

Individual Variation in the Effects of Dietary Cholesterol on Plasma Lipoproteins and Cellular Cholesterol Homeostasis in Man

STUDIES OF LOW DENSITY LIPOPROTEIN RECEPTOR ACTIVITY AND 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN BLOOD MONONUCLEAR CELLS

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ABSTRACT The effects of dietary cholesterol on plasma lipoproteins and cholesterol homeostasis in blood mononuclear cells have been examined in healthy adults. Addition of 1,500 mg of cholesterol to the daily diet of 37 subjects for 14 d was associated with a wide range of response of plasma total cholesterol concentration (from -6 to +75 mg/dl; mean change, +29 mg/dl; $P < 0.001$). Increases in plasma cholesterol reflected increased cholesterol concentrations in intermediate density lipoprotein (IDL; 1.006–1.019 g/ml), low density lipoprotein (LDL; 1.019–1.063 g/ml), and the HDL₂ subclass (1.063–1.125 g/ml) of high density lipoprotein, which on average accounted for 20, 58, and 22%, respectively, of the total increment. Similar responses occurred in 14 other subjects given 750 mg cholesterol per day for 28 d. Plasma apolipoprotein B concentrations in IDL and LDL also increased.

These effects on plasma lipoproteins were accompanied by three changes in freshly isolated blood mononuclear cells: (a) an increase in cell cholesterol content (mean change, +17%; $P < 0.01$); (b) suppression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity (-32%; $P < 0.001$); and (c)

reduction of LDL receptor activity (-74%; $P < 0.01$), quantified as the rate of degradation of ¹²⁵I-LDL to noniodide trichloroacetic acid-soluble material. These results provide the first direct evidence for the modulation of LDL receptor activity and HMG CoA reductase activity in a peripheral cell type in response to a dietary perturbation of human lipoprotein metabolism.

The percentage increase in LDL cholesterol was negatively correlated with the percentage decrease in HMG CoA reductase activity ($r = -0.49$, $P < 0.01$). An additional negative correlation existed between the increment in plasma cholesterol concentration and the capacity of cells to degrade ¹²⁵I-LDL after derepression by preincubation for 72 h in lipoprotein-deficient medium ($r = -0.74$, $P < 0.001$). Thus, differences between individuals in the responses of the plasma lipoproteins to dietary cholesterol appear to be related in part to differences in the capacity of peripheral cells to catabolize LDL and to down-regulate cholesterol synthesis.

INTRODUCTION

Dietary cholesterol is a determinant of the plasma cholesterol concentration in man (1). Considerable differences have been reported between individuals, however, in the magnitude of the change in plasma cholesterol induced by a given change in cholesterol consumption (1–5). Steroid balance studies have shown that there are also large differences between individuals in the extent to which dietary cholesterol is retained within body cholesterol pools (6).

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The biochemical basis of the variable effect of dietary cholesterol on plasma and tissue cholesterol in man has not been completely elucidated. Two metabolic responses to dietary cholesterol have been demonstrated in human subjects: suppression of total body cholesterol synthesis and increased fecal steroid excretion (6–9). At least part of the decrease in cholesterol synthesis occurs in the liver (10). Nestel and Poyser (9) noted that suppression of cholesterol synthesis (measured by steroid balance) was the greater metabolic response in subjects who showed only a small increase in plasma cholesterol, whereas in those who developed marked hypercholesterolemia an increase in steroid excretion was the predominant response.

In the rat the suppression of cholesterogenesis by dietary cholesterol occurs in several peripheral tissues, as well as in the liver (11). This effect probably occurs in response to an increase in the cellular uptake of chylomicron remnants (12) and low density lipoprotein (LDL),¹ the plasma LDL concentration being increased by cholesterol feeding in the rat (13). Tissue culture studies have now demonstrated receptors for LDL in the surface membranes of a variety of peripheral cells, including fibroblasts, smooth muscle cells, and lymphocytes (14). Binding of LDL to these receptors results in endocytosis of the lipoprotein followed by lysosomal degradation. Cholesterol released by hydrolysis of LDL cholesterol ester exerts three effects: (a) inhibition of LDL receptor synthesis, thereby reducing further LDL uptake; (b) inhibition of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase; and (c) stimulation of acyl coenzyme A: cholesterol acyltransferase, resulting in increased cellular cholesterol ester content. This "LDL receptor pathway" is thought to have two functions: the removal of LDL from plasma and the provision of peripheral cells with cholesterol for membrane synthesis. Evidence exists that the pathway operates *in vivo* (12, 15–17). It is not yet clear, however, whether or not the activity of LDL receptors in peripheral cells is a significant determinant of the plasma LDL concentration, or of its response to dietary change, in healthy humans. Nor has any direct evidence yet been presented for the modulation of LDL receptor activity or HMG CoA reductase activity in peripheral cells in response to dietary perturbation of plasma cholesterol concentration in man.

Although there is a considerable body of animal data, relatively little information has been published on the plasma lipoprotein changes induced by choles-

terol-rich diets in man. Studies in several species (13, 18–20) have demonstrated increases in very low density lipoproteins (VLDL) and LDL concentration in response to dietary cholesterol, and the appearance of two abnormal cholesterol-rich lipoproteins: a β -migrating particle of density < 1.006 g/ml (β -VLDL), and an α -migrating particle of density 1.02–1.09 g/ml containing apoproteins AI and E (HDL_c). What information is available from human studies suggests that the increase in plasma cholesterol induced by dietary cholesterol may be due largely to a rise in LDL concentration (21). Indirect evidence has been presented for the appearance of HDL_c in man (22).

The present study was undertaken with three objectives: (a) to further investigate the changes in plasma lipoproteins induced by dietary cholesterol in healthy humans; (b) to explore the associated effects on LDL receptor activity, HMG CoA reductase activity, and cholesterol content of blood mononuclear cells; and (c) to examine the relationships between the lipoprotein and metabolic measurements, in an attempt to gain further insight into the regulation of LDL catabolism and cellular cholesterol homeostasis in man.

METHODS

Experimental subjects. 51 healthy medical students and laboratory personnel (44 males, 7 females) participated in the study. Details of age, plasma lipid concentrations, relative body weight, and habitual cholesterol consumption are given in Table I. Habitual cholesterol consumption was assessed in 34 subjects by a dietitian by 7-d diet recall and use of food tables (23). For these subjects the proportion of calories derived from protein, carbohydrate, and fat averaged 13, 46, and 41%, respectively. Plasma cholesterol and glyceride concentrations in most subjects were below the 90th percentiles of an adult population in London (24); by these criteria two subjects had mild hypercholesterolemia, and two were hypertriglyceridemic. One subject, although normolipidemic, had β -migrating VLDL with a high cholesterol/glyceride molar ratio (1.54).

Experimental design. The experimental design was approved by the Ethics Committee of St. Thomas' Hospital. Two study protocols were followed. In study A the diets of 37 subjects were supplemented with six egg yolks per day (homogenized with orange juice) for 14 d. In study B 14 subjects were given a daily supplement of three egg yolks for 28 d. In each study consumption of the egg yolks was supervised by a dietitian. The subjects otherwise adhered to their habitual diets, and continued with their normal daily activities. Body weights remained essentially unchanged in 39 subjects in whom this was monitored, averaging 73.7 ± 2.8 kg (SEM) and 73.3 ± 2.8 kg at the beginning and the end of the study, respectively.

The cholesterol content per egg yolk, determined on a Technicon Auto Analyzer II (Technicon Instruments, Inc., Tarrytown, N. Y.) following chloroform:methanol (2:1, vol:vol) extraction of a sample of yolks, averaged 250 mg. Thus, the approximate daily cholesterol supplements were 1,500 mg in study A, and 750 mg in study B.

Plasma lipoprotein measurements. 10 ml of venous blood were collected without hemostasis, after a 14-h overnight fast, into disodium EDTA (1 mg/ml), and the plasma separated

¹Abbreviations used in this paper: HDL, high density lipoprotein; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

TABLE I
Ages, Plasma Lipids, Relative Body Weights, and Habitual Cholesterol
Intake of the Experimental Subjects

Study	No. of subjects	Age	Plasma cholesterol	Plasma glycerides	Percentage of ideal body weight*	Habitual cholesterol intake†
		yr	mg/dl			mg/dl
A	37	24±8 (19–47)	189±31 (131–268)	104±36 (35–380)	102±12 (86–143)	522±178 (217–827)
B	14	30±10 (19–60)	192±45 (139–271)	83±31 (22–143)	98±7 (84–108)	480±239 (234–978)

* Based on Metropolitan Life Insurance Tables (50).

† As assessed by 7-d recall of food intake (23).

Values are given as mean±SD (range).

Percentage of ideal body weight was quantified in only 25 subjects of study A; habitual cholesterol intake was quantified in 24 subjects of study A and 10 subjects of study B; other values refer to all subjects.

by centrifugation for 10 min. Lipoprotein fractions were then isolated by sequential preparative ultracentrifugation, as described by Havel et al. (25), between the following density ranges: <1.006 g/ml (VLDL), 1.006–1.019 g/ml (intermediate density lipoprotein, IDL), 1.019–1.063 g/ml (LDL), and 1.063–1.125 g/ml (HDL₂). The cholesterol content of the $d > 1.125$ g/ml infranate was considered to represent HDL₃. Density adjustments were made using NaCl and NaBr; ultracentrifugation was performed at 14°C in an angle-head rotor using an MSE Superspeed 50 centrifuge (MSE Scientific Instruments, Sussex, England); and the lipoprotein fractions were isolated by tube-slicing.

Cholesterol and triglyceride concentrations in whole plasma and in lipoprotein fractions were assayed by enzymatic procedures (Boehringer Mannheim Biochemicals, Indianapolis, Ind.; catalogue Nos.: 187 313 and 166 448). All measurements from a single subject were performed on the same day, after storage of the lipoprotein fractions at 4°C.

Losses of lipoproteins during ultracentrifugation were indirectly estimated from recoveries of cholesterol (mean 98%; range 89–107%) and glycerides (mean 92%; range 80–107%) relative to whole plasma values.

Total protein was assayed by the method of Lowry et al. (26), using crystalline bovine serum albumin as the standard. Apolipoprotein B (apo B) concentrations in VLDL, IDL, and LDL were measured by "rocket" electroimmunoassay (27), using 2% rabbit antiserum to human apo B (Boehringerwerke AG, Mannheim, West Germany) in 1.5% agarose-50 mM barbitone buffer (pH 8.6) and 10 vol/cm for 8–10 h. Standards (5 µl) of recentrifuged LDL of 1.030–1.050 g/ml d gave a linear response (rocket heights: 6–25 mm) over the range 40–250 µg apo B/ml. The antiserum showed no reaction against normal human HDL or serum from a patient with abetalipoproteinemia. The interassay coefficient of variation for apo B measurement was <5%.

Electrophoresis of plasma lipoproteins in agarose gel was performed as described by Noble (28). Acrylamide gel electrophoresis of the tetramethylurea-soluble apoproteins of VLDL was performed according to Kane et al. (29). Gels were fixed for 5 min in 12.5% (wt/vol) TCA, and stained overnight with Coomassie blue (0.01%, wt/vol). After destaining with 5% glacial acetic acid, the relative proportions of the different apoproteins were quantified by integrated

densitometry (Chromoscan 200; Joece-Loebl Ltd., Gateshead, England).

Isolation of mononuclear cells. Venous blood samples (100 ml) were obtained, after a 14-h overnight fast, immediately before and then 14 d after commencement of the dietary cholesterol supplement. 50-ml aliquots were defibrinated in sterile glass tubes, using the apparatus described by Wilson et al. (30). All subsequent manipulations of blood and mononuclear cells were carried out with sterile, siliconized glassware. Serum was separated from defibrinated blood by centrifugation at 2,500 rpm for 15 min (MSE model GF6 centrifuge). The packed cells were resuspended in an equal volume of preparative medium (Eagle's medium [Flow Laboratories Ltd., Irvine, Scotland] containing 0.22% [wt/vol] sodium bicarbonate, 100 U of penicillin/ml, and 100 µg of streptomycin/ml). 15 ml of the suspension was then layered onto 8 ml of Ficoll-Hypaque solution of density 1.077 g/ml (31), and centrifuged for 35 min at 1,750 rpm. The interphase layer of mononuclear cells was harvested, and washed twice in preparative medium by centrifugation at 1,400 rpm for 15 min. Finally, the cells were resuspended in RPMI 1640 culture medium (Flow Laboratories Ltd.) containing lipoprotein-deficient human serum (final protein concentration: 5 mg/ml) supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml). The interval between venepuncture and suspension of the cells in this medium was usually 3 h. Lipoprotein-deficient serum was prepared by ultracentrifugation of serum at a density of 1.215 g/ml for 48 h (25), followed by exhaustive dialysis against 0.15M NaCl, and sterilization by Millipore (0.45 µm) filtration (Millipore Corp., Bedford, Mass.)

Cell number was determined in a Coulter counter (Coulter Electronics, Hialeah, Fla.). Cell counts before and after exposure to an erythrocyte-lysing agent, Zaponin, revealed that a mean 9% of the total cells were erythrocytes. The yield of mononuclear cells from 100 ml of blood averaged 84×10^6 (range: 46×10^6 to 150×10^6). Differential counts of several hundred cells, using films stained with May-Grünwald/Giemsa stain, showed that ~95% of mononuclear cells were lymphocytes, the rest being monocytes. Essentially all of the cells were viable as measured by their ability to exclude trypan blue.

The possibility of significant contamination of the final

cell preparations with plasma proteins, including lipoproteins, was excluded in two ways. When the total mononuclear cells isolated from 20 ml blood were suspended in 1 ml Eagle's medium, no apo B could be detected in the suspension by electroimmunoassay, using the procedure already described. In other experiments, mononuclear cells were isolated from 20 ml blood, to which $\sim 5 \mu\text{Ci}$ of ^{125}I -albumin had been added, and suspended in 1 ml Eagle's medium. The total content of ^{125}I in the cell suspension was then measured by gamma counting, and found to be $<0.002\%$ of that originally added.

Derepression of mononuclear cells. Induction of maximum LDL receptor and HMG CoA reductase activities (derepression) was achieved by prolonged incubation of the cells in lipoprotein-deficient medium (17). Freshly isolated cells were suspended in RPMI 1640 culture medium containing 10% (vol/vol) lipoprotein-deficient serum at a cell concentration of $\sim 2 \times 10^6$ per ml. The suspensions were then divided into 15 to 20-ml aliquots in 250 ml siliconized conical flasks and left for 72 h in a humidified incubator (37°C , 5% CO_2 in air). Cell number, viability, and morphology remained constant.

HMG CoA reductase activity. Cell suspensions containing $\sim 10^7$ cells were centrifuged (1,400 rpm, 15 min) and the supernate discarded. The small number of erythrocytes in the cell pellet were removed by resuspension in 3 ml sterile distilled water for 10 s, followed by addition of 3 ml of twice-concentrated Dulbecco's phosphate-buffered saline A (Oxoid Laboratories Ltd., London, England) (32). The washed pellets were frozen in acetone-solid CO_2 , and stored at -70°C for subsequent assay.

All cell pellets from a given subject were assayed together using the same batches of substrate. Preparation of the post-mitochondrial supernatant fraction and assay of HMG CoA reductase activity were performed as described by Brown et al. (33). The [^{14}C]HMG CoA (New England Nuclear, Boston, Mass.) had a specific activity of 19–50 Ci/mol. The duration of each assay was 120 min. The amount of cell protein extract per assay was 100 μg ; the concentration of HMG CoA was 30–40 μM . Radioactivity was assayed in a Corumatic liquid scintillation counter (ICN, Hershham, England), with an efficiency of 78% for ^{14}C and 28% for ^3H . Recovery of [^3H]mevalonolactone internal standard averaged 59% (range 47–67%). Blank incubations were performed by adding 20 μl of 5 M HCl to the incubation mixture before the addition of ^{14}C -HMG CoA. In assays on freshly isolated cells collected during the control period, [^{14}C]mevalonate formation by the cell extracts averaged fivefold greater than the blank values. In many assays the mean values from incubations performed in duplicate were used for statistical analysis. The assay was shown to be linear with respect to time (0.5–2 h) and protein concentration (50–250 μg). The coefficient of variation for replicate assays using the same cell pellet was 3%. When duplicate measurements were made using cells collected on two different days (14–180 d apart) from seven subjects consuming their habitual diets, the results differed by 0–12% (mean, 9%).

LDL receptor activity of freshly isolated cells. The activity of high affinity LDL receptors in blood mononuclear cells was quantified from the capacity of the cells to degrade ^{125}I -labeled LDL to non-iodide TCA-soluble material in the presence and absence of excess unlabeled LDL (34). For these assays LDL was labeled with ^{125}I (Radiochemical Centre, Amersham, England) to a specific activity of 200–300 cpm/ng protein by a modification (34) of the iodine monochloride method of McFarlane (35), dialyzed against 0.15 M NaCl–0.3 mM Na_2EDTA , and sterilized by filtration (0.45 μm). Less than 5% of the total radioactivity in the final preparations was due to free iodide (quantified as TCA-soluble radioactivity) or to labeling of the lipid moiety

(soluble in chloroform: methanol, 2:1, vol:vol). For each experimental subject the same batch of ^{125}I -LDL, stored at 4°C under sterile conditions, was used for assays performed before and after dietary cholesterol supplementation. Previous studies have indicated that ^{125}I -LDL preparations stored for this length of time (14 d) under such conditions are suitable for receptor assays (36).

Cell suspensions in lipoprotein-deficient medium (see above), containing $4\text{--}7 \times 10^6$ cells/ml, were transferred in aliquots of 1.75 ml into 25- cm^2 polystyrene tissue culture flasks or 60 \times 15-mm polystyrene dishes (Corning Glass Works, Science Products Div., Corning, N. Y.). The cells were incubated (5% CO_2 in air, 37°C) for 4 h in a final volume of 2 ml with ^{125}I -LDL at a concentration of 25 μg protein/ml, in the presence and absence of a 25-fold excess of unlabeled LDL (34). Cell number and viability remained constant during the 4-h incubation, and there were no morphological changes on light microscopy. Blank values were obtained by performing identical incubations in the absence of cells. At the end of each incubation the flasks or dishes were immediately placed on ice to arrest ^{125}I -LDL degradation. The cell suspensions were then transferred into plastic centrifuge tubes with an additional 1 ml of culture medium, and sedimented by centrifugation in a refrigerated (4°C) centrifuge (MSE, Mistral 4L) at 2,500 rpm for 10 min. The culture medium was removed and assayed for non-iodide TCA-soluble material, as described below. Small numbers of erythrocytes in the cell pellets were removed by brief hypotonic lysis, as already described, after which the mononuclear cells were sedimented by centrifugation and the phosphate-buffered saline supernate discarded. (This procedure was shown not to significantly reduce the mononuclear cell number, as determined in a Coulter counter.) The cell pellets were then solubilized in 200 μl 1 M NaOH at room temperature for 12–18 h, and their protein content was measured by the method of Lowry et al. (26), using bovine serum albumin as standard.

The content of non-iodide TCA-soluble radioactivity in the culture medium was measured using the procedure originally described by Bierman et al. (37). TCA was added at a final concentration of 5% (wt/vol) to 2 ml of culture medium containing 0.5 ml "carrier" serum. The resultant precipitate was sedimented by centrifugation at 3,000 rpm for 20 min. Free iodide in the supernate was oxidized by mixing 1 ml with 50 μl of 30% H_2O_2 and 20 μl of 40% KI. After 5 min, molecular iodine was extracted with 2 ml chloroform, and the two phases separated by centrifugation at 4°C . An aliquot (500 μl) of the aqueous phase was then assayed for radioactivity in an LKB/Wallac gamma-counter (LKB Instruments, Inc., Rockville, Md.), counting with a standard error of less than $\pm 5\%$. Net degradation of ^{125}I -LDL to non-iodide TCA-soluble material by mononuclear cells was calculated as the difference between results obtained from identical incubations in the presence and in the absence of cells (36); freshly isolated cells collected during the control period gave mean values that were fourfold greater than the blank values. The component of cellular ^{125}I -LDL degradation that was competitively inhibited by a 25-fold excess of unlabeled LDL ($\sim 75\%$ of the total degradation) was considered to represent receptor-mediated catabolism (34).

LDL receptor activity of derepressed cells. The method used was identical to that described for freshly isolated cells, except that 2-ml cell suspensions containing $\sim 4 \times 10^6$ cells were incubated for 6 h with ^{125}I -LDL at a concentration of 10 μg protein/ml, in presence and absence of 25-fold excess LDL. High affinity degradation accounted for $\sim 90\%$ of total degradation. Cell viability and number did not change during the incubation.

The assay was shown to be linear with respect to time (1–6 h) and cell number. Precision was assessed by making duplicate measurements on cells collected from 49 fasted healthy subjects; the coefficient of variation was 7%.

Cell cholesterol content. Suspensions of $12\text{--}28 \times 10^6$ freshly isolated mononuclear cells in lipoprotein-deficient medium were centrifuged at 1,400 rpm for 15 min. Erythrocytes were removed from the cell pellet by hypotonic lysis (see above), and the resuspended mononuclear cells sedimented by centrifugation at 800 rpm for 15 min. Cell cholesterol content was then determined by gas-liquid chromatography, as described by Brown et al. (38), using 5 α -cholestane as internal standard. The analyses were performed in a Pye Unicam Series 104 instrument (Pye Unicam Limited, Cambridge, England), containing a 5-ft coiled glass column packed with 3% SE-30 in 80/100 mesh Chromosorb W.HP (Phase Separations Ltd., Queensferry, Wales) (column temperature, 280°C; carrier gas: argon, 75 ml/min). The detector response was linear up to 30 μg cholesterol/sample and sensitive to 0.5 μg . Recoveries, monitored with [^3H]cholesterol, were 90–95%. Serachol (Warner and Co. Ltd., Eastleigh, England), diluted with 0.15 M NaCl to a final concentration of 150 $\mu\text{g}/\text{ml}$, was used as standard. The intraassay coefficient of variation was 4.5% ($n = 18$).

RESULTS

Plasma lipoprotein changes induced by dietary cholesterol were examined in two studies.

Study A

Two blood samples were obtained on different days from all subjects while they were still consuming their habitual diets. The plasma cholesterol concentrations in these two base-line samples were not significantly different (mean difference, 3.6%). Similarly, plasma glyceride concentrations showed little difference (mean, 4.9%) on the 2 d.

Consumption of six egg yolks per day for 14 d increased the plasma total cholesterol concentration by 4–75 mg/dl in 32 of the subjects, had no effect in three subjects, and may have been associated with a small decrease in plasma cholesterol (–6 mg/dl) in two sub-

jects. The mean plasma cholesterol concentration for the 37 subjects was increased by 29 mg/dl ($P < 0.001$) (Table II). In contrast there was no significant change in plasma glyceride concentration (104 ± 10 vs. 98 ± 10 mg/dl). The change in plasma total cholesterol concentration was not significantly correlated with the base-line value (day 0), age, habitual cholesterol intake, or body weight.

Plasma lipoproteins were studied in 31 of the subjects. Since the lipoprotein responses of subjects in whom data were collected on habitual cholesterol intake and body weight were similar to those of individuals in whom this information was not obtained, the results from all subjects were pooled for analysis. The increases in plasma cholesterol concentration reflected increases in the concentrations of cholesterol in IDL, LDL, and HDL (Table II). Plasma VLDL cholesterol showed no statistically significant trend. Subfractionation of HDL into its two major subclasses, HDL₂ and HDL₃, was performed in 20 subjects. This showed that the rise in HDL cholesterol was confined to HDL₂ (Table II), the HDL₃ cholesterol being unchanged. On average, 78% of the increment in plasma cholesterol induced by dietary cholesterol reflected the increased cholesterol content of apo B-containing lipoproteins ($d < 1.063$ g/ml), the residual increment being accounted for by HDL₂.

In 12 subjects measurements were also made of the changes in plasma lipoprotein cholesterol following withdrawal of the egg yolks. 38 d after cessation of cholesterol-feeding the mean plasma cholesterol concentration (211 ± 8 mg/dl) had returned to the base-line value (209 ± 7 mg/dl); IDL and LDL cholesterol had returned to normal, and VLDL and HDL₃ cholesterol remained at base line. In contrast, the mean HDL₂ cholesterol concentration (21 ± 2 mg/dl) was still 17% higher ($P < 0.001$) than the base-line value (18 ± 1 mg/dl), although 16% lower than the value after 14 d of cholesterol supplementation (25 ± 2 mg/dl).

TABLE II
Plasma Lipoprotein Cholesterol Concentrations before and after Consumption of Six Egg Yolks Daily for 14 d (Study A)

	Plasma cholesterol concentration, mean \pm SEM(n)						
	Total	VLDL	IDL	LDL	HDL	HDL ₂	HDL ₃
	mg/dl						
Day 0	190 \pm 5 (37)	17 \pm 2 (31)	9 \pm 2 (31)	106 \pm 5 (31)	52 \pm 2 (31)	18 \pm 1 (20)	33 \pm 1 (20)
Day 14	219 \pm 1	15 \pm 2	15 \pm 2	124 \pm 4	58 \pm 1	25 \pm 1	34 \pm 2
P	<0.001	NS	<0.001	<0.001	<0.001	<0.001	NS

P values were obtained by paired *t* test.

NS, not statistically significant ($P > 0.05$).

Study B

Similar lipoprotein changes were observed in the 14 subjects who consumed a smaller cholesterol supplement (750 mg/d) for 28 days (Table III). The apo B concentrations in the different lipoproteins were also measured, and were found to be significantly increased in IDL and LDL, but not in VLDL. The changes in IDL cholesterol and apo B concentrations were positively correlated ($r = 0.52$; $P < 0.05$), as also were those in LDL cholesterol and apo B ($r = 0.44$; $0.05 < P < 0.10$). The cholesterol/apo B mass ratios in VLDL, IDL, and LDL were not significantly altered by the cholesterol-rich diet. In seven subjects these changes were associated with the appearance of β -migrating VLDL on agarose gel electrophoresis. This was accompanied by an increase in the ratio of apo E/apo C in VLDL, apo E accounting for $9.6 \pm 1.7\%$ and $12.9 \pm 2.1\%$ of tetramethylurea-soluble apoproteins before and after cholesterol-feeding respectively ($0.05 < P < 0.10$).

HMG CoA reductase activity. The effect of dietary cholesterol on HMG CoA reductase activity in freshly isolated and derepressed blood mononuclear cells was investigated in all 37 subjects of study A. Results obtained while the subjects were consuming their habitual diets (day 0) were compared with those obtained after 14 d of egg yolk supplementation.

Freshly isolated cells exhibited low levels of enzyme activity on day 0, and these were further reduced ($P < 0.001$) after 14 d of dietary cholesterol supplementation (Table IV). The percentage decrease in HMG CoA reductase activity was inversely related ($r = -0.49$, $P < 0.01$) to the percentage increase in LDL cholesterol concentration.

As would be anticipated, on both day 0 and day 14 cells that had been derepressed by preincubation for

TABLE III

Effects on Plasma Lipoproteins of Consuming Three Egg Yolks Daily for 28 d in 14 Subjects (Study B)

	Plasma concentration (mg/dl)		
	Day 0	Day 28	P
Total cholesterol	190 \pm 11	214 \pm 12	<0.001
VLDL cholesterol	12 \pm 3	13 \pm 2	NS
IDL cholesterol	9 \pm 2	15 \pm 2	<0.001
LDL cholesterol	106 \pm 8	122 \pm 10	<0.01
HDL cholesterol	50 \pm 2	58 \pm 2	<0.001
VLDL apo B	8.6 \pm 0.8	9.5 \pm 1.1	NS
IDL apo B	5.4 \pm 0.5	7.3 \pm 0.9	<0.01
LDL apo B	61 \pm 3.3	68.5 \pm 4.5	<0.001

Results are given as mean \pm SEM.

P values were obtained by paired *t* test; NS, not statistically significant ($P > 0.05$).

TABLE IV

Effects of Consuming Six Egg Yolks Daily for 14 d on the HMG CoA Reductase Activity of Blood Mononuclear Cells in 37 Subjects (Study A)

	HMG CoA reductase activity		
	Day 0	Day 14	P
	pmol of mevalonate formed/min/mg cell protein		
Freshly isolated cells	2.24 \pm 0.18	1.50 \pm 0.13	<0.001
Derepressed cells	10.3 \pm 0.84	10.1 \pm 0.66	NS

Results are expressed as mean \pm SEM.

P values were obtained by paired *t* test; NS, not statistically significant ($P > 0.05$).

72 h in lipoprotein-deficient medium had much higher HMG CoA reductase activities than did freshly isolated cells. The mean enzyme activity of derepressed cells collected on day 14 was similar to that of derepressed cells collected on day 0 (Table IV).

LDL receptor activity. The activity of LDL receptors in freshly isolated mononuclear cells was assayed in 12 subjects of study A. As shown in Table V, cholesterol feeding was associated with a striking reduction (by 74%) of the capacity of freshly isolated cells to degrade 125 I-LDL via high affinity LDL receptors during a 4-h incubation in vitro ($P < 0.01$).

In 18 subjects of study A the LDL receptor activities of mononuclear cells collected on days 0 and 14 were studied after derepression by incubation for 72 h in lipoprotein-deficient medium. Under these conditions the mean LDL receptor activities on the 2 d were not significantly different (Table V).

There was considerable variation between subjects in the capacity of mononuclear cells to degrade 125 I-

TABLE V

Effects of Consuming Six Egg Yolks Daily for 14 d on the High Affinity LDL Receptor Activity of Blood Mononuclear Cells (Study A)

	High affinity LDL receptor activity		
	Day 0	Day 14	P
	ng 125 I-LDL protein degraded/mg cell protein		
Freshly isolated cells (n = 12)	76.7 \pm 14.2	19.9 \pm 3.4	<0.01
Derepressed cells (n = 18)	1,812 \pm 141	1,788 \pm 140	NS

Results are expressed as mean \pm SEM.

P values were obtained by paired *t* test; NS, not statistically significant. Freshly-isolated cells were incubated for 4 h with 25 μ g 125 I-LDL protein/ml; derepressed cells were incubated for 6 h with 10 μ g 125 I-LDL protein/ml. See text for experimental details.

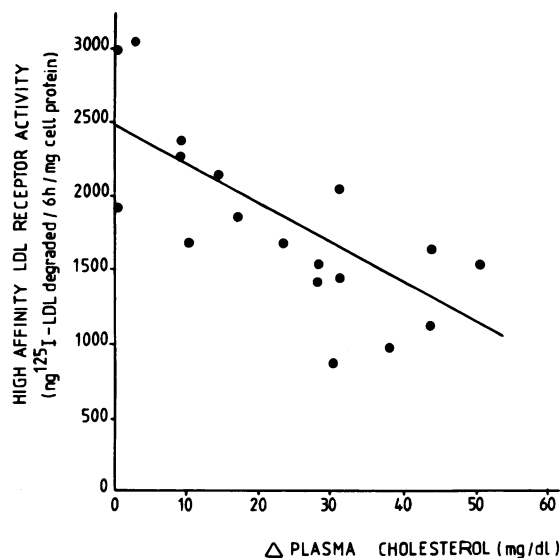


FIGURE 1 Relationship between the increment in plasma cholesterol concentration produced by consuming six egg yolks daily for 14 d and the LDL receptor activity of derepressed blood mononuclear cells collected immediately before the commencement of cholesterol feeding. (^{125}I -LDL concentration, $10\text{ }\mu\text{g}$ protein/ml. See text for experimental details.) $r = -0.74$, $P < 0.001$.

LDL, presumably reflecting differences in LDL receptor number per cell. The LDL receptor activity of cells was not significantly correlated with the plasma total cholesterol or LDL cholesterol concentrations, either before or after egg yolk feeding. There was a negative correlation, however, between the increment in plasma cholesterol and the LDL receptor activity of derepressed cells ($r = -0.74$; $P < 0.001$; Fig. 1). The relationship between the increment in LDL cholesterol and the LDL receptor activity of derepressed cells was weaker ($r = -0.51$; $P < 0.05$).

Cell cholesterol content. The cholesterol content of freshly isolated blood mononuclear cells was measured in 12 subjects of study A, immediately before and after 14 d of cholesterol feeding. Cell cholesterol increased by a mean of 17% from 11.1 ± 1.1 to $13.0 \pm 0.9\text{ }\mu\text{g}/\text{mg}$ cell protein ($P < 0.01$).

DISCUSSION

The addition of $\sim 1,500\text{ mg}$ cholesterol to the habitual daily diet of 37 healthy subjects for 14 d produced a mean increase in plasma cholesterol of $29\text{ mg}/\text{dl}$. This was similar to that predicted by the equation of Keys et al. (5). The responses in individual subjects differed considerably, however, ranging from a decrease of 6 to a rise of $75\text{ mg}/\text{dl}$. Consumption of 750 mg cholesterol daily for 28 d produced similar changes in 14 subjects. This was consistent with the report of Connor et al.

(1) that increasing the cholesterol intake beyond $500\text{ mg}/\text{d}$ produced little additional rise in the plasma cholesterol level.

The majority (58%) of the rise in plasma cholesterol was due to a rise in LDL cholesterol; increases in IDL and HDL₂ cholesterol accounted, respectively, for 20 and 22%. The apo B concentrations in IDL and LDL were also increased. These findings are consistent with those of Applebaum-Bowden et al. (21), who reported increases in the plasma total apo B and apo AI levels during egg yolk feeding in humans. These observations suggest that dietary cholesterol increases the plasma cholesterol level in man by increasing the number of IDL, LDL, and HDL₂ particles.

There is now unequivocal evidence that in normal humans LDL is derived from VLDL catabolism via IDL (39). By what possible mechanisms could dietary cholesterol have raised the IDL and LDL concentrations without increasing that of VLDL? One possibility is that both VLDL synthesis and catabolism were increased. A second is that, as in squirrel monkeys (40), the liver was stimulated to directly secrete cholesterol-rich particles within the IDL/LDL density range. A third possibility is that receptor-mediated catabolism of LDL was competitively inhibited by HDL_c. Mahley et al. (22) have already shown that the ability of human HDL to inhibit LDL binding by fibroblasts is enhanced during cholesterol-feeding. The same group has shown that canine HDL_c has, due to its content of apo E, a greater affinity for the LDL receptor than LDL itself (41, 42). It is possible that the increase in HDL₂ cholesterol in the present subjects reflected the appearance of HDL_c. The mechanism of this rise in HDL₂ cannot be determined from the present data, but one possibility is that it was secondary to increased chylomicron formation, since *in vitro* hydrolysis of VLDL triglyceride by lipoprotein lipase converts HDL₃ to HDL₂-like particles (43).

The changes in plasma lipoproteins were accompanied by an increase in the cholesterol content of freshly isolated mononuclear cells. This in turn was associated with two metabolic events in the cells: (a) a reduction of LDL receptor activity, as quantified by the degradation of ^{125}I -LDL; and (b) a reduction of HMG CoA reductase activity. Qualitatively similar changes have previously been shown to occur in a variety of peripheral cell types, including blood lymphocytes, in tissue culture in response to the addition of LDL to the incubation medium (14), operating to limit the LDL-induced rise in cell cholesterol content. The present findings, however, provide the first direct evidence for such modulation of the "LDL receptor pathway" in a peripheral cell type *in vivo* in response to a dietary perturbation in man. It is of interest that the mean increment in mononuclear cell cholesterol was less than that anticipated on the basis

of tissue culture studies (14), perhaps reflecting a greater efficiency of homeostatic mechanisms in vivo than in vitro.

There is evidence that cholesterol homeostasis in monocytes may differ quantitatively from that in lymphocytes (44). Although an effect of cholesterol feeding on the differential leukocyte count was not looked for in the present subjects, a change in the relative proportions of the two cell types in vivo would have had little or no effect on the metabolic characteristics of the mononuclear cell preparations examined in vitro, since the method of cell preparation removed most of the monocytes.

To what extent can the observed changes in the cholesterol content, HMG CoA reductase activity and LDL receptor activity of freshly isolated mononuclear cells be extrapolated to other peripheral cell types? It seems likely that the responses shown by such preparations do reflect the events occurring in many other nonhepatic tissues for several reasons. Firstly, the mechanisms of cholesterol homeostasis in lymphocytes maintained in vitro, including regulation of LDL receptor synthesis and HMG CoA reductase activity, have previously been shown to be similar to those of other peripheral cell types in culture (e.g., fibroblasts, smooth muscle cells) (14). Secondly, studies of human and animal peripheral lymph have indicated that the interstitial fluid of peripheral tissues contains all of the major lipoproteins of plasma (45, 46). Thirdly, the cholesterol concentration in human peripheral lymph, although lower than that in plasma, has been shown to be strongly correlated with the plasma cholesterol concentration (46). Thus, the diet-induced changes in plasma lipoprotein levels in the present study are likely to have been accompanied by parallel changes in interstitial fluid lipoproteins, and it is probable that most peripheral cells will have responded to these changes in a manner similar to that shown by freshly isolated lymphocytes.

Sterol balance studies have previously provided indirect evidence that cholesterol-feeding can result in a variable retention of cholesterol in human tissues (6). The present finding of an increase in the cholesterol content of freshly isolated blood mononuclear cells has now provided direct support for this concept. Steroid balance techniques have also repeatedly demonstrated down-regulation of whole body cholesterol synthesis during cholesterol feeding in humans, the other major response being an increase in fecal steroid excretion (6-9). The suppression of HMG CoA reductase activity in fresh mononuclear cells observed in the present subjects indicates that a proportion of the decrease in whole body cholesterol synthesis in response to dietary cholesterol occurs in nonhepatic cells.

The finding of a negative correlation between the percentage changes in LDL cholesterol concentration and HMG CoA reductase activity during cholesterol-feeding suggests that the magnitude of the rise in LDL concentration was related in part to the efficiency of regulation of cholesterol synthesis (assuming that cholesterol synthesis in mononuclear cells is regulated in a manner similar to that in quantitatively important sites such as the liver). Consistent with this finding is the report by Nestel and Poyser (9) that those individuals in whom plasma cholesterol was little increased by dietary cholesterol tended to show the greatest suppression of whole body cholesterol synthesis.

The decrease in the LDL receptor activity of freshly isolated mononuclear cells induced by dietary cholesterol is compatible with the increase in the fractional catabolic rate of ¹²⁵I-LDL observed in four subjects by Langer et al. (47) following withdrawal of dietary cholesterol. Before cholesterol feeding there was considerable variation between the present subjects in the LDL receptor activity of derepressed mononuclear cells. The inverse relationship between this value and the subsequent rise in plasma cholesterol level raises the possibility that the capacity of peripheral cells to catabolize cholesterol-rich lipoproteins (IDL, LDL, and HDL_c) via the LDL receptor may be a significant determinant of the magnitude of the effect of dietary cholesterol on plasma cholesterol concentration in man. Consistent with this proposal is the report by Connor and Jagannathan (48), who found that the mean increase in plasma cholesterol level induced by dietary cholesterol was greater in subjects with familial hypercholesterolemia than in normal subjects. On the other hand, Martin and Nestel (49) found that dietary cholesterol produced similar increments in plasma cholesterol concentration in normal and familial hypercholesterolemic children.

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