] Uptake of [^{3}H]cholesteryl oleoyl ether and [^{125}I]tyramine cellobiose tracers for human LDL was determined in the arterial wall of C57BL/6 mice that were fed with a normal chow or high fat coconut oil diet for 12 weeks. 24 h after LDL injection (200 μg of protein), the vascular system was thoroughly perfused and uptake of ^{3}H, ^{125}I-TC, and selective uptake was determined as described in Experimental Procedures. Results are expressed as calculated CE uptake per milligram of aorta ± SEM and as the percent contribution of LDL cholesteryl ester delivery to the arterial wall via selective uptake. *p* values are shown for differences in LDL uptake between mice fed with a normal chow or a high fat diet.

DISCUSSION

In the current studies, effects of FFA on selective CE uptake from LDL were examined in mouse macrophages, and potential mechanisms were elucidated. Our results showed that selective uptake from LDL was both time and LDL concentration dependent, and OA enhanced selective uptake from LDL. Since OA reduced cholesterol content in the plasma membrane, we speculated that the effect of fatty acids on increased selective uptake could be secondary to changes in the plasma membrane composition. Consistent with this hypothesis, ACAT inhibition abolished the effects of OA on enhancing selective uptake from LDL. Also, changes in cholesterol content in the plasma membrane in the absence of FFA also markedly affected selective uptake from LDL; increasing FC in the plasma membrane by ACAT inhibition reduced LDL selective uptake. Further evidence for plasma membrane cholesterol modulating selective uptake from LDL was obtained by studies with β-CD treatment, which depleted cholesterol from the plasma membrane and increased LDL selective uptake; saturation of the plasma membrane with cholesterol via cholesterol-saturated β-CD reduced selective uptake from LDL. These studies indicate that selective uptake from LDL is influenced by the cholesterol content in the plasma membrane and that increased selective uptake with OA is associated with alterations of plasma membrane cholesterol. Moreover, in C57BL/6 mice fed a high fat diet, a condition that leads to elevated plasma FFA levels, there was a marked increase in the contribution of selective uptake in total LDL-CE delivery compared to control mice, suggesting FFA-enhanced selective uptake could also occur in vivo and may enhance cholesterol delivery to the arterial wall.

In contrast to LDL selective uptake, neither FFA nor ACAT inhibition changed HDL selective uptake. Moreover, cholesterol depletion or enrichment by β-CD did not significantly alter HDL selective uptake, suggesting that HDL selective uptake is mediated by mechanisms different from for LDL.

The plasma membrane, as an initial CE acceptor, has been suggested to play an important role in selective uptake (*22*). This concept is supported by the ability of the plasma membrane to accommodate CE from plasma lipoproteins, as demonstrated by Morrison et al. (*52*). Their studies showed that the isolated plasma membrane as well as protein-free model PL bilayers was capable of accepting CE from lipoproteins. Since addition of cholesterol to the plasma membrane under normal condition causes reduced membrane fluidity, or a "stiff" membrane (*53*), such events could decrease the capacity of the plasma membrane as a CE reservoir. In contrast, depletion of cholesterol from the plasma membrane would increase the reservoir capacity and, hence, increase selective uptake. In keeping with this hypothesis, increased FC relative to PL reduced solubility of CE within model membranes (*27*, *28*). In cells treated with OA, we observed a 20% reduction in plasma membrane cholesterol content, with a concomitant reduction in FC to PL ratios. These changes are more than sufficient to double the capacity of lipid bilayers to accommodate cholesteryl esters (*27*). It is not known which areas of the plasma membrane contribute to non-SR-BI-mediated selective uptake. It is possible that CE transfer is facilitated in lipid-rich regions such as membrane rafts (*54*, *55*) compared to lipid-poor regions of the plasma membrane.

OA leads to reduced cholesterol in the plasma membrane because of enhanced intracellular cholesterol esterification. OA might also directly influence FC transport from the relatively cholesterol-rich plasma membrane. In our recent studies using NMR spectroscopy, cholesterol transfer from phosphotidylcholine model membranes was markedly enhanced by OA and other polyunsaturated fatty acids because of increased transfer rates of FC from cholesterol-rich to cholesterol-poor membranes (*56*). Thus, FFA may act not only to stimulate ACAT activity but also to facilitate FC removal from the plasma membrane, allowing accommodation of more CE. Although OA and other FFA can also stimulate PL synthesis, we did not observe any significant changes in cell PL content within the 8 h experimental periods used for most of our experiments. Nevertheless, it is possible that longer incubations with FFA (e.g., 18 h) could also enhance selective uptake by increasing PL synthesis and hence PL to cholesterol ratios in the plasma membrane.

Different effects of FFA and cholesterol on LDL and HDL selective uptake indicated distinct selective uptake pathways. Possible explanations include (a) selective uptake from both LDL and HDL was mediated by SR-BI, but subsequent processing (i.e., hydrolysis, distribution, etc.) was different, and/or (b) selective uptake from these lipoproteins is mediated by different pathways; SR-BI mediates HDL selective uptake, while most LDL selective uptake is mediated by other pathways. We favor the latter possibility since antimouse SR-BI antibodies inhibited selective uptake from HDL but had little effect on LDL selective uptake in J774 cells.

Another major difference between LDL vs HDL selective uptake was the effect of Pronase ± cyclohexamide treatment. Removal of most cell surface proteins by Pronase decreased LDL whole particle uptake but not selective uptake from LDL. In contrast, selective uptake from HDL was essentially abolished after Pronase treatment, in keeping with critical roles of SR-BI binding for most selective uptake from HDL but not LDL. Although a role of cell surface proteins in mediating LDL selective uptake cannot be completely excluded from these studies, other processes such as cell surface lipid−LDL lipid interaction may be important in determining LDL selective uptake.

Nevertheless, a small degree of inhibition of selective uptake from LDL in J774 cells by antimouse SR-BI antibodies suggests that at least two pathways for LDL selective uptake are available: SR-BI and a non-SR-BI pathway that is influenced by FC content in the plasma membrane. In our studies using macrophages, SR-BI contributed little to selective uptake from LDL. However, Swarnakar and colleagues demonstrated that SR-BI-transfected Cos cells had significantly increased selective uptake from LDL (*19*). We have previously hypothesized (*21*) that differences in how cells internalize and distribute cholesterol may relate to a varying role of SR-BI in LDL selective uptake. J774 cells, and the CHO cells used in previous studies (*21*), internalized and degraded LDL particles, and the majority of CE was internalized by endocytic pathways but only at low LDL concentrations (<25 μg/mL). At the LDL concentrations used herein (50−100 μg/mL), concentrations commonly found in interstitial fluid (*57*), substantial uptake of LDL-CE can occur via selective uptake after saturation of the LDL receptor (*17*, *21*). Although some selective uptake may occur via SR-BI in these cells, CE delivery via SR-BI may be masked by relatively large amounts of CE entering via endocytic pathways and non-SR-BI selective uptake pathways. In cells with high uptake of LDL-CE via LDL receptors (e.g., J774 cells), the selective uptake pathway may only become dominant after saturation of CE delivery via the LDL receptors, in keeping with our data showing increasing contribution of LDL selective uptake after 2−3 h incubation (Figures 3 and 4). In Cos cells, which have about 1/150 of LDL particle uptake compared to J774 cells, expression of SR-BI may also be an important mechanism for LDL CE delivery (*19*).

Our findings indicate that changes in the composition of the plasma membrane will have marked effects on selective uptake from LDL. We suggest that similar changes of the plasma membrane could occur in vivo. For example, during hydrolysis of triglyceride-rich particles at the arterial wall by lipoprotein lipase, higher local concentrations of released FFA could occur in microdomains and potentially lead to higher LDL selective uptake. Similarly, elevated plasma FFA level in diabetic states (*58*, *59*) with high FFA availability to tissues might enhance selective uptake in the artery, a process that could contribute to accelerated atherosclerosis in this disease (*60*). Consistent with this hypothesis, our studies in vivo demonstrated that high fat feedings associated with increased contribution of selective uptake to cholesterol delivery to the arterial wall. In other ongoing studies, we are finding that high fat diets can increase the contribution of LDL selective uptake to >50% of total CE delivery to the arterial wall in mice genetically modified for accelerated atherosclerosis (e.g., apolipoprotein E null mice) (unpublished observations). Although delineation of the contribution of selective uptake from LDL to the atherosclerotic process in humans and in animal models requires additional studies, we suggest that changes in the plasma membrane composition by FFA and cholesterol have important regulatory roles on this process not only in vitro but likely in vivo as well.

ACKNOWLEDGMENT

The authors gratefully acknowledge Inge Hansen and Fannie Kayserman for excellent technical assistance and Dr. Jan Breslow, Rockefeller University, for providing the mice used in experiments described in this paper.

REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1986) *Science 232*, 34−47.
2. Spady, D. K., Turley, S. D., and Dietschy, J. M. (1985) *J. Clin. Invest. 76*, 1113−1122.
3. Spady, D. K., Huettinger, M., Bilheimer, D. W., and Dietschy, J. M. (1987) *J. Lipid Res. 28*, 32−41.
4. Hiltunen, T. P., and Yla-Herttuala, S. (1998) *Atherosclerosis 137* (Suppl.), S81−S88.
5. Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N., Kataoka, M., Jishage, K., Ueda, O., Sakaguchi, H., Higashi, T., Suzuki, T., Takashima, Y., Kawabe, Y., Cynshi, O., Wada, Y., Honda, M., Kurihara, H., Aburatani, H., Doi, T., Matsumoto, A., Azuma, S., Noda, T., Toyoda, Y., Itakura, H., Yazaki, Y., and Kodama, T. (1997) *Nature 386*, 292−296.
6. Brown, M. S., and Goldstein, J. L. (1983) *Annu. Rev. Biochem. 52*, 223−261.
7. Han, J., Hajjar, D. P., Febbraio, M., and Nicholson, A. C. (1997) *J. Biol. Chem. 272*, 21654−21659.

8. Febbraio, M., Podrez, E. A., Smith, J. D., Hajjar, D. P., Hazen, S. L., Hoff, H. F., Sharma, K., and Silverstein, R. L. (2000) *J. Clin. Invest. 105*, 1049−1056.
9. Rumsey, S. C., Obunike, J. C., Arad, Y., Deckelbaum, R. J., and Goldberg, I. J. (1992) *J. Clin. Invest. 90*, 1504−1512.
10. Seo, T., and St. Clair, R. W. (1997) *J. Lipid Res. 38*, 765−779.
11. Galeano, N. F., Al Haideri, M., Keyserman, F., Rumsey, S. C., and Deckelbaum, R. J. (1998) *J. Lipid Res. 39*, 1263−1273.
12. Chait, A., and Wight, T. N. (2000) *Curr. Opin. Lipidol. 11*, 457−463.
13. Krieger, M. (1999) *Annu. Rev. Biochem. 68*, 523−558.
14. Williams, D. L., Connelly, M. A., Temel, R. E., Swarnakar, S., Phillips, M. C., Llera-Moya, M., and Rothblat, G. H. (1999) *Curr. Opin. Lipidol. 10*, 329−339.
15. Merkel, M., Kako, Y., Radner, H., Cho, I. S., Ramasamy, R., Brunzell, J. D., Goldberg, I. J., and Breslow, J. L. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 13841−13846.
16. Brissette, L., and Falstrault, L. (1994) *Biochim. Biophys. Acta 1213*, 5−13.
17. Rinninger, F., Brundert, M., Jäkle, S., Kaiser, T., and Greten, H. (1995) *Biochim. Biophys. Acta 1255*, 141−153.
18. Stangl, H., Cao, G., Wyne, K. L., and Hobbs, H. H. (1998) *J. Biol. Chem. 273*, 31002−31008.
19. Swarnakar, S., Temel, R. E., Connelly, M. A., Azhar, S., and Williams, D. L. (1999) *J. Biol. Chem. 274*, 29733−29739.
20. Green, S. R., and Pittman, R. C. (1991) *J. Lipid Res. 32*, 667−678.
21. Seo, T., Al Haideri, M., Treskova, E., Worgall, T. S., Kako, Y., Goldberg, I. J., and Deckelbaum, R. J. (2000) *J. Biol. Chem. 275*, 30355−30362.
22. Reaven, E., Tsai, L., and Azhar, S. (1996) *J. Biol. Chem. 271*, 16208−16217.
23. Rumsey, S. C., Galeano, N. F., Lipschitz, B., and Deckelbaum, R. J. (1995) *J. Biol. Chem. 270*, 10008−10016.
24. Chester, D. W., Tourtellotte, M. E., Melchior, D. L., and Romano, A. H. (1986) *Biochim. Biophys. Acta 860*, 383−398.
25. Kuo, P., Weinfeld, M., and Loscalzo, J. (1990) *Biochemistry 29*, 6626−6632.
26. Dusserre, E., Pulcini, T., Bourdillon, M. C., Ciavatti, M., and Berthezene, F. (1995) *Lipids 30*, 35−41.
27. Spooner, P. J., Hamilton, J. A., Gantz, D. L., and Small, D. M. (1986) *Biochim. Biophys. Acta 860*, 345−353.
28. Small, D. M., and Shipley, G. G. (1974) *Science 185*, 222−229.
29. Winegar, D. A., Salisbury, J. A., Sundseth, S. S., and Hawke, R. L. (1996) *J. Lipid Res. 37*, 179−191.
30. Temel, R. E., Trigatti, B., DeMattos, R. B., Azhar, S., Krieger, M., and Williams, D. L. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 13600−13605.
31. Higley, C. A., Wilde, R. G., Maduskuie, T. P., Johnson, A. L., Pennev, P., Billheimer, J. T., Robinson, C. S., Gillies, P. J., and Wexler, R. R. (1994) *J. Med. Chem. 37*, 3511−3522.
32. Tabas, I., Weiland, D. A., and Tall, A. R. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 416−420.
33. Bihain, B. E., Deckelbaum, R. J., Yen, F. T., Gleeson, A. M., Carpentier, Y. A., and Witte, L. D. (1989) *J. Biol. Chem. 264*, 17316−17321.
34. Rumsey, S. C., Stucchi, A. F., Nicolosi, R. J., Ginsberg, H. N., Ramakrishnan, R., and Deckelbaum, R. J. (1994) *J. Lipid Res. 35*, 1592−1598.
35. Christian, A. E., Haynes, M. P., Phillips, M. C., and Rothblat, G. H. (1997) *J. Lipid Res. 38*, 2264−2272.
36. Liscum, L., and Faust, J. R. (1989) *J. Biol. Chem. 264*, 11796−11806.
37. Liscum, L., and Collins, G. J. (1991) *J. Biol. Chem. 266*, 16599−16606.
38. Underwood, K. W., Andemariam, B., McWilliams, G. L., and Liscum, L. (1996) *J. Lipid Res. 37*, 1556−1568.
39. Underwood, K. W., Jacobs, N. L., Howley, A., and Liscum, L. (1998) *J. Biol. Chem. 273*, 4266−4274.
40. Mukherjee, S., Zha, X., Tabas, I., and Maxfield, F. R. (1998) *Biophys. J. 75*, 1915−1925.
41. Porn, M. I., Ares, M. P., and Slotte, J. P. (1993) *J. Lipid Res. 34*, 1385−1392.
42. Gotlib, L. J., and Searls, D. B. (1980) *Biochim. Biophys. Acta 602*, 207−212.
43. DeGrella, R. F., and Simoni, R. D. (1982) *J. Biol. Chem. 257*, 14256−14262.
44. Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466−468.
45. Knecht, T. P., and Pittman, R. C. (1989) *Biochim. Biophys. Acta 1002*, 365−375.
46. Kellner-Weibel, G., Geng, Y. J., and Rothblat, G. H. (1999) *Atherosclerosis 146*, 309−319.
47. Goodman, D. S., Deykin, D., and Shiratori, T. (1964) *J. Biol. Chem. 239*, 1335−1344.
48. Seo, T., Oelkers, P. M., Giattina, M. R., Worgall, T. S., Sturley, S. L., and Deckelbaum, R. J. (2001) *Biochemistry 40*, 4756−4762.
49. Kamp, F., Hamilton, J. A., Kamp, F., Westerhoff, H. V., and Hamilton, J. A. (1993) *Biochemistry 32*, 11074−11086.
50. Hamilton, J. A. (1998) *J. Lipid Res. 39*, 467−481.
51. Acton, S. L., Scherer, P. E., Lodish, H. F., and Krieger, M. (1994) *J. Biol. Chem. 269*, 21003−21009.
52. Morrison, J. R., Silvestre, M. J., and Pittman, R. C. (1994) *J. Biol. Chem. 269*, 13911−13918.
53. Gleason, M. M., Medow, M. S., and Tulenko, T. N. (1991) *Circ. Res. 69*, 216−227.
54. Simons, K., and Ikonen, E. (2000) *Science 290*, 1721−1726.
55. Simons, K., and Ikonen, E. (1997) *Nature 387*, 569−572.
56. Johnson, R. A., Worgall, T. S., Peng, S., Hamilton, J. A., and Deckelbaum, R. J. (2000) *FASEB J.*, A229.
57. Reichl, D., Simons, L. A., Myant, N. B., Pflug, J. J., and Mills, G. L. (1973) *Clin. Sci. Mol. Med. 45*, 313−329.
58. Golay, A., Swislocki, A. L., Chen, Y. D., and Reaven, G. M. (1987) *Metabolism 36*, 692−696.
59. Groop, L. C., Saloranta, C., Shank, M., Bonadonna, R. C., Ferrannini, E., and DeFronzo, R. A. (1991) *J. Clin. Endocrinol. Metab 72*, 96−107.
60. Carlsson, M., Wessman, Y., Almgren, P., and Groop, L. (2000) *Arterioscler., Thromb., Vasc. Biol. 20*, 1588−1594.

BI011949G