

Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate

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Abstract These studies examine the regulation of plasma low density lipoprotein (LDL)-cholesterol levels by varying quantities of dietary saturated and polyunsaturated triacylglycerols. At a constant load of 0.12% cholesterol and 20% triacylglycerol, substitution of polyunsaturated for saturated triacylglycerols caused LDL receptor activity to increase from 25% to 80% of control and reduced the LDL-cholesterol production rate from nearly 200% to 155%. These changes caused the plasma LDL-cholesterol concentration to decrease from nearly 190 to 50 mg/dl. When the dietary content of each triacylglycerol alone was incrementally increased, the saturated lipid suppressed receptor activity while the polyunsaturated triacylglycerol increased receptor-dependent LDL transport. The magnitude of these effects was quantitatively similar, although oppositely directed. However, the saturated triacylglycerol also caused a dose-dependent increase in the LDL-cholesterol production rate and markedly increased the plasma LDL-cholesterol level while the polyunsaturated lipid did not affect either of these. These independent effects were also evident in experiments where it was found that substituting polyunsaturated triacylglycerol for saturated lipid increased receptor activity significantly more than did simply reducing the dietary content of saturated triacylglycerol. ■ Thus, these studies show that triacylglycerols containing saturated or polyunsaturated fatty acids have effects on the major processes that regulate the plasma LDL-cholesterol level that are qualitatively and quantitatively distinct.—**Woollett, L. A., D. K. Spady, and J. M. Dietschy.** Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate. *J. Lipid Res.* 1992. **33**: 77–88.

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It is now well established that it is the lipid content of the diet in Western countries that is primarily responsible for the relatively high levels of plasma cholesterol carried in the low density lipoprotein (LDL) fraction. The plasma LDL-cholesterol concentration can easily be raised into the range of 75–250 mg/dl by feeding diets containing the usual small amounts of cholesterol (50–300 mg/1000 kcal intake) and large quantities of triacylglycerol (20–50 g/1000

kcal) found in human diets to experimental animals such as the male hamster (1, 2), cynomolgus monkey (3, 4), African green monkey (5), or baboon (6). However, it is equally clear that the magnitude of these effects is dependent in a complex way on the absolute quantity of both the cholesterol and triacylglycerol in the diet, as well as on the type of fatty acid contained in the triacylglycerol. For example, in both animals and humans, at any fixed level of triacylglycerol, the plasma LDL-cholesterol concentration varies directly with the level of dietary cholesterol intake (1, 7–9). On the other hand, when the load of both dietary cholesterol and triacylglycerol is fixed, the plasma cholesterol concentration is also markedly influenced by the chain-length and degree of saturation of the fatty acids in the triacylglycerol (2, 10–14).

Only recently have techniques become available that provide the means for understanding the physiological and molecular alterations that take place with lipid feeding that explain these changes in the steady-state level of LDL-cholesterol. When cholesterol alone is added to an essentially lipid-free diet, for example, there is a dose-related reduction in receptor-dependent LDL transport in the animals accompanied by a small increase in the rate of LDL-cholesterol formation. In the hamster, these two events account for the progressive increase in the plasma LDL-cholesterol concentration from 23 to 86 mg/dl as the dietary intake of cholesterol is increased from about 40 to 500 mg/1000 kcal of food intake (1). However, when such animals are fed a constant level of a saturated triacylglycerol such as hydrogenated coconut oil (HCO),

Abbreviations: HCO, hydrogenated coconut oil; SO, safflower oil; J^m , the maximal achievable rate of receptor-dependent LDL transport in an organ or the whole animal; J_r , the LDL-cholesterol production rate; K_m , the concentration of plasma LDL-cholesterol necessary to achieve half the velocity of J^m ; P, the proportionality constant for receptor-independent LDL transport in an organ or the whole animal; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein.

this same increase in dietary cholesterol causes the plasma LDL-cholesterol concentration to increase from 41 to 198 mg/dl. These marked changes are brought about by a progressive suppression of receptor-dependent LDL uptake and an increase in the LDL-cholesterol production rate. In contrast, when the constant load of dietary triacylglycerol is made up of largely unsaturated fatty acids such as safflower oil (SO), the suppression of receptor activity is reversed and there is little increase in the production rate. Hence, under these conditions the plasma LDL-cholesterol concentration increases from only 18 to 71 mg/dl as the load of dietary cholesterol is progressively increased from 40 to 500 mg/1000 kcal (1).

From observations such as these in both experimental animals and in humans, there is generally good experimental data supporting the recommendation that the levels of both cholesterol and triacylglycerol containing saturated fatty acids should be reduced in the diet (15). However, there is relatively little data on how quantitative changes in the dietary content of triacylglycerol, in general, and of the absolute amounts of saturated and unsaturated fatty acids, in particular, alter the specific cellular mechanisms that dictate steady-state plasma LDL-cholesterol levels. Thus, the present experiments were designed to investigate four specific questions in detail. First, under conditions of a constant intake of dietary cholesterol, what are the cellular effects of changing the absolute load of dietary triacylglycerol? Second, what is the physiological explanation for the common observation that the addition of saturated fatty acids to the diet is approximately two times as effective in raising the plasma LDL-cholesterol concentration as is an equal amount of polyunsaturated fatty acid in lowering this level? Third, under conditions of isocaloric substitution of polyunsaturated for saturated fatty acids, is the lowering of the plasma LDL-cholesterol level simply due to removal of the saturated lipids or is there an independent effect of the polyunsaturated lipids on the cellular mechanisms responsible for LDL clearance? Finally, under conditions of a constant load of dietary cholesterol, which is more effective in lowering the plasma LDL-cholesterol concentration, lowering the total content of dietary triacylglycerol along with the level of saturated fatty acids or, alternatively, keeping the level of triacylglycerol in the diet constant while substituting more unsaturated fatty acids for the saturated ones?

METHODS

Animals and diets

Male Golden Syrian hamsters (Charles River Lakeview, New Field, NJ) were housed in colony cages for

2 weeks before the experiments were started. Animals were subjected to 12 h of light and 12 h of darkness before and during the experiments, and measurements were made during the mid-dark phase of the cycle. Three types of diets were fed during the experiments. The control diet consisted of ground Wayne Lab Blox (Allied Mills, Chicago, IL) which contained 0.02% (w/w) cholesterol and 5.0% total lipids. The fatty acid composition of this control diet is presented elsewhere (1). In the cholesterol control diet, pure cholesterol dissolved in ethanol was added to the ground diet at a concentration of 0.12% which, after evaporation of the alcohol, gave a final dietary cholesterol content of 0.14%. In the third type of diet containing triacylglycerol, cholesterol (0.12%) was dissolved in the oils prior to mixing in a mechanical food blender. Triacylglycerol was added to these diets in various combinations and in varying amounts, expressed on a weight percentage basis. For example, a diet containing 10% hydrogenated coconut oil and 10% safflower oil contained 10 g of coconut oil, 10 g of safflower oil, and 80 g of ground Wayne Lab Blox. The fatty acid profiles of the two triacylglycerols used in these studies were determined by gas-liquid chromatography (16). The composition of hydrogenated coconut oil (HCO) was 11%, 7%, 50%, 16%, 7%, and 9% respectively, of the 8:0, 10:0, 12:0, 14:0, 16:0, and 18:0 fatty acids. Safflower oil (SO) contained 8%, 2%, 14%, and 76%, respectively, of the 16:0, 18:0, 18:1, and 18:2 fatty acids. In all studies, groups of animals received the control and cholesterol control diets as well as the appropriate triacylglycerol-containing diets ad libitum for 30 days at which time a variety of measurements were undertaken.

Lipoprotein preparations

Hamster LDL was isolated in the density range of 1.020–1.055 g/ml by preparative ultracentrifugation. The donor animals had been maintained on a low-cholesterol, low-fat control diet prior to harvesting the blood. Lipoproteins were labeled with either [¹²⁵I]tyramine cellobiose (17) or ¹³¹I (18). Lipoproteins were used within 24 h of preparation and were filtered through a 0.45- μ m Millipore filter immediately before use.

In vivo LDL clearance rates

Rates of tissue LDL clearance were determined in vivo using a primed-continuous infusion of [¹²⁵I]tyramine cellobiose-labeled LDL (19, 20). Ten minutes prior to termination of the 4-h infusion period, a bolus of ¹³¹I-labeled LDL was administered to each of the animals. The animals were then killed at 4 h by exsanguination through the abdominal aorta. The

liver was removed and the remaining carcass was frozen in liquid nitrogen and ground to a fine powder. Aliquots were then assayed for radioactivity. Clearance rates were determined by subtracting the tissue space of LDL at 10 min (^{131}I dpm per g of tissue divided by the ^{131}I dpm per μl of plasma) from the tissue space of LDL at 4 h (^{125}I dpm per g of tissue divided by the ^{125}I dpm per μl of plasma). These clearance rates were calculated as the μl of plasma cleared of its LDL content per h per g of tissue ($\mu\text{l}/\text{h}$ per g). In addition, these rates were multiplied by the whole liver and carcass weights in order to calculate LDL clearance by these two whole tissues ($\mu\text{l}/\text{h}$ per tissue).

In vivo LDL-cholesterol uptake rates

From these LDL clearance rates, uptake rates of LDL-cholesterol were determined. These uptake rates were expressed as the absolute mass of LDL-cholesterol that was taken up per h per g of tissue ($\mu\text{g}/\text{h}$ per g) and were calculated by multiplying the clearance rates of LDL ($\mu\text{l}/\text{h}$ per g) by the plasma LDL-cholesterol concentration ($\mu\text{g}/\mu\text{l}$). These rates, in turn, were multiplied by the whole liver and carcass weights to give the LDL-cholesterol uptake rates per whole tissue ($\mu\text{g}/\text{h}$ per tissue).

In vivo LDL-cholesterol production rates

Because these studies were performed during steady-state conditions where there was no detectable change in the plasma LDL-cholesterol concentration, the sum of the LDL-cholesterol uptake rates in all of the tissues of the animal must equal the rate of LDL-cholesterol production. Thus, LDL-cholesterol production rates were calculated by summing the LDL-cholesterol uptake rates in the liver and carcass, and these values were expressed as the μg of LDL-cholesterol produced per h per whole animal ($\mu\text{g}/\text{h}$ per animal). These rates were then normalized to a constant body weight of 100 g.

Calculations

The steady-state concentration of LDL-cholesterol is known to be determined by four separate parameters of LDL metabolism (1, 21). The rate at which LDL-cholesterol is taken up by a particular organ or by all of the tissues of the body (J_t) is equal to the sum of the rates of uptake by the receptor-dependent (J_d) and receptor-independent (J_i) transport processes in that organ or whole animal (2). Since J_d equals $(J^m C_1)/(K_m + C_1)$ and J_i equals PC_1 , it follows that J_t for any organ or for the whole animal can be calculated from the following expression (21):

$$J_t = \frac{J^m C_1 + PC_1 K_m + PC_1^2}{K_m + C_1} \quad \text{Eq. 1}$$

The parameter J^m is the maximal receptor-dependent transport velocity (a reflection of receptor number); K_m is the plasma concentration of LDL-cholesterol at which one-half of this maximal transport rate is achieved (a reflection of receptor affinity); C_1 is the concentration of plasma LDL-cholesterol in a given animal; and P is the proportionality constant for LDL transport by way of the receptor-independent pathway. The relationship between J_t and C_1 can be defined for a particular organ like the liver or the whole animal. Furthermore, in the latter case, this equation can be rearranged to give an expression that describes how the plasma LDL-cholesterol concentration will change in the whole animal given any alteration in receptor number (J^m) or LDL-cholesterol production since, in the steady-state, the rate of LDL-cholesterol removal from the plasma space (J_t) must equal the rate of entry into the plasma. This second expression is as follows:

$$C_1 = \frac{(J_t - J^m - PK_m) + \sqrt{(J_t - J^m - PK_m)^2 + 4PK_m J_t}}{2P} \quad \text{Eq. 2}$$

The specific values for each of these parameters in the liver and the whole animal were determined in each appropriate control group of hamsters and are given in the text. In each experiment, changes in receptor number (J^m) and production rate (J_t) are given relative to the absolute values for these two parameters experimentally determined in the appropriate control animals, i.e., they are expressed as a percentage of the respective values found in simultaneously run animals receiving the control diet.

Where appropriate, mean values ± 1 SEM are given. When comparing more than two dietary treatments, the data were analyzed by analysis of variance (22). Student's *t* tests were used ($P < 0.05$) to compare two treatments.

RESULTS

The first set of studies was designed to determine how the hepatic LDL receptor activity and production rate respond to a change in the degree of saturation of the dietary fatty acids under circumstances where the load of both triacylglycerol and cholesterol was kept constant. Groups of animals were fed diets that contained a constant amount of added cholesterol (0.12%) and triacylglycerol (20%) but the degree of saturation of the fatty acids in the triacylglycerol was varied over a wide range by mixing different proportions of a fully saturated triacylglycerol (HCO) and a highly unsaturated one (SO). After the 30-day feeding period, weight gain and liver weights were identical in all groups of animals receiving the diets containing

20% triacylglycerol where the lipids accounted for about 45% of caloric intake. As shown in Fig. 1, the addition of 0.12% cholesterol alone to the control diet raised the mean LDL-cholesterol concentration from 22 to 57 mg/dl. The further addition of 20% triacylglycerol that was fully saturated markedly increased this level to 190 mg/dl. However, as polyunsaturated triacylglycerol was systematically substituted for the HCO, the plasma LDL-cholesterol concentration progressively declined until it essentially equaled that seen in the cholesterol control animal when all of the triacylglycerol was SO.

Cholesterol carried in very low density lipoproteins (VLDL) also was affected by the dietary lipids. VLDL-cholesterol concentrations were elevated by the addition of 0.12% cholesterol from 12 to 51 mg/dl. When 20% HCO was added, the VLDL-cholesterol concentration increased to 235 mg/dl. As HCO was systematically replaced with SO, the concentration of VLDL-cholesterol progressively decreased to only 44 mg/dl when all of the triacylglycerol was polyunsaturated. Finally, even though the addition of cholesterol to these diets resulted in a small increase in the concentration of cholesterol carried in high density lipoproteins (HDL), the type of dietary triacylglycerol added to these diets had virtually no effect on the steady-state concentrations of HDL-cholesterol.

In order to elucidate the reasons for these marked effects, the rate of receptor-dependent LDL transport and the LDL-cholesterol production rates were next measured in similarly treated animals. As has been previously reported in a number of species, in the hamsters used in these studies, > 80% of the receptor-

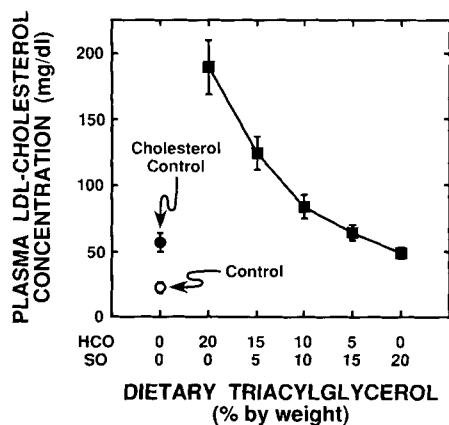


Fig. 1. Plasma LDL-cholesterol concentrations in animals fed a constant load of dietary triacylglycerol containing varying amounts of saturated and polyunsaturated fatty acids. The control diet contained no added cholesterol or triacylglycerol while the cholesterol control diet contained 0.12% added cholesterol. All other diets contained 0.12% added cholesterol and 20% added triacylglycerol. The composition of this triacylglycerol was systematically varied to contain different percentages of saturated (HCO) and polyunsaturated (SO) triacylglycerols. Each point represents the mean \pm 1 SEM for 18 animals in each experimental group.

dependent LDL transport detected in the whole animal was found to take place in the liver (19, 20, 23). Furthermore, as shown in Fig. 2, feeding the cholesterol control diet containing 0.12% cholesterol resulted in a decrease in both hepatic (B) and whole animal (A) LDL receptor activity to about 52% of the control value. This level, however, was markedly influenced by the degree of saturation of the added triacylglycerols: saturated triacylglycerols further suppressed receptor activity while substitution of polyunsaturated oil restored the rate of LDL transport. The addition of cholesterol to the diet also caused a significant increase in the LDL-cholesterol production rate (C) and this increase was even greater when 20% triacylglycerol was also added to the diet. Again, this increase was somewhat dependent upon the type of fatty acids present in the triacylglycerol. Fully saturated triacylglycerol increased the production rate to 203% of control, but this increase became smaller as the dietary triacylglycerol was made up of progressively more polyunsaturated fatty acids.

With data available on these two primary determinants of the plasma LDL-cholesterol, it was next possible to present graphically how alterations in the degree of saturation of the dietary lipids brought

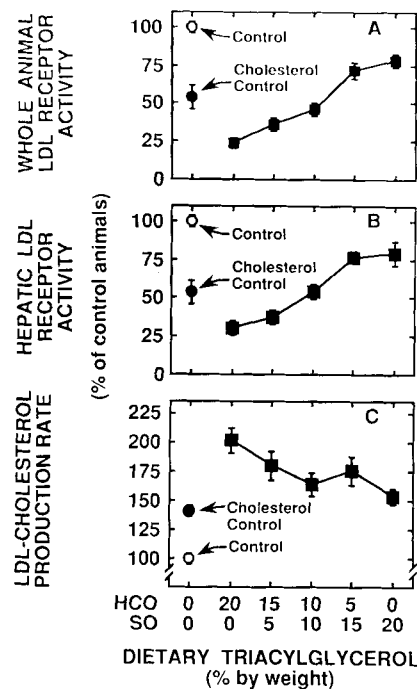


Fig. 2. LDL receptor activity in the whole animal (A) and liver (B) and LDL-cholesterol production rates (C) in animals fed a constant load of triacylglycerol with varying amounts of saturated and polyunsaturated fatty acids. The diets fed in this experiment were the same as described in Fig. 1. The absolute values for each of these parameters were determined and are expressed as a percentage of the corresponding values in the control animals fed a diet containing neither added cholesterol nor triacylglycerol. Each point represents the mean \pm SEM for 18 animals.

about these changes in the plasma cholesterol levels. The two theoretical curves shown in Fig. 3 were calculated using equation 2 in the Methods section. The absolute value of the LDL-cholesterol production rate (J_i) in control animals in these studies equaled $154 \pm 10 \mu\text{g/h}$ per 100 g animal while the absolute value for the maximal transport velocity of the receptor-dependent process (J^m) equaled $719 \pm 21 \mu\text{g/h}$ per 100 g animal. These two respective values were set at 100% for the LDL-cholesterol production rate and LDL receptor activity in Fig. 3. As is apparent, the modest increase in the plasma LDL-cholesterol concentration brought about by the cholesterol control diet was due to loss of nearly 50% of receptor activity and to a small increase in the LDL-cholesterol production rate. The extreme elevation seen when 20% HCO was added to this diet resulted from a doubling of the production rate and further loss of receptor activity. The marked effect of substituting polyunsaturated fatty acids on the LDL-cholesterol level resulted from a progressive increase in receptor activity, from about 25 to nearly 80% of control, and a small decrease in the LDL-cholesterol production rate, from about 200 to 155% of control.

Finally, in these same animals the level of cholesteryl esters in the liver also was strikingly affected by the degree of saturation of the fatty acids in the diet. As illustrated in Fig. 4, the addition of 0.12% cholesterol to the control diet raised the ester content nearly 60-fold, from 0.3 to 18.4 mg/g. This level was

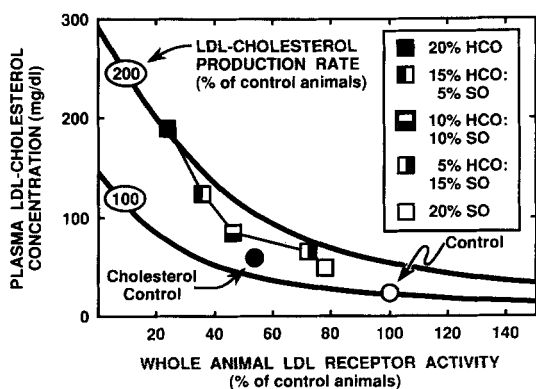


Fig. 3. Plasma LDL-cholesterol concentrations plotted as a function of whole animal LDL receptor activity and LDL-cholesterol production rates in animals fed a constant load of triacylglycerol with varying amounts of saturated and polyunsaturated fatty acids. The two solid curves show how the LDL-cholesterol concentration varies under circumstances where the LDL-cholesterol production rate is either 100% or 200% of the control value and where whole animal LDL receptor activity varies from 0 to 140% of control. These curves were calculated using the following parameter values (all normalized to a 100 g whole animal weight): J^m of $719 \mu\text{g/h}$, J_i of $154 \mu\text{g/h}$, K_m of 100 mg/dl and P of $1.08 \mu\text{g/h}$ per mg/dl . The mean experimental results obtained in the animals receiving diets containing neither cholesterol nor triacylglycerol (control), cholesterol only (cholesterol control), and various mixtures of triacylglycerols are superimposed upon these two theoretical curves.

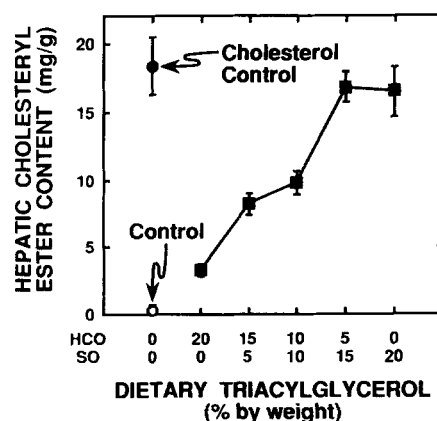


Fig. 4. Hepatic cholesteryl ester content in animals fed a constant load of triacylglycerol with varying proportions of saturated and polyunsaturated fatty acids. This figure shows the content of cholesteryl esters in the livers of the animals fed the same experimental diets described in Fig. 1. Each value represents the mean ± 1 SEM for determinations in 18 animals.

much lower in the presence of the saturated fatty acids and was restored when more unsaturated acids were fed. In contrast, there was only a slight increase in unesterified cholesterol levels in the liver when cholesterol was added to the diet (not shown) and this level was not influenced by the type of dietary triacylglycerol.

Thus, under the conditions of this first group of studies where the cholesterol (0.12%) and triacylglycerol (20%) content of the diet was kept constant, shifting from fully saturated fatty acids (predominantly 12:0 and 14:0) to polyunsaturated fatty acids (predominantly 18:2) profoundly altered several parameters of hepatic lipid metabolism. The saturated fatty acids markedly reduced the movement of cholesterol into the ester pool, suppressed receptor-dependent LDL transport into the liver, nearly doubled the LDL-cholesterol production rate, and raised the plasma LDL-cholesterol concentration to about 200 mg/dl. Substituting polyunsaturated fatty acids in the diet restored the flow of cholesterol into the hepatic ester pool, markedly increased hepatic receptor activity, marginally reduced the LDL-cholesterol production rate, and so lowered the plasma LDL-cholesterol concentration to only about 50 mg/dl.

These studies had the virtue of allowing observations of the effect of mixtures of different fatty acids under the circumstance of isocaloric intake. They did not, however, provide the means for discriminating quantitative or qualitative differences in the effects of these two types of triacylglycerols when fed individually. In the second group of studies, therefore, the quantitative effects of varying amounts of each of the two triacylglycerols alone were studied under circumstances where the intake of dietary cholesterol was again kept constant (0.12%). Thus, while caloric

intake varied in the different groups of animals, it was possible to compare the metabolic effects of each of the triacylglycerols at the same load and, therefore, at the same level of caloric intake. At any level of triacylglycerol feeding, the animals fed the saturated and unsaturated fatty acids achieved equal weight gains over the 30 days.

The marked difference in the effect of these diets on the plasma LDL-cholesterol concentration is shown in Fig. 5. When as little as 5% HCO was added to the cholesterol control diet, the LDL-cholesterol concentration essentially doubled, from 57 to 110 mg/dl. There was a dose-dependent, further increase until the plasma LDL-cholesterol concentration reached 190 mg/dl at a dietary HCO level of 20%. In contrast, the addition of 5, 10 and 20% SO to the diet had virtually no effect on the plasma LDL-cholesterol level. Thus, at the 20% level, saturated triacylglycerol alone resulted in a net increase in the plasma LDL-cholesterol concentration of 133 mg/dl while the same amount of polyunsaturated triacylglycerol caused an average decrease in the concentration of only 8 mg/dl.

Such a result, which has also been observed in human studies, might suggest that the polyunsaturated fatty acids have relatively little effect on LDL metabolism and that the results observed with isocaloric substitution (Fig. 1) simply resulted from reduction of the content of saturated fatty acids in the diet rather than from an independent effect of the polyunsaturated lipids. This possibility proved incorrect. As shown in Fig. 6, there was a dose-dependent, but oppositely directed, effect of both SO and HCO on LDL receptor activity. In the whole animal (A) and liver

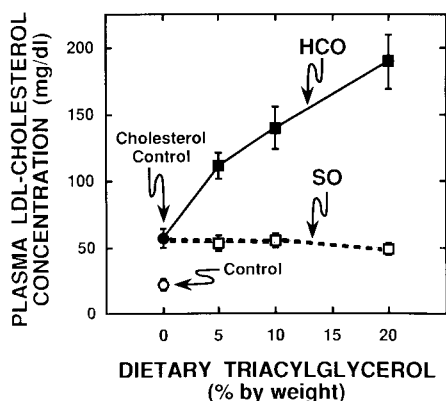


Fig. 5. Plasma LDL-cholesterol concentration in animals fed varying loads of triacylglycerol containing either saturated (HCO) or polyunsaturated (SO) fatty acids. Control animals were fed a diet containing no added cholesterol or triacylglycerol while the cholesterol control animals received a diet containing 0.12% added sterol. All other diets contained 0.12% added cholesterol and from 5 to 20% added saturated (HCO) or polyunsaturated (SO) triacylglycerol. Each point represents the mean \pm 1 SEM for 18 animals.

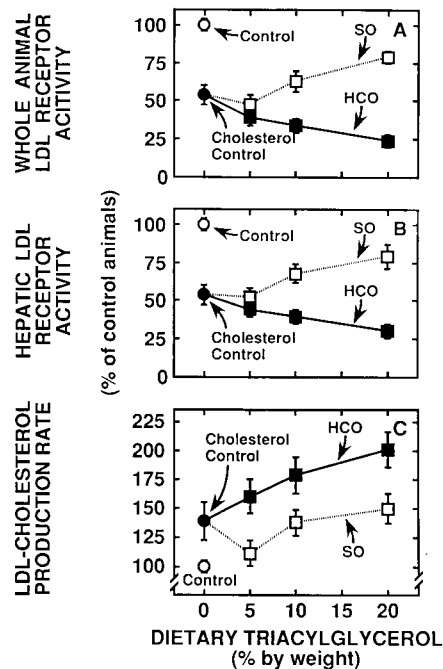


Fig. 6. LDL receptor activity in the whole animal (A) and liver (B) and LDL-cholesterol production rates (C) in animals fed varying loads of triacylglycerol containing either saturated (HCO) or polyunsaturated (SO) fatty acids. The diets fed in this experiment were the same as described in Fig. 5. Each point represents the mean \pm 1 SEM for 18 animals in each group.

(B), increasing amounts of saturated triacylglycerol led to progressive suppression of receptor-dependent LDL transport while increasing amounts of polyunsaturated triacylglycerol restored receptor activity. Furthermore, at the 20% level these effects were quantitatively similar, but oppositely directed, i.e., relative to the cholesterol control, there was approximately a 25% increase and a 25% decrease in receptor activity brought about by polyunsaturated and saturated triacylglycerols, respectively.

There was also a significant difference in the effect of these two lipids on LDL-cholesterol production rates (C). This parameter of LDL metabolism increased from about 140% of control to 203% as increasing amounts of saturated triacylglycerol were added to the diet. In contrast, the LDL-cholesterol production rates found at any level of SO feeding did not differ significantly from the values seen in the cholesterol control animals.

The manner in which these different effects of saturated and polyunsaturated triacylglycerols interacted to give the absolute changes in plasma LDL-cholesterol levels is illustrated in Fig. 7. Feeding increasing amounts of HCO resulted in further loss of LDL-receptor activity (the experimental points moved leftward on the graph) and the LDL-cholesterol production rate increased to 203% of control (the experimental points moved upward). Feeding increasing

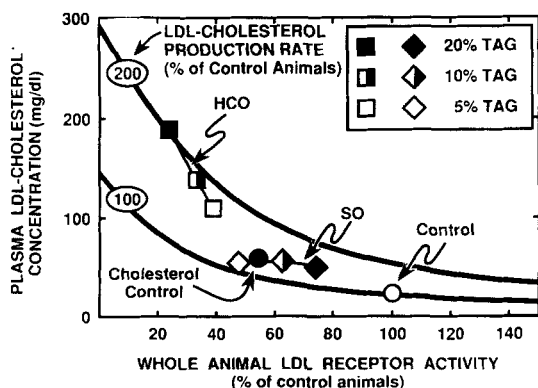


Fig. 7. Plasma LDL-cholesterol concentrations plotted as a function of whole animal LDL receptor activity and LDL-cholesterol production rates in animals fed varying loads of triacylglycerol (TAG) containing either saturated (HCO) or polyunsaturated (SO) fatty acids. The two solid curves show how the LDL-cholesterol concentration varies under circumstances where the LDL-cholesterol production rate is either 100% or 200% of the control value and where whole animal LDL receptor activity varies from 0 to 140% of control. Each point represents the mean \pm 1 SEM for 18 animals.

amounts of SO, on the other hand, caused a significant increase in LDL receptor activity but little alteration in the LDL-cholesterol production rate. Furthermore, these changes resulted in movement of the data points to the right where the receptor-dependent transport process was relatively unsaturated and, therefore, the plasma LDL-cholesterol concentration became insensitive to changes in receptor activity.

Finally, there also were marked differences in intrahepatic cholesteryl ester metabolism induced by these two triacylglycerols. As summarized in Fig. 8, the polyunsaturated triacylglycerol did not significantly change the cholesteryl ester content of the liver cell from that seen in the cholesterol control animals even though LDL receptor activity was increased (Fig. 6). Furthermore, the flow of cholesterol into the hepatic ester pool was markedly suppressed by feed-

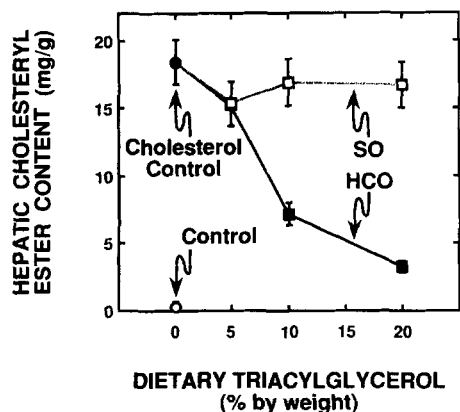


Fig. 8. Hepatic cholesteryl ester content in animals fed varying loads of triacylglycerol containing either saturated (HCO) or polyunsaturated (SO) fatty acids. Each point represents the mean \pm 1 SEM for 18 animals.

ing the saturated fatty acids of HCO even though LDL receptor activity was also suppressed by this experimental manipulation.

These two sets of data suggested that saturated and polyunsaturated fatty acids acted independently to regulate plasma LDL-cholesterol levels and that this independent action could be detected even in mixtures of these two types of triacylglycerol. This observation bears on the important question of human nutrition as to whether it is more effective to reduce the amount of saturated fat in the diet by simply reducing the total content of dietary triacylglycerol or, alternatively, whether it is preferable to essentially substitute polyunsaturated fatty acids for saturated ones while keeping constant, or actually increasing, the level of total dietary triacylglycerol. The third set of studies was designed to specifically address this possibility.

As shown in panel A, Fig. 9, the plasma LDL-cholesterol level in animals receiving a diet containing 0.12% cholesterol and 20% HCO averaged 189 mg/dl. This level decreased to 141 mg/dl when the triacylglycerol content of the diet was reduced to 10%, but declined further to 84 mg/dl when the dietary triacylglycerol content was kept constant at 20%, but half of the HCO was replaced by polyunsaturated fatty acids.

Similar findings were observed when the starting triacylglycerol level was 10% (B). The reasons for this marked difference in plasma LDL-cholesterol levels became apparent when receptor activity and production rates were measured under the same experimental conditions (Fig. 10). Hepatic LDL receptor activity was increased to a significantly greater extent (A) and LDL-cholesterol production was reduced to a greater

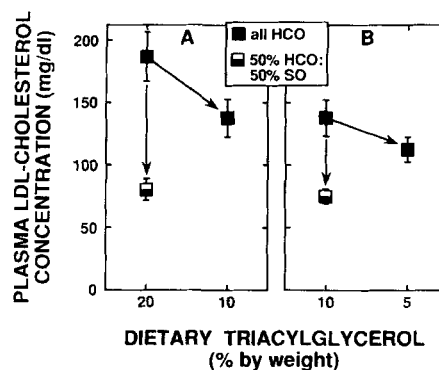


Fig. 9. The effect on the plasma LDL-cholesterol concentration of reducing the level of dietary saturated (HCO) fatty acids in the presence and absence of polyunsaturated (SO) fatty acids. In each experiment, animals were fed either 20% (A) or 10% (B) triacylglycerol containing all saturated fatty acids. In the other two groups in the two panels, the amount of saturated triacylglycerol was either reduced by half or was reduced by half and added to an equal amount of polyunsaturated triacylglycerol. Each point represents the mean \pm 1 SEM for 12 animals.

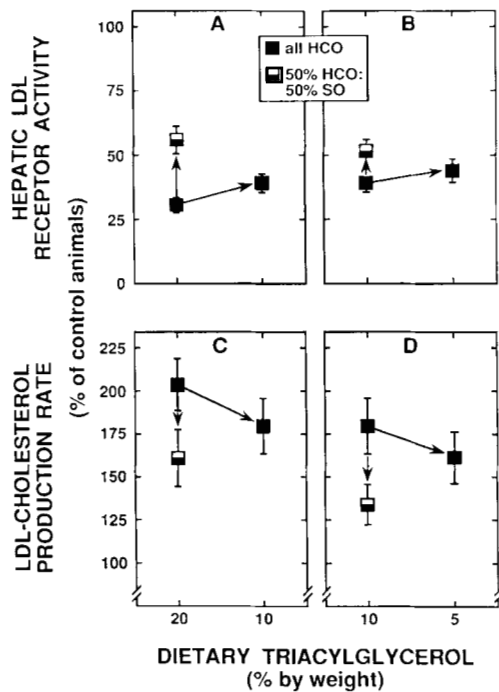


Fig. 10. Hepatic LDL receptor activity and LDL-cholesterol production rates after reduction in the dietary saturated triacylglycerols (HCO) in the presence and absence of polyunsaturated (SO) triacylglycerols. The different experimental groups were fed the diets described in Fig. 9. Each point represents the mean \pm 1 SEM for 12 animals.

degree (B) by keeping the dietary triacylglycerol content at 20% and substituting SO for half of the HCO than by simply reducing the dietary load of saturated triacylglycerol from 20 to 10%. Similar findings were evident at a starting dietary triacylglycerol concentration of 10% (B, D). Thus, as summarized in Fig. 11, the plasma LDL-cholesterol concentration clearly is

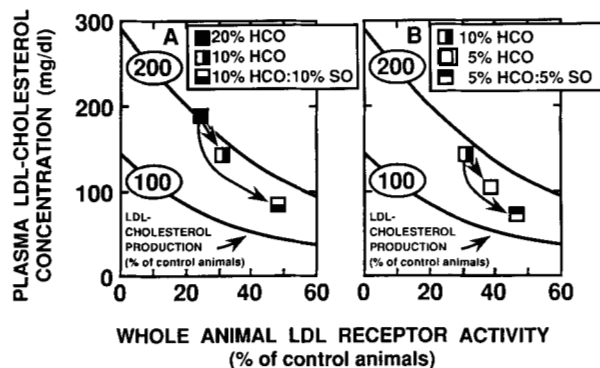


Fig. 11. The independent effects of saturated (HCO) and polyunsaturated (SO) triacylglycerols on plasma LDL-cholesterol concentration. The two solid curves show how the LDL-cholesterol concentration varies under circumstances where the LDL-cholesterol production rate is either 100% or 200% of the control value and where whole animal LDL receptor activity varies from 0 to 140%. The superimposed data points represent the experimental results obtained in the six groups fed different quantities of saturated and polyunsaturated fatty acids. Each point represents the mean value obtained in 12 animals.

more significantly reduced by adding polyunsaturated triacylglycerol to the diet while reducing the content of saturated lipid rather than by simply reducing the content of HCO.

DISCUSSION

In all species in which quantitative data are available, in the absence of dietary lipids the rate of LDL-cholesterol production is low and the capacity for receptor-dependent uptake into the liver is high. As a consequence of these relative rates, the plasma LDL-cholesterol concentration in these species is usually well below 50 mg/dl (1, 2, 20, 23). In the present studies, for example, the hamsters fed the control diet produced LDL-cholesterol (J_r) at only about 150 μ g/h per 100 g animal and had the capacity to remove this lipoprotein fraction from the plasma at the maximal velocity (J^m) of about 720 μ g/h per 100 g; thus, the steady-state LDL-cholesterol concentration in the plasma was in the range of 20–25 mg/dl. When sterols and triacylglycerols are present in the diet, most of the cholesterol and a portion of the fatty acids that are absorbed are delivered to the liver in the chylomicron remnant (24, 25). The presence of these lipids in the liver cell brings about metabolic alterations that ultimately lead to changes in both the LDL-cholesterol production rate and LDL receptor activity, and these changes, in turn, result in secondary alterations in the steady-state concentration of LDL-cholesterol in the plasma. The purpose of the present investigations was to quantitate how the three major groups of dietary lipids, i.e., cholesterol and saturated and unsaturated fatty acids, interact in the liver to regulate these metabolic events.

The addition of cholesterol alone to the diet, in amounts ranging from 50 to 400 mg/1000 kcal intake, causes only modest increases in the plasma LDL-cholesterol concentration in both experimental animals and in humans (1, 26–29). This is true even though increasing amounts of dietary sterol are associated with a dose-dependent increase in hepatic cholesteryl esters, suppression of maximal receptor-dependent LDL uptake and a small increase in the LDL-cholesterol production rate (1). The reason why the concentration of plasma cholesterol does not respond more dramatically to these metabolic alterations is shown by the theoretical curves in Fig. 3. As is apparent, the plasma LDL-cholesterol concentration does not increase linearly as receptor-dependent LDL transport is suppressed; rather, this parameter is very insensitive to changes in receptor activity and increases significantly only when J^m has decreased to less than about 50% of the control value.

Nevertheless, this net inflow of cholesterol into the liver is critically important in determining the quantitative response of the plasma LDL-cholesterol concentration to further alterations in these parameters of LDL metabolism. For example, doubling the production rate raises the plasma LDL-cholesterol concentration by only about 35 mg/dl if receptor activity equals 100% but by 135 mg/dl if J^m is reduced to only 20% of control. Thus, in the present studies the background level of dietary cholesterol was fixed at 0.12% and this resulted in hepatic cholesteryl esters of approximately 18 mg/g (Figs. 4 and 8), suppression of about half of hepatic and whole animal LDL receptor activity (Figs. 2 and 6), a 30–40% increase in the LDL-cholesterol production rate (Figs. 2 and 6), and, as a consequence of these events, a modest rise in the steady-state plasma LDL-cholesterol concentration to approximately 60 mg/dl (Figs. 1 and 5). Thus, any metabolic effect of triacylglycerols that either increased or decreased J^m or J_t could be readily detected and quantitated in this model.

When triacylglycerol containing saturated fatty acids was superimposed upon this background of dietary cholesterol intake, there were further marked alterations in each of these parameters that were related quantitatively to the level of triacylglycerol in the diet. For example, the flow of cholesterol into the pool of hepatic esters progressively decreased as the content of dietary saturated fatty acid was increased (Fig. 8). This change coincided with further suppression of LDL receptor activity (Fig. 6), a marked increase in the LDL-cholesterol production rate (Fig. 6), and a nearly fourfold rise in the steady-state plasma LDL-cholesterol concentration to about 200 mg/dl (Fig. 5). Clearly, however, this very marked increase in the plasma cholesterol level could not be attributed to any one of these changes but, rather, was due to the interaction of the alterations in three separate parameters. This is readily seen in Fig. 7 where it is apparent that the marked increase in the LDL-cholesterol concentration was the consequence of the effect of the changes in J^m and J_t induced by triacylglycerol feeding superimposed upon the changes in LDL receptor activity brought about by the background level of dietary cholesterol.

Very different results were obtained when 5%, 10%, and 20% triacylglycerol containing predominately unsaturated fatty acids was added to the background level of dietary cholesterol. Increasing amounts of these fatty acids had virtually no effect on the flow of cholesterol into the hepatic ester pool (Fig. 8) and the LDL-cholesterol production rate (Fig. 6) but did partially restore receptor-dependent LDL transport (Fig. 6). In fact, the unsaturated fatty acids were quantitatively as effective in restoring J^m as the sat-

urated lipids were in further suppressing hepatic receptor-dependent transport. Nevertheless, the net effect of these various changes on the plasma LDL-cholesterol concentration was very different under the conditions of these experiments. As seen in Fig. 7, restoration of receptor activity moved the data points to the right where the plasma LDL-cholesterol level becomes essentially independent of J^m . This, coupled with the lack of an effect on J_t , fully accounts for the fact that the concentration of LDL-cholesterol decreased only slightly (Fig. 5), even when 20% triacylglycerol containing unsaturated fatty acids was added to the cholesterol control diet. Thus, saturated fatty acids, which move the data points leftward and upward, always produce a quantitatively greater effect in elevating the plasma cholesterol level than do unsaturated lipids, which move the data points horizontally to the right, in lowering the plasma LDL-cholesterol concentration.

These studies illustrate that the effect of a given triacylglycerol on the plasma LDL-cholesterol level very much depends upon the net input of cholesterol into the liver and, hence, the baseline level of hepatic receptor activity. For example, when dietary cholesterol is very low and J^m is near 100%, the plasma LDL-cholesterol will change relatively little when even large quantities of either saturated or unsaturated fatty acids are added to the diet. In contrast, saturated fatty acids will markedly elevate, and unsaturated lipids will significantly lower, the circulating cholesterol levels if increased delivery of cholesterol to the liver has markedly lowered J^m and shifted the data points leftward to the steep part of the theoretical curves shown in Fig. 7. These studies also illustrate the hazards of attempting to judge the regulatory effects of dietary lipids (or other food products or pharmaceutical agents) on parameters of LDL metabolism when only the plasma LDL-cholesterol level is being followed. Depending upon the conditions of the experiment, it is entirely possible to have little effect on the steady-state plasma LDL-cholesterol concentration even though these products or agents have markedly changed J_t or J^m .

One of the characteristics that has been noted in previous experiments, as well as in the current studies, is that there is usually a reciprocal relationship between whole animal receptor activity and LDL-cholesterol production rates. Thus, J_t seems to increase or decrease in proportion to whether J^m activity has been suppressed or restored by changing the dietary content of cholesterol or saturated or unsaturated fatty acids (Fig. 6). In theory, lipids might alter LDL-cholesterol production by changing the rate of apoB synthesis and secretion from the liver, by changing the proportion of VLDL that is metabolized to LDL,

or by bringing about the formation of LDL particles that are more enriched with cholesterol relative to apoB. Studies in the African green monkey, however, have shown that feeding various combinations of cholesterol and triacylglycerol have no significant effects on either hepatic apoB mRNA abundance or the rate of apoB secretion in VLDL from the isolated, perfused liver (30). Cholesterol and triacylglycerol feeding does enrich the LDL particle with cholesteryl esters to a small extent (31, 32, and Woollett, L. A., D. K. Spady, and J. M. Dietschy, unpublished observations), but the major effect of lipid feeding on J_t appears to be articulated through changes in LDL receptor activity. In the receptor-deficient WHHL rabbit, for example, where hepatic secretion of VLDL is normal (33), there is delayed clearance of VLDL from the plasma and enhanced conversion to LDL (34) which, in turn, appears to account for the 5.6-fold increase in the LDL-cholesterol production rate seen in these homozygous animals (23). Thus, in the present studies the dose-dependent increase of J_t seen with saturated fatty acids and the relative decrease in these rates found with unsaturated lipids presumably primarily reflect the reciprocal changes in hepatic receptor activity brought about by the feeding of these lipids.

The next question of importance is whether these different effects of saturated and unsaturated fatty acids could be identified in the more physiological situation where mixtures of triacylglycerol were added to the cholesterol control diet. Under circumstances where total triacylglycerol intake was kept constant at 20% (approximately 45% of calories) and unsaturated lipids were substituted for the saturated fatty acids, the plasma LDL-cholesterol concentration was always reduced significantly more (Fig. 1) than was seen with a comparable reduction in dietary saturated fatty acids alone (Fig. 5). This was also true for restoration of receptor activity and reduction of the production rate (Fig. 2 versus Fig. 6). When tested directly, the additive effects of the two types of lipids were clearly evident. Reducing the dietary content of saturated fatty acids alone was significantly less effective in restoring J^m (Fig. 10), reducing J_t (Fig. 10), and lowering the LDL concentration (Fig. 9) than was a comparable reduction in the level of saturated lipids under circumstances where the total amount of dietary triacylglycerol was kept constant by adding unsaturated fatty acids. Thus, in the presence of dietary cholesterol, the independent effects of saturated and unsaturated fatty acids were clearly evident and algebraically additive. From the standpoint of lowering the plasma LDL-cholesterol level, these findings imply that it is more effective to keep the dietary triacylglycerol level high while substituting unsaturated for saturated lipids

than it is to lower the total dietary triacylglycerol level by simply reducing the content of saturated fatty acids. A similar conclusion was reached in earlier studies carried out in humans (26).

Finally, these studies provide additional information on how dietary fatty acids may act in the hepatocyte to signal regulation of receptor-dependent LDL transport. In general, a net inflow of cholesterol into the liver is associated with a reduction in LDL receptor mRNA abundance (30), a decrease in receptor-dependent LDL uptake (1), and an increased flow of sterol into the ester pool (1). The magnitude of these changes is directly related to the mass of cholesterol flowing into the liver (1) and, presumably, this information is communicated to the nucleus through some putative regulatory pool of cholesterol that reflects the size of the intracellular sterol pool and transmits this information to the sterol regulatory elements of the LDL receptor gene (35, 36). Since the effects of the dietary triacylglycerol described in these studies are very dependent upon the simultaneous flow of cholesterol into the liver, one possibility is that saturated and unsaturated fatty acids have differential effects on net sterol delivery to the liver from the peripheral organs or the intestine. However, direct measurements of cholesterol synthesis in the peripheral organs in these hamsters (unpublished observations) and sterol balance studies in man (37) have failed to reveal such differential effects. On the basis of such data it seems likely, therefore, that the regulatory effect of specific fatty acids may be exerted intracellularly, perhaps by altering the relationships between the putative regulatory pool and the other pools of sterol within the hepatocyte. For example, it is possible that saturated fatty acids accumulating in the liver are poor substrates for cholesteryl ester formation. This might lead, in turn, to a dose-dependent decrease in the flow of cholesterol into the ester pool (Fig. 8), over-expansion of the putative regulatory pool and, hence, disproportionate suppression of hepatic receptor activity (Fig. 6). It should be noted that such a series of events would lead to elevation of the plasma LDL-cholesterol concentration without any demonstrable change in net sterol balance across the liver or whole animal as appears, in fact, to be the situation (37). However, this relatively simple scenario does not explain why unsaturated fatty acids restore receptor activity under conditions where there is no apparent change in the cholesteryl ester pool in the liver cell (Fig. 8). Thus, future experiments must define which specific fatty acids in the triacylglycerols exert these regulatory effects and how the accumulation of these particular fatty acids alter intracellular cholesterol balance. ■■

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