Chronic dietary fat and cholesterol inhibit the normal postprandial stimulation of plasma cholesterol metabolism

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Abstract The response of parameters of plasma cholesterol metabolism was studied in baboons adapted either to a low-fat, low-cholesterol diet or a high-fat, high-cholesterol diet. Animals adapted to the low-fat diet responded to a single low-fat or high-fat meal, as do normal humans, by a stimulation of cholesterol transport from blood cells to plasma, a stimulation of esterification of cholesterol, and a stimulation of cholesteryl ester transfer to very low and low density lipoproteins. While fasting rates of esterification and transfer increased as a result of diet-induced hypercholesterolemia, the postprandial response was reversed, so that postprandial metabolism was characterized by a movement of cholesterol from plasma to blood cells, an inhibition of cholesterol esterification, and a net transfer of cholesteryl esters from VLDL and LDL to HDL. These data indicate that the effects of postprandial lipemia on plasma cholesterol metabolism critically depend upon fasting plasma cholesterol levels.— **Fielding, P. E., E. M. Jackson, and C. J. Fielding.** Chronic dietary fat and cholesterol inhibit the normal postprandial stimulation of plasma cholesterol metabolism. *J. Lipid Res.* 1989. **30:** 1211–1217.

Supplementary key words cholesterol transport • lecithin:cholesterol acyltransferase • cholesteryl ester transfer

There are major acute changes in plasma cholesterol metabolism during postprandial lipemia. Rates of cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) increase rapidly as triglyceride levels rise, then return to basal levels (1–3). When cell membranes are present, LCAT promotes an increased movement of free cholesterol out of these into plasma during postprandial lipemia (3). Concomitantly, during postprandial lipemia, there is an increased transfer of LCAT-derived cholesteryl esters into low and very low density lipoproteins (LDL and VLDL) (3,4). These changes, if comparable modifications occurred in vivo, would be expected to increase the flux of cholesterol from peripheral cells to plasma lipoproteins (particularly VLDL and LDL) for clearance by hepatic receptors (5). There is little or no comparable data on the metabolic response to postprandial lipemia in hypercholesterolemia, although fasting levels of LCAT activity and rates of transfer of preformed cholesteryl esters to VLDL and LDL have been reported to be increased in baboons and rabbits, respectively (6,7).

Stimulation by dietary fat of cholesterol transport from peripheral tissues to the liver (reverse cholesterol transport) might seem paradoxical, in view of the accumulation of tissue and plasma cholesterol that occurs with time in humans and lower primates as a result of diets rich in fat and cholesterol. One reconciliation of these different responses might be that the chronic overconsumption of fat and cholesterol eventually overwhelms or inhibits a normal and potentially beneficial stimulation of cellular cholesterol transport to plasma which accompanies postprandial lipemia.

The baboon is recognized as a model for human atherogenesis (8) and responds to increased dietary fat and cholesterol by an increase in plasma cholesterol concentration. In the present study, the acute (i.e., postprandial) and chronic effects of diet on plasma cholesterol transport, esterification, and transfer between lipoproteins in these animals were determined.

EXPERIMENTAL PROCEDURES

Study design

This study was carried out with 12 young male baboons (5–10 years, mean age 7.2 years) from the colony maintained at the Southwest Foundation for Biomedical Research, San Antonio, TX. The animals had been maintained for at least 8 weeks prior to the study on a chow diet

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

(Purina Monkey Chow, Ralston Purina Co., St. Louis, MO) containing 10% of calories as fat, 28% as protein, and 62% as carbohydrate, and <0.1 mg/g cholesterol (9). The animals were trained to consume a single daily early morning meal.

A cannula was surgically implanted into a femoral vein and led out through a tether system, so that blood could be drawn without anesthesia or sedation (10). The cannula was initially kept patent with heparin (5 units/ml), but 3 days before the study period, the heparin was withdrawn and the lines were kept open by an infusion of physiological saline only (2 ml/h). After recovery for 1–2 weeks, four series of measurements on the effects of postprandial lipemia were made, the first two when the animals were still adapted to the chow diet, the others when they had been fed a high-cholesterol, high-fat diet. This consisted of 40% of calories as fat, 20% as protein, and 40% as carbohydrate, and contained 4.0 mg/g of cholesterol (10).

In the first study on each animal, blood samples (10 ml) were taken after a 23-h fast, and 4, 8, and 12 h after consumption of the single, daily monkey-chow meal. The meal was eaten within 1 h, after which any uneaten food was removed. The average mass of food eaten was 420 ± 118 g. There was no significant difference in the amount of food eaten in this and the subsequent studies.

In the second study, carried out a week later, the same animals were fed a high-fat, high-cholesterol meal, again after a 23-h fast. Fasting, and 4, 8, and 12 h postprandial blood samples were again collected.

For the third study, the animals were adapted for 8 weeks to the high-fat, high-cholesterol diet. At the end of this period, after a 23-h fast, blood was drawn, and then following consumption of the high-fat, high-cholesterol meal, 4, 8, and 12 h postprandial blood samples were drawn.

Finally, in the last study, six of the animals in the preceding study, which had been adapted to the high-fat, high-cholesterol diet, were fed a single low-fat, low-cholesterol chow meal after a 23-h fast. Fasting, and 4, 8, and 12 h postprandial blood samples were again obtained.

All surgical procedures were performed by a veterinary surgeon, conducted in an aseptic manner, and executed under surgical anesthesia. All animal handling and sampling procedures were approved by the Institutional Animal Research Committee. The Southwestern Foundation for Biomedical Research is accredited by AAALAC.

Each 10-ml blood sample was drawn into 0.05 vol of 0.2 M sodium citrate solution (pH 7.0) and cooled immediately in ice water. Blood and plasma were used for measurement of plasma lipids and cholesterol metabolic activities, as described below. Plasma was obtained by centrifugation for 30 min at 2000 *g*, 0–2°C.

Rate of esterification of cholesterol in plasma

The initial free and esterified cholesterol content of plasma was determined in pentuplicate, using cholesterol oxidase and cholesterol esterase in a modified fluorimetric assay with 4-hydroxy-3-methoxyphenylacetic acid (11). The plasma was then incubated at 37°C, and the rate of esterification of cholesterol was determined as the rate of decrease of plasma free cholesterol. This rate (like those of the other assays below) was linear for at least 2 h.

Cholesterol transport into and out of blood cells

The rate of decrease in plasma free cholesterol in whole blood was measured by separating plasma from blood, before and after incubation of the whole blood for 2 h at 37°C. Since the total free cholesterol esterified was the same in the presence and absence of blood cells in these studies as in previous studies (3,12), the net rate of transport of the cholesterol from cells to plasma was estimated (12) as [decrease in plasma free cholesterol in the absence of blood cells] – [decrease in plasma free cholesterol in the presence of blood cells]. In some experiments, these estimates of cholesterol transport were extended by direct analysis of blood cell content. Cells were washed 5 × with phosphate-buffered saline solution (pH 7.4). Cellular lipids were extracted with chloroform and methanol, and cholesterol was determined as described above.

Cholesteryl ester transfer to VLDL and LDL

Before and after incubation at 37°C, portions of plasma were mixed with one-twentieth volume of 1 M $MgCl_2$ and dextran sulfate (10 mg/ml) to precipitate VLDL and LDL (13). The increase in supernatant cholesteryl ester (measured enzymatically as described above) represented the increase in HDL cholesteryl ester of the total increase in cholesteryl esters catalyzed by LCAT during the incubation period. Net transfer of cholesteryl ester to VLDL and LDL was therefore represented by [total increase in plasma cholesteryl esters] – [increase in HDL cholesteryl esters]. No direct synthesis of cholesteryl esters by LCAT on VLDL or LDL has been detected (Francone, Gurakar and Fielding, unpublished experiments).

While the enzymatic synthesis of cholesteryl esters by mammalian LCAT is always positive, net free and ester cholesterol movements between cells and plasma and among lipoproteins are at least reversible. In terms of the equations given above, a net uptake of free cholesterol from plasma lipoproteins into blood cells would have a negative sign, while the reverse would have a positive sign. Net transfer of preformed cholesteryl esters from VLDL and LDL to HDL would also have a negative sign, while net transfer from HDL to VLDL and LDL would have a positive sign. This convention is used throughout. Net movements of both free and ester cholesterol were found to change in direction in the course of dietary modifications.

Plasma triglyceride was measured as glyceride following the removal of phospholipids with silicic acid (14).

To better assess the total response of plasma lipid or metabolic values over the postprandial time period (12 h),

the area under the response curve, with the initial (fasting) value set at zero, was determined by triangulation. Total response was, therefore, the difference in the areas above and below the fasting value, and its units were those of concentration or rate multiplied by time.

The significance of differences between experimental values in the different experiments was determined by paired *t*-test. Differences with a P value of <0.05 were considered significant.

RESULTS

Plasma lipid levels

Initial plasma total cholesterol levels were similar to those previously reported (91 ± 24 mg/dl) (8). Following 8–10 weeks on the high-fat, high-cholesterol diet, cholesterol levels doubled on average (to 180 ± 52 mg/dl) while plasma fasting free cholesterol levels increased from 21.4 ± 10.1 mg/dl to 57.0 ± 17.2 mg/dl (an increase of 2.7-fold). There was no significant change in plasma cholesterol concentration during postprandial lipemia in any of the studies, a finding consistent with comparable human data (3) and reflecting the relatively high mass of total plasma cholesterol compared to dietary intake. Fasting plasma triglyceride levels were higher on average following adaptation to high dietary cholesterol (**Table 1**) but this increase in triglyceride levels did not reach statistical significance ($P > 0.05$). The extent of total response over the time period shown was determined by triangulation of the area under the response plot relative to the initial (fasting) value. Thus, increases in triglyceride concentration above this baseline had a positive value, any decrease below baseline had a negative value, and the total response was the sum of all areas (zero to 12 h postprandial). The units of the response were, therefore, mg/dl × h.

Not unexpectedly, postprandial lipemia following a meal containing 40% calories as fat was more marked than after a meal containing 10% calories as fat (Table 1). Twelve hours after the meal, plasma triglyceride levels had returned to close to their fasting values (**Fig. 1**). The response of plasma triglyceride levels to a given meal (expressed as the area under the response curve) did not differ on average whether the animal had been adapted to a chow or high-fat diet, even though plasma cholesterol levels were always higher in the second case (Table 1, lines 2 and 3). These results indicate that the change in plasma triglyceride over fasting levels in response to a given meal was independent of the fasting plasma cholesterol level with which it was associated.

Cholesterol transport between plasma and blood cells

In the fasting blood of animals adapted to the low-fat, low-cholesterol chow diet, there was little or no movement of free cholesterol between blood cells and plasma during incubation in vitro. On average, 4.3 nmol of cholesterol passed out of each ml of plasma per h into the blood cells, in addition to the loss of 19.7 nmol/per h that was esterified by LCAT. The rate of this transfer out of plasma into cells was correlated ($r = 0.60$) with plasma free cholesterol concentration.

During postprandial lipemia in the chow-adapted animals, the movement of cholesterol between blood cells and plasma was reversed: there was a relative increase in the movement of cholesterol from cells to plasma (**Fig. 2**, left panel). On average this stimulation was larger following the fat-rich meal compared to the chow meal (Table 1) ($P < 0.05$). Areas under the response curves were determined by triangulation as described above for plasma triglyceride.

A corresponding decrease in the cholesterol content of the blood cells was measured during postprandial lipemia (**Fig. 3**).

When the animals were adapted to the high-fat, high-cholesterol diet, a major change was evident in the fasting and postprandial balance of cholesterol between blood cells and plasma. In the hypercholesterolemic fasting blood, there was now a fourfold more active uptake of cholesterol from the plasma into the cells than in the

TABLE 1. Effects of postprandial lipemia on parameters of plasma cholesterol metabolism

Diet Period	n	Fasting TC[a]	Fasting TG[a]	TG Area[b]	Fasting LCAT[c]	LCAT Area[b]	Fasting Transport[c]	Transport Area[b]	Fasting Transfer[c]	Transfer Area[b]
1	12	91 ± 24	22 ± 8	35 ± 48	19.7 ± 10	51 ± 26	−4.3 ± 9.7	28 ± 38	−4.6 ± 7.3	15 ± 46
2	12	90 ± 23	24 ± 10	104 ± 44	20.7 ± 10	217 ± 256	−3.4 ± 9.2	55 ± 35	−8.4 ± 12.2	64 ± 51
3	12	180 ± 52	30 ± 10	114 ± 72	32.8 ± 9	−119 ± 91	−16.2 ± 6.6	−96 ± 83	15.7 ± 10.2	−76 ± 31
4	6	163 ± 48	26 ± 8	58 ± 44	35.2 ± 7	−218 ± 76	−12.3 ± 14.4	−73 ± 16	15.9 ± 12.1	−54 ± 37

The four diet periods are those described under Experimental Procedures (Study design). Values given are means ± 1 SD. The plasma TC in diet period #3 of those animals (n = 6) used for a fourth measurement was 166 ± 53 in diet period #4 (difference not significant from period #3).

[a]TC, TG: total cholesterol and triglyceride concentrations (mg/dl).

[b]Total increase in activity over 12 h compared to fasting (zero time) (nmol × h).

[c]Rate of esterification, transport between cells and plasma, and transfer to VLDL + LDL (nmol h^{-1}); a negative transport rate implies a net flux of cholesterol from plasma to the blood cells also present; a negative transfer rate implies a net flux of cholesteryl ester from VLDL and LDL to HDL, while a positive transfer rate implies a net flux of cholesteryl ester from HDL to VLDL and LDL.

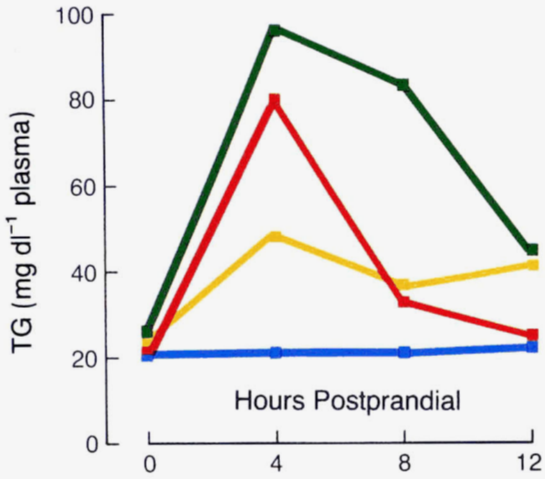

Fig. 1. Postprandial changes in plasma TG levels. Data are from a representative baboon. Total data are summarized in Table 1. Plasma was obtained from blood drawn after a 24-h fast (0 h) and at 4, 8, and 12 h postprandially. Blue: response to a 10% fat chow meal in the chow-adapted animal. Red: the response to a 40% fat meal in the chow-adapted animal. Green: the response to a high-fat, high-cholesterol meal after adaptation to this diet. Yellow: response to a 10% fat diet in an animal adapted to the high-fat, high-cholesterol diet.

blood of the chow-adapted animals (mean rate 16.2 ± 6.6 vs 4.3 ± 9.7 nmol ml^{-1} plasma h^{-1}) (Table 1) ($P < 0.01$). This uptake was correlated ($r = 0.68$) with the plasma free cholesterol level in the animals adapted to the high-fat, high-cholesterol diet. A further difference was seen during postprandial lipemia in the fat-adapted animals. Instead of stimulating cholesterol transport from blood cells to plasma, the same dietary stimulus, accompanied by a similar degree of lipemia (Fig. 1) had the opposite effect: during fat adsorption there was now a still greater uptake of free cholesterol from the plasma into the cells. These data mean that the net transport of cholesterol between cells and plasma is reversible; and that adaptation to a high-fat, high-cholesterol diet prevents the expected movement of cholesterol out of cells into plasma that is characteristic of the chow-adapted baboon (Table 1) and normal humans (3).

Cholesterol esterification rate

Initial activity was 19.7 ± 10.1 nmol cholesterol esterified ml^{-1} plasma h^{-1} (Table 1). When initially challenged with a 10% fat meal, a stimulation of plasma LCAT activity was seen in the chow-adapted animals (Table 1). This afterwards decreased again towards baseline (Fig. 2, center panel). The stimulation of LCAT over baseline was significant compared to fasting levels ($P < 0.05$). A greater stimulation on average was obtained when these animals were fed the high-fat meal (Table 1). The response to a high-fat meal was similar to that reported during postprandial lipemia in normal human plasma (1–3).

When the animals were adapted to the high-fat, high-cholesterol diet, fasting levels of LCAT activity were greatly increased compared to fasting rates in chow-adapted animals (32.8 ± 9.0 nmol vs 19.7 ± 10.0 nmol) (Table 1) ($P < 0.05$).

Fasting LCAT activity on both chow and high fat diets was positively correlated with plasma cholesterol levels ($r = 0.69$ and 0.55, respectively). While the endogenous cholesterol esterification rate in this study was lower than that previously reported with a radioisotope assay using lipid vesicles, the increased esterification rate in the present experiments, which accompanied adaptation to the high-fat, high-cholesterol diet, paralleled that reported earlier following chronic saturated fat feeding (6).

The fat-fed animals also showed a quite different response to postprandial lipemia, both to a chow or high-fat meal. Instead of rising, LCAT rates were inhibited during postprandial lipemia (Fig. 2, center panel).

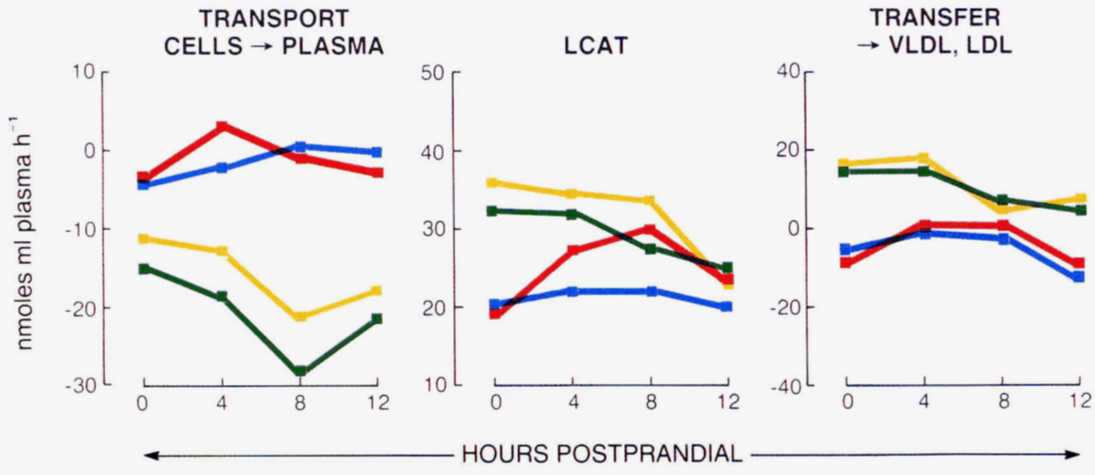

Fig. 2. Postprandial changes in cell-to-plasma cholesterol transport (left), cholesterol esterification (center), and cholesteryl ester transfer to VLDL and LDL (right). The colors, referring to different dietary periods, have the same significance as in Fig. 1. In Fig. 2 (left), positive values indicate net transport from cells to plasma, while negative values have the opposite connotation. In Fig. 2 (right), positive values indicate a net transfer from HDL to VLDL and LDL, while negative values have the opposite connotation. Values shown are means of individual rates at each time point.

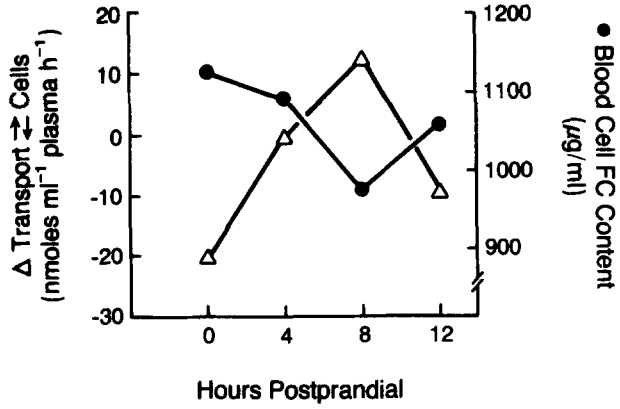

Fig. 3. Cholesterol balance between plasma and total blood cells during postprandial lipemia of a baboon adapted to the low-fat chow diet responding to a 40% fat meal. Circles: the cholesterol content of packed cells obtained by centrifugation of whole blood. Triangles: cholesterol net transport between plasma and blood cells, as described under Experimental Procedures.

Cholesteryl ester transfer to VLDL and LDL

In the fasting baboon adapted to the low-fat chow diet, there was a modest net transfer of cholesteryl esters from VLDL and LDL to HDL during incubation in vitro. In other words, the cholesteryl ester content of HDL increased at a rate somewhat greater than the total rate of cholesteryl ester synthesis by LCAT, augmented by cholesteryl esters already present in VLDL and LDL. This excess transfer to HDL is likely derived from preformed cholesteryl esters secreted in VLDL from the liver (15). On average, the net (i.e., excess) transfer of esterified cholesterol to HDL from VLDL and LDL was 4.6 ± 7.3 nmol ml^{-1} plasma h^{-1}, so that the total rate of increase of cholesteryl esters in HDL in vitro was the sum of this and the LCAT rate (4.6 + 19.7 = 24.3 nmol ml^{-1} plasma h^{-1}) (Table 1).

When the chow-adapted animals were fed a single fat-rich meal, there was a stimulation of cholesteryl ester transfer to VLDL and LDL compared to the baseline fasting value (Fig 2, right panel; Table 1). In some animals this increase was not sufficient to lead to a net transfer of cholesteryl esters to VLDL and LDL, even during postprandial lipemia; however, even in these animals, the rate of transfer from VLDL and LDL to HDL decreased during the postprandial response.

When the same animals had been adapted to the fat-rich, cholesterol-rich diet, very different kinetics were observed. Fasting plasma in all cases now catalyzed a substantial net transfer of esters from HDL to VLDL and LDL (mean rate 15.7 ± 10.2 nmol ml^{-1} plasma h^{-1}). That is, of the total of an average 32.8 nmol of cholesteryl ester synthesized by LCAT under these conditions, 15.7 nmol (or about one-half) was transferred to VLDL and LDL, while the remainder (32.8–15.7 or 17.1 nmol) was retained in HDL (Table 1).

Postprandial plasma in the fat-adapted animals also showed a quite different response than that observed earlier in the chow-fed baboons. Postprandial lipemia was accompanied not by an increase but by a net decrease in the rate of transfer of cholesteryl esters to VLDL and LDL. Typically, the rate of transfer of cholesteryl esters to HDL under these conditions exceeded the rate of synthesis of new cholesteryl esters by LCAT in the same plasma (Fig. 2, right panel; Table 1). Such "excess" cholesteryl esters are likely to represent lipids secreted in this form by the liver as VLDL, which are transferred to HDL during incubation in plasma (15).

Therefore, in the movement of cholesterol between blood cells and plasma, in the synthesis of cholesteryl esters in plasma, and in cholesteryl ester traffic between HDL and VLDL and LDL, the effect of postprandial lipemia was opposite in the chow-adapted baboons and in the same animals adapted to the high-fat, high-cholesterol diet. In the chow-adapted baboon, as in the normal human (3), postprandial lipemia stimulated the movement of cholesterol out of cells into plasma, and (as cholesteryl esters) into VLDL and LDL. In the fat-adapted animal, postprandial lipemia inhibited these reactions below fasting levels.

DISCUSSION

This study indicates that there are major changes in the metabolic handling of plasma cholesterol during postprandial lipemia that are induced by the chronic feeding of a diet rich in fat and cholesterol. If these results, obtained in nonhuman primates, are applicable to events in human plasma, they may provide an explanation, in part, for the accumulation of cholesterol in tissues that is associated with the increased consumption of these lipids in humans (16).

The effects of postprandial lipemia in chow-adapted baboons were generally similar to those reported earlier in humans (3), but with some important differences. The stimulation to cholesteryl ester synthesis by increased dietary fat under these conditions was substantial: over the 12-h period, the total of cholesterol esterified after a low-fat meal was the sum of the fasting level over the period plus the increment above the fasting level over the same period or (19.7 × 12) + 51 = 287 nmol ml^{-1} plasma. Following the 40% fat meal, it was (20.7 × 12) + 217 or, on average, 465 nmol ml^{-1} plasma, an increase of 62%. (Table 1). This stimulus was similar to that obtained in humans (3).

Blood cells functioned in vitro as cholesterol donors for the LCAT reaction, although not as efficiently as monolayers of fibroblasts (3) or particularly, endothelial cells (17) in studies with human plasma. It is not clear whether this represents a difference between cells or between species. An increased esterification of 217 nmol of cholesterol above initial fasting levels, over 12 h, in response to the

high-fat diet in the chow-adapted animals, was accompanied with the movement of 55 nmol of cholesterol out of the blood cells over the same period (Table 1). With human plasma and cultured cells, this relationship is almost quantitative (3).

Another difference was that, on average, only about 30% (64 of 217 nmol) of the extra cholesteryl esters generated by LCAT during the postprandial period in the chow-adapted animals was transferred to VLDL and LDL in plasma (Table 1). Essentially, 100% of such additional esters was transferred to VLDL and LDL in human plasma under the same conditions (3). It is quite likely that this difference reflects the much lower relative levels of VLDL and particularly LDL (compared to HDL) in baboon compared to human plasma (8). Not surprisingly, a chow meal containing 10% fat was less effective at stimulating transfer and other parameters of plasma cholesterol metabolism in the chow-adapted baboon than was the 40% fat meal. However, postprandial lipemia in normal baboon plasma (like that of humans) clearly increased the flow of cholesterol through LCAT to cholesteryl esters in VLDL and LDL.

Comparison of lines 2 and 3 in Table 1 shows the major changes found when the animals had been adapted to the high-fat, high-cholesterol diet. The extent of postprandial lipemia (in terms of the rise of plasma triglyceride above fasting values) was similar to that originally found in the chow-adpated baboons. All other rates changed substantially. Although fasting LCAT rates were increased in the fat-adapted animals, the inhibition of this activity now mediated by postprandial lipemia was sufficient to negate this effect over a 12-h period (cholesterol esterified = $(32.8 \times 12) - 119$ or 274 nmol ml^{-1} for the fat-adapted animal, vs. 465 nmol for the chow-adapted baboons) (see above).

In the fat-adapted animals, under both fasting and, particularly, postprandial conditions, there was a net movement of free cholesterol from the plasma into the blood cells. In view of other evidence that free cholesterol can move down a concentration gradient (18), it is likely that the uptake of cholesterol from plasma by blood cells represents diffusion. In vitro, uptake of such excess free cholesterol was by the blood cells. In vivo, other cells, such as endothelial and smooth muscle cells, would also be likely to take part in the clearance of excess of plasma free cholesterol. Other mechanisms (such as intracellular esterification, the turnover of red cells by the spleen, and the degradation of cholesterol by cells such as hepatocytes)

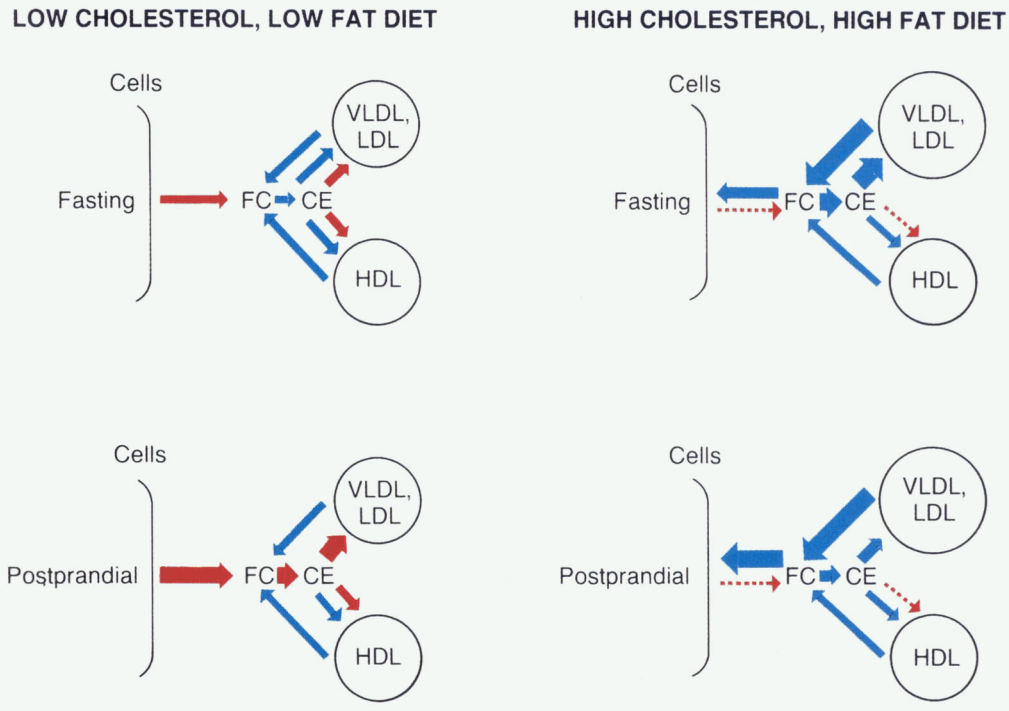

Fig. 4. An hypothesis to explain the different metabolic effects of increased rates of cholesterol esterification and transfer in fasting and postprandial lipemia in normal baboons and baboons adapted to a high-fat, high-cholesterol diet. Red: cholesterol derived from cell membranes. Blue: cholesterol newly derived from circulating plasma lipoproteins. The thickness of the indicators reflects the relative rate of transfer of free or esterified cholesterol between the different compartments. This model illustrates the concept of an increased flux of free cholesterol to LCAT from cell membranes (red arrow, postprandial lipemia on a low-fat, low-cholesterol diet) or from circulating plasma lipoproteins (blue arrow, fasting plasma) after adaption to the high-fat, high-cholesterol diet.

would contribute to free cholesterol removal. However, the transient movement of free cholesterol in and out of the blood cells, observed here by direct analysis, provides support for the concept (19) that these cells, among others, can act as a buffer modulating plasma free cholesterol demand by LCAT in whole body cholesterol homeostasis.

The present study shows that LCAT and cholesteryl ester transfer activities are increased to a similar extent under two different conditions: postprandially, in chow-fed normocholesterolemic animals, and in fasting, following the induction of dietary hypercholesterolemia. However, the sources of the extra free cholesterol esterified and transferred under the two circumstances are evidently very different. In the first case, much of the extra substrate used by LCAT originates from cell membranes, leading to a decrease in the cholesterol content of the cells. In the second case, the free cholesterol used is taken from the plasma lipoproteins. Thus, at similar LCAT rates, in one case cholesterol is unloaded from cells, and in the other it is cleared into the cells (**Fig. 4**).

The same situation applies to the rate of transfer of cholesteryl esters between HDL on the one hand, and VLDL and LDL on the other. The increased rate of transfer promoted by postprandial lipemia in normolipemic animals promoted a flux from HDL to VLDL and LDL. Postprandial lipemia in hypercholesterolemic animals promoted an increased transfer of cholesteryl esters from VLDL and LDL to HDL.

These data suggest that chronic dietary fat and cholesterol inhibit cellular cholesterol unloading and stimulate cholesterol loading, particularly postprandially. Further research will be required to identify the basis of these changes in plasma cholesterol metabolism at the molecular level. Preliminary analysis of cholesterol metabolism in normal plasma already indicates distinct pathways for the processing of cell-derived cholesterol (20), which may be modified in hypercholesterolemia. The present study demonstrates that reactions (cholesterol transport, cholesteryl ester transfer) reversible at different levels of plasma total cholesterol, determine whether a given diet accelerates the loading or unloading of cholesterol in cells. A knowledge of both plasma lipid levels and of catalytic factors of plasma cholesterol metabolism is required to predict the effect of dietary fat and cholesterol on plasma lipid metabolism.

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