Symposium: Evolution of Ideas about the Nutritional Value of Dietary Fat

Dietary Fatty Acids and the Regulation of Plasma Low Density Lipoprotein Cholesterol Concentrations[1,2]

John M. Dietschy

Department of Internal Medicine, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235–8887

ABSTRACT   Epidemiologic studies over the past 25 years have shown that the level of dietary fat intake is positively correlated with the average serum cholesterol value and mortality from coronary heart disease (CHD). A number of different investigators demonstrated that in addition to total fat, the fatty acid composition of diets influenced serum total cholesterol (TC) in humans. In general, saturated fatty acids were found to elevate the serum cholesterol concentration, and unsaturated fatty acids were found to decrease this value. The lipoprotein fraction most affected was the level of cholesterol carried in low density lipoprotein (LDL-C). It has now been demonstrated that the steady-state level of LDL-C is predominantly dictated by metabolic events in the liver. As the amount of dietary cholesterol entering the body is increased, there is expansion of the sterol pool in the liver cell and down regulation of LDL receptors (LDLR) that are primarily responsible for clearing LDL-C from the blood stream. When dietary cholesterol intake is kept constant, however, long-chain saturated fatty acids further suppress hepatic LDLR activity, whereas several unsaturated fatty acids have the opposite effect. These regulatory events depend upon the availability of the various fatty acids to shift intracellular cholesterol between a regulatory and storage pool of cholesterol, and this effect is mediated by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT).   J. Nutr. 128: 444S–448S, 1998.

KEY WORDS: • *plasma cholesterol* • *low density lipoprotein* • *dietary cholesterol* • *fatty acids*
• *acyl-CoA:cholesterol acyltransferase (ACAT)*

From a study of various populations worldwide, Ancel Keys reported in 1957 that dietary fat intake was correlated with the serum total cholesterol (TC) value and mortality from coronary heart disease (CHD) (Keys et al. 1957). In general, Western countries, including the United States, were high and Third World countries were low in all three parameters. These general findings have been confirmed in a number of subsequent epidemiologic studies over the past 25 years (Epstein 1989). This relationship is illustrated in **Figure 1** with curves that have been derived from two more recent publications. The solid and dashed curve is derived from an investigation of over 356,000 healthy males screened for the Multiple Risk Factor Intervention Trial (MRFIT) by the NHLBI and followed for 10 years (Expert Panel 1988, Stamler et al. 1986). The four data points at the lower end of the curve were calculated from a study of urban Chinese who, on average, have much lower serum TC concentrations and rates of death from

CHD than do Western populations. These latter data are important in that they show the existence of a strong correlation between rates of CHD death and the plasma TC concentration even in populations that traditionally have very low values for both of these parameters. These data suggest that the incidence of CHD death would be essentially zero at plasma TC concentrations below ~140 mg/dL, but above this level would increase in a nearly linear relationship to the serum cholesterol concentration.

EFFECT OF VARIOUS FATS ON SERUM CHOLESTEROL LEVELS

In the early 1950s, many investigators, including Groen in the Netherlands (Groen et al. 1952), Kinsell in California (Kinsell et al. 1952), Keys in Minneapolis (Keys et al. 1957) and Ahrens in New York (Ahrens 1957), studied the effect of varying the fatty acid composition of the diet on TC levels in humans. These and other investigations established that isocaloric substitution of unsaturated vegetable oils for animal fats and saturated vegetable oils caused the serum cholesterol concentration to decrease. The lipoprotein fraction most affected by these dietary shifts was the low density lipoprotein (LDL-C) cholesterol level.

In 1957, Keys et al. proposed a formulation describing the quantitative effects of varying the kind and amount of dietary
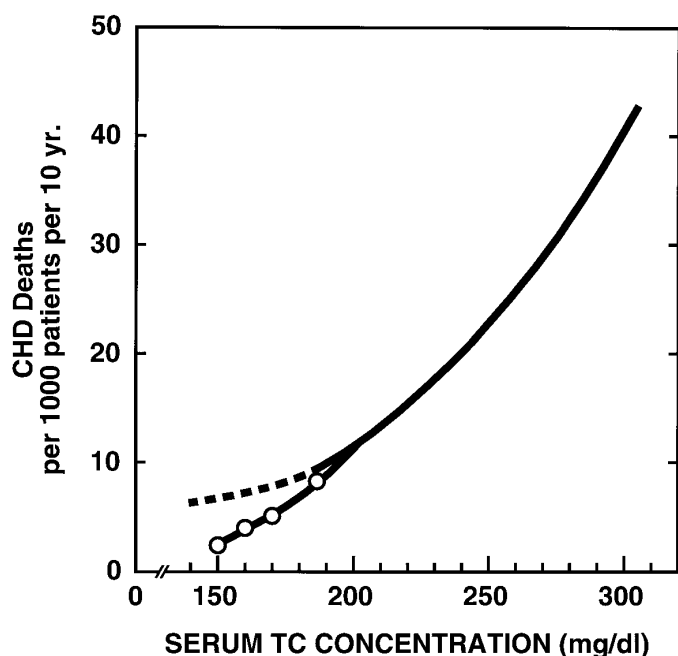
FIGURE 1 Death rates from coronary heart disease (CHD) as a function of serum total cholesterol (TC) concentrations. These curves were constructed after recalculating data from two sources and show the number of deaths per 1000 patients per 10 y. The solid plus dashed curve comes from an analysis of nearly 356,000 men (Expert Panel 1988, Stamler et al. 1986). The four data points in the lower curve were recalculated from a more recent study in urban Chinese (Chen et al. 1991). These data suggest that there is a direct relationship between the serum TC concentration and the rate of death from CHD and, further, that death due to CHD is very uncommon in individuals with a plasma TC concentration < 140 mg/dL.

fat on serum cholesterol levels in men (Keys et al. 1957). This formulation stated the following:

Δ cholesterol

$$= 2.7 \, (\Delta \text{ saturated fat}) - 1.3 \, (\Delta \text{ polyunsaturated fat})$$

The term for monounsaturated fatty acid was omitted because in these studies these particular lipids appeared to have little effect on the serum cholesterol concentration. This formulation, in effect, suggested that a decrease in saturated fatty acids was twice as potent in lowering the serum cholesterol concentration as was an equivalent increase in polyunsaturated fatty acids. Subsequent studies have shown that it is the component of cholesterol carried in LDL that is atherogenic and is affected by these lipids, whereas high density lipoprotein (HDL) appears to be an independent factor that protects against the development of atheroma.

NORMAL PHYSIOLOGY OF FATTY ACID METABOLISM

Fatty acids occur predominantly as esters of glycerol, i.e., triacylglycerol, in natural fats of animal and plant origin. There are many different types of fatty acids, but the most common include myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). The double bond in these naturally occurring unsaturated fatty acids is usually in the *cis* configuration but may be in the *trans* configuration in certain manufactured triacylglycerols.

Humans typically ingest 70–120 g of triacylglycerol per day.

Within the intestine, this lipid is rapidly digested to free fatty acids and monoglycerides, which are absorbed into the enterocyte, resynthesized to triacylglycerol, incorporated into the chylomicron and secreted into the intestinal lacteals. After reaching the blood stream, much of the triacylglycerol in the core of the chylomicron is hydrolyzed to free fatty acids, which are then taken up primarily by the adipose tissue and muscle. The remnant of the chylomicron, which still contains most of the dietary cholesterol that was absorbed, is rapidly cleared from the serum by specific receptors present in the liver. The hepatocyte, therefore, receives most of the cholesterol that is absorbed from the diet and, in addition, becomes enriched with the specific fatty acids that were in the triacylglycerol component of the diet.

As summarized in **Figure 2,** the liver is now known to be overwhelmingly important in the metabolism of two other classes of lipoprotein, VLDL and LDL. The VLDL particle is synthesized in the hepatocytes and functions to move triacylglycerol from the liver to the peripheral organs of utilization. The rate at which cholesterol carried in VLDL is secreted from the liver is known as the VLDL-C production rate (J_t^{VLDL}). A portion of the metabolized remnants of VLDL is apparently cleared directly back into the liver by LDL receptors (LDLR). The remaining remnants are converted to LDL at a rate designated the LDL-C production rate (J_t^{LDL}), and these are subsequently also taken up predominantly by the liver (Dietschy et al. 1993). The LDL receptor plays a critical role in this process (Brown and Goldstein 1986, Yamamoto et al. 1984), and nearly all of the LDLR activity that can be identified in the live animal or human is found in the liver (Dietschy et al. 1993, Spady et al. 1986). As is apparent in Figure 2, the steady-state concentration of LDL-C, therefore, is determined primarily by the LDL-C production rate, the level of hepatic receptor activity (J^m) and the affinity of the LDL particle for the LDLR (Dietschy 1997).

MECHANISMS OF REGULATION OF LDL-C LEVELS BY DIETARY CHOLESTEROL AND SPECIFIC FATTY ACIDS

Both dietary cholesterol and fatty acids have been shown to influence circulating concentrations of LDL-C in animals and humans. Presumably, these lipids must act by changing either hepatic LDLR activity, the LDL-C production rate or both. Recently, new data have become available that provide partial answers to two fundamental questions concerning this regulation: the manner in which the hepatocyte "senses" cholesterol concentration in the cell and whether dietary cholesterol and dietary fatty acids act through separate mechanisms or a single mechanism to regulate LDL-C concentrations. There is little doubt that LDLR activity is regulated by the sterol content of the cell. Recent studies have shown that such cells synthesize a precursor protein called sterol regulatory element binding protein (SREBP) that is attached to the nuclear envelope and endoplasmic reticulum (Sato et al. 1994). When the content of cell sterol is low, a fragment of this precursor protein is cleaved by a specific protease (Duncan et al. 1997). This fragment enters the nucleus where it binds and activates transcription of the LDLR gene. When the regulatory pool (C^R) of the cell is expanded, the cleavage of SREBP is inhibited, LDLR transcription is reduced and functional LDL receptor activity is lowered (Brown and Goldstein 1997, Duncan et al. 1997).

Recently, a model has been proposed that explains how both dietary cholesterol and fatty acid can alter hepatic LDLR activity through such a cellular mechanism (Spady et al. 1993).
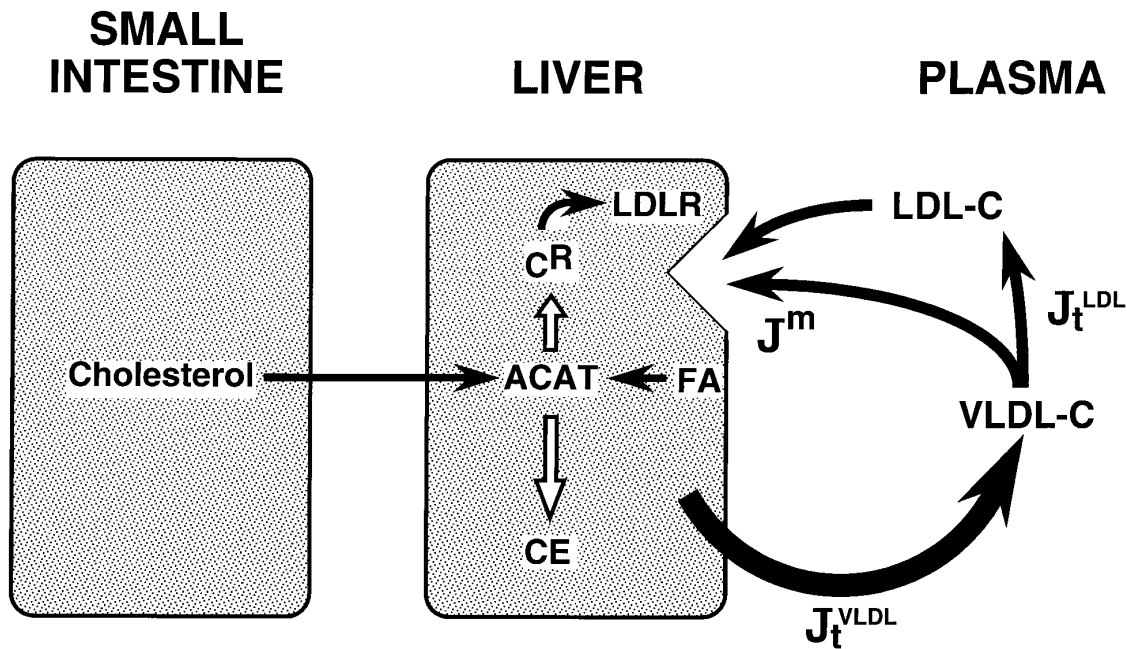
SMALL INTESTINE LIVER PLASMA

FIGURE 2 A model for the major steps that regulate steady-state concentrations of LDL-C. The function of VLDL is to transport triacylglycerol out of the liver. Because cholesterol is also present in this VLDL particle, each day an amount of VLDL-C is secreted that is expressed as the VLDL-C production rate (J_t^{VLDL}). After removal of much of the triacylglycerol core of this particle, a portion of the VLDL-C is directly removed from the plasma, principally through intervention of the LDL receptor (LDLR). The remainder is metabolized to LDL-C at a rate designated the LDL-C production rate J_t^{LDL}. The LDL-C is also principally removed from the plasma by means of the LDLR present in the liver. The levels of both the LDLR (J^m) and J_t^{LDL} appear to be dictated by the inflow of cholesterol from the intestine and the relative abundance of various fatty acids in the liver cell. Several of the long-chain length saturated fatty acids block the acyl CoA:cholesterol acyltransferase (ACAT) reaction and force dietary cholesterol into a regulatory pool (C^R) that then suppresses LDLR activity. In contrast, certain long-chain unsaturated fatty acids shift sterol away from the regulatory pool of cholesterol and enhance LDLR function. These shifts also influence the incorporation of cholesteryl esters (CE) into the VLDL particle. Thus, in the steady state, the concentration of LDL-C is influenced by total hepatic LDLR activity (J^m) and the VLDL-C and LDL-C production rates (Dietschy 1997, Meddings and Dietschy 1987).

This model assumes that both dietary cholesterol and dietary fatty acid alter the size of the cellular sterol regulatory pool (C^R) through their activity in driving the esterification enzyme acyl-CoA:cholesterol acyltransferase (ACAT)[3]. The activity of this enzyme is apparently constitutive, and the rate of the reaction is driven by both of its substrates, i.e., cholesterol and fatty acid. When excess cholesterol is delivered to the liver from the diet, there is presumably expansion of C^R with partial suppression of LDLR activity and a parallel increase in the steady-state concentration of cholesteryl esters (CE). Although the size of C^R cannot be measured directly, in this type of regulation there is always an inverse relationship between the steady-state concentration of CE in the cell and hepatic LDLR activity (Daumerie et al. 1992, Spady et al. 1993, Woollett et al. 1992b). LDLR activity varies linearly and inversely with the concentration of CE in the liver. In this situation, in which regulation is articulated by cholesterol alone, C^R and CE concentrations in the liver cell presumably increase in parallel so that their ratio remains constant (Spady et al. 1993).

This model can also be used to explain the regulatory effects of specific dietary fatty acids. This conclusion is supported by the recent important observation that the quantitative effect of dietary fatty acids is very dependent on the simultaneous inflow of cholesterol to the liver. This is illustrated by the two experiments shown in **Figure 3.** As is apparent, dietary cholesterol alone will raise the LDL-C concentration modestly (*left panel*). The simultaneous feeding of either saturated or unsaturated fatty acid will raise or lower, respectively, the LDL-C concentration achieved by the dietary cholesterol. However, the magnitude of the effect of saturated fatty acid, for example,

is quantitatively much greater when larger amounts of cholesterol are added to the diet. This effect was reproduced in a recent study in humans, as shown in the Figure 3, *right panel* (Fielding et al. 1995). The saturated and unsaturated fatty acids had virtually no effect on the LDL-C concentration in these subjects when there was little cholesterol in the diet, but the two types of lipid could be differentiated when the diet was supplemented with additional amounts of sterol. Incidentally, these two studies indicate how much greater is the cholesterol load in the typical animal experiment, compared with the typical human experiment. Nevertheless, these studies emphasize that fatty acids must be acting primarily through redistribution of the cholesterol pool in the liver cell.

It has recently been proposed that this regulation is articulated by the effect of specific fatty acids on the ACAT reaction (Fig. 2). When a fatty acid such as the 16:0 compound is fed along with cholesterol, sterol esterification is partially inhibited, the size of the CE pool is reduced and, presumably, C^R is expanded (Daumerie et al. 1992). As a consequence of this expansion, receptor mRNA concentrations are reduced and LDLR activity is partially suppressed. In contrast, if the liver is enriched with the preferred substrate for ACAT, i.e. oleic acid, cholesterol is apparently shifted from C^R into the CE pool and LDL receptor activity is increased (Daumerie et al. 1992, Spady et al. 1993). Under these circumstances, in which the cholesterol content of the diet is kept constant, certain long-chain fatty acids suppress hepatic LDLR activity, whereas others enhance the level of LDL-C uptake. In this case, the level of hepatic LDLR activity varies directly and essentially linearly with the steady-state concentration of CE.

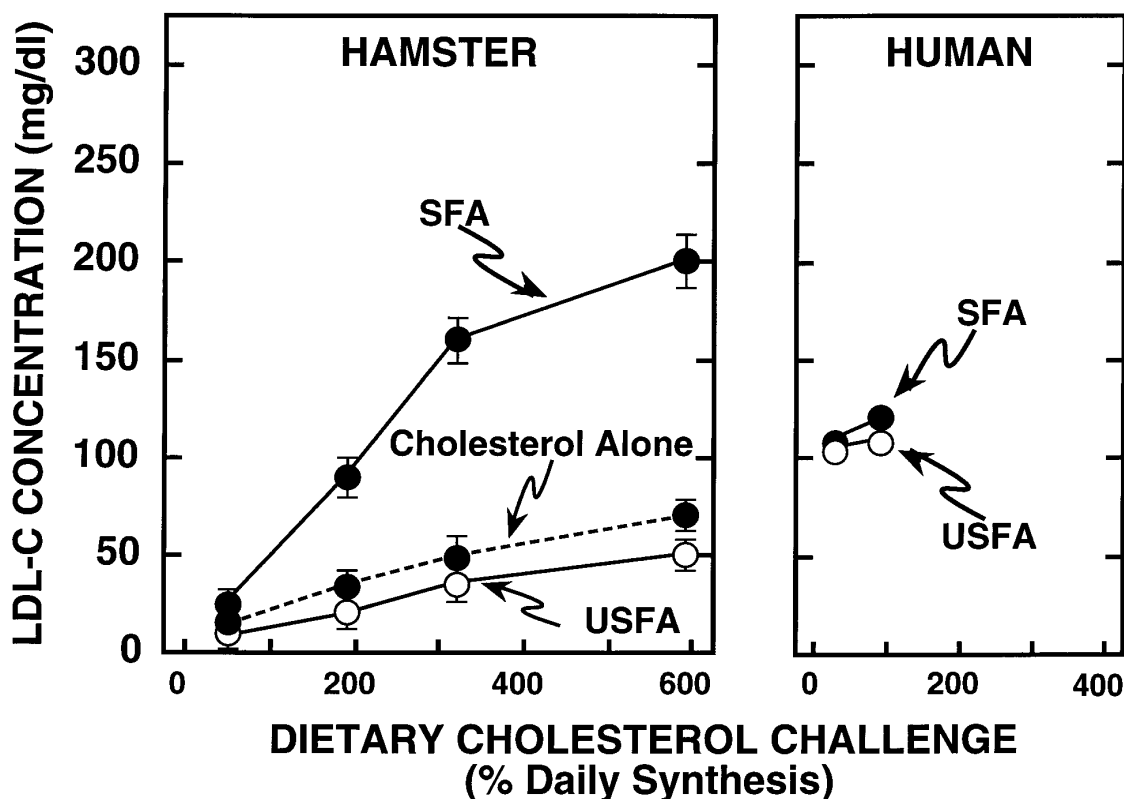A systematic study of pure triacylglycerols containing single

FIGURE 3 The dependency of fatty acid effects on LDL-C concentration on the level of dietary cholesterol intake. This diagram shows the absolute concentration of LDL-C achieved in hamsters or humans fed predominantly saturated fatty acids (SFA) or unsaturated fatty acids (USFA) under circumstances in which the amount of cholesterol in the diet was varied. The dietary cholesterol intake is presented as a percentage of the amount of cholesterol synthesized in the two species each day. This figure is constructed using data from two sources (Fielding et al. 1995, Spady and Dietschy 1988).

fatty acids has led to the conclusion that these compounds fall into three metabolic groups. The first group is made up of short- and medium-chain saturated fatty acids such as the 4:0, 6:0, 8:0 and 10:0 compounds, which are rapidly oxidized in the liver to acetyl CoA. These fatty acids do not alter the composition of the lipid pool in the liver, do not change the concentration of free or esterified cholesterol in the hepatocyte and do not alter hepatic LDLR activity. Thus, such fatty acids are biologically neutral with respect to regulation of the concentration of LDL-C. Interestingly, the long-chain saturated fatty acid, stearic acid (18:0), also appears to belong to this biologically neutral group (Woollett et al. 1992a and 1994). The second category of lipids includes the saturated fatty acids 12:0, 14:0 and 16:0 that do appear to inhibit cholesterol ester formation when fed with cholesterol. These fatty acids inhibit LDLR activity, enhance LDL-C production and increase the concentration of LDL-C in the serum beyond that seen with cholesterol feeding alone.

Finally, the third group of fatty acids is best exemplified by oleic acid, which is the preferred substrate for the ACAT enzyme. When fed along with dietary cholesterol, it markedly increases the cholesterol ester fraction in the liver, presumably reduces the size of the regulatory pool and significantly increases the level of hepatic LDLR activity (Daumerie et al. 1992). As a consequence, this monounsaturated fatty acid, along with other polyunsaturated compounds, lowers the circulating LDL-C level below that seen with cholesterol feeding alone.

Thus, as summarized in **Figure 4,** several of the common long-chain unsaturated fatty acids increase hepatic LDLR ac-

tivity and lower the LDL-C concentration, whereas other long-chain saturated compounds suppress receptor activity and elevate the circulating serum cholesterol concentration. Notably, both the 18:0 fatty acid and the 18:1 unsaturated fatty acid with the double bond in the *trans* configuration appear to be biologically neutral and have no effect on circulating LDL-C levels.

CONCLUSIONS

Although there is now little doubt that the total amount of dietary triacylglycerol and the composition of this fat play a significant role in elevating the plasma cholesterol concentrations in Western man, it has proved extremely difficult to significantly modify the pattern of dietary intake in the typical American. In several studies, for example, dietary modification has resulted in only 5–10% reductions in the TC or LDL-C concentrations. Such reduction would be expected to have only minor effects in reducing the level of CHD deaths (Fig. 1). Fortunately, a new class of pharmaceutical agents has become available, i.e., the 3-hydroxy-3-methylglutaryl (HMG) CoA reductase inhibitors or "statins," that are capable of lowering the circulating serum LDL-C levels by 25–60%. Three large clinical trials with these agents have now shown very significant reductions in coronary artery events and death (Pedersen et al. 1994, Sacks et al. 1996, Shepherd et al. 1995). Although the addition of dietary modification may be a useful adjunct to the therapy of these patients, it is now clear that the principal mode of therapy should be pharmacologic and should be directed at lowering
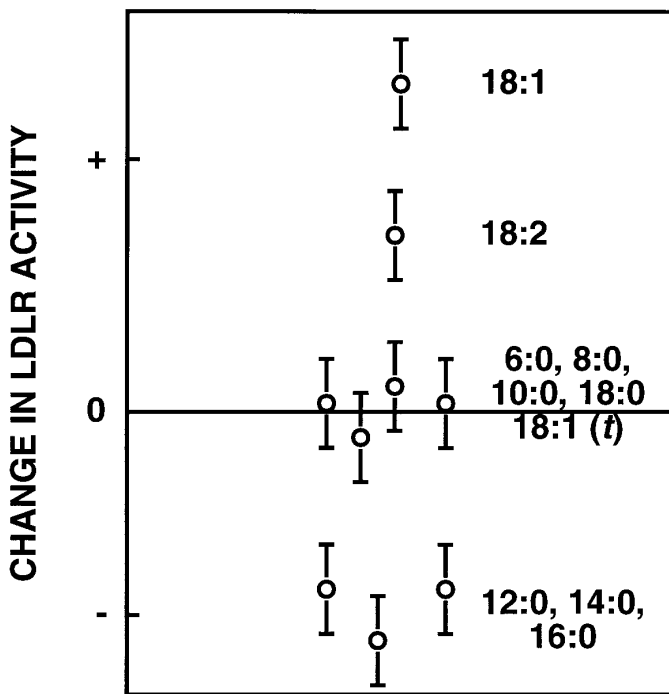
FIGURE 4 The relative change in hepatic LDL receptor (LDLR) activity in hamsters fed single, specific fatty acids. These data are derived from a variety of studies in which the intake of cholesterol was kept constant under circumstances in which the animals were fed identical amounts of single, specific fatty acids. Certain fatty acids increased (+) LDLR activity while others suppressed (−) the level of J^m. It should be emphasized that the absolute magnitude of these changes varies with both the level of cholesterol present in the diet and the amount of each fatty acid that was taken in each day.

the total plasma cholesterol concentration to below 200 mg/dL and the LDL-C level to below 100 mg/dL.

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