Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women

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Abstract Studies with animal models suggest that saturated fatty acids raise low density lipoprotein (LDL)-cholesterol levels by reducing LDL receptor-mediated clearance. To examine this directly in humans, we studied the effects of lowering dietary saturated fat on LDL-receptor abundance in peripheral mononuclear cells which reflects hepatic LDL-receptor status. Healthy males and females (n = 25) participating in the DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) Study consumed three experimental diets in a randomized cross-over design. Diets provided 34% fat, 15% saturated fatty acids (Average American Diet); 29% fat, 9% saturated fatty acids (Step-One Diet); and 25% fat, 6% saturated fatty acids (Low SAT Diet). Peripheral mononuclear cells were isolated from blood samples collected after 6 and 8 wk. An ELISA was used to quantify LDL-receptor protein in total cell membranes. LDL-receptor abundance increased by 10.5% after the Low SAT Diet ($P < 0.05$). This was associated with an 11.8% decrease in serum LDL-cholesterol ($P < 0.05$). A linear inverse relationship was observed between the percentage change in LDL-cholesterol and the percentage change in LDL-receptor abundance ($r = -0.59$; $P < 0.01$). In addition, LDL-receptor abundance also was correlated inversely ($P < 0.001$) with serum levels of LDL-cholesterol ($r = -0.747$) and apoB ($r = -0.593$). In summary, reducing dietary saturated fat is associated with an increase in LDL-receptor abundance of magnitude similar to the decrease in serum LDL-cholesterol. Thus, an important mechanism by which reductions in dietary saturated fatty acids decrease LDL-cholesterol in humans is through an increase in LDL-receptor number.—**Mustad, V. A., T. D. Etherton, A. D. Cooper, A. Mastro, T. A. Pearson, S. S. Jonnalagadda, and P. M. Kris-Etherton.** Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. *J. Lipid Res.* 1997. **38:** 459–468.

Supplemetary key words LDL cholesterol • LDL receptor • saturated fatty acids • mononuclear cells • ELISA assay

The results of numerous animal studies (1–10) have shown that the hypercholesterolemic effect of saturated fatty acids (SFA) is, in part, the result of a decrease in hepatic uptake of low density lipoprotein (LDL) from the circulation. Collectively, these studies suggest that changes in the hepatic LDL-receptor (LDLr) activity as a result of changes in the fatty acid composition of the diet are of primary importance in altering LDL-cholesterol (LDL-C) levels. Data from studies with hamsters (11) and pigs (12) provide support for an effect of dietary fatty acid modification on hepatic LDLr number. These studies have shown that dietary SFA decrease while dietary polyunsaturated fatty acids (PUFA) increase membrane-associated LDLr in the liver. To date, however, there is no direct evidence that this also occurs in humans.

To reduce the risk of coronary heart disease, public health recommendations have been made that all Americans over the age of two reduce their intakes of total fat and SFA. The evidence is clear that making these dietary changes will lower plasma total- and LDL-C levels, of which the latter is a powerful risk factor for

Abbreviations: LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; LDLr, LDL receptor; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MNC, mononuclear cells.

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coronary heart disease. Recently, Protocol 1 of the DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) Study[4] was conducted to examine the effects of reducing total fat and saturated fat on plasma lipids and thrombogenic factors in a diverse population (with respect to gender, age, race) of healthy adults (13). To determine whether the reduction in dietary SFA also affected LDLr protein abundance, this ancillary study was conducted with a subsample of subjects participating at The Pennsylvania State University Field Center. Because it was not feasible to obtain human liver samples, we assessed the effects of these dietary manipulations on LDLr number on peripheral mononuclear cells (MNC). Among the advantages of studying MNC is their ready accessibility and the fact that the regulation of lipoprotein uptake in MNC appears to resemble that of hepatocytes (14–17). Peripheral MNC have proven to be a valuable model for studies of diet and pharmacological interventions on LDLr activity (18–24). Thus, we elected to use these cells to investigate the effects of decreasing dietary SFA on LDLr protein abundance in healthy male and female participants of the DELTA Study.

METHODS

A detailed description of the design of the DELTA Study is presented elsewhere (13). Methods pertinent to the present study are described below.

Subjects

This study was conducted with 25 subjects ages 22–65 years (mean age, 37). Of these, 15 were male (2 African-American) and 10 were female [7 premenopausal (1 African-American) and 3 postmenopausal]. All subjects were healthy as determined from extensive medical questionnaires, physical examination, and blood tests. Baseline total-cholesterol levels (mean 215 ± 35 mg/dl (SD)) were between the 25th and 90th percentile for age, race, and gender (25). Plasma triglycerides (100 ± 30 mg/dl) and HDL-cholesterol (HDL-C) levels (51 ± 11 mg/dl) were below the 90th percentile and above the 10th percentile, respectively. This study was approved by The Pennsylvania State University Institutional Review Committee.

Study design

The DELTA Study used a randomized, double-blind, three-way crossover design. Each diet period was 8 weeks in length, with breaks of 4–6 weeks between diet periods. All meals and snacks were provided to participants; every subject ate two meals each weekday in a supervised cafeteria setting. Other weekday meals, snacks, and all weekend meals were packaged and eaten by participants at a time and place of convenience. A self-selected meal that met the guidelines of a Step-One Diet was offered as an option to subjects (on Saturday evening), if desired, to enhance compliance with the experimental diet protocol. All food was weighed or measured and diets were prepared individually for each participant based upon their caloric needs. Subjects were weighed daily during the week and adjustments were made so that weight did not change more than ± 1 kg during the study.

Blood samples were collected once/wk during weeks 6 and 8 from subjects who had fasted 12–14 h. The average of these values for lipids/lipoproteins and LDLr protein abundance was used in the present study. Blood for the lipid and lipoprotein analyses was collected into silicone gel-coated tubes for subsequent serum preparation. Blood (30 ml) for the mononuclear cell isolation was collected into tubes containing heparin. All samples were processed immediately.

Diets

The diets fed in this study were: an Average American Diet (AAD) that provided 34% of calories from fat and 15% from SFA; a Step-One Diet with 29% of calories from fat and 9% from SFA; and a very low saturated fat diet (Low SAT) with 25% of calories from fat and 6% from SFA. All experimental diets provided 275 mg of cholesterol, 13% and 6% of calories from monounsaturated and polyunsaturated fat, respectively. Dietary carbohydrate was 48%, 55%, and 59% of calories on the AAD, Step-One, and Low SAT Diets, respectively. All diets provided 15% of calories from protein.

The nutrient composition of the experimental diets was validated by the Food Analysis Laboratory Control Center located at Virginia Polytechnic and State University. In addition, nutrient composition of the experimental diets was monitored throughout the study to assure that nutrient specifications were met.

Serum lipid determination

Serum was obtained by centrifugation at 1500 *g* for 30 min. Aliquots (0.5 ml) were stored in cryovials at −80°C until the end of the study when all samples were analyzed. Serum total cholesterol, LDL-C, HDL-C, and triglyceride levels were quantified by enzymatic analysis at the Mary Imogene Bassett Research Institute in Cooperstown, NY (13). HDL-C was determined after precipitation of apolipoprotein B-containing lipoproteins with dextran-sulfate (MW 50,000). LDL-C levels were calcu-

[4]The DELTA Study is a multicenter clinical nutrition trial funded by the National Heart, Lung, and Blood Institute, 1992–1996.

lated. Measurements of apolipoprotein B (apoB), apolipoprotein A-I (apoA-1) were performed using rate immunonephelometry (Beckman Array, Brea, CA); Lp[a] was measured using Macra Lp[a] ELISA (Strategic Diagnostics, Newark, DE).

Mononuclear cell isolation

Mononuclear cells were isolated by centrifugation on a Ficoll gradient as described by Boyum (26). Thirty ml of whole blood was diluted with 15 ml of Hank's buffered salt solution (HBSS) without Ca^{2+} and Mg^{2+}. The diluted sample was underlaid with 10 ml of Hypaque-Ficoll (Sigma Chemical Co., St. Louis, MO) and centrifuged at room temperature for 30 min at 500 *g*. The interface containing the mononuclear cells was removed with a pipette, diluted with 10 ml HBSS, and centrifuged at 4°C for 10 min at 600 *g*. The cell pellet was washed twice and resuspended in 10 mM Tris-buffer (150 mM NaCl, 1 mM $CaCl_2$, pH 7.4) and frozen at −70°C until the end of the study when all samples could be assayed together. Total cell membranes were prepared after homogenizing the cell suspension on ice using a polytron (setting 6 for 20 sec). The homogenate was centrifuged at 800 *g* for 4 min (4°C) and the supernatant was centrifuged at 100,000 *g* for 60 min. The pellet was resuspended in 0.25 M sucrose, 1 mM EDTA, 20 mM Tris-HCl (pH 7.4) and 100 mM leupeptin, and used for the assay of LDLr.

LDL receptor protein assay

An enzyme-linked immunosorbent assay (ELISA) was used to quantify LDLr protein using the procedure described by May et al. (27). The polyclonal rabbit anti-rat LDLr IgG antibody used in this assay has been characterized for the measurement of LDLr in human cells (27) and does not recognize the LDLr-related protein nor the oxidized LDLr (28), both of which are present on mononuclear cells.

The ELISA assay conditions were optimized using 2 μg of solubilized membrane protein per well. Samples were incubated overnight at 37°C in 96-well plates (Immulon-2, Dynatech, Chantilly, VA). After blocking with bovine serum albumin [BSA; 3.0% in phosphate-buffered saline (PBS)], the anti-LDLr IgG was added in excess at a concentration of 100 μg/ml. The amount of adherent antibody was determined using a second alkaline phosphatase-conjugated IgG (Zymed Laboratories, San Francisco, CA) and the color reaction was quantified in a microelisa reader by absorbance at a dual wavelength of A405/490. Nonspecific binding was determined in parallel samples using normal rabbit IgG (Zymed Laboratories, San Francisco, CA) and was typically less than 20% of specific binding. The nonspecific binding was subtracted from the total binding to determine specific binding and the absorbance units of the unknown samples were compared to that obtained from the standard curve. To construct a standard curve, plates were coated with excess goat anti-rabbit IgG (1 μg/ml in PBS) and anti-LDLr IgG was added to triplicate wells (0.078, 0.156, 0.312, 0.625, 1.25, 2.5, and 5.0 ng/100 μl in PBS containing 0.3% BSA and 1 mM $CaCl_2$). After incubation and washing, alkaline phosphatase was quantified calorimetrically and a standard curve was developed (**Fig. 1**). Typically, the *r* was always greater than 0.95. The absorbance of unknown samples was expressed as ng anti-LDLr antibody bound.

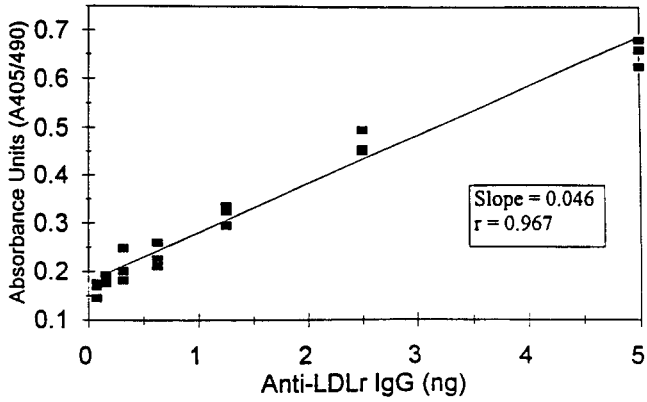

Fig. 1. A typical standard curve obtained using the ELISA assay for LDL receptor (LDLr) protein. To construct the standard curve, ELISA plates were coated with excess goat anti-rabbit IgG (1 μg/ml in phosphate-buffered saline) and anti-LDLr IgG was added to triplicate wells (0.78, 0.156, 0.312, 0.625, 1.25, 2.5, 5 ng/100 μl phosphate-buffered saline). The amount of adherent anti-LDLr IgG was determined using a second IgG conjugated to alkaline-phosphatase, and the color reaction was quantified using a dual wavelength absorbance of A405/490.

All assays were performed using a single antibody preparation. The intra-assay variation in LDLr protein abundance averaged 4.8% ± 2.7% (n = 6). The inter-assay variability of triplicate determinations of a control membrane preparation measured within each assay was 7% ± 3%; in order to compare the results of samples within and across diet periods that were assayed on separate days, a correction factor was used based on the control value.

Statistics

Differences between the endpoint measurements as affected by diet were analyzed with analysis of variance (ANOVA). Tukey's test was used to determine significant differences between the three experimental test diets. Finally, regression analysis was performed and Pearson's correlation coefficients were determined to evaluate the relationship between LDLr protein levels and plasma lipid and lipoprotein concentrations. Relationships between the change in plasma lipids in re-

TABLE 1. Effect of reducing dietary total fat and saturated fatty acids on plasma total and lipoprotein cholesterol and apolipoproteins

Parameter	Experimental Diets		
	AAD	Step-One Diet	Low SAT Diet
		MG/DL	
TC	215 ± 31[a]	203 ± 31[ab]	195 ± 27[b]
LDL-C	144 ± 26[a]	133 ± 25[ab]	127 ± 23[b]
HDL-C	51 ± 11[a]	47 ± 11[a]	44 ± 9[a]
TG	104 ± 36[a]	115 ± 46[a]	112 ± 41[a]
ApoB	132 ± 26[a]	127 ± 27[a]	125 ± 23[a]
ApoA-I	140 ± 20[a]	132 ± 20[a]	126 ± 18[a]
Lp[a]	23 ± 22[a]	26 ± 24[a]	27 ± 24[a]

Values are means ± SD (n = 25 subjects). AAD, Average American Diet; TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; apoB, apolipoprotein B-100; apoA-I, apolipoprotein A-I; Lp[a], lipoprotein [a].

[ab]Means within rows with different superscripts are significantly different ($P \leq 0.05$).

sponse to diet and the change in LDLr protein abundance were determined similarly.

RESULTS

Effects on serum lipids

As expected, there were significant effects of diet on several lipid endpoints. Compared to the AAD, reducing total and saturated fat was associated with decreases in total-, LDL-C, and HDL-C levels (**Table 1**). Significant reductions in total- and LDL-C (approximately 10–12%) were observed only after consumption of the Low SAT Diet compared to the AAD ($P \leq 0.05$). This is in contrast to the results reported from the larger multicenter DELTA Study with 103 subjects (29) that demonstrated significant ($P \leq 0.01$) reductions in these endpoints after both the Step-One Diet and the Low SAT Diet. Similarly, the decrease in HDL-C, apoA-I and apoB and the increase in Lp[a] and triglycerides in response to the lower fat and saturated fat diets were not statistically significant, although the trends observed are consistent with those changes that were significant ($P \leq 0.01$) in the larger study (29).

Effects on mononuclear cell LDLr protein abundance

LDLr protein on peripheral MNC was higher when subjects consumed the lower fat diets (**Fig. 2**). Compared to the AAD, LDLr protein abundance was increased by 6% after the Step-One Diet and 9% ($P \leq 0.05$) after the Low SAT Diet. These changes are similar in magnitude (but, as would be expected, in an opposite direction) to the decrease in LDL-C levels in response to the lower fat diets. Although the increase in LDLr protein abundance between the AAD and the Step-One Diet was not significant, multiple regression analysis showed a significant and linear inverse relationship between the intake of dietary SFA on the controlled diets and LDLr protein abundance on peripheral MNC (i.e., the slope of the regression coefficient for SFA, % kcals, was negative, Fig. 2).

Correlation between serum lipids and apolipoproteins and LDLr on MNC

Total cholesterol ($r = -0.687$; $P < 0.001$), LDL-C ($r = -0.747$; $P < 0.001$), and apoB ($r = -0.593$; $P < 0.001$) were significantly and negatively correlated with LDLr protein on MNC (**Figs. 3A and 3B,** and **Fig. 4A,** respectively). These linear relationships were statistically significant ($P \leq 0.001$) when the combined data were analyzed (as shown in Figs. 3 and 4), as well as when analyzed separately for each individual (n = 25) within each diet period ($P \leq 0.05$; data not shown). Other serum lipids such as HDL-C, triglycerides, apoA-I, or Lp[a] were not associated with LDLr protein abundance (Figs. 3C and 3D, Figs. 4B and 4C).

To further examine the relationship between LDLr and LDL-C, correlational analysis was conducted between the percentage change in LDLr abundance and percentage change in LDL-C as a result of decreasing dietary saturated fat. As can be seen in **Fig. 5,** there was a significant association ($r = -0.59$; $P < 0.01$) between the change in LDLr and the change in LDL-C. The regression equation shows that for every 1% increase in LDLr protein abundance there is a 0.73% decrease in LDL-C. This relationship is statistically significant when the combined data are analyzed for all diet combinations (n = 75), as well as when analyzed separately (not shown) for changes between the AAD and Step-One Diet ($r = -0.53$, $P < 0.05$; n = 25) and AAD and Low SAT Diet ($r = -0.63$, $P < 0.05$; n = 25). There was no significant correlation between the change in LDLr and LDL-C between the Step-One and Low SAT diets ($r = -0.25$, ns; n = 25).

DISCUSSION

The present study provides direct evidence that decreasing dietary SFA increases the number of LDLr available for removing LDL from the circulation in normocholesterolemic, healthy men and women. That the changes in LDLr were similar in magnitude to the decrease in LDL-C levels suggests that reducing dietary SFA lowered LDL-C levels primarily by increasing LDLr number. These data are consistent with isotope-kinetic

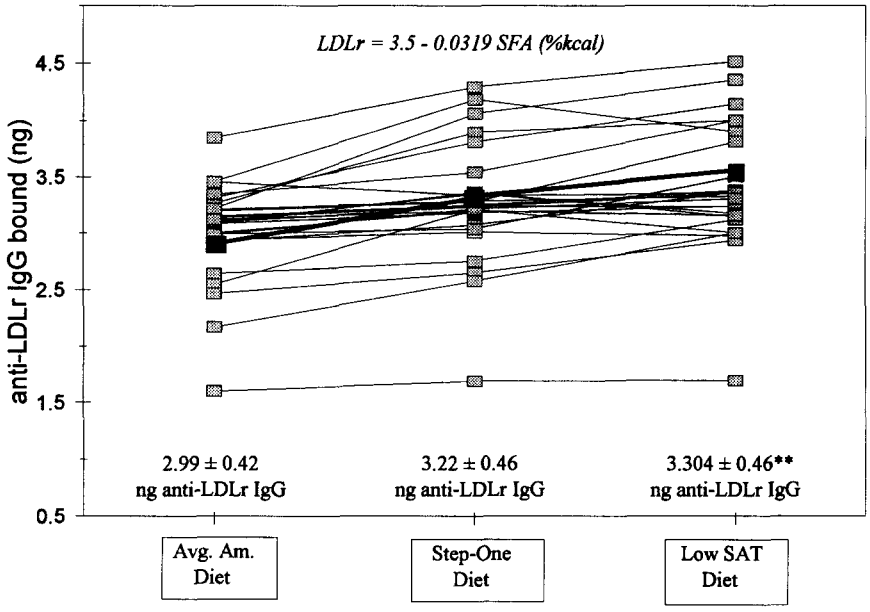

Fig. 2. Line graph showing response of LDLr protein abundance (ng anti-LDLr IgG bound) as measured on peripheral MNC in individuals consuming diets that differed in total and saturated fat. Analysis of variance and follow-up pairwise (Tukey's) test showed a significant increase in LDLr protein abundance between the Average American Diet (AAD) and the Low SAT Diet (** = $P \leq 0.05$). The thick line represents the average response for LDLr protein abundance for the (n = 25) participants. The regression equation represents relationship between changes in LDLr protein abundance as dietary SFA was progressively decreased in the three experimental diets.

studies in humans (30, 31) and animals (1, 2, 5, 6, 10) that demonstrate that the LDL-raising action of SFA is the result of impaired hepatic uptake of LDL from the circulation. Furthermore, these data are consistent with animal studies that show that large changes in dietary SFA can markedly suppress hepatic LDLr levels (11, 12).

Peripheral MNC have been used extensively in studies where diet or pharmacological interventions on LDLr binding activity are of interest (18–24). The 'qualitative' effects of these treatments on MNC LDLr activity generally are consistent with those predicted from animal studies of LDLr activity on hepatic tissues; however, there often is no clear relationship between the changes in LDL-C in response to the interventions and changes in LDLr activity. For example, a number of studies reported that the receptor-mediated degradation of ^{125}I-labeled LDL increased from 2-fold (20, 22, 24) to 16-fold (21) in response to the interventions while the LDL-C levels generally decreased less than 20%. In addition, individual data from normocholesterolemic subjects fail to show any clear relationship between LDLr activity and plasma LDL-C or apoB levels (22). Collectively, these studies suggest that direct quantification of LDLr levels in MNC may be a better estimate of hepatic LDLr status than measuring LDLr activity in vitro. LDLr activity can be influenced by the composition of the LDL particle (32) or perhaps other factors associated with the limitations inherent to measuring LDL binding in vitro (33), especially in circumstances where the number of LDLr is low (27). Thus, while measuring LDLr activity may be useful in assessing generalized or qualitative effects of treatments on LDLr status, the predictive value of these measures in defining the effects of dietary factors on LDLr status is limited. While the increases we observed in LDLr protein abundance on MNC between the AAD and Step-One Diet (7.7%, n.s.) and between the AAD and Low Sat Diet (10.5%, $P < 0.05$) were modest, they nonetheless were similar in magnitude to the decreases in LDL-C levels (i.e., 7.6% and 11.8%, respectively). We also observed a highly significant and inverse relationship between the percentage change in LDLr protein abundance and the percentage change in serum LDL-C ($r = -0.59$; $P < 0.01$; Fig. 5). That is, those individuals who had the greatest increase in LDLr protein abundance in response to reductions in dietary saturated fat also had the greatest decrease in LDL-C levels. This relationship was observed across the wide range of responses to changes in saturated fat intakes. The exception was that there was no significant relationship between these parameters when the participants changed from the Step-One Diet to the Low Sat Diet (Fig. 5). This exception likely reflects the small range of changes in both

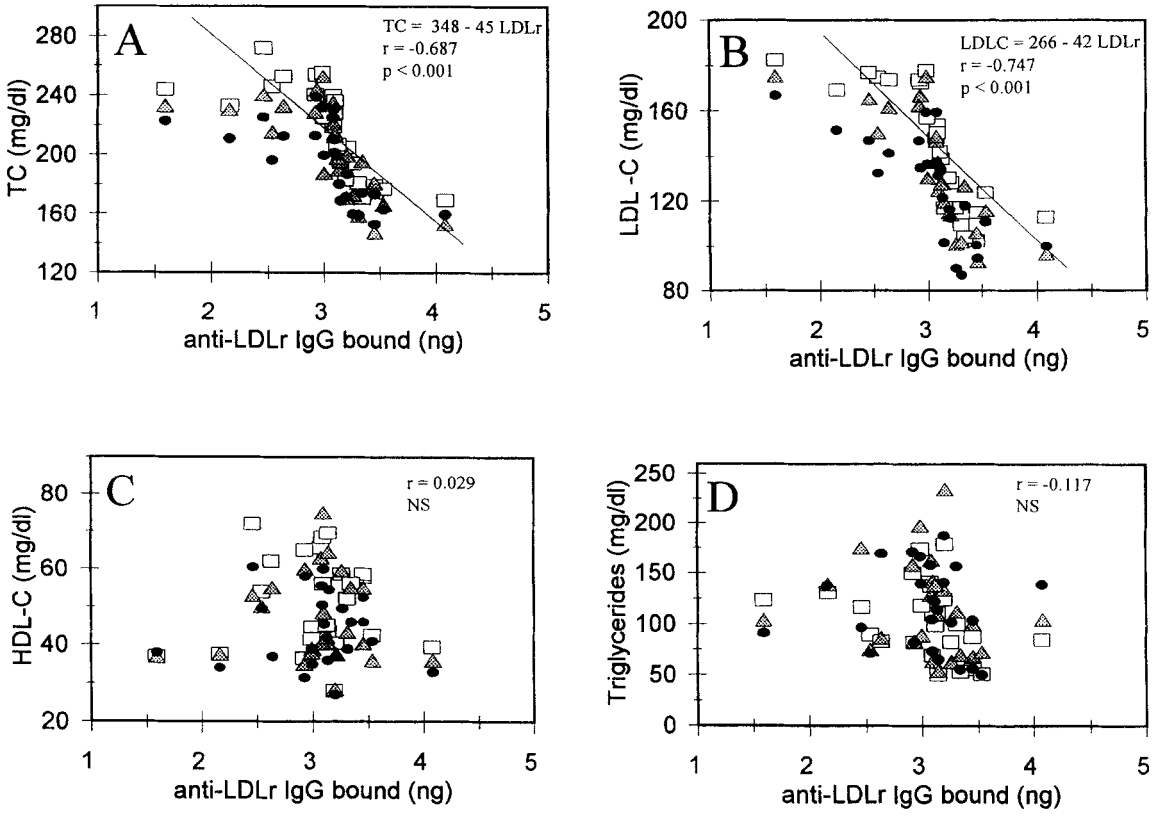

Fig. 3. Correlations between LDL receptor (LDLr) protein abundance (ng anti-LDLr IgG bound) on mononuclear cells and serum total lipids and lipoprotein cholesterol (mg/dl). Data are from individual subjects (n = 25) on Average American Diet (square), Step-One Diet (triangle), and Low SAT Diet (circle). The regression equation (listed if significant), correlation coefficient (*r*), and level of significance for all subjects' data combined are noted. Plots A, B, C, and D display the correlation between LDLr protein abundance and serum total cholesterol (TC), LDL-C, HDL-C, and triglycerides, respectively.

LDL-C and LDLr as a result of decreasing saturated fat from 9% to 6% of calories rather than any mechanistic differences in the regulation of LDL-C. Taken together, these data support the hypothesis that a major mechanism by which dietary SFA affects plasma LDL-C in humans is mediated through changes in LDLr number.

As expected, we found a wide range of LDLr protein levels in our participants and we also observed a wide range of responses to dietary SFA reduction (Fig. 2). It has been hypothesized that the cholesterolemic response to dietary SFA (in particular C16:0) might be dependent upon the individual's LDLr status or extent of LDLr down-regulation (34). In this study, however, there was no relationship between the baseline level of LDLr protein abundance (assessed on the AAD/control diet) and the predicted cholesterolemic response to reductions in dietary SFA (data not shown). Similarly, there was no relationship between serum LDL-C levels and the LDLr response to diet; specifically, the LDL-C levels of those individuals who decreased or had no change in LDLr in response to the dietary intervention (Fig. 2) were spread across the range of LDL-C levels of the study group. Thus, these data show that changes in LDL-C are primarily dependent upon the extent to which the LDLr protein is influenced by changes in the fatty acid composition of the diet.

The inverse relationship between plasma LDL-C, apoB-100, and LDLr abundance, independent of diet (Figs. 3B and 4B), is not surprising. Although the synthesis and secretion of apoB-100 and the amount of VLDL converted to LDL, as well as the amount of VLDL removed directly from plasma, also represent ways of influencing circulating LDL concentrations (35), there is a large body of evidence which demonstrates that once formed, the lifetime of LDL in the plasma is predominantly determined by the abundance of LDLr (35–37). Furthermore, uptake of LDL by the LDLr pathway in normal humans has been shown to range from 50% (30) up to 80% (38) of total LDL uptake. Our data with circulating MNC that express LDLr are consistent with other studies that have reported a strong linear relationship between LDLr protein abundance in hepatic membranes and plasma LDL-C in humans (39, 40).

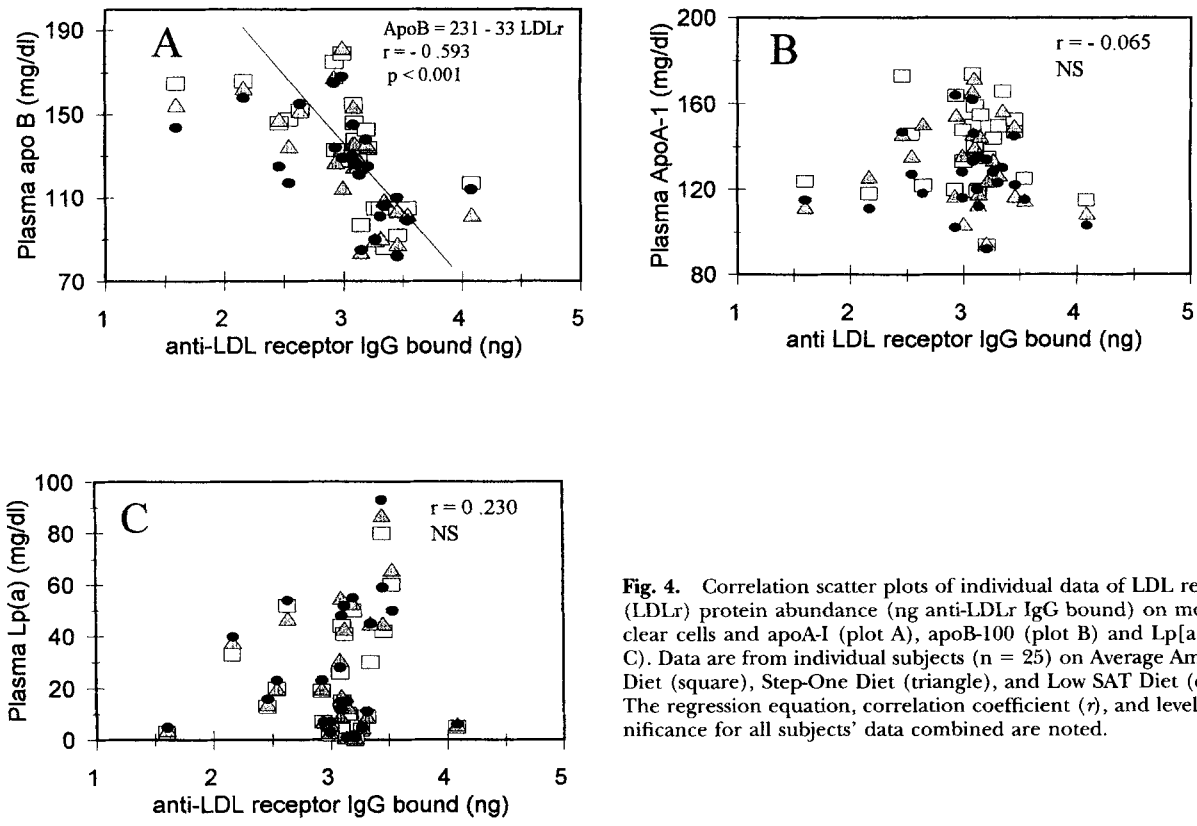

Fig. 4. Correlation scatter plots of individual data of LDL receptor (LDLr) protein abundance (ng anti-LDLr IgG bound) on mononuclear cells and apoA-I (plot A), apoB-100 (plot B) and Lp[a] (plot C). Data are from individual subjects (n = 25) on Average American Diet (square), Step-One Diet (triangle), and Low SAT Diet (circle). The regression equation, correlation coefficient (r), and level of significance for all subjects' data combined are noted.

Because the plasma LDL-C concentration (average 130 mg/dl) is much higher than the K_d of LDL for the LDLr [equivalent to 1.54 mg/dl (41)], it is unlikely that the decrease in plasma LDL-C in response to a reduction in SFA would cause the up-regulation of LDLr protein abundance in MNC (or that the serum LDL-C level determines the LDLr in these circulating cells). Although the precise mechanisms by which fatty acids affect LDLr protein abundance are unclear, we (12) and others (11) have demonstrated in different animal models that large changes in dietary SFA can decrease LDLr mRNA levels in the liver, providing evidence that dietary fatty acids affect LDLr at the level of gene expression. Although the molecular mechanisms by which fatty acids affect LDLr protein and mRNA abundance are unknown, it is possible that they act directly by affecting mRNA stability and/or transcription rate of the LDLr gene as has been shown for other genes (42–44), or perhaps indirectly through altering the regulatory pool of sterols known to control LDLr gene transcription (36). With respect to the latter point, studies in hamsters (45) and different cell types (46) suggest that SFA suppress the esterification of free cholesterol to the inert cholesterol ester pool. Our own work with pigs, however, showed that SFA have a repressive effect on hepatic LDLr protein and mRNA abundance independent of changes in hepatic free cholesterol and cholesterol ester content (12). Another hypothesis is that SFA may alter the composition and physical properties of the endoplasmic reticulum, thereby interfering with the proteolytic release of membrane-bound sterol regulatory-element binding protein(s) (SREBP) that plays an important role in transcriptional regulation of the LDLr gene (47). Recently, a 10 kDa cysteine protease, CPP32, that releases SREBP from its transmembrane anchor has been shown to also require proteolytic cleavage from an inactive 32 kDa precursor (48). It will be of interest to determine whether dietary fatty acids have any influence on the activity of these proteases or on other related mechanisms of sterol-regulated transcription of the LDLr gene.

In summary, the present study has demonstrated that measuring LDLr protein abundance from MNC membranes is useful for quantitatively assessing LDLr status in healthy adult men and women. We showed that LDLr abundance in MNC is exclusively associated (inversely) with serum LDL-C and apoB-100 levels. Furthermore, our study is the first to show in humans that reductions in total fat and saturated fat increase LDLr protein abundance in direct proportion to the decrease in se-

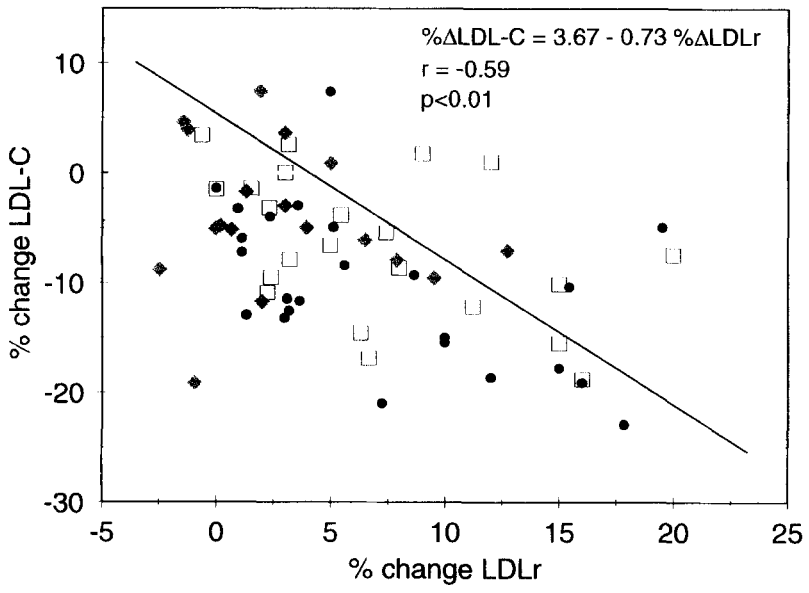

Fig. 5. Correlation scatter plots of individual data of LDL receptor (LDLr) protein abundance (ng anti-LDLr IgG bound) on mononuclear cells and the percentage change in LDLr protein abundance and the percentage change in serum LDL-C levels between the Average American Diet and the Step-One Diet (square), Average American Diet and Low SAT Diet (circle), and Step-One Diet and the Low SAT Diet (diamond). The regression equation, correlation coefficient (r), and level of significance for all data (n = 75) are indicated.

rum total and LDL-C levels. Thus, an important mechanism by which fatty acids affect LDL-C in healthy men and women is mediated through changes in LDLr number.

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