ELSEVIER

Journal of Nutritional Biochemistry 13 (2002) 330–336

THE JOURNAL OF Nutritional Biochemistry

Oleic, linoleic and linolenic acids enhance receptor-mediated uptake of low density lipoproteins in Hep-G2 cells

B.H. Simon Cho[a,*], Ryowon Choue Dokko[a], Byung Hong Chung[b]

[a]*Division of Nutritional Sciences, University of Illinois, Harlan E. Moore Heart Research Foundation, 503 South Sixth Street, Champaign, IL 61820, USA*

[b]*Atherosclerosis Unit, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 27 June 2001; received in revised form 21 November 2001; accepted 11 January 2002

Abstract

In the present study, the binding, internalization and degradation of low-density lipoprotein (LDL) was investigated in Hep-G2 cells treated with 18:0, 18:1, 18:2 and 18:3. In non-treated control cells, the surface binding (heparin-releasable) of ^{125}I-LDL progressed in a saturable manner reaching equilibrium within 2 h, amounting 24.0 ± 1.1, 29.5 ± 1.3 and 31.4 ± 2.8 (ng/mg cell protein) at 1, 2 and 4 h, respectively. The cells rapidly internalized ^{125}I-LDL reaching a plateau at 2 h (72.4 ± 6.3/1 h, 96.7 ± 4.3/2 h and 100.8 ± 4.6 ng/mg protein/4 h, respectively). The degradation of internalized LDL progressed slowly during the first hour of incubation reflecting the time required to an uptake and delivery of LDL to the cellular lysosomes. The levels of degraded LDL discharged into the medium then increased rapidly in a linear manner after the initial lag period, amounting 16.8 ± 1.2, 51.8 ± 7.0 and 118.2 ± 5.7 ng/mg protein at 1, 2 and 4 h, respectively. The treatment of cells with of 1.0 mM of fatty acids for 4 h resulted in a significant increase in the surface binding of ^{125}I-LDL compared to the control (34.9 ± 3.0), but it was significantly lower in cells exposed to 18:0 (48.2 ± 2.0) than to 18:1 (56.8 ± 5.1), 18:2 (56.0 ± 3.5) and 18:3 (57.8 ± 6.0 ng/mg protein/4 h) ($P < 0.05$). The levels of degraded LDL in cells remained nearly the same regardless of fatty acid treatments, but degraded LDL levels in the medium were much higher in cells exposed to 18:1 (167.6 ± 10.1), 18:2 (159.8 ± 7.7) and 18:3 (165.1 ± 14.7) than to 18:0 (142.1 ± 8.4) and the control (121.2 ± 3.4 ng/mg protein/4 h) ($P < 0.05$). The present finding that 18:1 is equally effective in enhancing the receptor-mediated LDL uptake and its degradation as those of 18:2 and 18:3 suggests that the major action of 18:1 in lowering LDL-cholesterol levels also involves an increased clearance of LDL via hepatic LDL-receptors.

Keywords: Fatty acids; Hep-G2 cells; Low-density lipoprotein; LDL receptor; LDL uptake

1. Introduction

In humans, plasma low-density lipoprotein (LDL) is the major transport vehicle for cholesterol and its elevation is regarded as one of the principal risk factors for the development of atherosclerotic vascular disease [1,2]. Hepatic LDL receptor activity comprises an important aspect of LDL and cholesterol metabolism [3,4]. It has been known that the major regulator of LDL-receptor synthesis is the amount of cholesterol within a cell [5]. Intracellular cholesterol inhibits the HMG-Co A reductase activity and down-regulates the LDL-receptor synthesis in the liver cells [6,7]. The LDL uptake depends upon the interaction between the hepatocyte membrane and LDL, and changes in membrane fluidity alter LDL binding to LDL receptors [8,9].

Plasma cholesterol concentrations are strongly influenced by the quantity and composition of fat in the diet [10,11]. Dietary saturated fats have been suggested to amplify the action of intracellular cholesterol in suppressing the synthesis of LDL receptors thereby increasing the concentration of plasma LDL cholesterol [12]. The substitution of polyunsaturated fat for saturated fat in the diet results in a reduction of plasma LDL-cholesterol levels [13] and an increase in hepatic LDL receptor activity [14]. Several mechanisms have been proposed to account for the changes in plasma and lipoprotein cholesterol concentrations resulting from the intake of different dietary fats [11], however, it is still a pressing question in respect to the physiological action of individual fatty acids in a complex equilibrium between the rate of lipoprotein production and its turnover.

* Corresponding author. Tel.: +1-217-256-8171; fax: +1-217-256-8171.

E-mail address: b-cho@staff.uiuc.edu (B.H.S. Cho).

0955-2863/02/$ – see front matter
PII: S0955-2863(02)00174-2

In our previous studies, the cellular uptake of 18:0, 18:1, 18:2 or 18:3 was found to be almost the same and it has provided an useful in vitro model to compare the metabolic activities of C-18 fatty acids [15]. Although dietary fats have shown to affect the LDL receptor activity [12–14], the independent effect of individual fatty acids is rather difficult to obtain primarily because these dietary fats consist of various saturated and unsaturated fatty acids. Since few studies have examined the effects of individual fatty acids on the receptor-mediated LDL uptake and its catabolism, the present study was undertaken to investigate the effects of 18:0, 18:1, 18:2 or 18:3, which differ in the degree of unsaturation, on the receptor-mediated uptake, internalization and degradation of LDL in Hep-G2 cells. The human hepatoma-derived cell line Hep-G2 was chosen in the present studies because these cells have been reported to retain many normal hepatic metabolic functions, lipoprotein synthesis, and cholesterol metabolism [16].

2. Materials and methods

2.1. Materials

The human hepatoma cell line Hep-G2 was obtained from American Type Culture Collection (ATCC, Rockville, MD). ^{125}I (carrier-free form, 10 mCi in 0.1 ml of 0.1 N NaOH) was purchased from New England Nuclear (Boston, MA). Bovine serum albumin (BSA) (essentially fatty acid-free), Dulbecco's Modified Eagle's Medium (DMEM), penicillin/streptomycin, fetal bovine serum (FBS), and thrombin were purchased from Sigma Chemical Co. (St. Louis, MO). Stearic acid (18:0), oleic acid (18:1ω9), linoleic acid (18:2ω6) and linolenic acid (18:3ω3), sodium salts (99% + pure), were purchased from Nu Check Prep (Elysian, MN). All other chemicals and solvents were of analytical grade.

2.2. Cell culture

Hep-G2 cells were grown in DMEM supplemented with 10% (v/v) FBS, penicillin (100 units/ml) and streptomycin (100 (g/ml). Stock cultures were maintained in T-25 flasks at 37°C under a humidified atmosphere of 95% air and 5% CO_2. The medium was changed every third day. When cells were grown to 70–80% confluency, fresh cultures were initiated. For experiments, cells from stock cultures were dissociated with a 0.25% trypsin–0.10% EDTA solution and an aliquot of cell suspension (6×10^5) was seeded into each well in 6-well culture plates. On day 4, when the cultures were subconfluent, cells were washed with Ca^{2+}-, Mg^{2+}- free Hank's balanced salt solution (HBSS) and incubated with 1.0 ml of DMEM containing 10% lipoprotein-deficient serum for 24 h prior to each experiment. All experiments were performed on day 5 while the cells were actively growing.

2.3. Preparation of fatty acid/albumin complexes

Stock solutions of fatty acid/albumin mixture were prepared under aseptic conditions as described previously [15]. Briefly, 20 μmoles of a fatty acid, sodium salt (18:0, 18:1, 18:2, or 18:3) were dissolved in 1.0 ml of sterilized water at temperatures between 25–75°C. Five μmoles (300 mg) of essentially fatty acid-free BSA were dissolved in 4.0 ml of DMEM culture medium (pH 7.4). The warm fatty acid solution was then added to BSA solution dropwise while stirring, and mixed with DMEM containing 10% lipoprotein-deficient serum in a final concentration of 1 mM. All fatty acid/albumin (4/1 molar ratio) solutions were optically clear and prepared freshly just prior to the experiment.

2.4. Preparation of low-density lipoprotein (LDL) and lipoprotein-deficient serum (LPDS)

Pooled human blood from normolipidemic subjects was obtained from the local blood bank. The lipoproteins were isolated by sequential preparative ultracentrifugation [17]. For LDL isolation, the lipoprotein fraction of density $<$1.019 g/ml was first removed, and the remaining fraction was then adjusted to a density of 1.063 g/ml and the LDL fraction was isolated by centrifugation at 135,000 $\times$ g for 36 h at 4°C in a Beckman model L-50 preparative ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). After dialysis, the isolated LDL was sterilized by passage through a Millipore filter (0.45 μm). The protein content of LDL was determined by the method of Lowry et al. [18]. The LDL suspension was adjusted to a protein concentration of 20 mg/ml with 0.15 M NaCl and stored at 4°C. The sterilized LDL was used within a week.

For LPDS preparation, the fraction remained after LDL isolation was adjusted to a final density of 1.21 g/ml and centrifuged at 135,000 $\times$ g for 36 h at 4°C. After removal of all lipoproteins of density $<$1.21 g/ml, the remaining lipoprotein-deficient fraction was dialyzed at 4°C against 0.15 M NaCl for 48–72 h and converted to LPDS by incubating it with thrombin at a final concentration of 10 NIH units/ml at 4°C for 24 h. The resulting clot was removed by centrifugation at 18,000 $\times$ g for 2 h at 4°C. LPDS was then sterilized by passage through a Millipore filter (0.45 μm). The total cholesterol content of LPDS was $<$5% of the level in whole plasma.

2.5. Radioiodination of LDL

The isolated LDL was labeled with ^{125}I (carrier-free form, 10 mCi in 0.1 ml of 0.1 N NaOH) based on Bilheimer's modification [19] of the iodine monochloride method as described by Goldstein et al. [20]. The iodinated LDL solution was purified by Sephadex G-25 column chromatography and dialyzed against 0.15 M NaCl until the final dialyzate had less than 3,000 cpm/ml. The labeled LDL solution was sterilized by passage through a Millipore filter

(0.45 μm). Analysis of ^{125}I-labeled LDL revealed that 98.2% of radioactivity was precipitated by 10% trichloroacetic acid and 3.2% was extracted with chloroform-methanol (2:1, v/v). The specific activity was 337 cpm/ng of LDL protein.

2.6. LDL-uptake assay

The LDL uptake, which is a sum of cellular membrane binding and internalization, was determined as described by Goldstein et al. [20]. The cells grown in monolayer in 6-well clustered dishes (4 days) were incubated with DMEM containing 10% lipoprotein-deficient serum (LPDS) for 24 h as described above. In experiments with fatty acids, cells were treated with serum-free DMEM containing 1.0 mM of a respective fatty acid/albumin complex or 0.25 mM of albumin alone as a control at 37°C for 4 h. The cells were then incubated with 10 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS with or without 500 μg/ml of unlabeled LDL at 37°C for 4 h. After incubation, the medium was removed for measurement of ^{125}I-labeled degradation products as described below, and cells were washed with ice-cold buffer containing 0.15 M NaCl, 50 mM Tris-HCl and 2 mg/ml of bovine serum albumin according to the method of Goldstein et al. [20]. The cells were then incubated with 2.0 ml of ice-cold buffer containing 50 mM NaCl, 10 mM HEPES and 10 mg/ml of heparin for 60 min at 4°C. The buffer solution was then collected and an aliquot was counted for determination of the amount of ^{125}I-LDL released from the cell surface (heparin-releasable ^{125}I-LDL). The cells were dissolved in 1.0 ml of 0.1 N NaOH by incubation at room temperature for 15 min. Aliquots of the cell suspension were counted to determine the amount of ^{125}I-LDL that had been internalized by the cells (heparin-resistant ^{125}I-LDL), and were analyzed for the protein content by the method of Lowry et al. [18].

2.7. Assay for degradation of ^{125}I-LDL

Aliquots of cells suspension and medium were added to glass tubes containing 0.5 ml of 50% trichloroacetic acid to precipitate undegraded ^{125}I-LDL. After incubation at 4°C for 30 min, the precipitated material was removed by centrifugation at 1,000 × g. An aliquot of the supernatant was mixed with 10 μl of 40% potassium iodide as carrier, followed by the addition of 40 μl of 30% hydrogen peroxide. The ^{125}I-iodine, which was converted from ^{125}I-iodide ions by hydrogen peroxide, was extracted with chloroform. Aliquots of aqueous layer were counted for radioactivity.

2.8. Assay for surface binding of ^{125}I-LDL

The surface binding of LDL was determined at 4°C because the binding of LDL continues, while receptor-mediated endocytosis ceases at this temperature [20]. The cells grown in monolayer in 6-well clustered dishes (4 days) were incubated with DMEM containing 10% lipoprotein-deficient serum (LPDS) for 24 h as described above. In experiments with fatty acids, cells were treated with serum-free DMEM containing 1.0 mM of 18:0, 18:1, 18:2 or 18:3 complexed with albumin or 0.25 mM of albumin alone as control for 4 h at 37°C. Prior to binding experiments, cells were initially placed in a refrigerator (4°C) for 30 min. The cells were then incubated with 10, 20 and 50 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS with and without 500 μg/ml of unlabeled LDL at 4°C for 2 h. After incubation, cells were washed with ice-cold buffer containing 0.15 M NaCl, 50 mM Tris-HCl and 2 mg/ml of bovine serum albumin according to the method of Goldstein et al. [20]. The cells were then incubated with 2.0 ml of ice-cold buffer containing 50 mM NaCl, 10 mM HEPES, and 10 mg/ml of heparin for 60 min at 4°C. The buffer solution was collected and an aliquot was counted for determination of the amount of ^{125}I-LDL released from the cell surface (heparin-releasable ^{125}I-LDL). The cells were dissolved in 1.0 ml of 0.1 N NaOH by incubation at room temperature for 15 min and aliquots of the cell suspension were used to determine the protein content and radioactivity. The receptor-mediated LDL uptake (specific binding) was calculated by subtracting the radioactivity bound in the presence of 500 μg/ml of unlabeled LDL (nonspecific binding) from that in its absence (total binding).

2.9. Statistical analysis

Values are presented as means ± SD. Data were analyzed by analysis of variance (ANOVA) and Duncan's multiple range test. Differences between variables were considered significant at $P < 0.05$.

3. Results

The surface binding, internalization and degradation of ^{125}I-LDL in non-treated control cells at 37°C is shown in Fig. 1. The surface binding (heparin-releasable) of ^{125}I-LDL progressed in a saturable manner reaching equilibrium within 2 h, amounting 24.0 ± 1.1, 29.6 ± 1.5 and 31.4 ± 2.8 (ng/mg of protein) at 1, 2 and 4 h, respectively. The surface bound ^{125}I-LDL accounted about 15.1% of the total ^{125}I-LDL uptake during the 4 h incubation period. The cells rapidly internalized ^{125}I-LDL (heparin-resistant) reaching a plateau at 2 h (72.4 ± 6.3/1 h, 96.7 ± 4.3/2 h and 100.8 ± 4.5 ng/mg protein/4 h, respectively). The degradation of internalized LDL progressed slowly during the first hour of incubation reflecting the time required to an uptake and delivery of LDL to the cellular lysosomes. The levels of degraded LDL discharged into the medium then increased rapidly in a linear manner after the initial lag period, amounting 16.7 ± 1.2, 51.7 ± 7.0 and 118.2 ± 5.7 ng/mg protein at 1, 2 and 4 h, respectively. On the other hand, in the presence of excess amount of non-labeled LDL (500

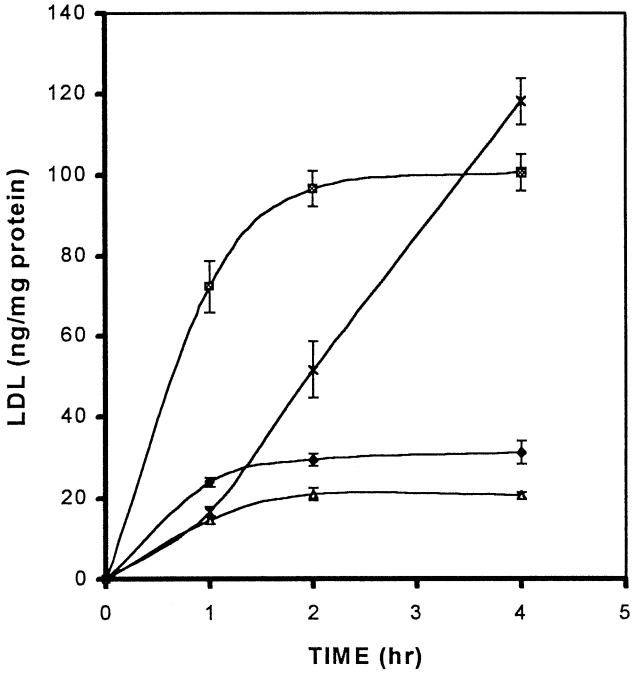

Fig. 1. Binding, internalization and degradation of ^{125}I-LDL in Hep-G2 cells. The cells were grown in DMEM containing 10% fetal bovine serum at 37°C for 4 days, and then cells were preincubated for 24 h in DMEM supplemented with 10% lipoprotein-deficient serum (LPDS). Afterward, cells were incubated with 10 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS at 37°C for 4 h. The symbols represent for surface binding (◆—◆), internalization (■—■), degradation products in cells (△—△), and medium (x—x). The values are mean ± S.D. (n = 6).

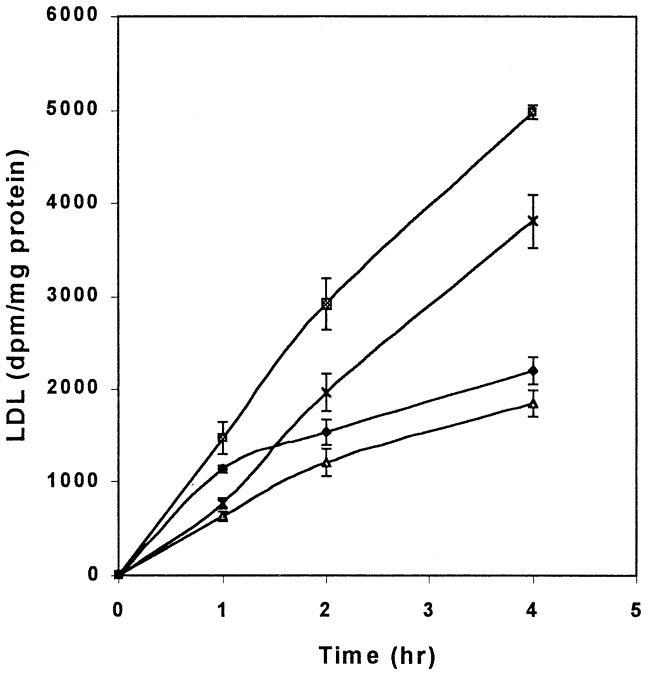

Fig. 2. Binding, internalization and degradation of ^{125}I-LDL in the presence of 500 μg/ml of unlabeled LDL in Hep-G2 cells. The cells were grown in DMEM containing 10% fetal bovine serum at 37°C for 4 days, and then cells were preincubated for 24 h in DMEM supplemented with 10% lipoprotein-deficient serum (LPDS). Afterward, cells were incubated with 10 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS with 500 μg/ml of unlabeled LDL at 37°C for 4 h. The symbols represent for surface binding (◆—◆), internalization (■—■), degradation products in cells (△—△), and medium (x—x). The values are mean ± S.D. (n = 6).

μg/ml), the surface binding, internalization, degradation of LDL progressed almost linearly during the 4 h treatment period (Fig. 2).

The total surface, specific and non-specific bindings of ^{125}I-LDL in cells incubated with 10, 20 and 50 μg/ml of ^{125}I-LDL at 4°C were shown in Fig. 3. The total LDL-binding at 4°C represents exclusively the cell surface binding, because the internalization of LDL by receptor-mediated endocytosis is inhibited at this temperature. The total surface binding of LDL by cells increased steadily with increasing amounts of 10, 20 and 50 μg/ml of ^{125}I-LDL present in the medium at 4°C (16.2 ± 1.2, 22.5 ± 1.0 and 30.2 ± 2.5 ng/mg protein/2 h, respectively). The specific binding of ^{125}I-LDL also increased, but the rate of increase became slow down after 20 μg of ^{125}I-LDL. However, the non-specific binding of LDL increased linearly (1.5 ± 0.1, 2.7 ± 0.2 and 6.6 ± 0.1 ng/mg protein/2 h), and the non-specific binding comprised about 9.3%, 12.0% and 21.9% of the total binding at 10, 20 and 50 μg/ml of ^{125}I-LDL concentrations, respectively.

The surface binding (heparin-releasable), internalization (heparin-resistant) and degradation of ^{125}I-LDL (10 μg) in cells treated with 18:0, 18:1, 18:2 or 18:3 at 37°C are shown in Table 1. The examination of cell viability in the preliminary experiment revealed that average cell counts were similar between the control and all fatty acid groups, and cell viability was greater than 95% assessed by Trypan blue exclusion. The light microscopic examination revealed the presence of numerous intracellular lipid droplets in cells incubated with fatty acids, but no other morphological abnormalities were observed in those cells. The treatment of cells with of 1.0 mM of fatty acids for 4 h resulted in a significant increase in the surface binding of ^{125}I-LDL compared to the non-treated control (34.9 ± 3.0) ($P < 0.05$). However, the surface binding of ^{125}I-LDL was significantly lower in cells exposed to 18:0 (48.2 ± 2.0) than to 18:1 (56.8 ± 5.1), 18:2 (56.0 ± 3.5) and 18:3 (57.8 ± 6.0 ng/mg protein/4 h). The internalization of ^{125}I-LDL was significantly higher in cells exposed to 18:1 (141.4 ± 7.2), 18:2 (142.0 ± 8.1) and 18:3 (146.4 ± 11.3), than to 18:0 (114.4 ± 3.5) and control (103.5 ± 5.5) ($P < 0.05$). The levels of LDL degradation in cells remained nearly the same regardless of fatty acid treatment, but the levels of LDL degradation product discharged into the medium were much higher in cells exposed to 18:1 (167.6 ± 10.1), 18:2 (159.8 ± 7.7) and 18:3 (165.1 ± 14.7) than to 18:0 (142.1 ± 8.4) and the control (121.1 ± 3.4 ng/mg protein/4 h) ($P < 0.05$). The total surface, specific and non-specific bindings of ^{125}I-LDL (10 μg) in cells treated with 18:0, 18:1, 18:2 or 18:3 at 4°C in absence and presence of 500 μg of unlabeled LDL are shown in Table 2. The total surface

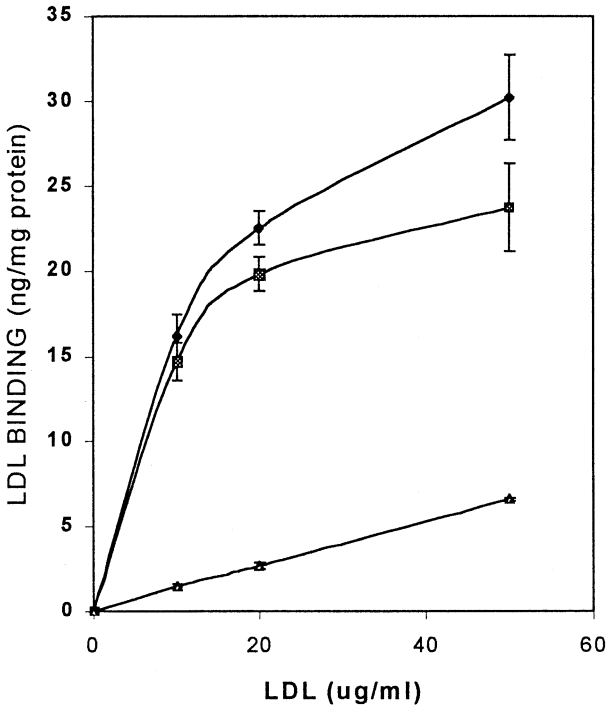

Fig. 3. Surface, specific and non-specific bindings of ^{125}I-LDL in cells incubated with various concentrations of ^{125}I-LDL at 4°C. The cells were grown in DMEM containing 10% fetal bovine serum at 37°C for 4 days, and then cells were preincubated for 24 h in the DMEM supplemented with 10% lipoprotein-deficient serum (LPDS). Afterward, cells were incubated with 10, 20 and 50 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS with and without 500 μg/ml of unlabeled LDL in cells at 4°C for 2 hr. The symbols represent for surface binding (◆—◆), specific binding (■—■) and non-specific binding (△—△). The values are mean ± S.D. (n = 6).

and specific bindings of ^{125}I-LDL were significantly higher in cells treated with fatty acids than in the control ($P < 0.05$). Among fatty acids, the total surface and specific bindings were significantly lower in cells exposed to 18:0 than to 18:1, 18:2 and 18:3 ($P < 0.05$). In non-specific binding of ^{125}I-LDL, however, no significant differences were noted regardless of fatty acids treatments. In overall, the treatment of cells with fatty acids significantly increased the receptor-mediated specific binding, internalization and degradation of ^{125}I-LDL, but cells exposed to 18:0 was significantly lower than to 18:1, 18:2 and 18:3 in those parameters ($P < 0.05$).

4. Discussion

In the present study, Hep-G2 cells were found to be very active in the receptor-mediated binding, internalization, and degradation of human low-density lipoprotein (LDL), which is consistent with findings of others [21,22]. The receptor-mediated catabolism of LDL consists of three stages that include the initial binding of LDL to high affinity receptors on the cell surface, internalization through endocytosis, and degradation in lysosomes [23]. The LDL binding to high-affinity cell surface receptors progressed in a saturable manner reaching equilibrium within 2 h, which is in agreement to the findings of others [22,24]. In contrast to the high affinity binding process, the nonspecific binding of LDL is characterized by an apparent lack of saturability and is proportional to a concentration of LDL in the medium [21,25]. In the present study, the non-specific binding progressed linearly and accounted 9.3%, 12.0% and 21.9% of the total surface binding at 10, 20 and 50 μg/ml of ^{125}I-LDL concentration in the medium, respectively.

In the present study, the treatment of Hep G-2 cells with fatty acids significantly increased the receptor-mediated binding, internalization and degradation of ^{125}I-LDL. However, the receptor-mediated LDL uptake was found to be significantly lower in cells treated with 18:0 than with 18:1, 18:2 and 18:3. These results may be relevant to findings of clinical and animal studies in which dietary saturated fatty acids decrease receptor-mediated clearance of LDL, thereby increasing plasma LDL cholesterol levels [26,27]. The available evidence suggests that substitution of unsaturated fatty acids for saturated fatty acids results in an increase in LDL-receptor activity [14,28]. Recent studies also reported that dietary linoleic acid increases and palmitic acid de-

Table 1
Binding, internalization and degradation of ^{125}I-LDL in cells treated with 18:0, 18:1, 18:2 or 18:3[1,2]

^{125}I-LDL	None	Fatty acids			
		18:0	18:1	18:2	18:3
		(ng/mg cell protein/4 h)			
Total binding	280.0 ± 8.7[c]	326.0 ± 11.5[b]	388.0 ± 13.5[a]	379.9 ± 12.6[a]	393.1 ± 29.5[a]
Surface bound	34.9 ± 3.0[c]	48.2 ± 2.2[b]	56.8 ± 5.5[a]	56.0 ± 3.9[a]	57.8 ± 6.0[a]
Internalized	103.5 ± 5.5[b]	114.4 ± 3.5[b]	141.4 ± 7.2[a]	142.0 ± 8.1[a]	146.4 ± 11.3[a]
Degraded/cell	20.4 ± 1.3	21.6 ± 1.7	22.2 ± 2.8	22.1 ± 1.5	21.9 ± 1.0
Degraded/medium	121.1 ± 3.4[c]	142.1 ± 8.4[b]	167.6 ± 10.1[a]	159.8 ± 7.7[a]	165.1 ± 14.7[a]

[1] The cells were grown in DMEM containing 10% fetal bovine serum at 37°C for 4 days, and then cells were preincubated for 24 h in the DMEM supplemented with 10% lipoprotein-deficient serum (LPDS). Afterward, cells were treated with 1 mM of 18:0, 18:1, 18:2, or 18:3 for 4 h, followed by incubation with with 10 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS at 37°C for 4 h. The surface-bound and internalized LDL represents the heparin-releasable and heparin-resistant LDL, respectively.

[2] Values are the mean ± SD (n = 6). Letters sharing different superscript letters in the same row are significantly different ($P < 0.05$).

Table 2
Surface, specific and non-specific bindings of ^{125}I-LDL in cells treated with 18:0, 18:1, 18:2 or 18:3[1,2]

^{125}I-LDL	None	Fatty acids			
		18:0	18:1	18:2	18:3
		(ng/mg cell protein/2 h)			
Surface binding	17.0 ± 0.8[c]	22.1 ± 1.4[b]	25.3 ± 2.0[a]	25.2 ± 1.7[a]	25.7 ± 1.5[a]
Specific binding	15.5 ± 0.7[c]	20.4 ± 2.3[b]	23.7 ± 1.9[a]	23.5 ± 1.7[a]	24.0 ± 1.1[a]
Non-specific binding	1.5 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.2

[1] The cells were grown in DMEM containing 10% fetal bovine serum at 37°C for 4 days, and then cells were preincubated for 24 h in the DMEM supplemented with 10% lipoprotein-deficient serum (LPDS). Afterward, cells were treated with 1 mM of 18:0, 18:1, 18:2 or 18:3 for 4 h, followed by incubation with 10 μg/ml of ^{125}I-LDL in DMEM containing 10% LPDS with and without 500 μg/ml of unlabeled LDL at 4°C for 2 h.
[2] Values are the mean ± SD (n = 6). Letters sharing different superscript letters in the same row are significantly different ($P < 0.05$).

creases hepatic LDL receptor protein and LDL receptor mRNA levels [29].

The regulation of hepatic receptor activity by saturated or unsaturated fatty acids has also been postulated through an effect of a specific fatty acid on acyl-CoA: cholesterol acyltransferase (ACAT) reaction [30,31]. Upon LDL uptake by the liver, free cholesterol is released by the hydrolysis of LDL-cholesteryl ester, and this lipoprotein-derived cholesterol consequently regulates cellular cholesterol metabolism by suppressing HMG-CoA reductase. As the level of intracellular free cholesterol rises, the microsomal ACAT is stimulated to reesterify excess cholesterol and synthesis of new LDL receptors is inhibited [32]. Therefore, factors that raise intracellular cholesterol appear to suppress the formation of receptors, while the reduction of cholesterol level within the cell stimulates receptor synthesis. When the cells become enriched with stearic acid, the cholesterol esterification reaction is partially inhibited because the saturated fatty acid is a less favorable substrate for ACAT activity, and this results the expansion of regulatory free cholesterol pool while reducing the cholesteryl ester pool size. The expansion of free cholestrol pool size causes a reduction of receptor RNA concentration and a diminished LDL receptor activity by a mechanism of cholesterol feed back inhibition. On the other hand, when the cells are enriched with unsaturated fatty acids, ACAT reaction is enhanced resulting a decrease in free cholesterol pool size [28].

In our previous study [15], it was noted that the treatment of Hep-G2 cells with 1 mM of 18:0 for 4 h resulted about 330 nmoles/mg cell protein of its cellular uptake. Although a portion of 18:0 is converted to 18:1 by desaturation process, the presence of an excessive amount of 18:0 could unfavorably affect the microsomal ACAT activity. In fact, incorporation of 18:0 into cholesteryl ester was found to be fairly low (8.6% from 5.6%), whereas a marked incorporation of 18:0 was noted in triglyceride (30.0% from 8.4%) and phospholipid (21.3% from 11.8%) compared to the untreated control [15]. The total cholesterol levels in cells treated with fatty acids were found to be in the order of 18:0 > 18:1 > 18:2 = 18:3. Thus, the elevation of cellular cholesterol concentration resulted from 18:0 treatment could unfavorably affect the LDL receptor synthesis, and the overall effect of 18:0 may be similar to that of palmitic acid, which has been reported to decrease LDL receptor activity by regulating LDL-receptor gene expression [33, 34]. In addition, the changes in the fatty acid composition of LDL-phospholipid with saturated fatty acids could also modify the surface coat of lipoproteins containing apolipoprotein B, influencing the binding of apo B to the cellular LDL receptors [9]. Dietary fat-induced changes in LDL-receptor activity have been correlated with changes in the composition and physical properties of cell membranes [27]. Thus, the alteration of the fluidity of cell membrane harboring LDL-receptors by enrichment of phospholipids with 18:0 could also affect the normal function of LDL receptors within the cell membrane, possibly by reducing the binding or internalization of circulating LDL [9].

In summary, the treatment of Hep-G2 cells with fatty acids significantly increased the receptor-mediated binding, internalization and degradation of ^{125}I-LDL, but cells exposed to 18:0 was significantly lower than to 18:1, 18:2 and 18:3 in those parameters. It is also noteworthy that despite difference in the degree of unsaturation, the LDL uptake and its degradation in cells treated with 18:1 was almost the same as those of 18:2 and 18:3. The present finding that 18:1 is equally effective in enhancement of the receptor-mediated LDL uptake as those of 18:2 and 18:3 suggests that the major action of 18:1 in lowering LDL-cholesterol levels also involves an increased clearance of LDL via hepatic LDL-receptors as the other polyunsaturates.

Acknowledgments

This work was supported in part by US Public Health Service Grant (NIH HL60306) and by the fund from the Harlan E. Moore Charitable Trust.

References

[1] J.L. Goldstein, M.S. Brown, The low-density lipoprotein pathway and its relation to atherosclerosis Ann Rev Biochem 46 (1977) 897–930.
[2] S.M. Grundy, Cholesterol and coronary heart disease, J Am Med Assoc 256 (1986) 2849–2858.

[3] P.T. Kovanen, M.S. Brown, S.K. Basu, D.W. Bilheimer, J.L. Goldstein, Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hyper-cholesterolemia of cholesterol-fed rabbits, Proc Natl Acad Sci USA 78 (1981) 1396–1400.
[4] C.J. Packard, L. McKinney, K. Carr, C. Shepherd, Cholesterol feeding increases low density lipoprotein synthesis, J Clin Invest 72 (1983) 45–51.
[5] P.A. Dawson, S.L. Hofmann, D.R. van der Westhuyzen, T.C. Sudhof, M.S. Brown, J.L. Goldstein, Sterol-dependent repression of low density lipoprotein receptor promotor mediated by 16-base pair sequence adjacent to binding site for transcription factor sp1, J Biol Chem 263 (1988) 3372–3379.
[6] M.S. Brown, S.E. Danna, J.L. Goldstein, Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts, J Biol Chem 249 (1974) 789–796.
[7] V.A. Kosykh, S.N. Preobrazhensky, V.O. Ivanov, V.S. Repin, Primary culture of human hepatocytes: evidence for specific LDL uptake, J Cell Biol 97 (1983) 466a.
[8] A.D. Attie, R.C. Pittman, Y. Wantanable, Low density lipoprotein receptor deficiency in cultured hepatocytes of the WHHL rabbit, J Biol Chem 256 (1981) 9789–9792.
[9] J. Loscalzo, J. Fredman, R.M. Rudd, I. Barsky-Vasserman, D.E. Vaughan, Unsaturated fatty acids enhance low density lipoprotein uptake and degradation by peripheral blood mononuclear cells, Arteriosclerosis 7 (1987) 450–455.
[10] F.H. Mattson, S.M. Grundy, Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man, J Lipid Res 26 (1985) 194–202.
[11] S.M. Grundy, M.A. Denke, Dietary influences on serum lipids and lipoproteins, J Lipid Res 31 (1990) 1149–1172.
[12] D.K. Spady, J.M. Dietschy, Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster, Proc Natl Acad Sci USA 82 (1985) 4526–4530.
[13] D.R. Illingworth, W.S. Harris, W.E. Connor, Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans, Atherosclerosis 4 (1984) 270–275.
[14] D.K. Spady, L.A. Woollett, Interaction of dietary saturated and polyunsaturated triglycerides in regulating the processes that determine plasma low density lipoprotein concentration in the rat, J Lipid Res 31 (1990) 1809–1819.
[15] R.C. Dokko, B.H.S. Cho, B.H. Chung, Cellular uptake of stearic, oleic, linoleic, and linolenic acid and their effects on synthesis and secretion of lipids in Hep-G2 cells, Int J Biochem & Cell Biol 30 (1998) 65–76.
[16] N.B. Javitt, Hep G2 cells as a resource for metabolic studies: lipoprotein, cholesterol, and bile acids, FASEB J 4 (1990) 161–168.
[17] R.J. Havel, H.A. Eder, J.H. Bragdon, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J Clin Invest 34 (1955) 1345–1353.
[18] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J Biol Chem 193 (1951) 267–275.
[19] D.W. Bilheimer, S. Eisenberg, R.J. Levy, The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observation, Biochim Biophys Acta 260 (1972) 212–221.
[20] J.L. Goldstein, S.K. Basu, M.S. Brown, Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. Methods Enzymol 98 (1983) 241–260.
[21] L. Havekes, V. Van Hinsbergh, H.J. Kempen, The metabolism in vitro of human low density lipoprotein by the human hepatoma cell line, Hep-G2, Biochem J 214 (1983) 951–958.
[22] G.Y. Wu, C.H. Wu, V.A. Rifici, R.J. Stockert, Activity and regulation of low density lipoprotein receptors in a human hepatoblastoma cell line, Hepatology 4 (1984) 1190–1194.
[23] R. Mahley, W.T.L. Innerarity, R.E. Pitas, Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine rich and B apoproteins, J Biol Chem 252 (1977) 7279–7287.
[24] A.M. Leichtner, M. Krieger, A.L. Schwartz, Regulation of low density lipoprotein receptor function in a human hepatoma cell line, Hepatology 4 (1984) 897–901.
[25] P.A. Soltys, D.W. Portman, J.P. O'Malley, Binding properties of high-density lipoprotein subfractions and low-density lipoproteins to rabbit hepatocytes, Biochim Biophys Acta 713 (1982) 300–314.
[26] J. Shepherd, C.J. Packard, S.M. Grundy, D. Yeshurun, A.M. Gotto, Jr., O.D. Taunton, Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man, J Lipid Res 21 (1980) 91–99.
[27] P.C. Kuo, M.A. Rudd, R. Nicolosi, J. Loscalzo, Effect of dietary fat saturation and cholesterol on low density lipoprotein degradation by mononuclear cells of cebus monkeys, Arteriosclerosis 9 (1989) 919–927.
[28] D.K. Spady, J.M. Dietschy, Interaction of dietary cholesterol and triglycerides in regulation of hepatic low density lipoprotein transport in the hamster, J Clin Invest 81 (1988) 300–309.
[29] V.A. Mustad, J.L. Ellsworth, A.D. Cooper, P.M. Kris-Etherton, T.D. Etherton, Dietary linoleic acid increases and palmitic acid decreases hepatic LDL receptor protein and mRNA abundance in young pigs, J Lipid Res 37 (1996) 2310–2323.
[30] C.M. Daumerie, L.A. Woollett, J.M. Dietschy, Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools, Proc Natl Acad Sci USA 89 (1992) 10797–10801.
[31] D.K. Spady, L.A. Woollett, J.M. Dietschy, Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids, Ann Rev Nutr 13 (1993) 355–381.
[32] M.S. Brown, P.T. Kovanen, J.L. Goldstein, Regulation of plasma cholesterol by lipoprotein receptors, Science 212 (1981) 628–635.
[33] L.A. Woollett, D.K. Spady, J.M. Dietschy, Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster, J Clin Invest 89 (1992) 1133–1141.
[34] R.J. Srivastava, H. Ito, M. Hess, N. Srivastava, G. Schonfeld, Regulation of low density lipoprotein receptor gene expression in HapG2 and Caco2 cells by palmitate, oleate, and 25-hydroxycholesterol, J Lipid Res 36 (1995) 1434–1446.