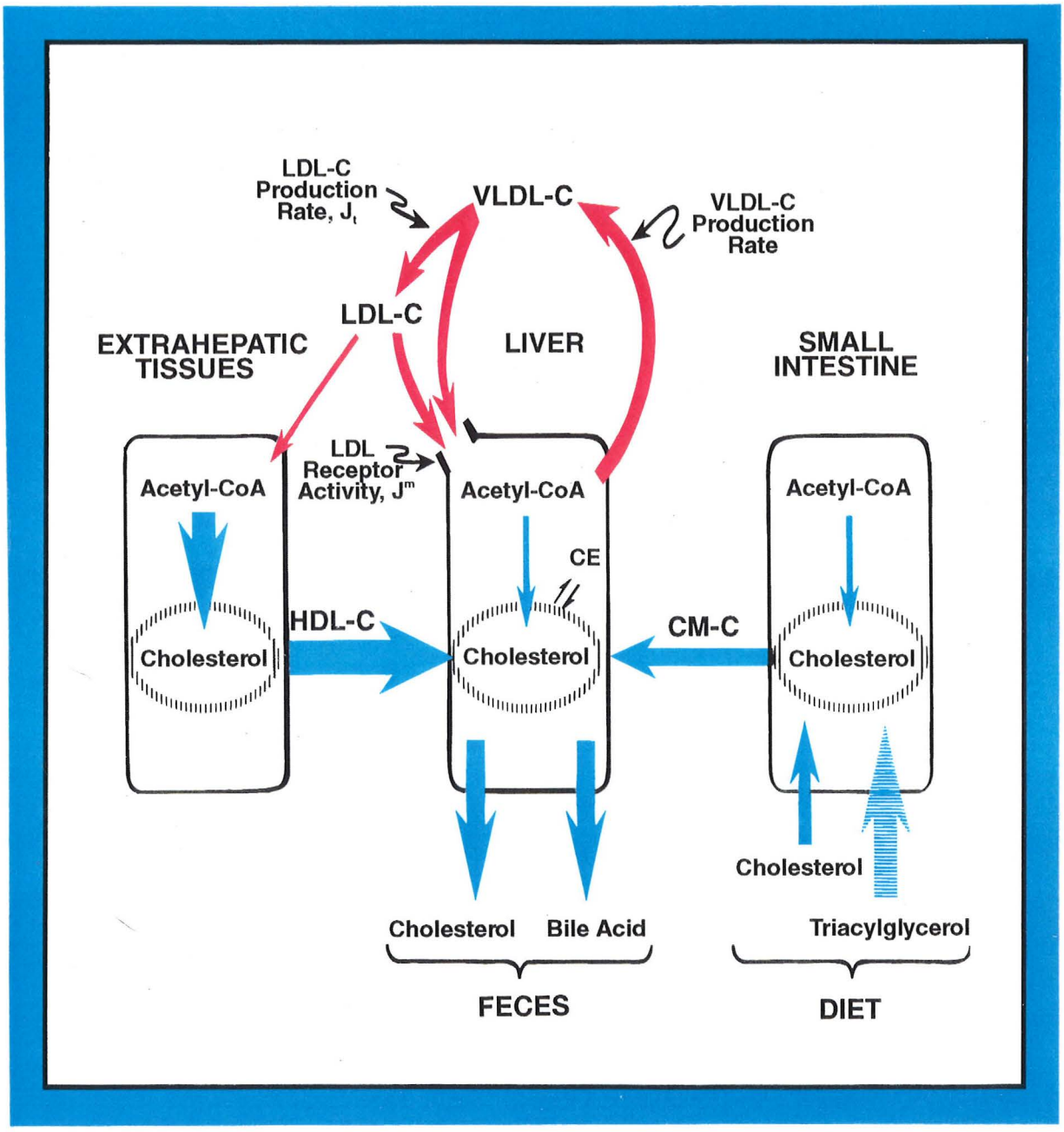
# DOES A LOW PLASMA CHOLESTEROL CONCENTRATION CAUSE MADNESS?





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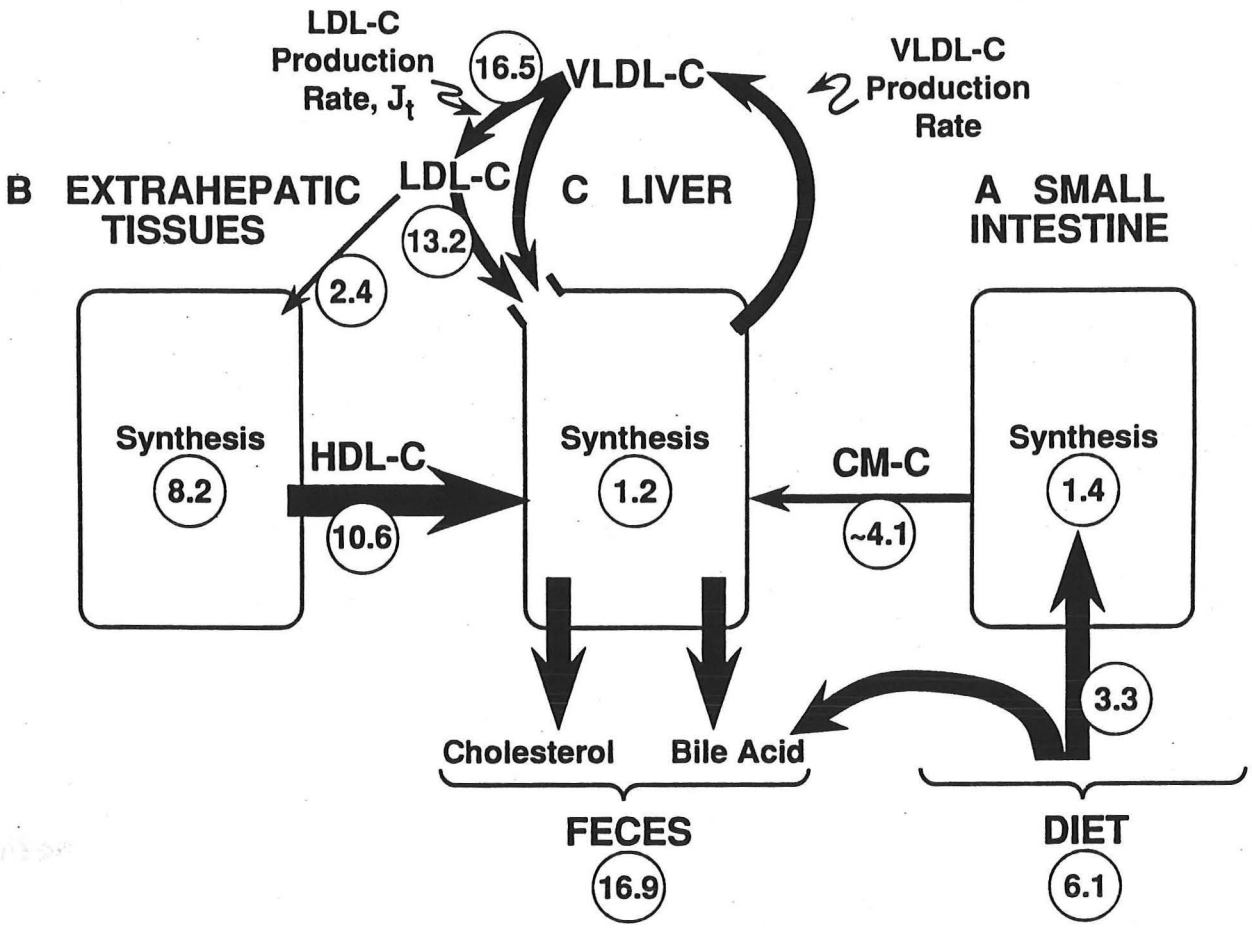
## I INTRODUCTION

Over the past twenty years compelling evidence has accumulated that the incidence of atherosclerosis is directly proportional to the circulating level of cholesterol carried in low density lipoproteins (LDL-C). Furthermore, when the LDL-C concentration is decreased by dietary or pharmacological manipulations, there appears to be a decrease in clinical diseases associated with atherosclerosis. However, in a number of series it appears that there is an increase in non-cardiovascular deaths from illnesses such as stroke or various cancers and, in particular, from violent deaths involving suicide, auto accidents and murders. The question has been raised, therefore, whether lowering the circulating plasma cholesterol level below a certain critical value may lead to disordered brain metabolism with depression, more aggressive behavior and an increased incidence of violent deaths. This protocol reviews the validity of this concept.

## II GENERAL FEATURES OF CHOLESTEROL MOVEMENT ACROSS THE VARIOUS ORGANS OF THE PRIMATE.

Before reviewing the data that deal with these new epidemiological findings, it is first necessary to review the general features of cholesterol and lipoprotein metabolism in the primate. As emphasized in **Figure 1**, the liver plays the central role in two separate, but related, processes which are responsible for maintaining cholesterol balance across individual organs and the whole animal, and for regulating the steady-state concentration of LDL-C in the circulating plasma. Recent data have established that virtually every tissue synthesizes sterol from acetyl-CoA and, in addition, cholesterol may be absorbed into the body from dietary sources. In the fetus or immature animal there must be a net accumulation of approximately 1.5 - 2.0 g of cholesterol for each kg of tissue that is added to the body during growth. In the adult animal that is not growing, however, an amount of cholesterol equal to that which is newly synthesized and absorbed across the small intestine must be excreted each day since the content of sterol in the body remains virtually unchanged over the life of the animal. While small amounts of cholesterol are lost through sloughing of skin and intestinal epithelial cells and by conversion to various steroid hormones, the major excretory pathway involves the secretion of sterols into bile by the hepatic parenchymal cells. This process includes both the direct secretion of cholesterol itself as well as the trans-canalicular movement of bile acid, the metabolic end-product of cholesterol degradation by the liver.




Thus, as illustrated in **Figure 1**, an amount of cholesterol equal to that newly synthesized each day in the extrahepatic tissues (B) must be transported through the plasma, presumably carried in high density lipoproteins (HDL-C), to the liver. Similarly, the sterol that is either synthesized or absorbed in the small intestine (A) must also reach the liver, although in this case the chylomicron particle (CM-C) is the primary carrier in the plasma. In the steady-state, where the weight of the animal is constant and no changes are occurring in the cholesterol concentration in any tissue compartment, the absolute rate of cholesterol synthesis and absorption must equal the absolute rate of cholesterol and bile acid excretion in the feces (plus the small amounts of sterol lost from the skin or converted to various hormones). Thus, the daily turnover of cholesterol in any species, expressed as the mg of cholesterol entering and leaving the body pool per day per kg body weight, can be quantified by either measuring the absolute rate at which sterol is being synthesized and absorbed in the intact animal or by measuring the absolute rate at which cholesterol and bile acid are being excreted in the feces.

The liver also plays the central role in the metabolism of cholesterol carried in very low density lipoproteins (VLDL-C) and LDL. Although the principal function of the VLDL particle appears to be the transport of triacylglycerol from the liver to the peripheral organs for oxidation or storage, this particle also contains both free and esterified cholesterol; thus, sterol is secreted from the liver in this

particle at a velocity defined as the VLDL-C production rate (**Figure 1**). During the metabolism of the VLDL particle with removal of a large portion of its triacylglycerol core, a remnant is formed and a portion of the VLDL is also converted to LDL at a velocity defined as the LDL-C production rate ($J_t$). Both the LDL-C and the VLDL remnants appear to be largely cleared from the plasma by LDL receptors located in the liver. The level of hepatic LDL receptor activity ($J^m$) is markedly influenced by net cholesterol balance across the liver and by the types of fatty acids reaching the hepatocyte from the diet. When hepatic LDL receptor activity is suppressed, less of the VLDL remnants is cleared by the liver and there is a corresponding increase in the LDL-C production rate and an elevation of the circulating LDL-C concentration. Conversely, when LDL receptor activity is increased, the LDL-C production rate is decreased and the LDL-C concentration in the plasma is lowered.

The numbers shown in circles in **Figure 1** illustrate the magnitude of each of these fluxes in a primate-like Cynomolgus monkey. These figures represent the mg of cholesterol moving through each of the pathways each day per kg body wt and are very similar to those figures established in humans. In such primates about 80% (8.2 mg/day per kg) of the cholesterol that is synthesized in the body is synthesized in the extrahepatic organs. Lesser amounts are synthesized in the liver and small intestine. Each day about 6 mg/day per kg of cholesterol is eaten in the diet of which approximately half is absorbed into the body. Thus, the animal must excrete in the feces an amount of cholesterol (16.9 mg/day per kg) equal to that synthesized in all of the tissues of the body and that eaten in the diet. Under these conditions about 16.5 mg/day per kg is converted from VLDL-C to LDL-C and approximately 80% of this is immediately cleared back into the liver. Since only 2.4 mg/day per kg of LDL-C is delivered to the extrahepatic tissues, approximately 10.6 mg/day per kg of sterol must be delivered from the extrahepatic tissues to the liver carried in HDL. The amount of cholesterol and, particularly, triacylglycerol in the diet affects both LDL-C production and LDL receptor activity and so alters the steady-state concentration of LDL-C in the plasma.

## III FORMATION OF THE ATHEROMA.

Abundant data, mainly from various epidemiological studies, have shown that the development of atherosclerotic disease is directly proportional to the steady-state concentration of LDL-C in the plasma and inversely proportional to the concentration of cholesterol carried in high density lipoproteins (HDL-C). The LDL

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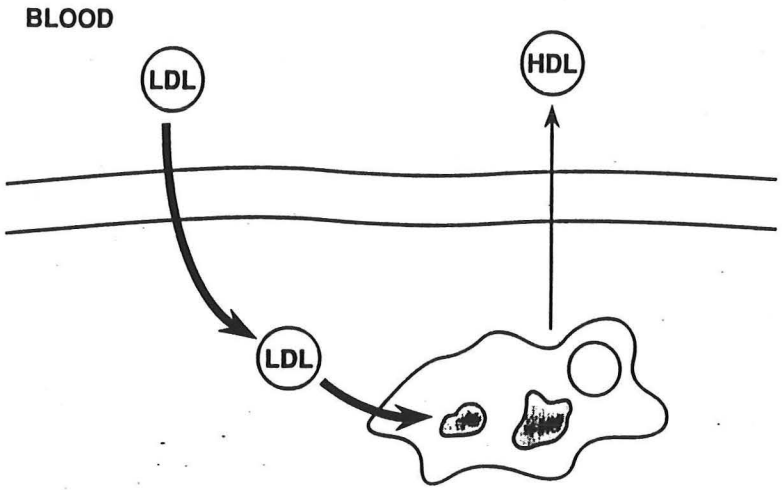


molecule is apparently able to very slowly penetrate the endothelial lining of the arterial system. Certain components of this particle may undergo subtle oxidation in such a way that the particle is recognized by a scavenger receptor present on tissue macrophages. This scavenger receptor (PNAS 76:333, 1979) is separate from the LDL receptor, although both were discovered by Brown and Goldstein. With the recruitment of additional monocytes from the circulating blood, the uptake of this modified LDL leads to the accumulation of "foam" cells that are filled with cholesteryl esters. This process of early fatty-streak formation clearly begins in childhood and the percentage of the surface area of the vessel that is involved in this process is directly proportional to the concentration of LDL-C in the child. As the lesion matures there is hyperplasia of fibroblasts and smooth muscle cells so that the lesion begins to protrude into the lumen of the vessel, and a fibrous cap is formed over the top of the fat-laden cells. The atheroma slowly increases in size over many years and may lead to overt oclusion when the fibrous cap ruptures and clot formation occurs. Thus, actual infarction of an organ involves a series of complex interactions including atheroma formation, atheroma rupture, clot formation and arterial smooth muscle spasm. All evidence now indicates that the underlying lesion, the atheroma, begins to form in childhood, and the rate of its formation is directly proportional to the concentration of LDL-C in the plasma.

## IV THE QUANDARY: DOES LOWERING THE PLASMA CHOLESTEROL CONCENTRATION INCREASE MORTALITY BY TRAUMA WHILE DECREASING CARDIAC DEATHS?

Over the last 20-30 years a series of different studies have suggested that the incidence of clinically evident atherosclerosis is related to the plasma cholesterol concentration. Furthermore, additional interventional studies indicated that

actively lowering the plasma cholesterol level decreased the incidence of atherosclerotic disease and, in some cases, actually reversed the process of atheroma formation. Generally, such data took the form of the relationship shown in **Figure 3**. In a large population some end-point of clinical atherosclerosis was defined; commonly this was the onset of angina or a myocardial infarction which heralded the onset of clinical coronary artery disease. The incidence of such disease, commonly corrected for age and sex, was then plotted against the plasma cholesterol concentration. Initially, many of these series were referred to the plasma total cholesterol concentration while more recent series have all been related to the concentration of cholesterol carried in LDL. In nearly all of these clinical studies a relationship such as that shown in **Figure 3** has been found: the risk of developing clinical heart disease, or some other manifestation of atherosclerosis, is directly related to the plasma total (LDL) cholesterol concentration.

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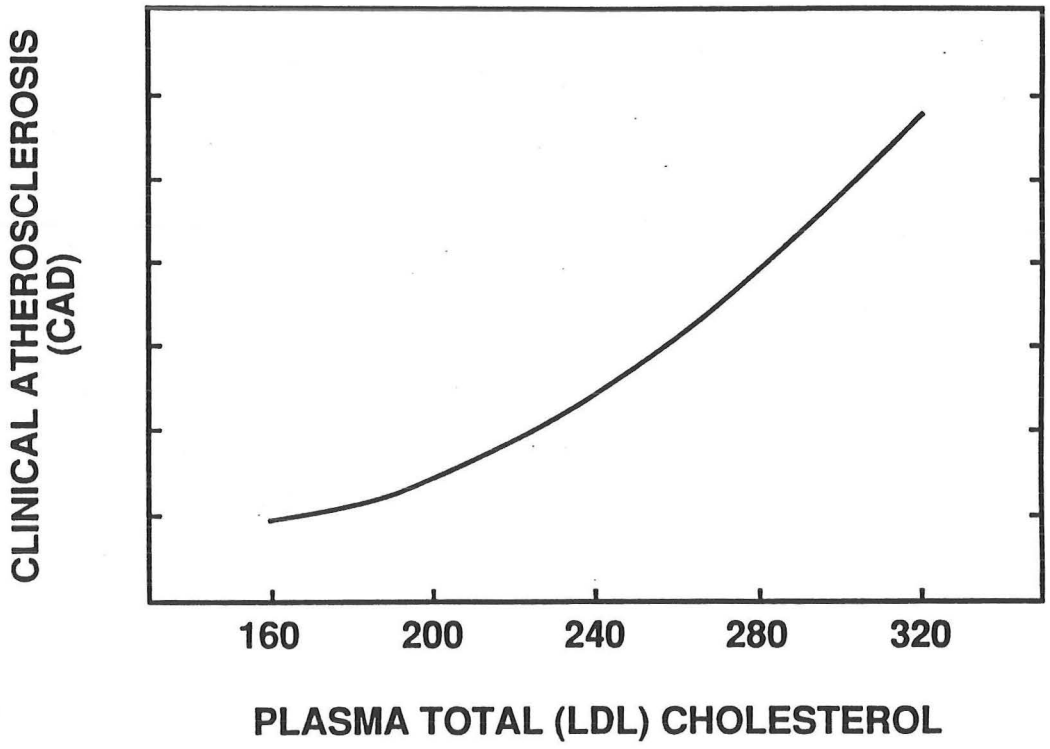

While this relationship was observed in nearly all of the major clinical series, in several of these clinical trials there appeared to be an increased rate of death in the patient groups with the lowest cholesterol levels. Some of these deaths were attributable to cancer and stroke, but many appeared to be due to trauma. This second group consisted of patients who were murdered, committed suicide or died in a variety of violent ways. Furthermore, while lowering the plasma cholesterol concentration unequivocally decreases the incidence of clinical atherosclerotic disease, it was difficult in some of these series to demonstrate that actual mortality was reduced by manipulating the plasma lipid level. Thus, as illustrated in **Figure 4**, when mortality from all causes was plotted as a function of the plasma

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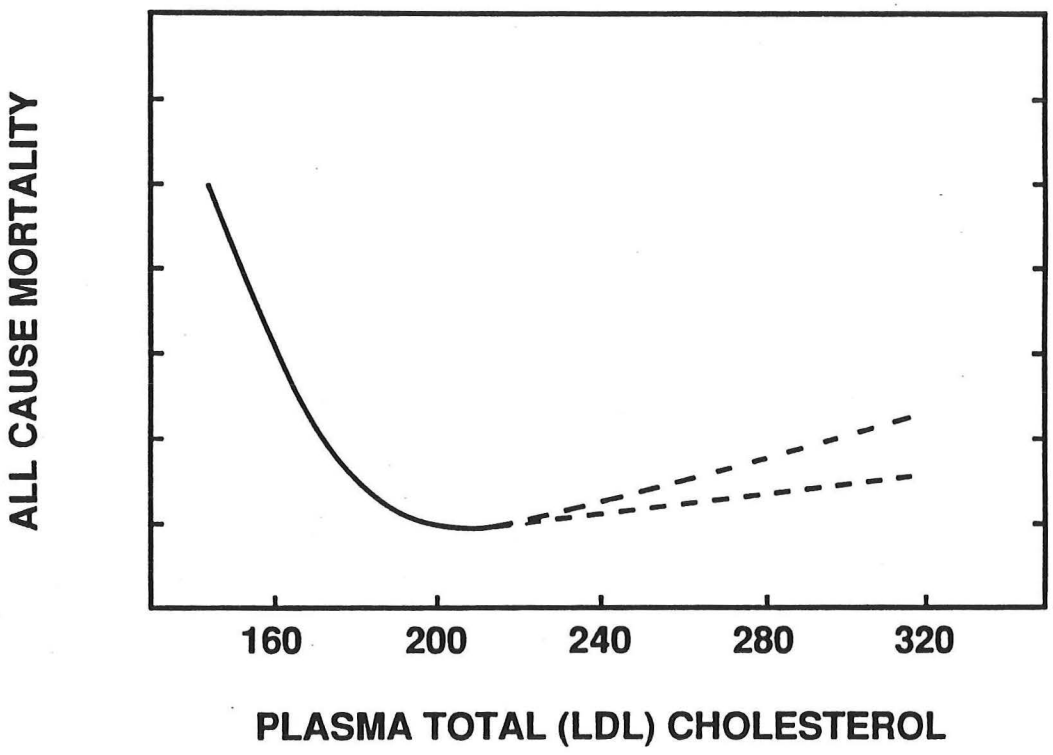


cholesterol level a "U" shaped curve often emerged. Thus, as the plasma cholesterol level was progressively lowered, there was probably a decrease in patient mortality until the plasma cholesterol level reached an intermediate value, but then the mortality rate begin to increase again as the cholesterol concentration was reduced to still lower levels. Thus, the current quandary emerged as to 1) whether reducing high plasma cholesterol concentrations actually reduced the number of patient

deaths from atherosclerotic disease and 2) whether low plasma cholesterol levels were actually associated with an increased number of none-cardiac deaths that resulted from suicide, trauma, cancer and other illnesses. This possibility has been widely popularized in the medical and lay press during the last year by one or two epidemiologists and cardiologists who, on the basis of these observations, have implied that plasma cholesterol screening and treatment should be stopped since there is little demonstrable effect on all-case mortality and, in fact, more individuals may be dying as a result of a low plasma cholesterol level than are dying of the consequences of a high LDL-C concentration. These few investigators have been widely quoted in the lay press, particularly in England, Germany and Canada, and serious questions have been raised even in this country concerning the effectiveness of these cholesterol lowering programs.

## V DATA SUPPORTING A REDUCTION IN CARDIAC EVENTS AND MORTALITY BY LOWERING THE PLASMA CHOLESTEROL CONCENTRATION.

Several very early studies clearly outlined the various risk factors that were associated with the development of clinical cardiovascular disease or were associated with death due to CHD. In the Framingham Study, for example, the eight-year probability of developing a cardiovascular disease (per 1,000) was markedly dependent upon the plasma total cholesterol concentration as well as the presence or absence of glucose intolerance, systolic hypertension, cigarette smoking and left ventricular hypertrophy. In the absence of any other risk factor, the probability increased from <1 to 3.9/1000 as the plasma cholesterol concentration increased from 185 to 335 mg/dl. However, at the highest plasma cholesterol concentration this probability increased to 23/1000 in patients with glucose intolerance and systolic hypertension, to 35 in such patients who also smoked, and to 60 in such patients who also manifested left ventricular hypertrophy. However, within any of these risk groups the absolute risk of developing cardiovascular disease was very dependent upon the plasma cholesterol level. Thus, in the patient group with glucose intolerance, hypertension and cigarette smoking, the relative risk could be reduced nearly seven-fold by reducing the plasma cholesterol concentration from 335 mg/dl to 185 mg/dl.

Similar associations were found in the MRFIT Study where the age-adjusted CHD death rate/1000 men/6 years increased from approximately 3 to 18 as the plasma total cholesterol concentration increased from 140 to 300 mg/dl. This

investigation, as well as several other studies, suggested that a "threshold" level of cholesterol was being reached at about 200 mg/dl: below this level the incidence of CHD disease did not dramatically decrease as the plasma cholesterol level was further reduced. However, similar data have recently been gathered in over 9,000 Chinese men and women living in urban Shanghai, China. The overall level of the plasma cholesterol concentration and the incidence of coronary heart disease was substantially lower in this population than in the American and European populations that had been studied earlier. Nevertheless, as illustrated in **Figure 5**, the relative risk of CHD was again directly related to the mean total cholesterol level in this population. Most importantly, there was no evidence of a "threshold" effect. Thus, the relative risk of heart disease appeared to decrease as an almost linear function of the total plasma cholesterol concentration, even in this population that traditionally had much lower circulating plasma cholesterol levels. There was no significant relationship between the serum cholesterol concentration and deaths due to stroke or various cancers.

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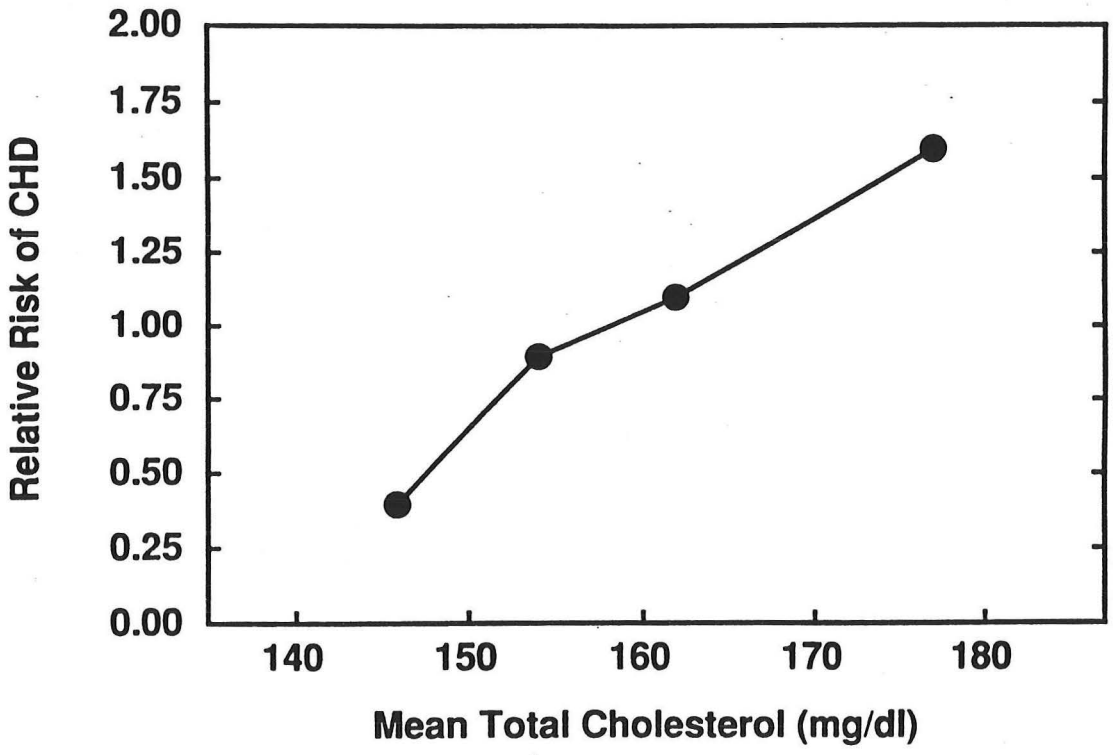


These studies as well as a number of other investigations, imply that the risk of heart disease, and therefore the incidence of atherosclerosis in the coronary vessels, is directly related to the average cholesterol concentration and, further, that

this is a time dependent process. Direct support for this conclusion comes from the PDAY research group which has quantitatively measured the level of atherosclerosis in young people who died abruptly from trauma. In these investigations it was shown that the percentage of the arterial system involved in early fatty streaks was directly proportional to the plasma LDL-C concentration. Furthermore, in a more recent analysis of these data (**Figure 6**), it is clear that this

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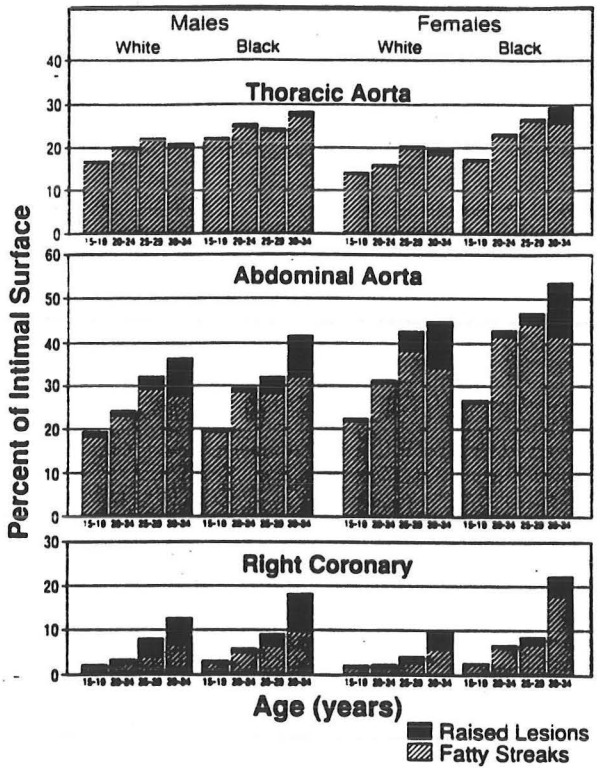


process is time dependent. In over 1,500 individuals, varying in age from 15 to 34 years, involvement of the right coronary artery markedly increased with age. Furthermore, there was progressive conversion of early fatty streaks to raised atheromatous lesions in these young people. These findings were essentially the same in males and females, whites and African-Americans. Clearly, the inescapable conclusion from all of these studies is that fatty streak and atheroma formation begins in childhood and progressively expands throughout life. Furthermore, the rate of increase in these atherosclerotic plaques as a function of time is directly dependent upon the steady-state concentration of LDL-C profusing the arterial lining.

With the recognition of this association between clinical heart disease and the plasma cholesterol level, a number of intervention trials have been undertaken during the last twenty years. Unfortunately, many of these studies were carried out

in an era when it was difficult to lower the plasma of LDL-C concentration more than 10-20 percent. As summerized in **Table I**, for example, many of these studies were carried out by dietary manipulation or the use of agents or operative procedures that increased cholesterol loss from the body. Feeding bile acid sequestrant or ileal passby increases hepatic LDL receptor activity and lowers the plasma LDL-C concentration (**Figure 1**). These various procedures have been utilized in a variety of studies, **Table II**, to observe the effect of such treatments on various parameters of lipoprotein and coronary artery metabolism.

**Table I**

- Lower dietary cholesterol
- Switch from saturated to unsaturated fatty acids
- Bile acid sequestrants
- Ileal resection
- Agents that block cholesterol absorption
- Agents that inhibit cholesterol synthesis
- Combinations
- Agents of unknown mechanisms

**Table II**

| Trial | Diet/ drug/ other | Primary/ secondary | Single/ multi-factor | Open/ blind | M/F | Age range (yr) | Mean age (yr) | Follow up (yr) | Baseline serum cholesterol (mmol/l) |
|---|---|---|---|---|---|---|---|---|---|
| MRFIT | Diet | Primary | Multi | Open | M | 35–57 | 46 | 6–8 | 6.55 |
| Hjermann et al | Diet | Primary | Multi | Open | M | 40–49 | 45 | 6–7½ | 8.42 |
| WHO fact | Diet | Primary | Multi | Open | M | 40–59 | 48 | 5–6 | 5.60 |
| Acheson and Hutchinson | Drug | Secondary | Single | Blind | M+F | - | - | ≤7 | 7.46 |
| Carlson et al | Drug | Secondary | Single | Open | M+F | ≤70 | 59 | 3½ | 6.40 |
| Coronary Drug Project | Drug | Secondary | Single | Blind | M | 30–64 | 54 | 4½–8 | 6.45 |
| Dorr et al | Drug | Secondary | Single | Blind | M+F | 18+ | 54 | 3 | 7.95 |
| Dayton et al | Diet | Secondary | Single | Open | M | 55+ | 66 | ≤8 | 6.06 |
| Leren | Diet | Secondary | Single | Open | M | 30-64 | 56 | 5 | 7.67 |
| MRC | Diet | Secondary | Single | Open | M | ≤60 | - | 2–7 | 7.05 |
| Woodhill et al | Diet | Secondary | Single | Open | M | 30–59 | 49 | 2–7 | 7.31 |
| LRC-CPPT | Drug | Primary | Single | Blind | M | 35–59 | 47 | 7–10 | 7.23 |
| WHO (clofibrate) | Drug | Primary | Single | Blind | M | 30–59 | 45 | 5.3 average | 6.47 |
| Frick et al | Drug | Primary | Single | Blind | M | 40–55 | 47 | 5 | 7.47 |
| Frantz et al | Diet | Primary | Single | Open | M+F | ALL | - | 5 | 5.36 |
| Miettinen | Other | Primary | Multi | Open | M | 40–55 | 48 | 5 | 7.12 |
| Gothenburg | Other | Primary | Multi | Open | M | 47–55 | - | 10 | 6.48 |
| POSCH | Other | Secondary | Single | Open | M+F | - | 51 | 9.7 | 6.50 |
| DART | Diet | Secondary | Single* | Open | M | ≤71 | 56 | 2.0 | 6.48 |

Some of these trials involved primary prevention of heart disease while others were devoted to secondary prevention. These studies involved a variety of genetically different populations, both men and women, and utilized various endpoints for defining differences in the treated and control groups. In one of the earliest clinical trials of this type, The Lipid Research Clinics Coronary Primary Prevention Trial, followed patients for 7.4 years using cholestyramine resin as the active drug. This compound only reduced the plasma LDL-C level by 13% but reduced the incidence of myocardial infarction by approximately 24%. This study gave rise to the relationship that a 1% decrease in the plasma LDL-C level can produce a 2% reduction in coronary events.

The results of many of these trials have been recently analyzed by Dr. Ingar Holme, **Figure 7**, for example, shows the decrease in CHD risk relative to the

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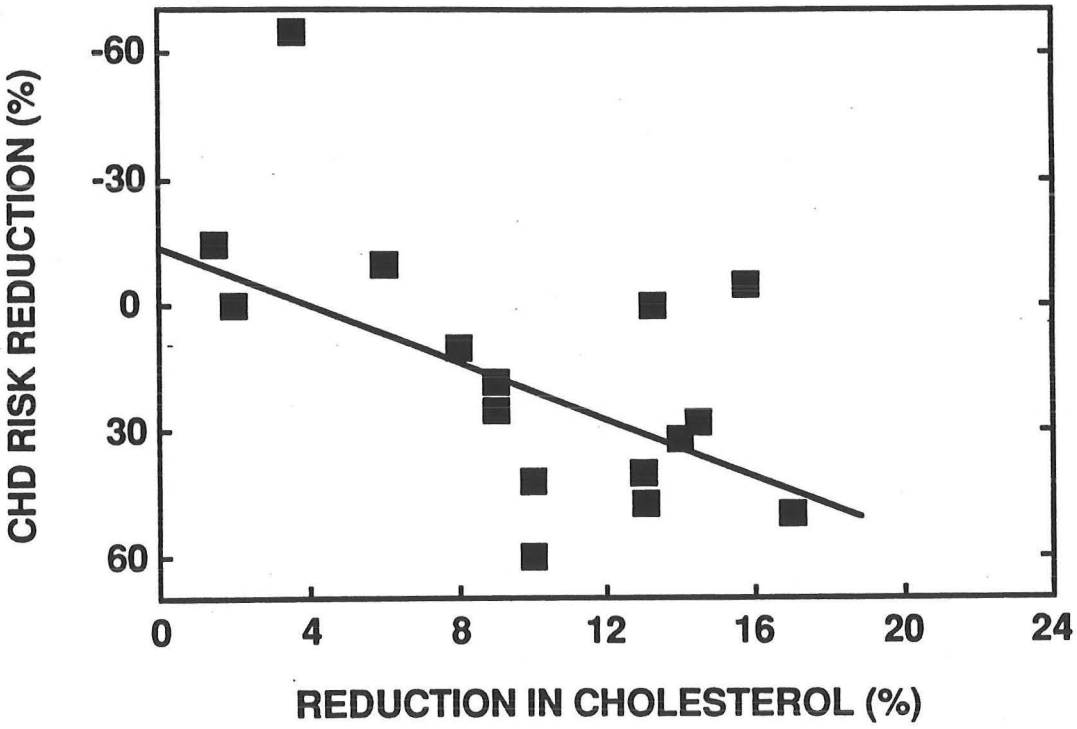


reduction in plasma cholesterol concentration achieved when 16 of these trials were combined. On average, these combined studies gave a decrease in CHD risk of 2.5% for each 1.0% reduction in the plasma total cholesterol level. Thus, these 16 combined studies gave essentially the same results as originally reported in the LRC investigation. That this reduction in CHD risk is associated with a change in the morphology of coronary arteries is illustrated by the data in **Figure 8**. As is

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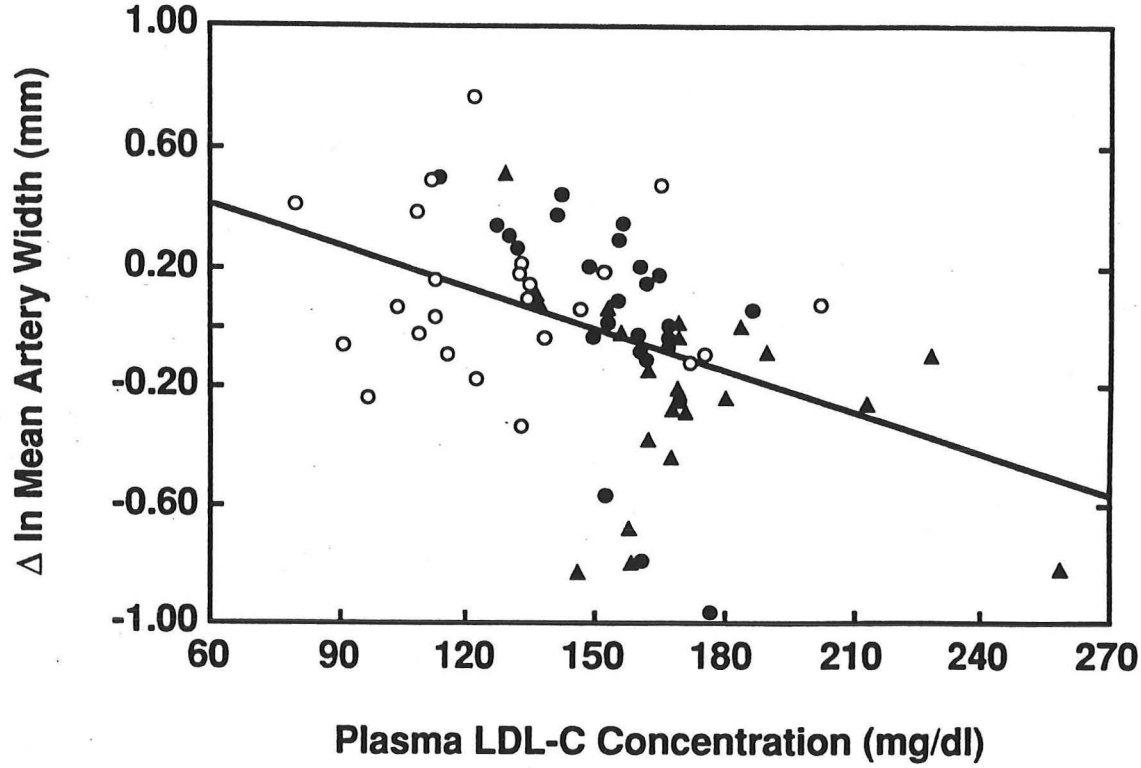


apparent, mean artery width actually decreased in the control group (▲), remained unchanged in the group treated with diet alone (●), and increased in the group more aggressively treated with drugs (○). In fourteen of these studies mortality data were also available as a function of the percentage reduction in the serum cholesterol (**Figure 9**). As is apparent, there was an inverse relationship between total mortality and the percent reduction in cholesterol levels, but this lowering of the mortality rate did not become significant until at least an 8-9% reduction in the plasma cholesterol was achieved.

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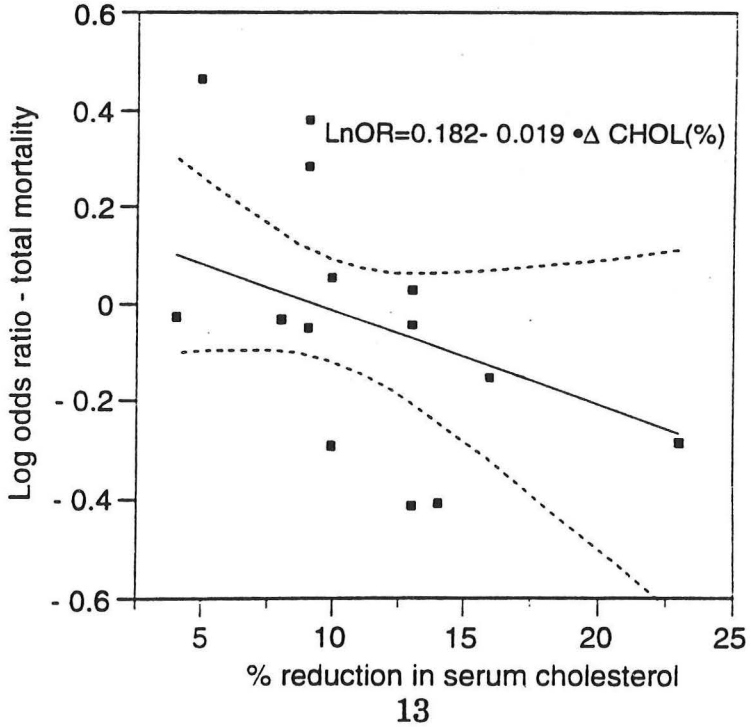

Thus, individual studies, as well as this analysis of combined studies unequivocally established that lowering the plasma cholesterol level lowers the risk of CHD, stops or even reverses the development of atherosclerosis and, minimally, reduces overall mortality. It should be emphasized that these effects are all the more surprising considering that in most of these series the reduction in the plasma cholesterol level was relatively minor, in the range of 10-20% and, for practical purposes, did not even lower the plasma cholesterol levels into the range seen in Japanese or Chinese populations and did not begin to approach the concentrations of LDL-C that are considered to be "normal" in nonhuman primates and other experimental animals.

## VI DATA IMPLYING THAT TRAUMATIC DEATHS ARE INCREASED AT LOW PLASMA CHOLESTEROL LEVELS.

While these data appeared to demonstrate unequivocally the beneficial effects of lowering the plasma cholesterol level, there were several bothersome features evident in even the earliest clinical trials. In the LRC Prevention Trial, for example, the risk of death from all causes was only slightly, and not significantly, reduced after feeding cholestyramine, and there appeared to be a greater number of violent and accidental deaths in the treatment groups. This also was true of the Scandinavian Study using the cholesterol lowering drug Gemfibrozil. The treated group appeared to have a higher rate of fatal accidents, homicides and suicides in the treatment group compared to the control group. When such data are formally plotted, as in **Figure 10**, the relative risk of an individual dying, therefore, appears to be high at both high plasma cholesterol levels and at low sterol levels. Thus, in this study performed in 8,000 Japanese-Americans in the Honolulu Heart Study, there was clearly an increase in all-cause mortality in patient groups in the third, fourth and fifth quintile plasma cholesterol levels. However, there was also an increase in mortality seen in those patients in the very low cholesterol quintile.

Findings such as these caught the attention of several epidemiologists and cardiologists who postulated that lowering the plasma cholesterol value below a certain critical level led to major physiological disruptions, particularly in the central nervous system that was causally related to aggressively behavior and increased incidents of dramatic deaths. This was formulated into a hypothesis by Hyman Ingelberg and published in Lancet (1992). As shown in **Figure 11**, this hypothesis had three parts. First, that, in fact, there was a statistically significant increase in violent deaths in patients with a low plasma cholesterol concentration.

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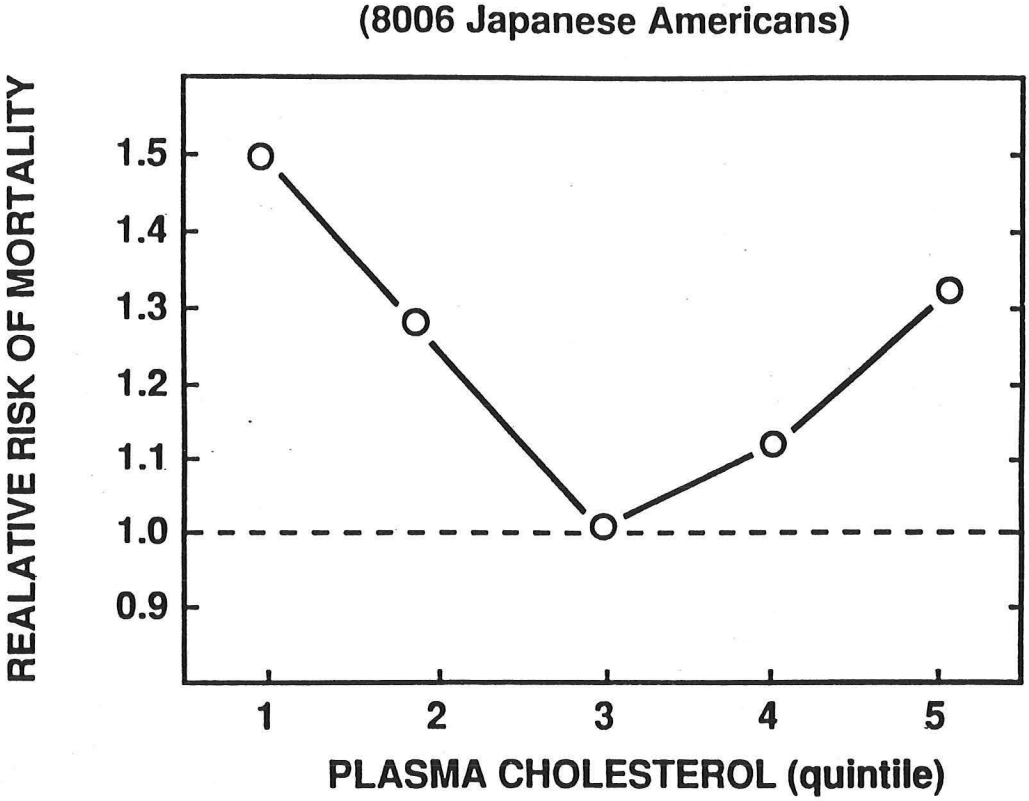



HYMAN ENGELBERG

(11) Primary prevention trials which have shown that the lowering of serum cholesterol concentrations in middle-aged subjects by diets, drugs, or both leads to a decrease in coronary heart disease have also reported an increase in deaths due to suicide or violence • • • • Low membrane cholesterol decreases the number of serotonin receptors. Since membrane cholesterol exchanges freely with cholesterol in the surrounding medium, a lowered serum cholesterol concentration may contribute to a decrease in brain serotonin, with poorer suppression of aggressive behavior.

Lancet 1992; 339:727-729.

Second, that the cholesterol content of brain was an equilibrium with the cholesterol content of plasma. And, third, that the reduced content of brain cholesterol led to a decrease in brain serotonin receptors and, hence, aggressive behavior. This hypothesis was then picked up in an article published in the British Journal of Psychiatry (1993) entitled "Low Serum Cholesterol And Suicide" and evaluated by a panel of psychiatrists. It should be noted that this paper contained no data on any parts of this hypothesis. Nevertheless, this information was picked up by the lay press and spawned a series of articles in Germany, Scandinavia, Canada and, particularly, Great Britain. These articles often suggested that it was dangerous to lower the plasma cholesterol level and that such lowering was associated with erratic behavior, violence and death. This subsequently led to several editorials implying that it was more dangerous to lower the plasma cholesterol level than to leave these levels untreated. One author called for a moratorium on all cholesterol testing.

Despite the lack of objective data, there were several peripheral investigations quoted that seemed to imply a connection between violent behavior and plasma total cholesterol levels. One such study looked at depressive symptoms in older men and is summarized in **Figure 12**. In this study groups of elderly males were administered a psychological test, The Beck Depression Inventory, which allegedly measures psychological depression. Scores of 21-30 apparently indicate moderate depression. As is evident in this figure, in patients in the 8th and 9th decade of life there was a slightly higher score in those patients with a low-plasma cholesterol level. None of these patients achieved the scores necessary to diagnose clinical depression, and many of the patients in this low cholesterol group had significant weight loss during the study. It is unclear, therefore, whether there was any causal relationship between the plasma cholesterol levels and the slight increase in the depression index.

| Plasma Cholesterol | 60-69 | 70-79 | 80-89 |
|---|---|---|---|
| Low | 5.08 * | 7.30 | 9.33 |
| Normal | 5.06 | 6.01 | 6.02 |
| Borderline | 4.68 | 5.65 | 6.60 |
| High | 4.54 | 5.65 | 6.92 |

*Beck Depression Inventory

(12)

In a second study that is quoted to support this thesis, serum cholesterol levels were measured in a group of murderers who resided in a psychiatric clinic in

(13)

| | Habitual Violence Under Alcohol | Intermittant Explosive Behavior | Murders Without Habitual Violence | Antisocial Behavior |
|---|---|---|---|---|
| Serum Cholesterol | 207 ±43 | 204 ±41 | 252 ±35 | 231 ±30 |

Helsinki. As shown in **Figure 13** these 280 homosuicidal offenders were divided into those who were habitually violent when drunk, those who were intermittently violent when drunk, those individuals who committed murders but were not habitually violent and those murderers who simply manifested antisocial behavior. As is evident, those individuals who were habitually or intermittently violent when drunk had somewhat lower total plasma cholesterol levels than those who murdered, but in a less violent way. The more violent patient groups clearly contained a large number of individuals classified as having schizophrenia, paranoid psychosis, and a variety of other serious psychiatric disorders. There was no data on dietary intake at the time these plasma cholesterol levels were drawn, nor were many other variables controlled.

California Psychological Inventory (CPI)

(14)

| | Socialization | |
|---|---|---|
| | Hi | Lo |
| Self-Acceptance | | |
| Hi | 237 | 221 |
| Lo | 279 | 217 |

A third quoted study is outlined in **Figure 14** and shows the results of administering the California Psychological Inventory to a group of 34 California firemen. As is apparent, the plasma cholesterol concentration was somewhat higher in the small number of individuals who, on the basis of this test, appeared to have a low level of self-acceptance. The conclusion of this small study was that "the man who is particularly adherent to social norms, places high value on being dependable and conscientious, and is self-critical is more likely to have a higher serum cholesterol level".

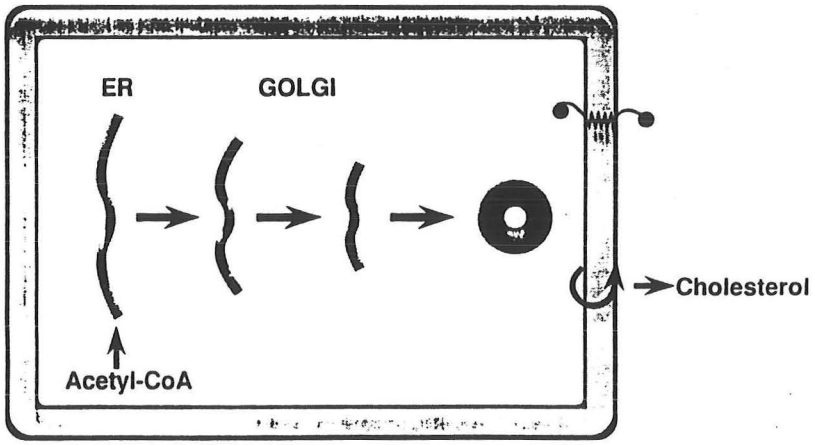
In addition to these three human studies, several animal studies are also quoted which imply, for example, that high fat diets make monkeys more "passive" and less aggressive and that serotonin release in the central nervous system may also be altered by high fat diets.

On balance, these are all extraordinarily weak studies. None of the human studies, for example, made any attempt to control dietary intake, level of activity, genetic background, etc., Similarly, the animal studies are not adequately controlled and, in fact, the level of serotonin receptors in the central nervous system was not directly measured using modern techniques. Nevertheless, these types of very borderline studies have been combined with the data suggesting an increase in non-cardiac deaths in the low plasma cholesterol groups to support this hypothesis that the plasma cholesterol level somehow affects brain function and aggressive behavior. In contrast to these studies, there are now abundant direct measurements of how plasma cholesterol is cleared by different organs in the body and to what extent such clearance takes place in the central nervous system.

## VII NORMAL CHOLESTEROL MOVEMENT THROUGH THE PLASMA AND INTO VARIOUS ORGANS.

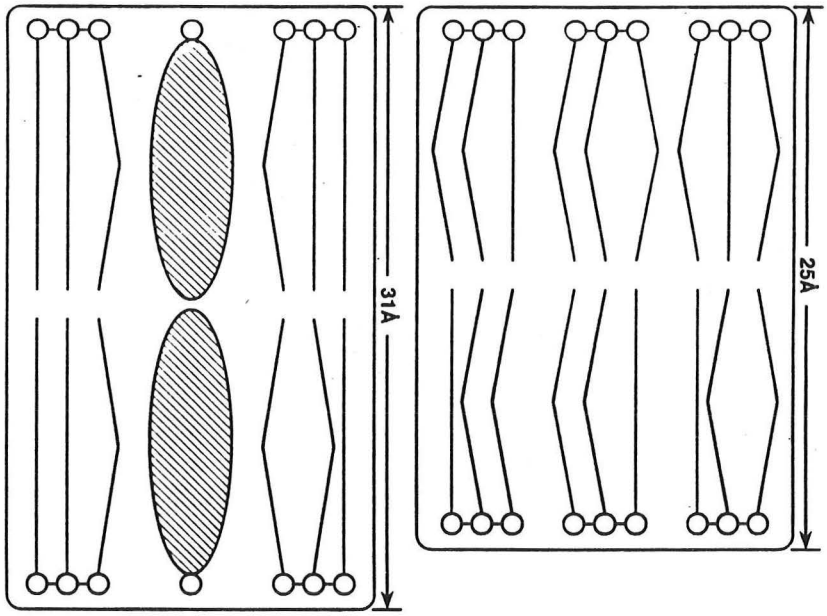
Cholesterol is a critical structural component of all cells. A constant supply of this molecule is required during cell division and growth and, even in the nondividing cell, sterol is being constantly "turned-over". As illustrated diagramatically in **Figure 15**, the typical cell is surrounded by a cholesterol-rich

(15)



plasma membrane while the membranes that make up the endoplasmic reticulum and other membranous structures of the cell are relatively poor in cholesterol content. Such membranes, that consist primarily of phospholipids, are very fluid and allow proteins to move and fold within their structure. When cholesterol is inserted into such membranes, as illustrated in **Figure 16**, there is hydrophobic

(16)



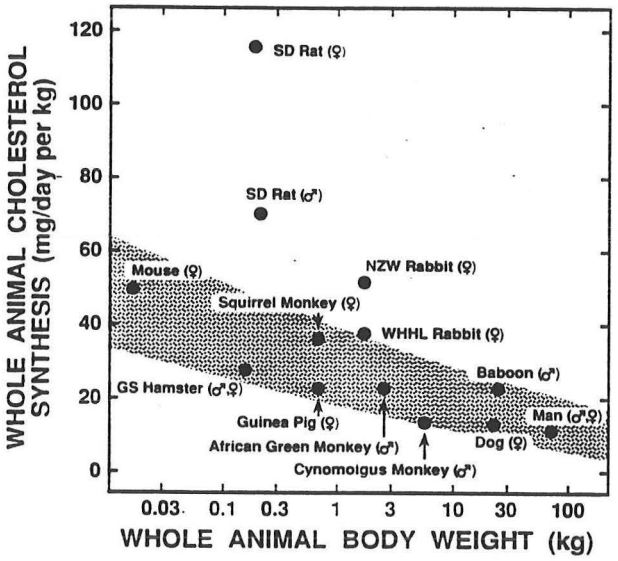

interactions between the sterol nucleus and the saturated and unsaturated fatty acids that are attached to the phospholipids. As a consequence of this interaction the membrane becomes more rigid and fluidity is reduced, and the membrane thickens by approximately 6Å. It is of interest that recent data have shown that proteins that become localized and function in the endoplasmic reticulum and Golgi apparatus have hydrophobic transmembrane-spanning regions that are approximately 5 residues shorter than those proteins that ultimately become inserted into the cholesterol-rich plasma membrane.

Thus, in general terms, every cell must have a source of cholesterol. This sterol is synthesized from acetyl-CoA on the endoplasmic reticulum (**Figure 15**) and then transported through the Golgi apparatus to the plasma membrane. In this manner the plasma membrane becomes relatively rigid, has the appropriate fluidity for supporting membrane bound enzymes and transporters, and has the appropriate thickness for the localization of these proteins. The cholesterol that is in the outer leaflet of the membrane readily dissociates if an external protein acceptor is present. This is followed by rapid "flip-flop" of cholesterol from the inner membrane

to the outer surface. Thus, each day the cell must synthesize an amount of cholesterol equal to that which is lost to the external environment. In general, the rate of sterol turn-over in the different cell types is proportional to the rate of metabolic turn-over of a particular cell.

It is of considerable importance, therefore, to understand the rates of cholesterol synthesis in the different organs of different animal species and man. In the past, measuring rates of sterol synthesis in vivo in an animal or in man involved the difficult process of quantifying the rates of cholesterol and bile acid output in the feces each day. More recently it has been demonstrated that these rates can be measured directly by administering animals water labeled with either tritium or deuterium. Since approximately 22-25 μg atoms of the hydrogen from water are incorporated into each μmol of sterol, it is possible to calculate the absolute rate of sterol synthesis occurring in vivo from the rates of tritium or deuterium incorporation into cholesterol. Utilizing both of these techniques, rates of cholesterol synthesis have now been measured in a number of different species and these are summarized in **Figure 17**. In nearly all of these studies, the animals

(17)




were on diets very low in cholesterol and triacylglycerol so that these rates of synthesis essentially equal the rate at which sterol is being turned-over each day in vivo in these animals.

As is apparent, the rate of cholesterol synthesis per kg of body weight varies markedly with the size of the animal: man synthesizes about 10 mg/day per kg while the mouse makes approximately 50 mg/day per kg. Many other species, including four non-human primates, have intermediate rates of sterol synthesis

between these two extreme values. The shaded area in this figure delineates a group of animals where the rate of sterol synthesis decreases by approximately 10 mg/day per kg for each ten fold increase in body weight. Thus, just as the basal metabolic rate and the turnover of proteins in various species varies inversely with the logarithm of body weight, so also the synthesis and turnover of cholesterol through the cellular membranous structures is markedly influenced by the size of the animal.

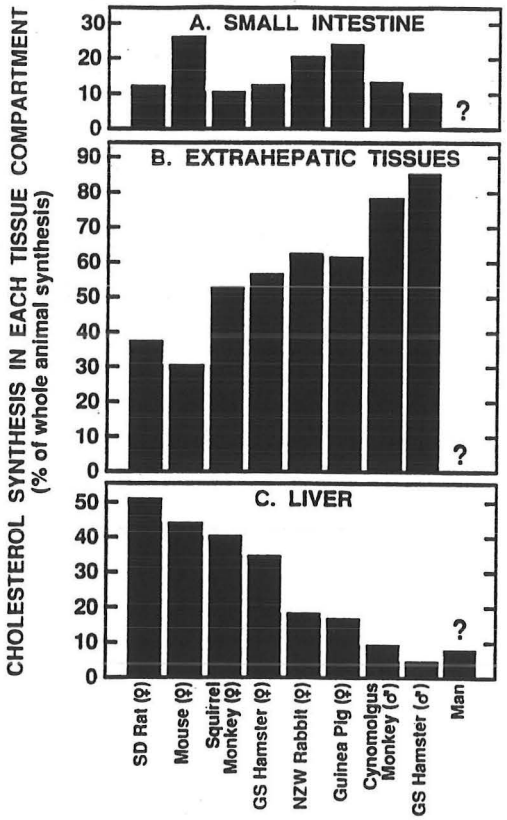
While **Figure 17** shows the rates of synthesis in sexually mature members of different species these rates vary in the same species when measured in the fetus or newborn, pregnant, obese and aged animal. Such comparative data are partially available in three species including the SD rat, NZW rabbit and man. In the developing fetus, where most of the cholesterol needed for new tissue growth comes from de novo synthesis, the rate of whole animal synthesis is 3-4 fold higher than in the sexually mature member of that species. Similarly, the newborn has a rate of synthesis per kg of body weight that is about twice that of the adult. The pregnant animal and obese human have slightly higher rates of whole body synthesis than their respective lean, non-pregnant controls.

Thus, these data illustrate the remarkable adaptability of the sterol biosynthetic pathway to meet the changing needs of the organism for cholesterol to support tissue growth and for membrane remodeling. Throughout the life of the rat, for example, there is a nearly ten fold change in the rate of whole animal synthesis, from approximately 470 mg/day per kg in the rapidly developing fetus to only 50 mg/day per kg in the aged adult. Clearly, the full range of sterol requirements in the body can be met by the biosynthetic pathway from conception to death, even in the total absence of dietary cholesterol.

It is still commonly believed that the liver is the major site for this biosynthetic activity in the whole animal. This concept arose from early studies where assays of rates of sterol synthesis were performed in vitro using various $^{14}C$-labeled precursors like [$^{14}C$]acetate. Such studies commonly revealed that the majority of the biosynthetic activity that could be demonstrated in all of the tissues of the body by these in vitro assays was accounted for by the activity observed in the liver. However, it became clear that many of these $^{14}C$-labeled substrates were poorly taken up and metabolized to [$^{14}C$]acetyl-CoA in the extrahepatic tissues. Furthermore, the specific activity of the [$^{14}C$]acetyl CoA pool that is the immediate precursor for sterol biosynthesis was disproportionately (relative to the liver) diluted in many of these tissues by the intracellular generation of large amounts of

unlabeled acetyl-CoA. As a result of all of these technical problems, it was demonstrated that the rates of synthesis in the extrahepatic organs had been systematically underestimated by, in some tissues, as much as 90-95% .

(18)




With the advent of techniques that circumvented these artifacts, it became possible to measure absolute rates of synthesis not only in the whole animal in vivo, but the contribution of each organ to such whole animal synthesis could also be quantified. **Figure 18** summarizes such data in seven species where rates of sterol synthesis have been measured in vivo under circumstances where the animals had been fed diets low in cholesterol and triacylglycerol. This diagram shows the contributions of the small intestine (A) and liver (C) to whole animal synthesis while the contributions of the remaining extrahepatic organs have been combined into a single value (B). Under these circumstances where dietary cholesterol intake was essentially zero, the liver contributes, at most, 40-50% of the cholesterol synthetic activity found in the female SD rat, mouse and squirrel monkey. However, this contribution is significantly less in the other species and amounts to <20% in the NZW rabbit, guinea pig, cynomolgus monkey and male GS hamster. Estimates of the importance of the liver in man also suggest that this organ is a relatively minor contributor to whole body synthesis.

Data such as those shown in **Figure 18**, however, are very much influenced by the conditions under which the measurements were made since marked changes in rates of cholesterol synthesis are induced by any condition that alters net sterol balance across a particular organ or across the whole animal. Furthermore, since it is the cholesterol pools in the intestinal epithelial cell and liver that are most influenced by such manipulations, it is the rates of sterol synthesis in these two particular organs that respond to changes in sterol balance. Thus, for example, if net sterol input into the body is increased, e.g., by adding small amounts of

cholesterol to the diet, then there is marked suppression of the rate of hepatic cholesterol synthesis, partial suppression of intestinal synthesis and virtually no change in synthesis in the extrahepatic organs. Conversely, if net sterol loss from the body is increased, e.g., by blocking the intestinal absorption of bile acids or cholesterol or by feeding soluble fibers, the rate of cholesterol synthesis in the liver and, to some extent, in the intestine increases to compensate for this loss while the rate of synthesis in extrahepatic organs again remains essentially unchanged.

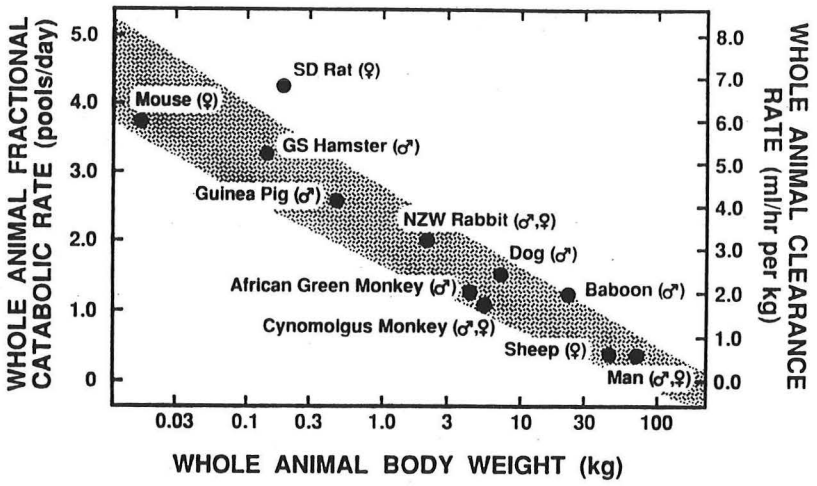
Thus in the steady state the absolute rate of cholesterol synthesis in the liver must always equal the absolute rate of sterol excretion in the feces minus the absolute rate of cholesterol delivered to the liver from the intestine and extrahepatic tissues. Hence, hepatic synthesis is necessarily suppressed when net sterol delivery from the intestine to the liver is increased and is markedly elevated when net sterol loss in the feces is enhanced. The data in **Figure 18** reflect the particular situation where all of the animals were fed commercial diets containing constant amounts of dietary fiber and various nutrients, but essentially no cholesterol. If an additional amount of soluble fiber or a bile acid sequestrant were added to the diets of each of these animals to increase fecal sterol loss, then synthesis in the liver, but not in the extrahepatic tissues, would increase and the relative contribution of hepatic cholesterol synthesis to whole animal synthesis would be higher. In contrast, if cholesterol were added to the diets of each of these species in the small amounts usually present in Western human diets, i.e., an amount equal to 30-60% of the daily sterol turn-over rate, then synthesis in the liver, but not in the extrahepatic organs, would be partially suppressed. In this situation the relative hepatic contribution to whole animal cholesterol synthesis would be very small, even in the rat, mouse and squirrel monkey. Thus, under dietary conditions equivalent to those found in Western man, the extrahepatic tissues probably account for >80% of whole animal sterol synthesis in virtually every species that has been studied.

The second problem of importance concerns the role of the liver in determining the steady-state concentration of LDL-C in the plasma. As illustrated in **Figure 1**, LDL-C is formed primarily from the metabolism of VLDL-C. In the past it has been suggested that some LDL-C may also be secreted directly by the liver, although a recent analysis of this possibility suggests that this latter pathway is relatively unimportant or may not exist at all. In the steady state, the rate at which LDL-C is removed from the plasma and degraded by all of the tissues of the body must equal the LDL-C production rate ($J_t$). Thus, one way in which to express the rate of LDL-C uptake is as an absolute rate of transport having the units of mg

of LDL-C taken up by the various organs each day per kg of body weight. The amount of LDL-C removed from the plasma can also be expressed as a classical clearance value. The absolute rate of LDL-C removed from the plasma each hour divided by the plasma LDL-C concentration yields the LDL-C clearance rate which describes the ml of plasma entirely cleared of its LDL-C content per hr per kg of body weight. Finally, either the absolute rate of LDL-C transport out of plasma or the clearance rate can be expressed as a fraction of the LDL-C pool or the plasma volume, respectively, present in 1 kg of body weight. This calculation yields a term called the fractional catabolic rate which describes the fraction of the LDL-C pool removed from the plasma each hr or day. Thus, the rate at which LDL-C is removed from the plasma can be expressed in three different ways, i.e., the absolute rate of LDL-C transport (mg/day per kg), the LDL-C clearance rate (ml/hr per kg) and the LDL-C fractional catabolic rate (pools/day). The first two values must be normalized to a constant body weight, e.g., 1 kg, while the third is independent of body weight. Different methods are available for quantifying directly the absolute rate of LDL-C transport in the whole animal, the whole animal LDL-C clearance rate and the fractional catabolic rate: however, it should be emphasized that once one of these values has been quantified, the other two can be calculated.

The rate of LDL-C removal from the plasma space has now been measured in at least eleven species and these are summarized in **Figure 19**. These rates are expressed as both the whole animal fractional catabolic rate and clearance rate, and are plotted against the logarithm of the weight of each species. Most animals, except man, were on diets low in cholesterol and triacylglycerol. As is apparent, the effect of body size on LDL-C turnover is even greater than on whole animal cholesterol synthesis. Small animals such as the mouse, GS hamster and SD rat, for example, degrade about 4 pools of LDL-C per day while man removes from the plasma only about 0.4 pools/day. Stated differently, one kg of man will clear only about 0.6-0.7 ml of plasma per hr of its LDL-C content while one kg of these small animals will clear 6-7 ml/hr. The shaded area in **Figure 19** delineates a group of animals where the fractional catabolic rate and the clearance rate decrease by approximately 1.1 pools/day and 1.8 ml/hr per kg, respectively, for each 10-fold increase in body weight. Also of note is the fact that the rate of LDL-C clearance in the female SD rat is much closer to the main-stream group of animals than is this species with respect to cholesterol biosynthesis. Furthermore, it is of interest that this relationship is made up of animals that are omnivorous, carnivorous and

(19)

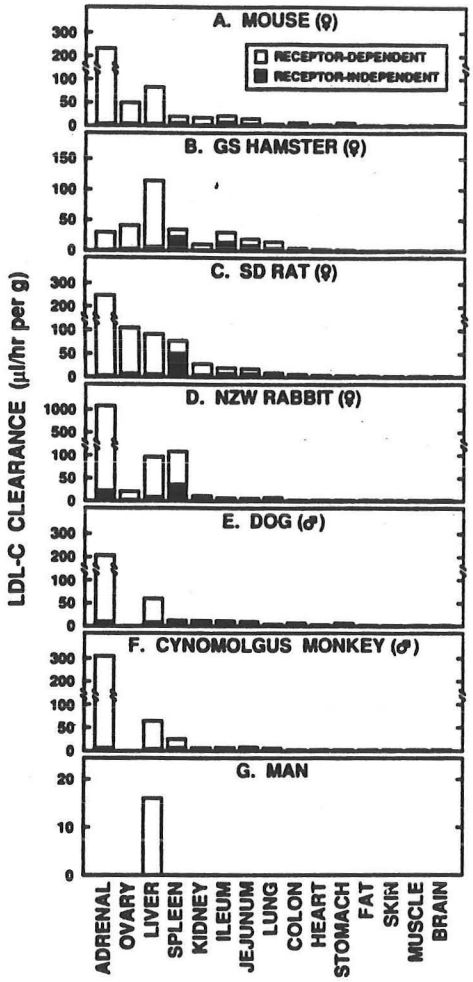



herbivorous. Included within the herbivorous group is at least one species of ruminant.

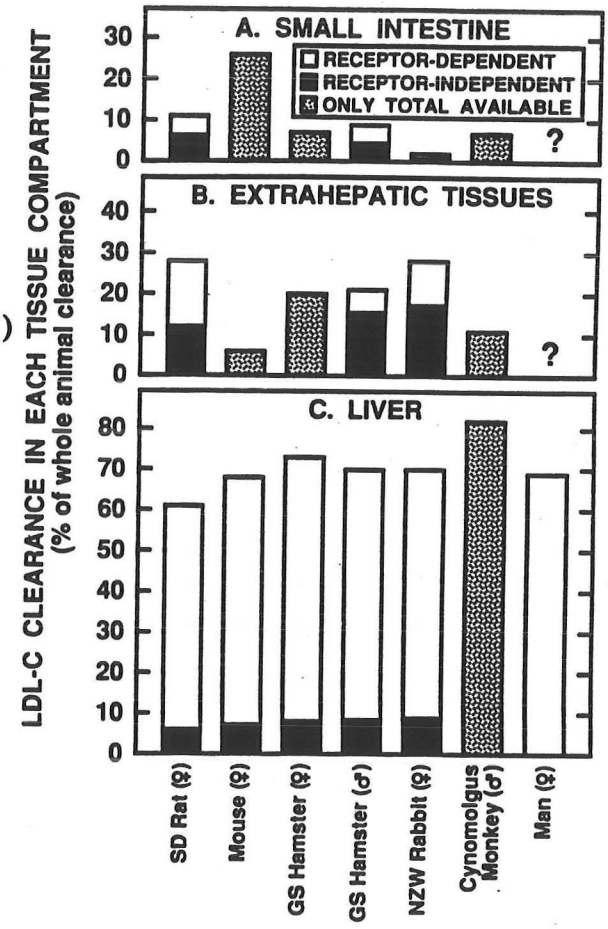
Extensive investigations have now established that two separate processes account for these rates of removal of LDL-C from the plasma space. One of these processes depends upon the LDL receptor mechanism and so is receptor-dependent. The second mechanism is poorly understood but is not dependent upon the activity of the LDL receptor and so is commonly described as being receptor-independent. In virtually all species about 75% of the LDL-C that is removed from the plasma is removed by the receptor-dependent process while the remaining 25% is accounted for by receptor-independent transport. In animals or humans that genetically lack LDL receptor activity, however, this receptor-independent process accounts for all LDL-C uptake.

The next question of importance is which tissues in the body account for these rates of receptor-dependent and receptor-independent LDL-C uptake observed in the whole animal. With the development of radiolabeled markers for LDL that are retained within the different organs and short-term, steady-state infusion techniques using both homologous and derivatized LDL-C, it has become possible to determine the absolute rates of receptor-dependent and receptor-independent LDL-C transport into every organ in the live animal. **Figure 20** summarizes the available data of this type and shows the rates of receptor-dependent and receptor-independent transport into fifteen different tissues of six species. The calculated rate of receptor-dependent uptake in the human liver is also shown. These data are expressed as clearance rates and describe the μl of plasma cleared of its LDL-C content each hr per g of each organ.

(20)




(21)




As is apparent, the general profile of LDL transport is the same in all species. First, only three organs consistently manifest high rates of LDL-C transport per g of tissue: the liver and two endocrine tissues, the adrenal gland, and the ovary. Furthermore, it is clear that these high rates are achieved only because there is a large component of receptor-dependent uptake in these three organs. The magnitude of this component in the adrenal gland and ovary, however, varies markedly among the different species regardless of size, while receptor-dependent transport in the liver varies inversely with animal weight. Thus, in small animals such as the mouse, GS hamster and SD rat, hepatic LDL-C clearance equals approximately 100 µl/hr per g, but this value drops to about 60 µl/hr per g in the dog and cynomolgus monkey and to only 15-17 µl/hr per g in man. There is a second group of tissues where receptor-dependent transport can also be clearly identified, yet the overall rates of LDL-C uptake are usually low. This group includes the spleen, kidney, small intestine, lung and colon. Finally, there is a third group of

organs that include adipose tissue, skin, muscle and brain, where it is essentially impossible to detect any LDL-C uptake, either receptor-dependent or receptor-independent.

Except for the receptor-dependent transport rate calculated for the liver in a young transplant patient (G), no detailed clearance data are available in vivo in man. Heparin-specific LDL binding to homogenates of various human tissues has, however, been reported. To the extent that such binding data reflect receptor-dependent LDL transport, these data generally agree with the actual clearance rates illustrated in **Figure 20**. Homogenates of organs such as brain, adipose tissue, muscle and skin have little specific binding while tissues such as spleen, kidney, small intestine and colon manifest significantly greater specific interaction with LDL. The two endocrine tissues, the adrenal gland and ovary, bind the greatest amount of LDL. Unfortunately, heparin-specific LDL-binding in hepatic membranes in this study was very low. However, these particular measurements were carried out in tissues obtained from older patients who presumably were maintained on diets containing cholesterol and saturated fatty acids, and many of these subjects had tumors and were subjected to the trauma of surgery. It is to be anticipated, therefore, that hepatic LDL-C clearance in the livers of such subjects would be suppressed and the rates of transport in these individuals would certainly not be comparable to those shown in **Figure 20** which were all obtained in healthy, young, sexually mature animals maintained on diets essentially free of lipids.

The key to understanding LDL-C turnover in the whole animal or man revolves around the relative importance of each of these tissues for clearing LDL-C from the plasma. The absolute rate of LDL-C uptake into each organ can be calculated by multiplying the rate of receptor-dependent and receptor-independent LDL-C transport per g of tissue by the weight of each organ in each species. The sum of the uptake rates in the individual organs equals the rate of LDL-C turnover independently determined in the whole animal. The relative importance of each organ to whole animal LDL-C degradation can then be calculated, and these data are summarized in **Figure 21** for all species in which such measurements have been made. In five species maintained on diets low in cholesterol and triacylglycerol, it is clear that the liver accounts for the uptake and degradation of about 70% of the LDL-C that is turned over in the whole animal each day (C). A similar figure can be calculated from the limited data available in man. The absolute level of hepatic LDL-C uptake in these various animals is dictated by two variables: in going from small animals such as the mouse to the larger primates 1) the rate of clearance into

the liver decreases from ~120 to ~15 μl/hr per g and 2) the relative size of the liver decreases from ~5.5 to ~1.5% of body weight. Yet despite these major variables, the liver is the overwhelmingly important site for LDL-C clearance from the plasma in every species in which quantitative data are available. The small intestine (A) and all of the remaining extrahepatic tissues (B) together account for the degradation of the remaining ~30% of LDL-C. Furthermore, within this latter group large and important organs such as muscle and the central nervous system appear to take up virtually no cholesterol from the circulating LDL-C pool. Thus, in the whole animal, and presumably in man, most cholesterol is synthesized and utilized in the extrahepatic organs while most of the cholesterol carried in LDL is taken up into the liver.

Two other important conclusions come from the data shown in **Figure 21**. In the whole animal, 72-78% of LDL-C degradation is receptor mediated. Of this receptor-dependent transport identifiable in the whole animal, ~80% takes place in the liver of all species in which data are available. In contrast, 22-28% of LDL-C is removed from the plasma in the whole animal by the receptor-independent process, and ~70% of this transport activity is located in the intestine (A) and other extrahepatic tissues (B). Thus, any genetic or environmental factor that reduces receptor-dependent LDL-C transport in the liver will necessarily partially shift the burden of LDL-C clearance in the whole animal from the liver to the extrahepatic organs.

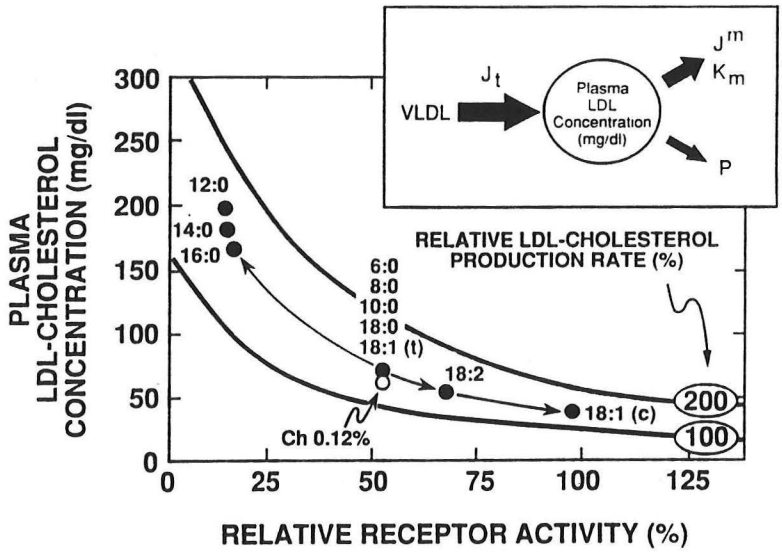
As is the case with the data on cholesterol synthesis in the various organs, the data in **Figure 21** apply only to the particular situation where animals are maintained on commercial diets essentially free of cholesterol and triacylglycerol. Any dietary addition that would enhance net sterol loss from the body may increase receptor-dependent LDL-C transport into the liver and so increase the relative importance of this organ as a site for LDL-C clearance in the whole animal. Conversely, the addition of certain lipids to the diets of these animals may lead to suppression of hepatic receptor-dependent transport and a decrease in the importance of the liver to whole animal LDL-C degradation.

From these data it appears that there is little or no biological use for LDL-C so that it may be appropriate to consider LDL-C one of the remnants of VLDL metabolism that should be cleared from the plasma as rapidly as possible. Thus, the two classes of apo B-containing lipoproteins would manifest similar functions and behaviors. The chylomicron particle functions primarily to move triacylglycerol from the intestinal epithelial cell to the peripheral sites of utilization and storage,

and the remnant of this particle is rapidly cleared from the plasma by the liver, primarily through utilization of the chylomicron remnant receptor. In a similar fashion, VLDL functions primarily to move triacylglycerol from the liver to the same peripheral sites of utilization and storage, and its remnants are also rapidly cleared from the plasma by the liver, principally through intervention of the LDL receptor (**Figure 1**). Thus, in the absence of dietary lipids, the LDL-C concentration is well below 40 mg/dl in virtually every species in which such data are available and these include the rat, hamster, guinea pig, various non-human primates and the human infant. Even this low level is attributable to the fact that hepatic LDL-C clearance occurs at rates of only about 100 µl/hr per g while the hepatic clearance of chylomicron remnants takes place at rates in excess of 20,000 µl/hr per g. If hepatic LDL receptor activity is increased only modestly, to ~500 µl/hr per g, the concentration of LDL-C, like the concentration of chylomicron remnants, decreases essentially to zero.

When these experimental animals, or humans, are placed on typical human diets containing cholesterol and triacylglycerol, there are significant changes in the rates of receptor-dependent LDL-C transport in the liver. The relationship between receptor-dependent LDL-C transport and the steady-state level of LDL-C in the plasma is illustrated by the insert in **Figure 22**. As is apparent in this insert, the concentration of LDL-C in the plasma is primarily determined by the rate at which LDL-C is being produced, i.e., the LDL-C production rate, $J_t$, and the rate at which

(22)



LDL-C is removed from the plasma by receptor-dependent transport, $J^m$. The quantitative relations between the steady-state LDL-C concentration, receptor activity and production rate are shown by the theoretical curves in this figure.

In virtually every species that has been studied, including man, the addition of small amounts of cholesterol to an essentially lipid-free diet leads to a series of changes in hepatic sterol metabolism that ultimately results in modest elevations of the plasma LDL-C concentrations. Cholesterol that is present in the diet is partially absorbed and delivered to the liver in the chylomicron remnant (**Figure 1**). The initial metabolic effect of this absorbed sterol is to suppress the rate of cholesterol synthesis to essentially zero. As additional amounts of dietary cholesterol enter the liver they are almost immediately esterified to fatty acids, particularly the 18:1(9*c*) compound, and deposited in the cytosol as biologically inert, cholesteryl esters. The rate of this reaction, which is catalyzed by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT), appears to be driven by the amount of excess cholesterol in the cytosol. Thus, the small pool of unesterified sterol that is the putative regulator of hepatic receptor activity appears to vary directly with the steady-state level of cholesteryl esters in the cell. When this steady-state is achieved, therefore, the concentration of cholesteryl esters varies directly, and receptor activity varies inversely, with the amount of cholesterol absorbed from the diet. As receptor activity is progressively suppressed, simultaneous increases in LDL-C production are also observed.

Thus, the effects of cholesterol feeding in the experimental animal or man on sterol and LDL-C homeostasis (**Figure 1**) can be summarized as follows. First, it is cholesterol itself that very likely is the component of the diet that causes these physiological effects. Second, where this mechanism is operative measurement of net sterol balance in the animal or human, i.e., the total amount of cholesterol synthesized in all of the tissues and absorbed across the small intestine, will always reveal a net increase in sterol delivery to the liver. Third, in response to this net increase in input, both the putative regulatory pool and the esterified pool of cholesterol increase in parallel so that $J^m$ decreases inversely and linearly with respect to the concentration of cholesteryl esters as net delivery of sterol into the liver is progressively increased. Fourth, the dose-dependent suppression of $J^m$, together with small increases in $J_t$, fully account for the modest increases in the plasma LDL-C concentration observed with cholesterol feeding. Fifth, because of expansion of this putative regulatory pool, the liver becomes even less important as a site for whole animal sterol synthesis, and a greater proportion of LDL-C

clearance must take place in the extrahepatic organs. Sixth, these changes, in turn, lead to a decrease in the whole animal LDL-C fractional catabolic and clearance rates, and a decrease in the percentage of LDL-C that is removed from the plasma by receptor-dependent transport.

Finally, it should also be noted that each one of these alterations can be reversed if net negative sterol balance is induced across the liver by, for example, increasing the content of soluble fiber in the diet or by feeding agents that specifically block bile acid and/or cholesterol absorption. Such maneuvers are invariably signaled by a decrease in the content of hepatic cholesteryl esters and $J_t$ and by an increase in the rate of hepatic cholesterol synthesis and $J^m$. The liver becomes relatively more important as a site for sterol synthesis, and receptor-dependent LDL-C clearance in the whole animal and the fractional catabolic rate increase.

Triacylglycerol is the second major lipid component of human diets and may account for 30-50% of total caloric intake in many individuals. When added to a diet essentially free of cholesterol, triacylglycerols generally have less effect on the plasma LDL-C level than when dietary sterol is present in significant amounts. In the presence of dietary cholesterol, however, these lipids profoundly affect hepatic sterol metabolism and the parameters of LDL-C transport. Once absorbed, triacylglycerols are also carried in the chylomicron particle (**Figure 1**). The majority of the fatty acids in this lipoprotein are taken up by muscle and adipose tissue as the chylomicron is metabolized in the extrahepatic organs by lipoprotein lipase. However, a portion of the triacylglycerol is retained and delivered directly to the liver as the chylomicron remnant is cleared, and, in addition, fatty acids bound to albumin are constantly being circulated from the extrahepatic stores to the liver. Thus, the hepatocyte becomes enriched with the fatty acids present in the triacylglycerol of the diet and this, in turn, may markedly alter the relative activities of the various hepatic metabolic pathways in which the fatty acyl-CoA derivatives are utilized. In general, the metabolic effects of these lipids depend very much on whether they are saturated fatty acids of medium or long chain length or are unsaturated.

Recent data have identified which of the specific fatty acids present in mixed triacylglycerols are capable of exerting these regulatory effects in the liver. As summarized in **Figure 22**, if animals are fed diets containing a fixed amount of cholesterol and triacylglycerol, the changes observed in the parameters of LDL transport in the liver vary markedly with the type of fatty acid making up the

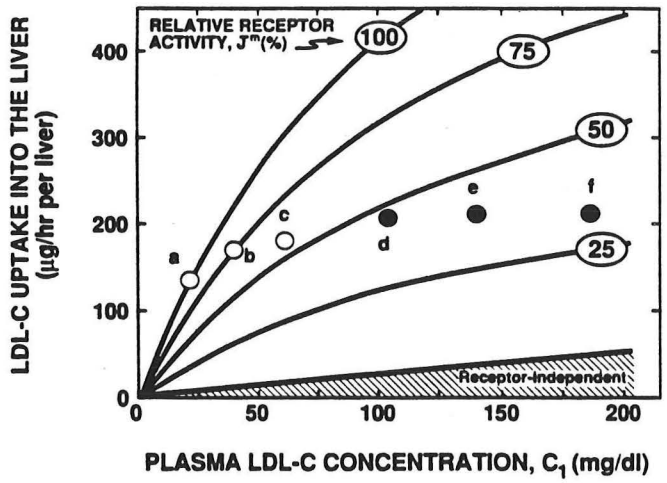
triacylglycerol. The saturated fatty acids 6:0, 8:0 and 10:0 are rapidly metabolized to acetyl-CoA by the liver cell and so do not alter the pattern of fatty acids in the various hepatic lipid pools. They do not change hepatic cholesteryl ester levels, nor do they alter $J^m$, $J_t$ or $C_1$. Thus, as shown in **Figure 22**, these particular lipids are biologically neutral with respect to LDL metabolism. In contrast, when the saturated fatty acids 12:0, 14:0 and 16:0 are fed they enrich the hepatic lipid pools, suppress cholesteryl ester formation, further reduce $J^m$ and markedly increase $J_t$. As a result of these changes the plasma LDL-C increases from the level of ~65 mg/dl seen with cholesterol feeding alone to nearly 200 mg/dl. The monounsaturated fatty acid 18:1(9*c*), on the other hand, is active in restoring $J^m$ and reducing $J_t$ so that $C_1$ drops to only about 50 mg/dl. These changes take place under circumstances where the content of cholesteryl esters in the liver cell markedly increases. This ability to up-regulate hepatic receptor activity is lost, however, if the double bond in this monounsaturated fatty acid is either converted to the *trans* configuration or is fully saturated. Thus, the 18:0 and 18:1(9*t*) fatty acids are biologically neutral and do not alter any parameter of LDL metabolism beyond that attributable to the cholesterol also present in the diet. Similar results have been reported in human studies where the ability of the 18:1(9c) fatty acid to lower the plasma LDL-C level is lost when the lipid is fully saturated or converted to the *trans* configuration. It should again be emphasized that all of these regulatory events occur under circumstances where there is no detectable change in the percentage of dietary cholesterol that is absorbed or in net cholesterol delivery to the liver.

Thus, fatty acids reaching the liver and enriching the various substrate pools also exert major regulatory effects on hepatic LDL metabolism that can be summarized as follows. First, since the magnitude of the effects of fatty acids is proportional to the amount of cholesterol also present in the diet, regulation of LDL receptor gene transcription by these lipids must be exerted indirectly, probably through an alteration in the size of the putative regulatory pool of cholesterol in the liver. Second, in contrast to regulation brought about by cholesterol feeding, these marked changes in $J^m$ caused by fatty acid feeding occur under circumstances where there is no observable change in either cholesterol absorption or synthesis and, hence, no demonstrable change in net sterol balance across the liver or whole animal. Third, the active saturated fatty acids that suppress $J^m$ also suppress steady-state cholesteryl ester levels in the liver cell while those that actively restore $J^m$ increase the size of this ester pool. Thus, in contrast to the regulation exerted by dietary cholesterol, with fatty acid feeding, $J^m$ varies directly with the steady-state

size of the cholesteryl ester pool. Fourth, the 12:0, 14:0, and 16:0 fatty acids suppress $J^m$ and increase $J_t$ while the 18:1(9*c*) compound has the opposite effect. The changes in these two parameters are sufficient to explain the large alterations in $C_1$ that are induced by these lipids. Many other fatty acids are biologically neutral and exert no regulation on LDL metabolism. Fifth, feeding these fatty acids has no effect on cholesterol synthesis and so does not change the relative importance of the liver for whole animal synthesis. However, the relative importance of the liver for whole animal LDL clearance and the percentage of LDL that is cleared by receptor-dependent transport are altered by particular fatty acids, depending upon whether that fatty acid increases or decreases $J^m$.

Finally, this regulation of $J^m$ and $J_t$ by dietary cholesterol and triacylglycerol has important implications with respect to absolute rates of cholesterol flux across the liver and extrahepatic organs. These implications are very different under the conditions that exist in the live animal compared to in vitro studies performed in isolated cell systems. **Figure 23**, for example, illustrates how the absolute rate of LDL-C uptake varies as receptor activity in the liver is progressively suppressed. The four theoretical curves show how the rates of LDL-C transport would vary with changes in the plasma LDL-C concentration under circumstances where $J^m$ in the liver is reduced from 100% to 75, 50 and 25% of the control value. The data points labeled a-f represent actual experimental measurements of LDL-C uptake where $J^m$

(23)




was suppressed by cholesterol and saturated fatty acid feeding. In the control situation (a) $J^m$ equals 100%, about 130 µg of LDL-C is transported into the liver each hour, and this velocity is achieved at a plasma LDL-C concentration of ~20 mg/dl. If these hepatocytes were studied under in vitro conditions where $C_1$ was kept constant at 20 mg/dl and $J^m$ was progressively suppressed, then the uptake of

LDL-C would progressively decrease in direct proportion to the reduction in receptor activity, i.e., under these in vitro conditions where $C_1$ is constant the rate of LDL-C transport across the liver would be determined directly by $J^m$.

This is never the case, however, in the live animal or man. Under such in vivo conditions the rate at which LDL-C is being formed remains constant, or actually increases, as receptor activity in the liver is suppressed by dietary lipids. Thus, as hepatic receptor activity is suppressed, the plasma LDL-C concentration necessarily increases until a velocity of transport equal to or greater than that seen in the control animals is achieved. Point d, for example, illustrates the experimental result when cholesterol and a small amount of saturated lipid is fed until a new steady state is achieved. As is evident, even though $J^m$ is decreased to ~45% of control, the velocity of LDL-C uptake actually increased slightly to ~200 μg/hr. This occurred because $C_1$ progressively increased until a velocity of LDL-C transport equal to the rate of LDL-C production was achieved in the new steady state, even though only ~45% of the LDL receptors were present in the liver. In the control situation (a) this velocity was achieved at a value of $C_1$ equal to only about 20% of $K_m$ whereas it was necessary for $C_1$ to increase to a value essentially equaling the $K_m$ of the receptor-dependent transport process when $J^m$ was partially suppressed. As is apparent from the other data points, these same adaptations take place at all other levels of lipid feeding and suppression of $J^m$; in all cases $C_1$ will increase to maintain the flux of LDL-C across the liver at a nearly constant value. Thus, in contrast to the in vitro situation where $C_1$ is usually kept constant, under in vivo conditions the rate of LDL-C transport across the liver is determined directly by the LDL-C production rate, $J_t$, and not by hepatic receptor activity, $J^m$.

These observations have very important implications with respect to the relationship between the plasma cholesterol level and sterol balance across various tissues. As $J^m$ in the liver is progressively suppressed by feeding greater and greater amounts of cholesterol and saturated lipids, the plasma LDL-C concentration increases dramatically while LDL-C flux across the liver and the extrahepatic tissues that also possess receptor-dependent transport remains nearly constant. Viewed differently, if such an animal or human is treated with a pharmaceutical or dietary manipulation that lowers the plasma LDL-C level, for example, from 200 to 50 mg/dl, there is essentially no change in net LDL-C transport across the various organs of the body (**Figure 23**). Thus, changing the dietary intake of cholesterol, fatty acid or soluble fiber markedly alters the plasma LDL-C concentration but, for practical purposes, does not change cholesterol

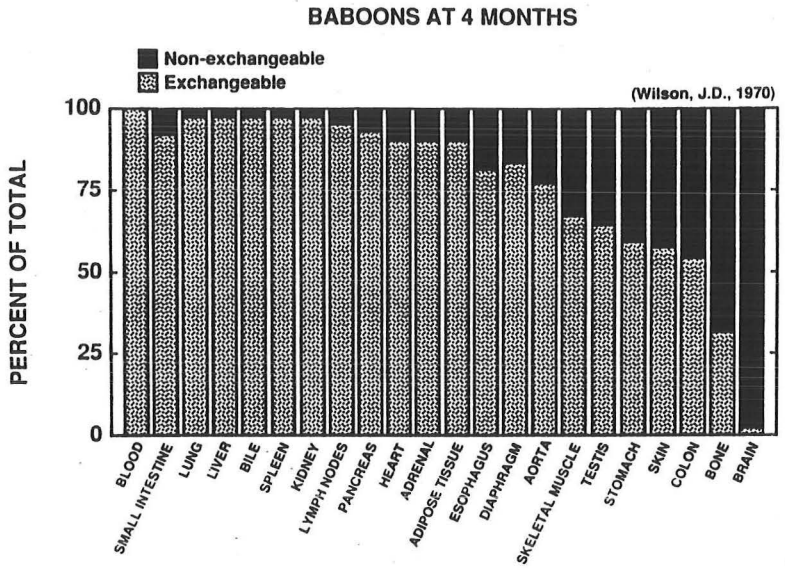
homeostasis across any tissue. Only the endothelial lining of the vascular system, therefore, is subject to the detrimental effects of high plasma cholesterol levels and only the physiology of these cells is influenced when the plasma LDL-C level is dramatically lowered.

## VIII THE SPECIAL CASE: THE BRAIN.

One of the primary hypotheses concerning the relationship between plasma cholesterol levels and behavior is that the cholesterol content of the brain is somehow affected by the circulating level of cholesterol carried in lipoproteins. Certainly the central nervous system tissues are unique among the extrahepatic tissues in that the various portions of the brain have much higher contents of cholesterol than virtually any other tissue in the body. There is now considerable data as to the source for this cholesterol. While most older studies have shown relatively low rates of cholesterol synthesis in the central nervous system of the mature animal, this is not true in the fetus and newborn. The central nervous system undergoes differentiation at a very early fetal age. During this time recent studies have shown exceptionally high rates of cholesterol synthesis in the brain. These rates parallel the rapidly increasing relative weight of the central nervous system in the fetus. These rates are somewhat lower after the birth of the animal although they are still among the highest rates found in the developing newborn. As the central nervous system approaches its adult size, there is progressive further suppression of the rate of cholesterol synthesis until in the adult rates of new sterol synthesis in the brain are almost undetectable. Clearly, then, synthesis is the major source, if not the only source, for the rapidly growing brain in the fetus and newborn animal. In contrast, it has been impossible to detect the uptake of cholesterol carried in LDL from either the maternal circulation to the fetal brain or between the plasma pool and the brain of an adult animal. As illustrated in **Figure 20**, for example, under circumstances where tissues like the liver and endocrine glands rapidly clear LDL-C from the plasma there is no detectable uptake of this lipoprotein-cholesterol by the mature nervous system.

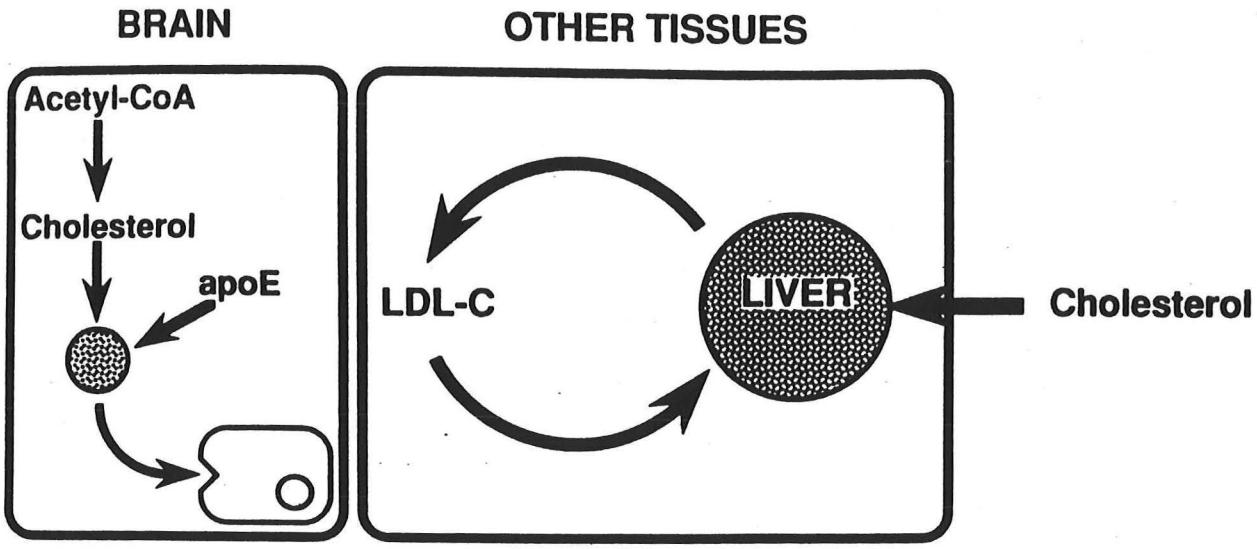
Not only is there essentially no net uptake of lipoprotein into the brain from the plasma space, but, in addition, there is virtually no molecular exchange of cholesterol between the central nervous system compartment and the remainder of the body. This is illustrated by the data in **Figure 24** in which the exchangeable pool of cholesterol in each major organ was quantified over a four month period in

(24)




the baboon. As is apparent, a large percentage of the cholesterol in nearly every organ is rapidly exchangeable. In contrast, the great majority of the cholesterol that is within the central nervous system is non-exchangeable. Thus, not only has it been impossible to demonstrate net transfer of cholesterol from LDL-C into the brain but, in addition, the cholesterol that has been synthesized in situ in the central nervous system appears to be virtually fixed and non-exchangeable with the other cholesterol pools in the body. Finally, animal experiments have been carried out in which the plasma cholesterol concentration in the pregnant female has been varied by feeding various lipid-containing diets. The content of cholesterol in the fetal and newborn brains of the offspring of these animals show no differences in cholesterol content. Similarly, changing the steady-state level of LDL-C in the adult animal has no effect on the cholesterol content of the brain of that same animal.

Thus, the overwhelming conclusion from these experiments is that de novo synthesis is the major, if not the sole, source for the sterol that is used to construct the brain and, further, there is virtually no exchange of this sterol with the remaining cholesterol pools in the body. Thus, as illustrated diagramatically in **Figure 25**, it appears that the central nervous system is virtually isolated from the other tissues of the body with respect to cholesterol metabolism. Most of the sterol that is required for the developing brain in the fetus and juvenile animal comes from de novo cholesterol synthesis. These rates of synthesis decline toward zero as the brain reaches its mature size. The brain clearly does synthesize certain apo proteins such as apoE and clearly possesses cells with LDL receptors. The possibility exists, therefore, that apoE-particles containing cholesterol may be used to move sterol between various cell types within the central nervous system but




(25)

there is no evidence at this time that circulating LDL-C from the plasma in any way contributes to the brain cholesterol pools. Furthermore, there is no evidence that the content of cholesterol in the central nervous system is altered by changes in the steady-state LDL-C concentration.

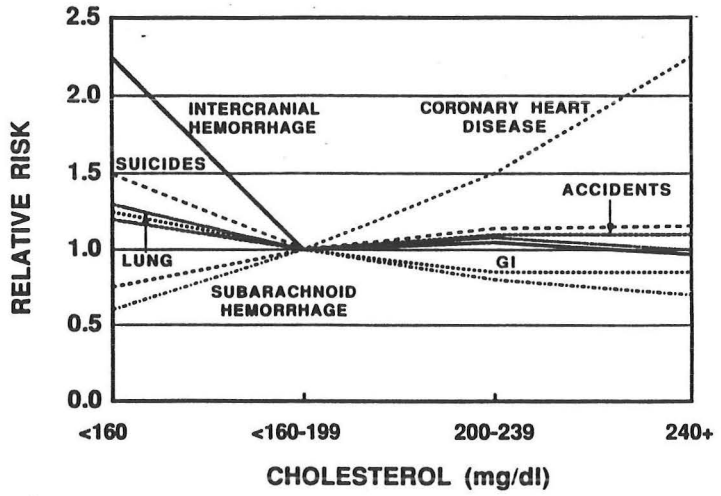
## IX WHAT ACTUALLY KILLS PATIENTS WHO HAVE LOW PLASMA CHOLESTEROL LEVELS.

From the extensive experimental data available and reviewed above there is no evidence that changes in circulating plasma cholesterol levels alter cholesterol homeostasis in the brain. Two questions therefore remain: 1) Why is it that the mortality rate due to CHD is not more dramatically lowered by reducing the plasma cholesterol concentration and 2) Is the incidence of traumatic deaths really significantly increased in patients with low cholesterol levels?

First, as discussed earlier, analysis of the various combined studies clearly indicates that mortality due to CHD does decrease as the plasma cholesterol level is reduced. It should be emphasized that in almost all of the individual studies reported to date the period of follow-up has been only 5-8 years. In addition the degree of cholesterol reduction has been very modest. In most studies the apparent beneficial effect of plasma cholesterol lowering on mortality rate does not begin to become evident until after 4-5 years of treatment. Thus, as emphasized by Keech

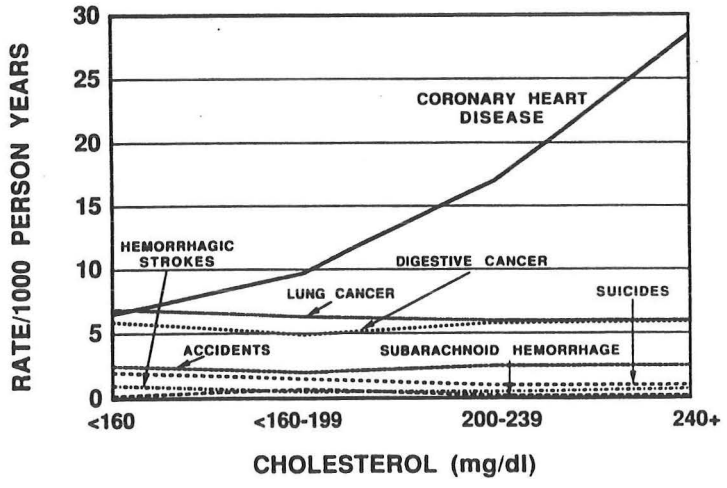
(Oxford University), none of these studies has sufficient statistical power to prove a beneficial effect on these mortality rates. He concludes that "the evidence for clinically important reduction in CHD risk by lowering cholesterol is overwhelming statistically significant (5 standard deviations from zero effect)". More definitive data on reductions in mortality rates will require investigations utilizing the much more effective new cholesterol-lowering agents that are capable of reducing the plasma cholesterol level 40-50%.

(26)




Second, there are a number of new data that bear on the second question of whether there is an unusual risk of non-cardiovascular diseases in the patient populations with low cholesterol levels. As illustrated in **Figure 26**, relative risk analysis typically gives these "U" shaped curves. These data, however, are normalized to individual patients. Thus, a few deaths due to trauma in the small number of cases in the low cholesterol group have a disproportionate effect on these statistics. When data such as those in **Figure 26** are plotted as absolute risk data as was done in **Figure 27**, these small numbers of traumatic deaths essentially

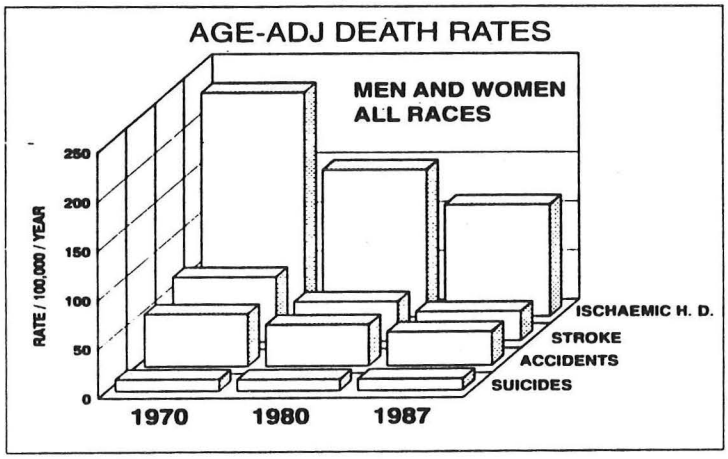
(27)



disappear. Thus, the risk of dying of cardiac disease per 1,000 person years continuously increases from the lowest plasma cholesterol level to the highest. In contrast the risk of dying from various cancers, suicides and accidents is essentially constant and independent of the plasma cholesterol concentration.

That lowering the plasma cholesterol concentration is not associated with an increase in traumatic deaths is also suggested by gross population statistics in the United States. Over the last thirty years there has been a progressive fall in the average plasma cholesterol concentration in the American population. As shown in **Figure 28**, during this same period there has been a progressive decrease in the age adjusted death rate from ischemic heart disease. During this same period the death rates from stroke, accidents and suicides have also diminished. While several different events have contributed to these statistics (for example, better treatment of hypertension, better highways and safety devices in automobiles), if the reduction in the plasma cholesterol concentration were causally related to traumatic deaths, one would have anticipated an increasing rate of suicides and accidents. This has clearly not occurred in the United States.

(28)




Another point that should be emphasized is that in those few studies in which there was an apparent increase of violent deaths in patients treated with cholesterol-lowering agents, the level of plasma cholesterol values achieved with treatment was still significantly higher than the average plasma cholesterol concentration in Chinese and Japanese groups. Clearly there is no increase in traumatic deaths in these Asian populations. Furthermore, as discussed above, there is no increased incidents of cancers or strokes in these populations. It is difficult to understand, therefore, how the minor reductions in plasma cholesterol

achieved in the various interventional studies could have been casually related to a change in aggressive behavior.

Still, there was a concern about these small numbers of patients. As a consequence, an investigative team from the Center for Drug Evaluation and Research of the Food and Drug Administration was sent to investigate these traumatic deaths in both the LRC and the Helsinki Heart Study. Three examples of the kinds of deaths that occurred in these studies are shown in **Figure 29**. These

**Example 1.**

**A 53-year-old man (allocated to gemfibrozil treatment) died after being run over by a train. He was heavily drunk (blood alcohol 3.2%). He had lain down on the tracks. The death was classified as suicide. This patient had dropped out of the study more than 4 years earlier.**

**Example 2.**

**(29)**

**A 51-year-old man (allocated to gemfibrozil treatment) was shot by his neighbor's son. The death was classified as homicide. This patient had dropped out of the study 1 year earlier.**

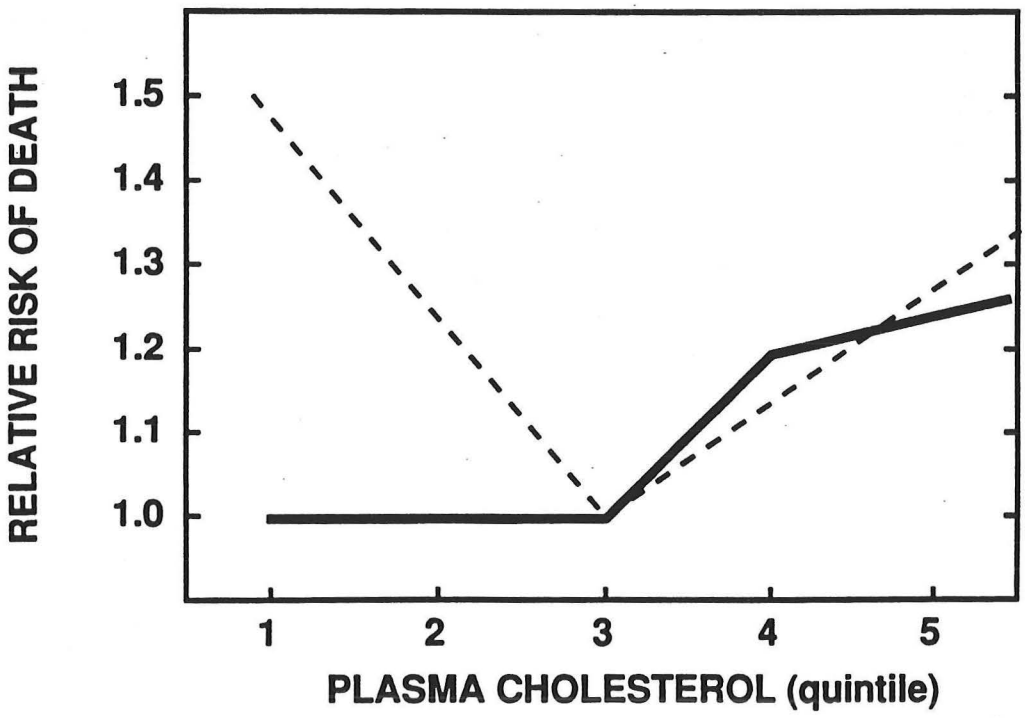
**Example 3.**

**A 49-year-old man (allocated to gemfibrozil treatment) was driving a crop harvester on a sloping field. The crop harvester turned over and the driver was crushed. The death was classified as accident. There was no blood alcohol, and the patient had adhered to drug treatment.**

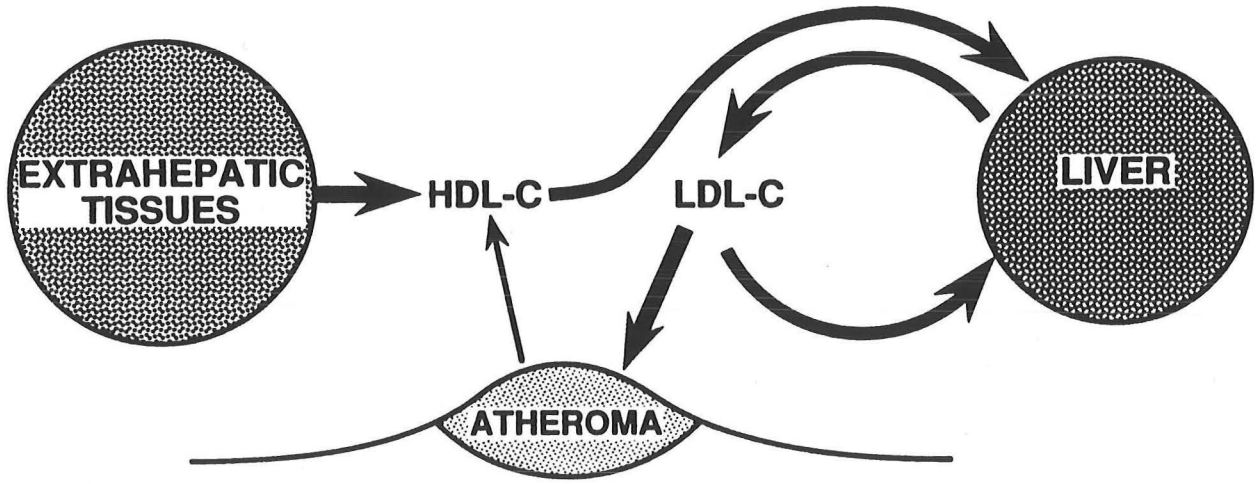
investigators found that many of the deaths occurred in individuals who had stopped taking their medication months-to-years before the accidents. Many of the individuals had preexisting psychiatric disease. Many were the victims of crime, not the instigators. These investigators concluded therefore that "little evidence remains to support the hypothesis that cholesterol-lowering drugs are causally associated with deaths due to homicides, suicides and accidents in these trials".

Finally, the dramatic "U" shaped curve previously discussed for the Honolulu Heart Study has recently been reanalyzed. These investigators found that an unusual number of these Japanese-Americans with low plasma cholesterol concentrations had preexisting disease. Many had had gastrectomy, bowel resections, alcoholism, hepatic cirrhosis, various tumors or were very heavy smokers. When these individuals with preexisting disease were excluded from the series, as shown in **Figure 30**, the apparent increase in mortality rates disappeared in those groups with a low plasma cholesterol concentration while the increasing risk of death from coronary artery disease persisted in the high cholesterol groups. Thus, all of the objective data strongly suggest that there is no causal relationship between lowering the plasma cholesterol level and an increasing incidence of cancer or traumatic deaths.

(30)



Thus, as illustrated in **Figure 31**, it now seems apparent that there are very effective ways to prevent or even reverse atheroma formation by manipulating the LDL-C concentration and, to a lesser extent the HDL-C concentration. As has been emphasized in this review the extent of atheroma formation clearly is a time dependent process that is related to the steady-state concentration of LDL-C in the plasma. These new generation of pharmaceutical agents make it possible to dramatically lower the LDL-C concentration and so retard the development of atheroma formation and, in a secondary way, actually reverse atheroma size. Changes in the circulating level of LDL-C should have no effect on the normal physiology of cholesterol balance across the extrahepatic tissues, in general, and the brain, in particular. Since these new agents are 3-4 times more potent than the agents used in many of the initial controlled trials, it is to be anticipated that this form of therapy will dramatically lower the risk of CHD and the mortality rate associated with heart disease.




**(31)**

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