In Vitro Regulation of Low-Density Lipoprotein Receptor Interaction by Fatty Acids

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Low-density lipoprotein (LDL) receptor binding is the initial step in receptor-mediated clearance. Dietary fat composition is known to affect LDL clearance, but the mechanism of the effect is unknown. We have examined the effects of altered membrane fatty acid composition, as might occur when specific dietary fats are consumed, on LDL binding using a Chinese hamster ovary (CHO) line that constitutively expresses the human LDL receptor. Binding of pooled human LDL to its receptor was compared in cells enriched with various fatty acids. Binding affinity was greater (lower K_d) for cells grown in 16:0-, 18:0-, or 18:1-enriched media than for those grown in 18:2 ($P < .0001$). The apparent receptor number (Bmax) was lower for cells enriched in saturated fatty acids and 18:1. Fluidity was assessed by measuring diphenylhexatriene (DPH) fluorescence anisotropy (r_s). Cells enriched in 18:1 or 18:2 were the most fluid ($P < .003$). The correlation between binding and fluidity ($r = .24$, $P = .27$) was weak and did not appear to explain the effects of fatty acid modification on LDL receptor binding. Thus, it appears that cellular enrichment in 16:0, 18:0, and 18:1 increases binding affinity by affecting properties other than membrane fluidity. Changes in Bmax may also contribute to the observed differences in LDL binding.

DIET CAN HAVE a major impact on the concentration of circulating low-density lipoprotein (LDL).[1-6] Decreasing saturated fat intake and increasing dietary fiber generally decreases elevated plasma LDL concentrations. Other diet modifications, such as a reduction in cholesterol or an increase in polyunsaturated fat intake, may also decrease LDL cholesterol concentration. The mechanism of the effect of changes in dietary fatty acids on LDL concentration is still unknown. Results of metabolic studies designed to determine how diet decreases LDL cholesterol have not been consistent.[5,6] Some studies find that most of the effect of fat saturation is on LDL production,[7,8] whereas others find mainly changes in clearance.[9-11] One recent study showed that diets enriched in monounsaturated and polyunsaturated fat reduced LDL production as compared with saturated fat, but only polyunsaturated fat increased LDL clearance.[12]

When LDL clearance is altered by diet, this is functionally achieved via changes in receptor-mediated uptake. The LDL receptor accounts for approximately 75% of LDL clearance in vivo.[13,14] Non–receptor-mediated clearance accounts for only a small percentage of LDL clearance and does not appear to be responsive to diet modification.[10,15] Therefore, diet interventions that alter LDL cholesterol concentration might be expected to affect LDL receptor–mediated uptake. There are several ways in which this could be accomplished. Dietary changes may modify LDL particle composition, thus altering the affinity of the particle for the receptor.[11,16] Diet may also alter the cell membrane and thereby influence the cellular environment in which the LDL receptor exists, thus altering the number of receptors or affinity for the LDL particle.[11] To better understand the various effects of dietary fat composition on LDL metabolism, we have used an in vitro modification to parallel the effects of diet on the liver and tested these effects independent of changes in the LDL particle, as would occur in a diet study. Thus, this study was designed to examine the effect of changes in fatty acid composition of the cell membrane on LDL binding.

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MATERIALS AND METHODS

Pooled Human LDL

LDL was isolated from 6 U outdated frozen plasma from the American Red Cross by sequential ultracentrifugation.[17] Each unit was thawed and adjusted to density 1.019 g/mL with NaBr (d 1.470 g/mL). Very-low-density and intermediate-density lipoproteins were removed by ultracentrifugation in a 60-Ti rotor at 55,000 rpm for 20 hours at 15°C. The bottom fraction was collected and pooled, and density was increased to 1.063 g/mL. After centrifugation in a 60-Ti rotor at 55,000 rpm for 20 hours at 15°C, LDL was collected in the top 7 mL of each tube. LDL was pooled and dialyzed against buffer A (150 mmol/L NaCl solution with 0.1 mmol/L disodium EDTA and 1 mmol/L NaH_2PO_4, pH 7.4). The LDL was then sterilized by filtration through a 0.22-μm filter (Millex-GV, Bedford, MA), and protein content was determined.[18] Aliquots were frozen at −80°C.[19] The LDL used in all of these experiments was from a single large pool. Frozen human LDL has been shown to yield the same results as fresh LDL.[19]

Radioiodination of LDL

LDL was iodinated with ^{125}I using a modification[20] of the iodine monochloride method reported by MacFarlane.[21] Frozen pooled LDL (0.75 mL at 8 mg/mL) was diluted to 1 mL with 1 mol/L glycine-NaOH buffer, pH 10, and placed in an ice bath. Five millicuries of $Na^{125}I$ in 0.05 mL 1N NaOH was added with rapid mixing. The reaction was initiated by addition of 0.1 mL iodine monochloride, 2.64 mmol/L. The mixture was allowed to stand for 5 minutes on ice, followed by dilution with 1.35 mL buffer A. The labeled LDL was dialyzed against 1 L phosphate-buffered saline, pH 7.4, for 21 to 24 changes until the dialysate was less than 2,000 cpm/mL. Specific activity was 334 ± 71 cpm/ng protein (mean ± SD), and 94% ± 1.5% of the radioactivity was protein-

associated as determined by assessment of lipid-bound radioactivity[22] and percent free iodine.

Preparation of Fatty Acid–Supplemented Newborn Calf Lipoprotein-Deficient Serum

A single lot of newborn calf serum was used. Each bottle of serum was thawed, and solid KBr was added to increase the density to 1.215 g/mL. After ultracentrifugation for 48 hours at 55,000 rpm at 15°C in a 60-Ti rotor, the top 15 mL containing the lipoproteins was removed and discarded. The bottom of the tubes (lipoprotein-deficient serum [LDS]) was pooled for dialysis against 36 L buffer A, as previously described.[19] After dialysis, LDS was sterilized by filtration through a 0.22-μm filter, and an aliquot was taken for protein and cholesterol measurements. Cholesterol content was less than 5 mg/dL.[23]

Pure (> 95%) fatty acids were mixed with equimolar amounts of NaOH and heated to 90°C for 10 to 15 minutes to prepare the soluble sodium salt. The fatty acid salt solution was cooled to 55°C and added to stirred newborn calf LDS at 55°C (final concentration, 1 μmol fatty acid/mL LDS). The fatty acid–supplemented newborn calf LDS was then adjusted to 50 mg protein/mL with sterile saline. Preparations were made using the following fatty acids: 16:0, 18:0, 18:1, and 18:2.

Cells

The Chinese hamster ovary (CHO) cell line TR715-19, a gift from Drs M.S. Brown and J.L. Goldstein, was used in this study.[24,25] TR715-19 is a CHO line into which cDNA for human LDL receptor was inserted by cloning into the pcDX vector. The LDL receptor sequence without its native promoter region was positioned downstream of the enhancer and early promoter regions of the SV40 virus. These cells contain no native LDL receptor and express four times more human LDL receptors per cell than fibroblasts.[19] The cells were maintained in medium A (Ham's F-12 nutrient mixture containing 20 mmol/L HEPES, pH 7.4, 2 mmol/L L-glutamine, 20 μmol/L mevinolin, 200 μmol/L mevalonate, 4% vol/vol newborn calf LDS, and 1% vol/vol fetal calf serum), as described previously.[19] Cells cultured under these conditions are referred to as regular. Stock cultures were subcultured in fatty acid–modified medium A, in which the 4% (vol/vol) newborn calf LDS was replaced by fatty acid–modified newborn calf LDS. Cells were subcultured for at least 1 month before experiments were performed.

Cells were plated (day 0) for binding experiments at 7.5×10^4/60-mm^2 petri dish in 3 mL of the appropriate fatty acid–modified medium A. On day 3, cells were switched to medium C containing 5% fatty acid–modified newborn calf LDS, 0.1 μg/mL 25-hydroxycholesterol, and 10 μg/mL cholesterol. Experiments were performed on day 4.

Measurement of LDL Binding, Internalization, and Degradation

Assays were conducted as described by Davis et al[25] and Goldstein et al.[26] Cells were switched on the day of the experiment (day 4) to medium F (Eagle's minimum essential medium, 10 mmol/L HEPES, pH 7.4, 24 mmol/L bicarbonate, 1% vol/vol nonessential amino acids, and 10% pooled human LDS). The final volume in each dish contained 1 μg/mL of ^{125}I-LDL protein and various concentrations of unlabeled LDL of 0, 1.5, 3.0, 6.0, 9.0, 19.0, 39.0, and 59.0 μg LDL protein/mL to determine displacement. Nonspecific binding was determined with 500 μg protein/mL. After a 2-hour incubation, the dishes were transferred to an iced metal plate in the cold room, and after 5 minutes, the incubation medium was removed to tubes containing 0.5 mL 50% (wt/vol) trichloroacetic acid (TCA) for determination of LDL degradation.

The cells were washed rapidly three times with 3 mL ice-cold buffer B (150 mmol/L NaCl, 50 mmol/L Tris hydrochloride, pH 7.4, and 2 mg/mL bovine serum albumin), after which the cells were incubated twice for 10 minutes each with 3 mL buffer B. The final wash was a rapid wash with 3 mL buffer C (150 mmol/L NaCl with 50 mmol/L Tris hydrochloride, pH 7.4) followed by addition of 2 mL buffer D (50 mmol/L NaCl, 10 mmol/L HEPES, pH 7.4, and 10 mg/mL heparin). The cells were incubated at 4°C for 1 hour with gentle shaking. After incubation, a 1-mL aliquot of buffer D from each dish was removed and ^{125}I radioactivity was measured to determine binding. The remaining buffer was removed, 2 mL 0.2-mol/L NaOH was added, and the cells were dissolved overnight at 4°C. A 1-mL aliquot was removed and counted to determine LDL internalization, and 0.25 mL was assayed for protein content by a modification[18] of the Lowry assay. Tubes containing the TCA-medium mixture were centrifuged at 2,500 rpm for 20 minutes, and a 1-mL aliquot of TCA-soluble supernatant was removed. The supernatant was mixed with 10 μL 40% (wt/vol) KI, followed by addition of 40 μL 30% hydrogen peroxide. After vortexing, the tubes stood for 10 minutes, followed by addition of 2 mL chloroform with vigorous mixing. A 0.5-mL aliquot of the aqueous upper layer was removed for counting to determine LDL degradation.

Binding data were analyzed by Scatchard analysis, and K_d and apparent receptor number (Bmax) were calculated.[27] Internalization and degradation were determined at 60 μg LDL protein/mL. To determine whether a difference in the rate of receptor recycling occurred, the internalization index for these experiments was calculated[26] using the following formula: amount of LDL internalized + (amount degraded/amount bound).

Isolation of Plasma Membrane

Confluent flasks of fatty acid–enriched cells were trypsinized on the day following the binding assays for fluidity measurements and plasma membrane isolation. Cells were subject to the same growing conditions as for the binding assay, with an overnight exposure to medium C. Cell homogenates were mixed with 55% sucrose (wt/vol) and adjusted to a final concentration of 44% sucrose to obtain the plasma membrane.[28] The 44% sucrose–cell homogenate was overlayered with 42% sucrose and then centrifuged at 4°C for 2.5 hours at 25,000 rpm in an SW28 rotor. The plasma membrane layer was removed and frozen at −80°C for determination of fatty acid composition.

Fatty Acids

Lipids were extracted by an adaptation[29] of the method reported by Sperry and Brand.[30] Fatty acyl composition was determined by capillary gas chromatography of the corresponding methyl esters prepared by transesterification with methanolic HCl.[29] Chromatography was performed using a Hewlett-Packard Model 5890A gas chromatograph (Wilmington, DE). The instrument was equipped with a dual flame-ionization detector, a Model 7673A automatic sampler, and a Model 3396A integrator. Chromatography was performed with a Supelco SP-2560 fused-silica, 30-m × 0.25-mm ID capillary column (Bellefonte, PA) with a 0.20-μm thick film.

Fluidity Measurement

Membrane fluidity was assessed by measuring fluorescence polarization of a nonpolar hydrocarbon probe inserted into cell membranes. Cell membranes were labeled by incubation of trypsinized cells with diphenylhexatriene (DPH) in Dulbecco's phosphate-buffered saline, pH 7.4. After a 1-hour incubation at 37°C, fluidity

Table 1. Fatty Acid Composition (mol %) of the CHO Cells

Fatty Acid	Regular	16:0	18:0	18:1	18:2
14:0	2.84 ± .19[a]	2.80 ± .17[a]	1.77 ± .06[b]	1.34 ± .22[b]	1.49 ± .16[b]
16:0	25.15 ± .71[b]	32.80 ± 1.0[a]	16.75 ± .77[c]	16.89 ± 1.4[c]	19.15 ± .97[c]
16:1	6.40 ± .45[b]	11.56 ± .81[a]	3.35 ± .28[d]	4.13 ± .35[cd]	2.35 ± .22[d]
18:0	12.93 ± .60[b]	10.27 ± 1.2[b]	16.97 ± 1.2[a]	11.19 ± 1.7[b]	12.37 ± .60[b]
18:1	40.06 ± 1.3[c]	33.18 ± 1.9[c]	47.37 ± 3.2[b]	59.23 ± 3.4[a]	13.84 ± 1.4[d]
18:2	4.17 ± .23[b]	3.02 ± .28[b]	2.86 ± .19[b]	1.98 ± .11[b]	45.79 ± 2.7[a]
18:3	0.33 ± .01	0.33 ± .03	0.34 ± .11	0.12 ± .07	0.18 ± .01
20:0	0.24 ± .03[b]	0.21 ± .02[b]	0.43 ± .03[a]	0.26 ± .06[b]	0.14 ± .05[b]
20:3	0.39 ± .11	0.27 ± .02	1.89 ± 1.6	0.53 ± .23	0.13 ± .01
20:4	2.50 ± .05[a]	2.02 ± .11[b]	2.09 ± .15[b]	1.54 ± .04[c]	1.12 ± .06[d]
20:5	0.33 ± .02	0.36 ± .02	0.38 ± .04	0.05 ± .02	0.74 ± .39
22:5	1.61 ± .13	0.95 ± .08	1.55 ± .54	0.61 ± .06	0.63 ± .17
22:6	1.00 ± .06[a]	0.64 ± .06[b]	0.69 ± .09[b]	0.18 ± .11[c]	0.30 ± .12[c]
24:0	0.71 ± .11	0.49 ± .08	0.63 ± .22	0.61 ± .13	1.00 ± .05
24:1	0.76 ± .19	0.61 ± .06	0.37 ± .06	0.73 ± .14	0.44 ± .05

NOTE. Results are the mean ± SD. Values followed by different superscripts are significantly different at $P < .05$.

was measured in 3° to 5° increments across a range of temperatures from 37° to 4°C. Steady-state fluorescence polarization measurements, with excitation and emission wavelengths of 366 and 430 nm, were made using methods developed by Shinitzky and Barenholz.[31] All measurements were made with the SLM 4800 fluorometer (Rochester, NY) in the steady-state mode, and the data are reported as anisotropy values.

Data Analysis

The data (mean ± SD) are mean values of five experiments per fatty acid treatment. The data were tested for normality. An ANOVA was then performed on normally distributed data using the General Linear Models (SAS, Cary, NC) procedure for all measurements, and where differences were found, the Student-Neuman-Keuls multiple-range test was used. If the data were not normally distributed, as in the case of the internalization index average, the Kruskall-Wallis test was used. Correlations were assessed using Pearson's correlation analysis.

RESULTS

Cells and Plasma Membrane Fatty Acid Composition

No differences in cell growth were observed between cells grown in the various fatty acid–enriched media. Cells have been subcultured for over a year in the various fatty acid–enriched media. Fatty acid compositions of the plasma membranes showed the expected enrichment pattern (Table 1).

LDL Binding

Displacement curves from cells grown in saturated fatty acids showed greater binding affinity than from those grown in medium supplemented with polyunsaturated fatty acid. An example is shown in Fig 1.

Transformation of the data by Scatchard analysis showed a significant difference in the K_d between the various fatty acid–enriched cells ($P < .0001$; Table 2). The K_d was lower for the saturated fatty acids and oleate than for 18:2.

Bmax was also significantly different between fatty acids ($P < .0001$). Bmax was lowest in cells enriched with 16:0 and 18:0, intermediate in 18:1 cells, and highest in 18:2 cells (Table 2). Figure 2 shows the Scatchard plot for 16:0- and 18:2-enriched cells.

Internalization and Degradation

Internalization of LDL was highly correlated with binding ($r = .73$, $P < .0001$), and a similar trend for differences due to treatment was found for internalization ($P < .0001$) (Table 2). To determine whether internalization was related to the rate of receptor recycling, the internalization index was calculated. Because the index is different at each LDL concentration, the mean internalization index was calculated for each concentration of unlabeled LDL. No differences in the mean internalization index were found ($P = .35$), suggesting that the amount of LDL bound to the surface was the major regulator of internalization. No significant difference was found in the amount of LDL degraded between any of the fatty acid–enriched cells

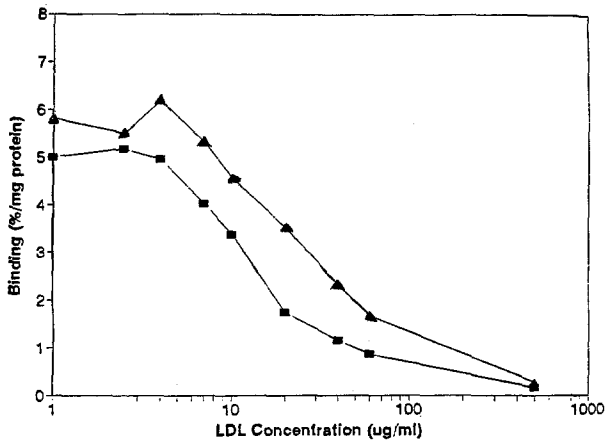

Fig 1. Example of a displacement curve of LDL binding to cells enriched in 16:0 (■) and 18:2 (▲). A 2-hour incubation at 37°C was performed. The final volume in each dish contained 1 μg/mL of ^{125}I-LDL protein and various concentrations of unlabeled LDL, 0, 1.5, 3.0, 6.0, 9.0, 19.0, 39.0, and 59.0 μg LDL protein/mL, to determine displacement. Nonspecific binding was determined with 500 μg protein/mL.

Table 2. LDL Binding Data

Fatty Acid	K_d (μg/mL)	Internalization (ng/mg protein/2 h)	Degradation (ng/mg protein/2 h)	Bmax (μg/mg protein)	Mean Internalization Index	DPH37 (r_s)
16:0	7.4 ± 2.2[a]	438.7 ± 237.5[a]	463.8 ± 274.8	446.6 ± 121.2[a]	2.98 ± 1.17	0.124 ± 0.012[ab]
18:0	7.6 ± 2.6[a]	566.3 ± 52.4[ab]	527.7 ± 249.7	491.8 ± 88.9[a]	2.73 ± 0.94	0.129 ± 0.016[b]
18:1	9.4 ± 2.4[a]	774.3 ± 138.8[b]	924.9 ± 273.9	791.9 ± 95.2[b]	3.23 ± 0.20	0.102 ± 0.014[a]
18:2	17.9 ± 2.4[b]	1,098.4 ± 183.4[c]	1,133.6 ± 638.2	1,379.2 ± 121.7[c]	3.51 ± 1.03	0.113 ± 0.015[ab]
Regular	15.7 ± 1.6[b]	1,104.2 ± 293.5[c]	930.0 ± 295.1	1,129.5 ± 408.2[c]	3.84 ± 0.63	0.153 ± 0.014[c]

NOTE. Results are the mean ± SD from 5 experiments each at 37°C for 2 hours. Values followed by different superscripts are significantly different at $P < .05$.

($P = .07$); however, the trend was similar to that for internalization.

Membrane Fluidity

Membrane fluidity, expressed as DPH anisotropy at 37°C, was significantly ($P < .0001$) affected by fatty acid enrichment of the cells. Cell membranes enriched in the unsaturated fatty acids 18:1 and 18:2 were more fluid than membranes from 16:0- and 18:0-enriched cells. Although fluidity increased as LDL receptor affinity increased, fluidity and binding affinity were not significantly correlated ($r = .24$, $P = .27$; Fig 3). Fluidity was not correlated with Bmax ($r = .08$), indicating that differences in Bmax were not related to fluidity.

DISCUSSION

LDL Binding

This study has demonstrated that fatty acid composition of cell membranes can influence LDL binding. Cells enriched in 16:0 and 18:0 had greater binding affinity and decreased Bmax as compared with those grown in 18:2. Thus, based on these data, consuming a diet enriched in saturated fat would be expected to increase affinity of the LDL receptor in the liver and perhaps decrease the number of LDL receptors. The binding affinity of 18:0- and 18:1-enriched cells was similar, probably because cells grown in 18:0-enriched media desaturated much of the 18:0 to 18:1.

In vivo studies have shown that feeding saturated fat as compared with polyunsaturated fat decreased LDL receptor activity. Saturated fat was associated with a lower fractional catabolic rate (FCR) for LDL,[10,11] and the lower FCR is due to a decrease in the receptor-mediated–clearance pathway.[10-12] However, the mechanism of the changes in clearance is not fully understood.

Several studies designed to determine the mechanism of the effect of saturated fat on LDL metabolism suggest that dietary fat affects LDL metabolism through its effect on LDL receptor number.[11,32-35] Fernandez et al[11] found greater LDL binding in guinea pigs fed corn oil diets; binding correlated with FCR, but binding affinity was not different between fatty acids. Differences in binding were attributed to increases in receptor number with the corn oil treatment. Similarly, several in vivo and in vitro studies have reported that saturated fat decreases the concentration of mRNA for the LDL receptor.[34-39] Our observations give support to the theory that saturated fat consumption results in a reduction in the uptake and clearance of LDL, but the mechanism is undetermined in this cell model.[40]

On the other hand, a few dietary studies have reported differences in LDL binding affinity.[41,42] These are studies in which LDL was isolated and binding examined in vitro. These studies show that differences in LDL receptor interaction can occur when LDL is modified by diet.

The effect of fatty acid modification of cells in vitro on LDL uptake has been examined by others. Gavigan and Knight[43] reported that LDL degradation was increased in fibroblasts cultured for 1 week in medium enriched with

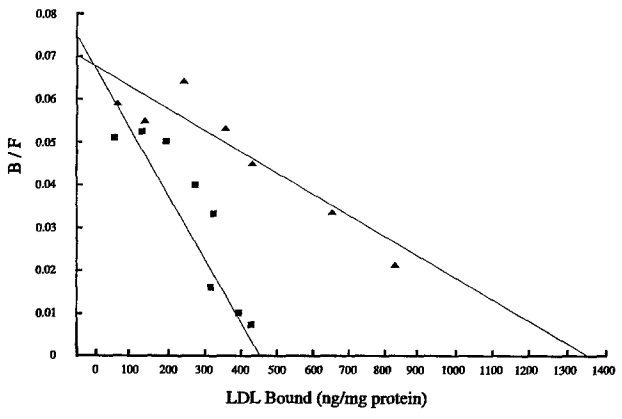

Fig 2. Scatchard plot of LDL binding to cells enriched in 16:0 (■) and 18:2 (▲). The mean of 5 experiments for 16:0- and 18:2-enriched cells and the lower K_d and Bmax of cells treated with 16:0 are shown.

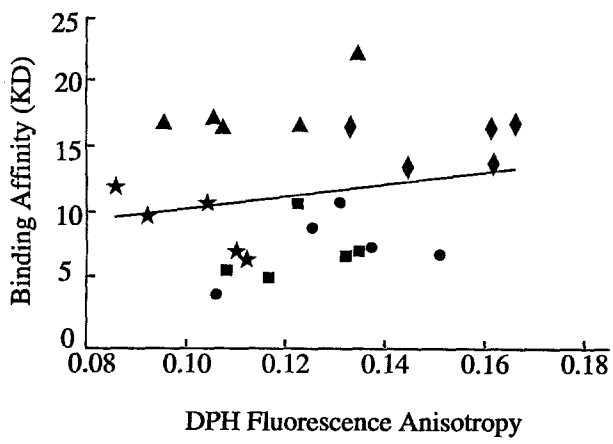

Fig 3. Correlation between anisotropy and binding affinity ($r = .24$). 16:0 (■), 18:0 (●), 18:1 (★), 18:2 (▲), and regular (◆).

linoleic acid as compared with media enriched with oleic or palmitic acid. There was a trend toward increased amounts of LDL binding to cells grown in unsaturated fatty acid. Similarly, Loscalzo et al[44] showed increased LDL uptake in monocytes incubated with medium containing 18:2 for 1 hour. Neither binding affinity nor receptor number were examined in either of these studies.

Kuo et al[45] examined LDL binding in fatty acid–enriched HepG2 hepatocytes. They found, as we did, that saturated fatty acids result in a higher binding affinity than 18:2. However, they reported that oleic acid had the highest K_d, whereas in the present study the K_d for oleic acid was lower than for linoleic acid. In addition, their results showed that palmitic acid enrichment resulted in the highest Bmax, whereas we found that linoleic acid supplementation was associated with the highest Bmax. Therefore, although the trends for binding affinity were similar to the present study, the results for Bmax were not. Results of these two studies may differ because of the different cell types used.

Fluidity

Fatty acid modification of the cell membrane increased membrane fluidity in all cases. The increase in fluidity is likely due to effects of fatty acid supplementation in general and less to the type of fat. Berlin et al[46] reported an analogous result for women fed diets differing in total amount of fat and type of fat. Erythrocyte fluidity was greater on the high-fat diets, whereas the polyunsaturated to saturated ratio appeared to have little effect. The differences in fluidity between fatty acids were similar to those reported by Kuo et al,[45] who observed that cells enriched in either oleic or linoleic acid were more fluid than cells grown in saturated fatty acids.

The relationship between fluidity and binding affinity was weak and did not reach significance. Yet there may have been some contribution of fluidity to the differences observed in binding affinity. Differences in fluidity could cause orientation of the receptor in the cell membrane to be altered such that either a different number or orientation of active sites was available. However, because the correlation between fluidity and binding was low, it is likely that other properties of the fatty acids contributed to the large changes in binding affinity. Kuo et al[45] found a stronger relationship between binding and fluidity, and therefore concluded that membrane fluidity explained most of the fatty acid–induced changes in LDL binding. Differences between results of these two studies may be due to the large differences that were obtained for Bmax in CHO cells.

Results for the LDL receptor are similar to several studies of fatty acid modification of the membrane environment of the insulin receptor.[47-49] Berlin et al[47] reported that the type of dietary fat affected insulin binding affinity and receptor number, yet the effect appeared to be only weakly related to measures of fluidity. Others have reported similar results for the insulin receptor, with saturated fats associated with increasing affinity but decreasing receptor number.[48-50] Thus, fatty acid modification of cell membranes appears to alter insulin and LDL receptor binding in a similar way.[51]

No effects of the various fatty acids were seen on cell growth or cell size. Similar results were reported by Rintoul et al,[52] who found that CHO cells grown in fatty acid–enriched media grew as well as cells grown in medium containing LDS.

This study, conducted in a cell in which regulation of LDL receptor number is under the control of the SV40 promoter, has shown that the binding affinity of LDL is altered by fatty acid modification of the membrane. Cells grown in saturated fatty acids have a greater binding affinity than those grown in polyunsaturated fatty acids. On the other hand, the apparent number of LDL receptors, as determined by Scatchard analysis, was altered by fatty acid modification of the cell membrane. Cells grown in saturated fatty acids appeared to have fewer receptors than those grown in polyunsaturated fat. These data suggest that the type of dietary fat may influence LDL metabolism via changes in the cell membrane independent of any effect on the LDL particle. In addition, since analogous results have been reported in studies of the insulin receptor, these results point to a more general effect of changes in cell fatty acids on membrane functions. Changes in fluidity may explain some of the decrease in LDL binding when cells are grown in saturated fatty acids, but do not appear to explain all of the effects. These results may explain some of the reduction in LDL clearance observed when saturated fat is consumed.

Although it is clear that the main effects of the fatty acids were at the cell surface, we cannot separate the effects on binding affinity from those on Bmax in these experiments. Further studies will be required to examine the possible mechanisms of the observed changes in LDL receptor number.

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