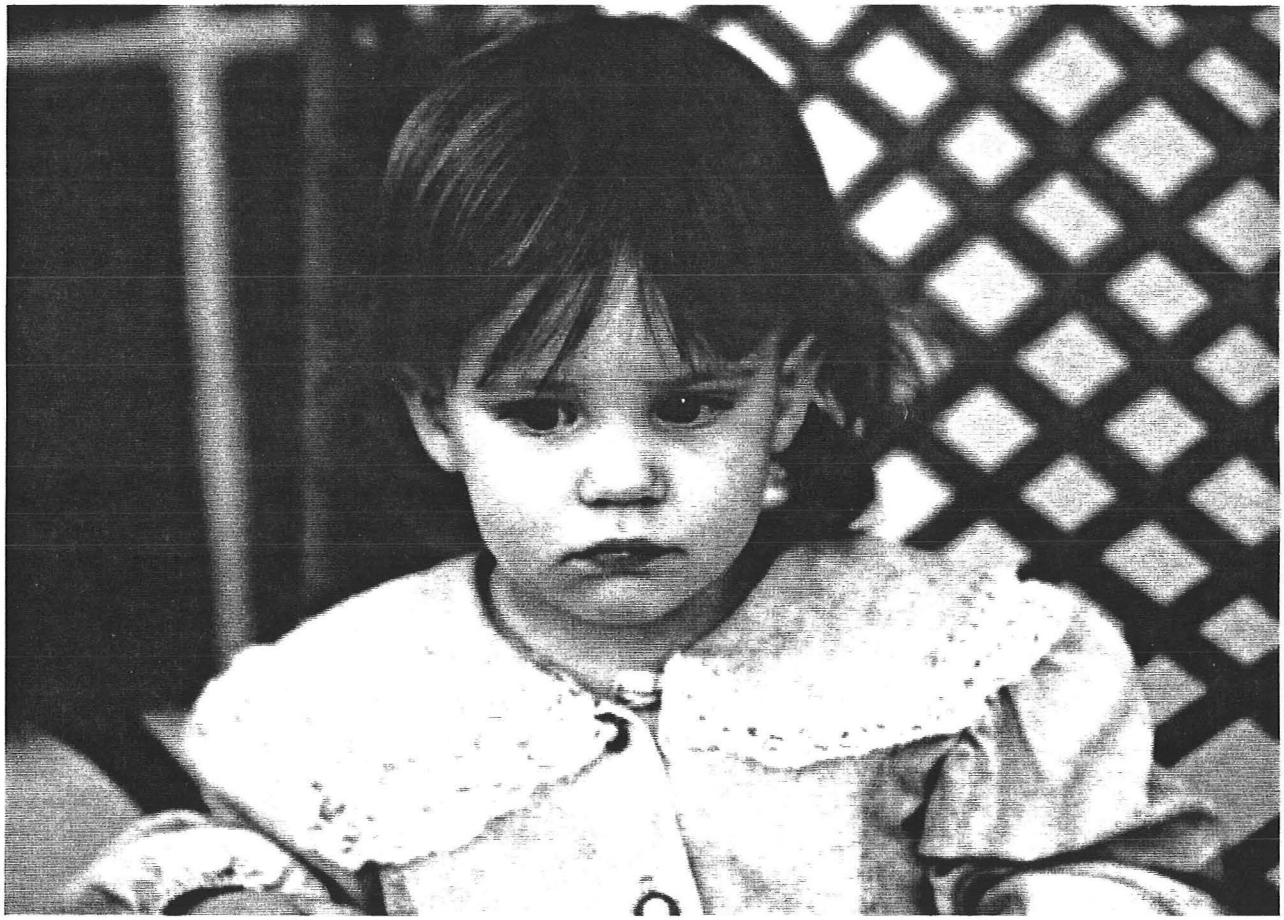
Medical Grand Rounds

CAN CORONARY HEART DISEASE BE ELIMINATED FROM THE POPULATION IN OUR LIFETIME?



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I. INTRODUCTION

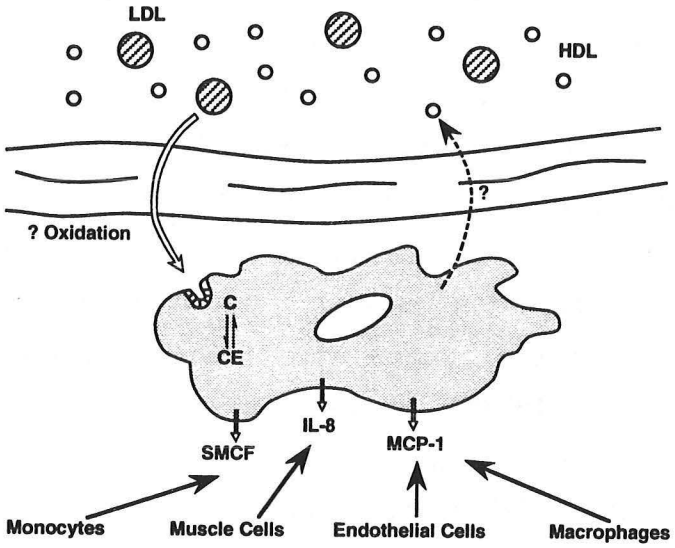
Over the past ten years there has been the introduction of at least three groups of pharmaceutical agents that have altered the outcome of major clinical diseases in remarkable ways. In each case, these agents have been directed at a very specific enzyme or transporter in parts of the gastrointestinal tract. Introduction of H2 blockers, for example, provide the first highly effective means for regulating hydrogen ion secretion into the stomach. These agents significantly altered the therapeutic approach to patients with peptic ulcer disease and greatly reduced the need for gastric surgery. More recently, pharmaceutical agents that bind irreversibly to the proton pump have proved to be highly effective in treating reflux esophagitis and its complications and have greatly reduced the need for anti-reflux surgery. A third group of compounds, the HMG CoA reductase inhibitors (statins) are remarkable pharmaceutical agents that now appear to be capable of markedly reducing the need for various cardiac procedures and, in addition, appear to actually significantly reduce the incidence of clinical coronary heart disease. These agents partially inhibit the rate limiting enzyme for cholesterol biosynthesis in the liver. As a consequence of this effect, the liver cell increases its rate of synthesis of specific receptor molecules (the LDL receptor) on its cell surface that are responsible for the removal of the remnants of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) from the circulating plasma. As a consequence of this increase in LDLR activity, the rate at which LDL is formed (the LDL-C production rate) is reduced and the rate at which LDL is removed from the plasma (the LDL-C fractional catabolic rate) is increased and, consequently, the steady-state concentration of LDL-C is reduced. Data derived from both experimental animals and epidemiological studies in humans have provided essentially irrefutable evidence that the magnitude of atherosclerosis formation in coronary arteries, and the magnitude of clinical coronary artery disease, is related directly to the level of circulating total cholesterol (TC) and LDL-C concentrations. Recent large clinical trials have provided new, impressive data that lowering these circulating cholesterol levels with the statins profoundly lowers the incidence of clinical coronary events, death from coronary causes, the need for a variety of coronary operative procedures, hospitalization days related to coronary disease and overall mortality rates. The

results of these new studies, as well as older studies, are reviewed in this protocol, and these data provide the basis for markedly reducing the incidence of coronary artery disease within the American population.

II. THE MACROPHAGE AND ATHEROMA FORMATION

It is now recognized that the cholesterol-loaded macrophage is one of the major hallmarks of early atherosclerosis and is very likely involved in the initiation and maturation of the more advanced atherosclerotic lesion (**Figure 1**).

Figure 1



In general, such monocytes apparently take up minimally altered LDL from the plasma. This is associated with the release of a number of chemotactic factors that recruit additional monocytes and smooth muscle cells to the area which expands the lesion and leads to encroachment upon the vessel lumen. Much is now known about various steps involved in this process. Early in the pathogenesis of these lesions, monocytes are apparently attracted to subendothelial locations in areas of arteries subjected to hydrodynamic stress. These cells possess a "scavenger" receptor that is capable of taking up large amounts of LDL that have been minimally altered by oxidation. This can lead to large quantities of cholesterol ester being stored in these cells, and this process may activate the synthesis of a number of chemotactic factors. One of these, smooth muscle cell chemotactic factor (SMCF), is made constitutively in such

cells while other factors such as IL-8 is induced by oxidized LDL. While the genesis of these lesions is not fully understood, it is likely that these chemotactic factors are responsible for recruitment of additional monocytes, smooth muscle cells and fibroblasts into the incipient atherosclerotic lesion. Presumably, the further uptake of minimally modified LDL leads to increased accumulation of cholesterol esters within these cells and the development of classical foam cells.

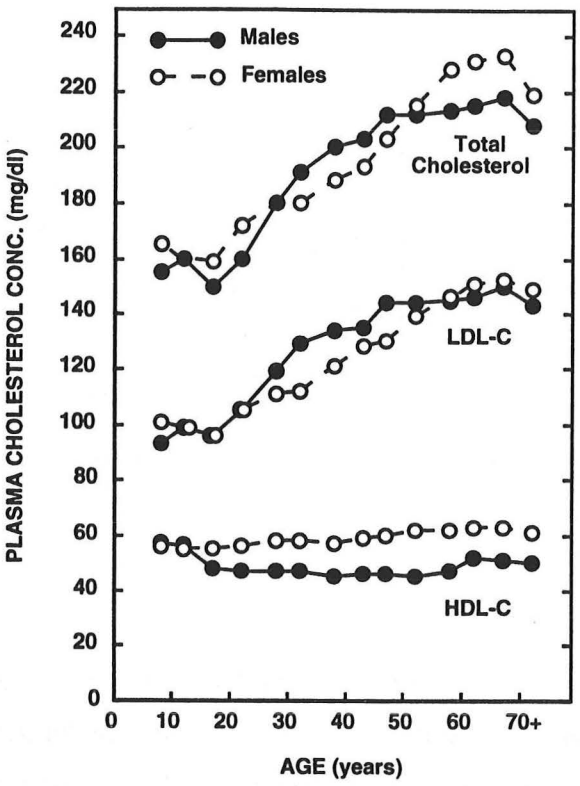
The earliest lesion within the arteries can be identified by appropriate lipid staining and consists of lipid laden macrophages and proliferating smooth muscle cells that protrude only slightly into the arterial lumen. With time, these lesions mature into the typical fibrous plaque in which there is continued proliferation of smooth muscle cells and monocytes, the formation of cellular debris and a thickened fibrous cap. This lesion may eventually become complicated when there is hemorrhage into the adjacent arterial wall, rupture of the plaque itself or clotting within the residual lumen above the plaque. While the molecular details of this process still remain to be elucidated, what is clear is that development of the atheromatous lesion is directly related to the steady-state concentration of LDL-C in the plasma and to the duration of time that this concentration has been maintained. In addition, in a manner that is very poorly understood, the development of this atheromatous process is inhibited by high concentrations of circulating high density lipoproteins (HDL).

III. DISTRIBUTION OF PLASMA CHOLESTEROL CONCENTRATIONS IN DIFFERENT HUMAN POPULATIONS

There is now little doubt that the magnitude of atheroma formation in a given individual is directly related to the concentration of LDL-C (and other apoB containing lipoproteins) present in the plasma. In general, the total concentration of cholesterol (TC) present in the plasma is a function of the LDL-C concentration. Many of the earlier epidemiological studies did not differentiate TC from LDL-C. Nevertheless, except for some relatively rare special conditions, elevations in TC are almost always brought about by elevations in the LDL-C.

This relationship, and the remarkably high plasma cholesterol concentrations present in the American population, are illustrated by the data in **Figure 2.** As is evident in this diagram, from birth to death, the HDL-C

Figure 2



concentrations remain essentially constant although the values in males tend to be lower than those in females. The LDL-C concentrations are remarkably high in young humans and, as is evident in **Figure 2**, progressively climb with aging. The LDL-C concentrations tend to be somewhat lower in females until menopause when these values become essentially the same in men and women. As a consequence of these changes in the American population the total plasma cholesterol is very high, even in children, and progressively increases into the range of 200-240 mg/dl in older individuals. These levels, particularly of LDL-C, are higher than seen in any other species and in most other humans in the world and undoubtedly are the major determinant for the high rates of coronary artery disease seen in the United States.

This conclusion is supported by a number of epidemiological studies. For example, in the Yandapu-Enga tribal community of New Guinea, the plasma TC concentrations in adults are essentially half of those in the United States (**Figure 3**). This is due to the fact that their plasma LDL-C concentrations (**Figure 4**) average approximately 80 mg/dl while those in the United States approach 140-160 mg/dl. These individuals live in the village of Turkisenta at approximately 7,000 feet. These people are heavy users of tobacco (73% in the males) and ate diets

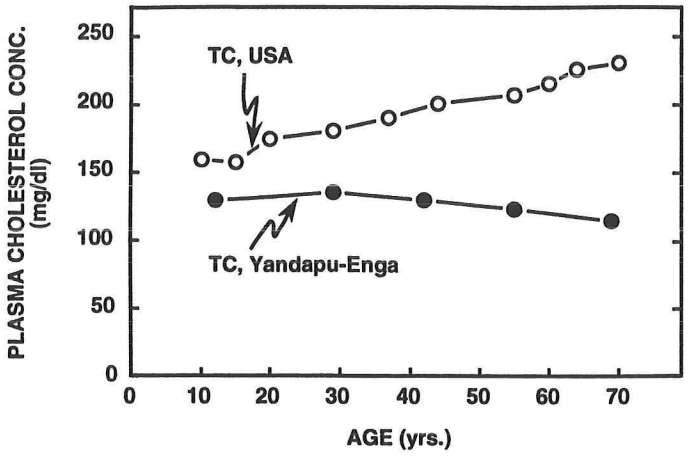


Figure 3

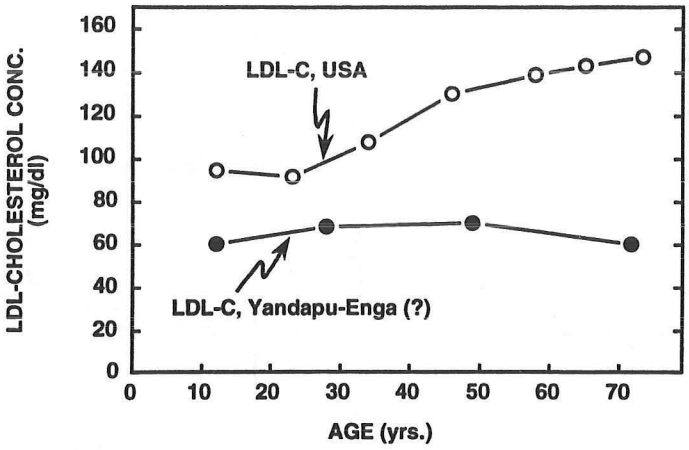
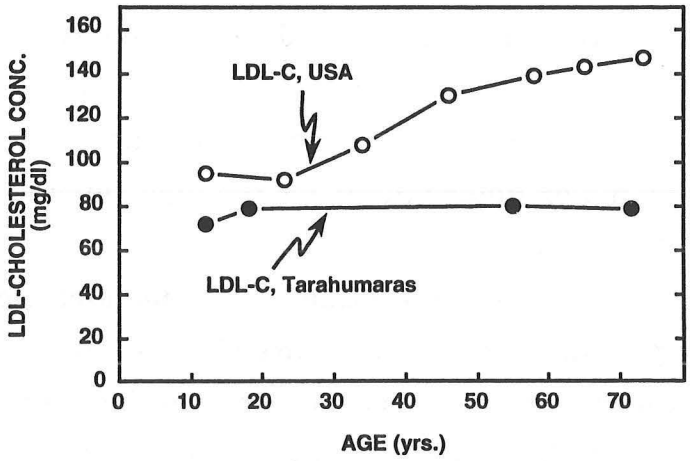


Figure 4

based primarily on the cultivation of wild yams. Carbohydrates supply 90% of their calories while fat intake is about 3%. While a low percentage of these people had valvular heart disease, there was virtually no hypertension, cardiovascular disease, peripheral vascular disease, angina or other evidence of coronary artery disease.

These striking findings are not due to a peculiar genetic background in these people because similar findings have been reported in other tribal groups like the Tarahumara (**Figure 5**) where the mean plasma TC concentration is

Figure 5



about 125 mg/dl and where the LDL-C values are again about half of those seen in the United States. Like the Yandapu, these individuals are mountain dwellers and carbohydrates make up 75% of their caloric intake. Corn and beans, rather

than yams, are the source for this carbohydrate. Despite the fact that the mean plasma LDL-C levels were low in this population, the plasma cholesterol correlated directly with dietary cholesterol intake, as is true in all other appropriate experimental animals maintained on diets low in triacylglycerol. The Tarahumara, like the Yandapu, have virtually no atherosclerosis that can be detected clinically.

The frequency distribution of the plasma TC concentrations in societies like the Tarahumara are shown in **Figure 6** and contrasted with the frequency distribution of these values in the American population. One can superimpose upon these curves the frequency distribution for large populations like the 1.2 billion Chinese. It is clear from such comparisons that most humans in the world have plasma TC concentrations that vary between 100-200 mg/dl (LDL-C concentrations of 60-90 mg/dl) while the population of Americans (and Europeans) is distinctly abnormal. For practical purposes, essentially 85% of the

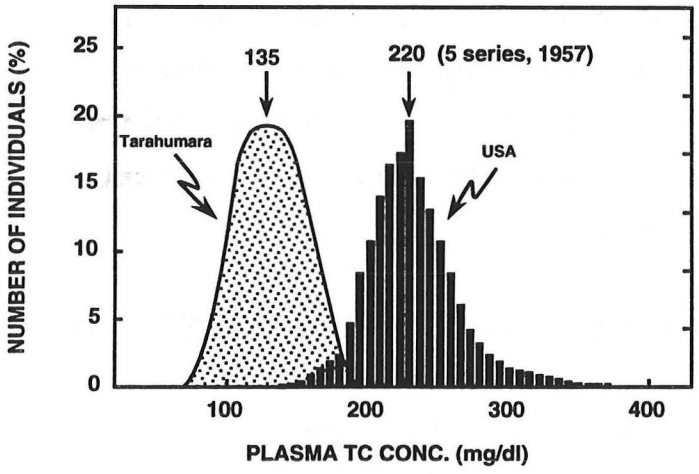


Figure 6

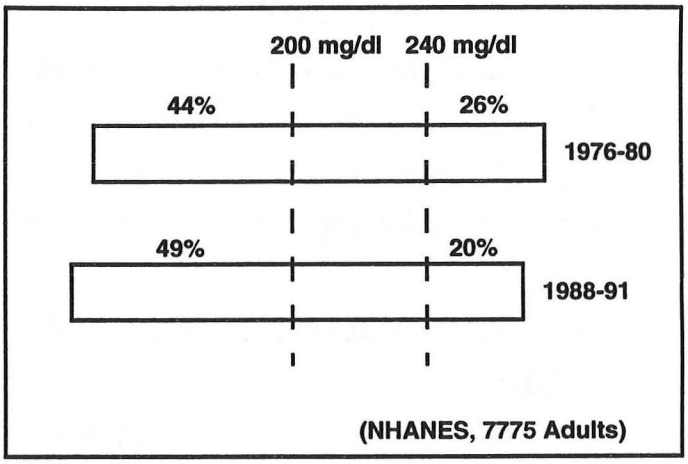


Figure 7

American population is hypercholesterolemic. Clearly, this abnormality is a function of environment and not genes. When individuals of the same genetic background as the Tarahumara are moved to the United States and adopt US eating patterns, the plasma cholesterol essentially doubles and there is a high incidence of coronary artery disease. In San Antonio, for example, very careful diet and disease surveys have shown that the eating patterns are identical in individuals from the same neighborhood who are either derived from the

caucasoid gene pool or the Amerindian pool, and both of these groups of individuals have indistinguishable high plasma cholesterol levels and incidences of coronary artery events. Similar data are available from other groups that have immigrated into the United States from areas of low coronary disease. The Tarahumara have cholesterol intakes of about 1 mg/day per kg body weight while Americans have an intake of 3-5 mg/day per kg body weight. About 10% of the caloric intake of the Tarahumara is from triacylglycerol whereas in the United States 35-50% of calories are derived from triacylglycerol. Over the past 20 years, a very vigorous campaign by the American Heart Association and the National Institutes of Health has probably brought about a small change in these dietary patterns in the United States. These small alterations in cholesterol and triacylglycerol intake probably account for the very modest leftward shift in the frequency distribution of plasma TC concentrations in the American population shown in **Figure 7**. It should be emphasized, however, that even in 1990 about half of the US population had a plasma TC concentration greater than 200 mg/dl. Thus, the conclusion that must be reached is that the great majority of Americans are grossly hypercholesterolemic when compared to the majority of the world's population or to a variety of other mammals and primates, and this hypercholesterolemia is almost certainly primarily the result of enviromental factors, i.e., the types of diets that are consumed.

IV. THE EVOLUTION OF ATHEROSCLEROSIS IN CHILDREN AND YOUNG ADULTS

One of the earliest papers that recognized the high incidence of atherosclerosis in coronary arteries reported the results of 300 autopsies in young men (22 years of age, on average) who died of battle wounds in Korea. Approximately 77% of the hearts in these individuals showed some degree of gross evidence of coronary atherosclerosis. The findings varied from fatty streaks to fibrous plaques producing variable degrees of luminal narrowing. Similar findings were seen in the Vietnamese War where in 105 autopsies of battle casualties approximately 45% had evidence of gross atherosclerosis. Studies such as these then gave rise to major investigations of the age-dependent appearance of coronary atherosclerosis in children and young adults. One of these

investigations was the Bogalusa Heart Study in which quantitative measurements were made of coronary artery involvement with atherosclerosis in children and young adults who had died acutely as a result of trauma. These studies confirmed that atherosclerosis was already prevalent in children and young adults and, further, that the percentage involvement of the arterial surface area was essentially a linear function of the plasma LDL-C concentration, even in these youngsters. One of the very interesting findings was that lipid-laden macrophage foam cells were found in the intima of about 35% of infants during the first 8 months of life. These cells tended to disappear only to reappear at about the time of puberty when more substantial accumulations of foam cells were seen in the coronary arteries.

Figure 8 summarizes some of the results found in approximately 1500 young individuals between the ages of 15 and 34 years. As is apparent, there was an age-dependent increase in the percent of the intimal surface area that was

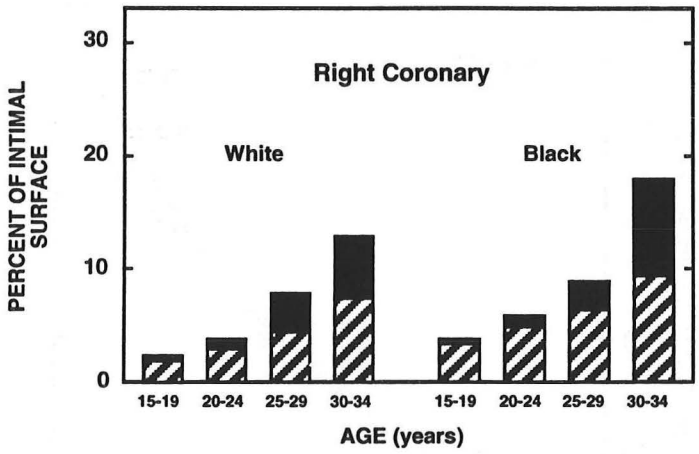


Figure 8

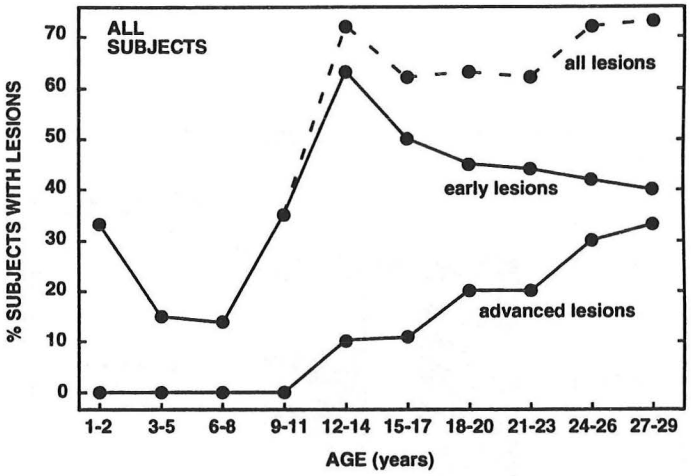


Figure 9

involved in the abdominal aorta and in the right coronary artery. The cross-hatched areas represent early fatty streaks while the black portion of the bars represents more mature, raised lesions. Still other data are summarized in **Figure 9**. It is particularly interesting that 35% of infants had early fatty streaks in their coronary arteries. These tended to disappear in later years only to reappear at puberty. More mature, fibrous lesions were rare before the age of 9,

but then progressively increased in incidence until approximately one-third of individuals at the age of 29 had such lesions. Thus, by this age, fully 70% of the subjects have some type of atherosclerotic lesion in their coronary arteries and half of these were advanced fibrous plaque lesions. Thus, not only are the majority of Americans hypercholesterolemic, but, in addition, the majority also have atherosclerosis that begins very early in life and progresses to involve a greater percentage of the intimal surface as the individual ages.

V. RELATIONSHIP OF CORONARY ATHEROSCLEROSIS AND CORONARY EVENTS TO PLASMA CHOLESTEROL LEVELS

If, as these data indicate, the level of coronary atherosclerosis is dependent upon both the level of plasma cholesterol (LDL-C) and the age of the patient, then there should also be a relationship between manifest clinical coronary artery disease and the plasma lipid levels. Over the past 30 years an abundance of data has unequivocally supported this relationship. One of the earliest studies is

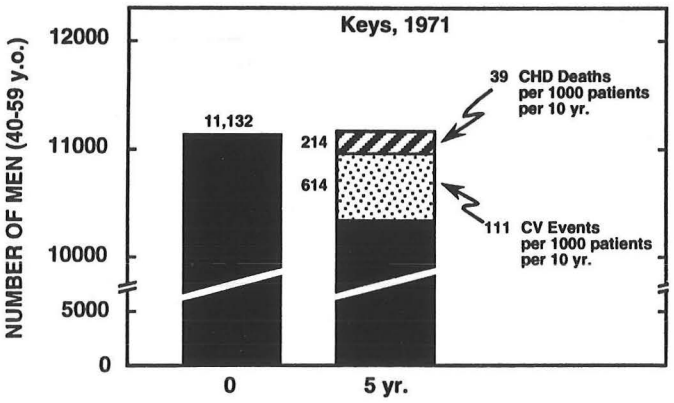


Figure 10

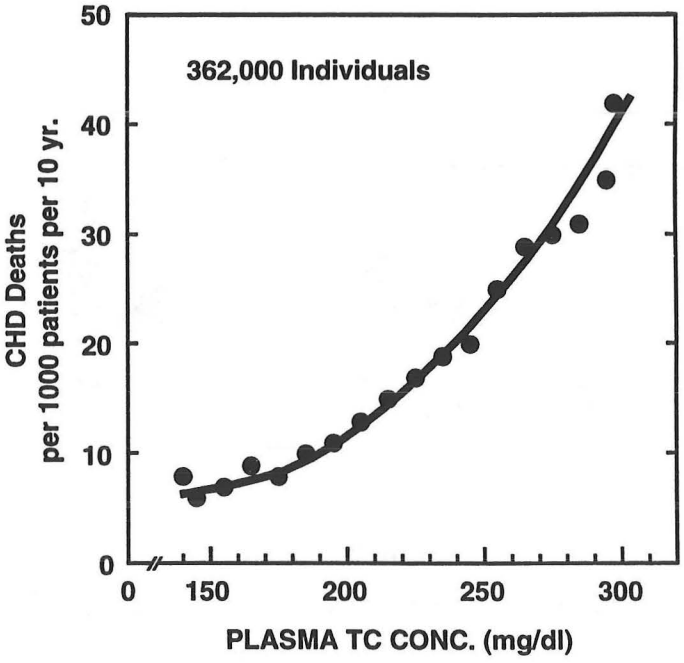


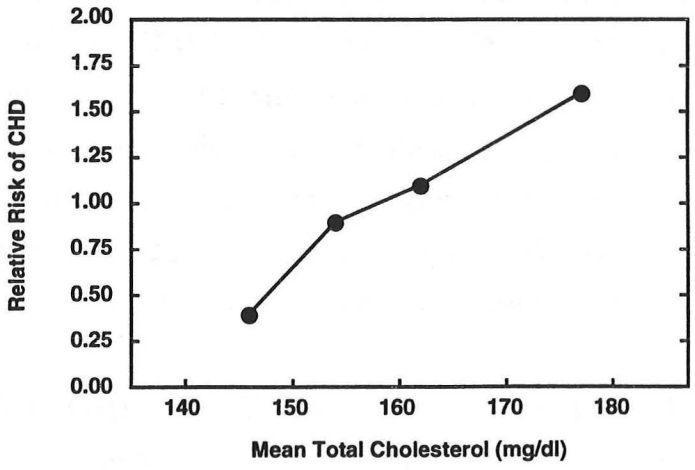
Figure 11

illustrated in **Figure 10** where Dr. Keys and his colleagues in 1971 followed 11,132 individuals for five years. During this time 614 individuals had a coronary event, and 214 died. Thus, the incidence of coronary events in this group was 111 per

1,000 patients per 10 years. A far more elegant and detailed study of this relationship came from the Multi-Risk Factor Intervention Trial (MRFIT). As shown in **Figure 11**, from a study of the 362,000 men, in the age range of 35-57 years, in the early 1970s, there was an unequivocal relationship between death due to coronary artery disease and the steady-state plasma TC concentration. This death rate varied from approximately 7 per 1000 individuals per 10 years at a plasma cholesterol concentration of 140 mg/dl to 42 per 1,000 individuals per 10 years at a TC concentration of 300 mg/dl. Thus, there was approximately a six-fold increase in the death rate in those individuals with the highest TC concentration versus those with a concentration of 150 mg/dl.

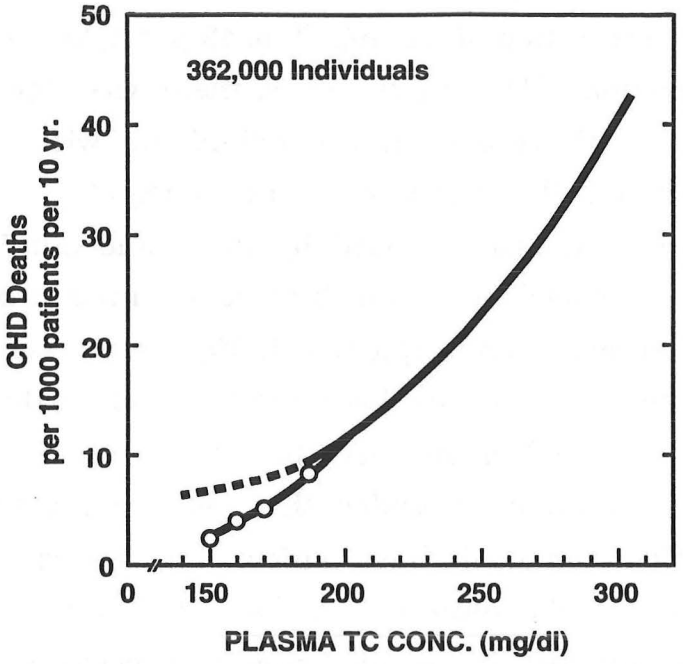
One of the important issues raised by these and similar studies was whether there was a "threshold" below which there was little or no change in the risk of coronary artery disease. As is apparent in **Figure 11**, these data suggested that below a value of about 200 mg/dl there was relatively little decrease in the death rate. However, this end of the curve has the fewest individuals and, in addition, it is now clear from other studies that Americans with these very low plasma TC concentrations commonly have confounding, underlying diseases that result in excess mortality. The nature of the lower end of this curve has more recently been directly examined by detailed studies directed by Richard Pito in 9,000 urban Chinese men and women, aged 35-64. As illustrated in **Figure 12**, the range of TC concentrations in these individuals was very low and varied

Figure 12



from about 145 to 180 mg/dl. As is apparent, the relative risk of CHD was essentially a linear function of the plasma TC level, even at these very low plasma cholesterol levels. Specifically, there was no evidence in these studies of a threshold below which relative risk of CHD leveled off at a constant value. From the raw data presented in this study, it is also possible to calculate the absolute

Figure 13



CHD deaths in each quartile of these 9,000 Chinese individuals. These data are plotted at the lower end of the MRFIT curve and are shown in **Figure 13**. As is apparent, these absolute death rates appear to extend the MRFIT curve in an essentially linear manner such that the death rate from CHD approaches zero at a plasma TC concentration of approximately 140-150 mg/dl. It is noteworthy that this is the plasma TC concentration commonly encountered in groups like the Tarahumara and Yandapu, populations in which there is essentially no coronary artery disease. These data suggest that in free living populations there is essentially a linear relationship between the rate of death from CHD and the plasma TC concentration between the levels of 150 mg/dl and 300 mg/dl.

VI. THE REGULATION OF PLASMA CHOLESTEROL CONCENTRATIONS

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. The typical cell is surrounded by a cholesterol-rich plasma membrane while the membranes that make up the endoplasmic reticulum, mitochondria and other membranous structures in the cell are relatively poor in cholesterol content. Such membranes that consist primarily of phospholipid are very fluid and allow proteins to move and fold within their structure. When cholesterol is inserted into such phospholipid membranes there are hydrophobic 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 6 Å. 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 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 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 turnover in the different cell types is proportional to the rate of metabolic turnover of a particular cell.

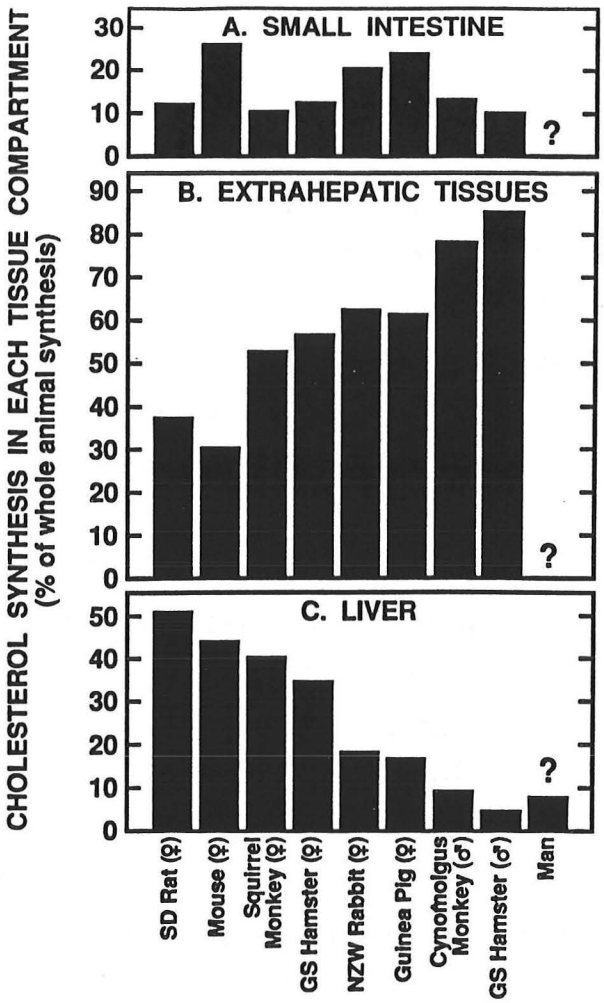
Because every cell requires a continuous supply of cholesterol, this sterol is synthesized actively in virtually every organ system in the body. Humans, for example, must synthesize about 10 mg/day per kg body weight while a small animal like the mouse synthesizes approximately 100 mg/day per kg. Many other species, including non-human primates, have intermediate rates of cholesterol synthesis between these two extremes.

Within any species, the rate of synthesis is also related to the age of the

animal. In the fetus and newborn animal, for example, rates of cholesterol synthesis are often 4-6 times higher than in the adult, per kg body weight. Similarly, higher rates of cholesterol synthesis are seen in the pregnant animal and in the obese human. Such data illustrate the remarkable adaptability of the sterol biosynthetic pathway for meeting changes in the needs of the organism for cholesterol to support tissue growth and for membrane remodeling. No mammal has an absolute requirement for exogenous or dietary cholesterol. Rather, the changing requirements for cholesterol during pregnancy and growth and development in the youngster are met by remarkable alterations in rates of cholesterol synthesis. 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 is 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 have been systematically underestimated, and, in some tissues, by as much as 90%. With the advent of new techniques that circumvented these artifacts, absolute rates of cholesterol synthesis can now be measured in the whole animal or human in vivo. The data in **Figure 14** summarize such information from seven species where rates of sterol synthesis have been measured in vivo under circumstances where the animals were fed diets low in cholesterol and triacylglycerol. This diagram shows the contribution of the small intestine (A) and liver (C) to whole animal synthesis while the contributions of the remaining extrahepatic organs

Figure 14



have been combined into a single value (B). Under these circumstances where dietary cholesterol intake was nearly zero, the liver contributes, at most, 40-50% of the cholesterol synthetic activity found in the rat, mouse and squirrel monkey. However, this contribution is significantly less in other species and amounts to < 20% in the rabbit, guinea pig, cynomolgus monkey and Golden Syrian 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 14**, 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 these 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 synthesis, partial suppression of intestinal synthesis and virtually no change in synthesis in the extrahepatic organ. 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 rates of cholesterol synthesis in the liver and, to some extent in the intestine, increase to compensate for this loss while the rates of synthesis in the extrahepatic organs remain 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 delivery 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 sterol loss in the feces is enhanced.

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 15**, 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. Thus, one way in which to express the rate of LDL-C uptake is as an absolute rate of transport having the units 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 removal 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 hour per kg of body weight. Finally, either the absolute rate of LDL-C transport out of the plasma or the clearance rate can be expressed as a fraction of the LDL-C pool or the plasma volume, respectively, present in one kg of body weight. This calculation yields a term called the fractional catabolic rate

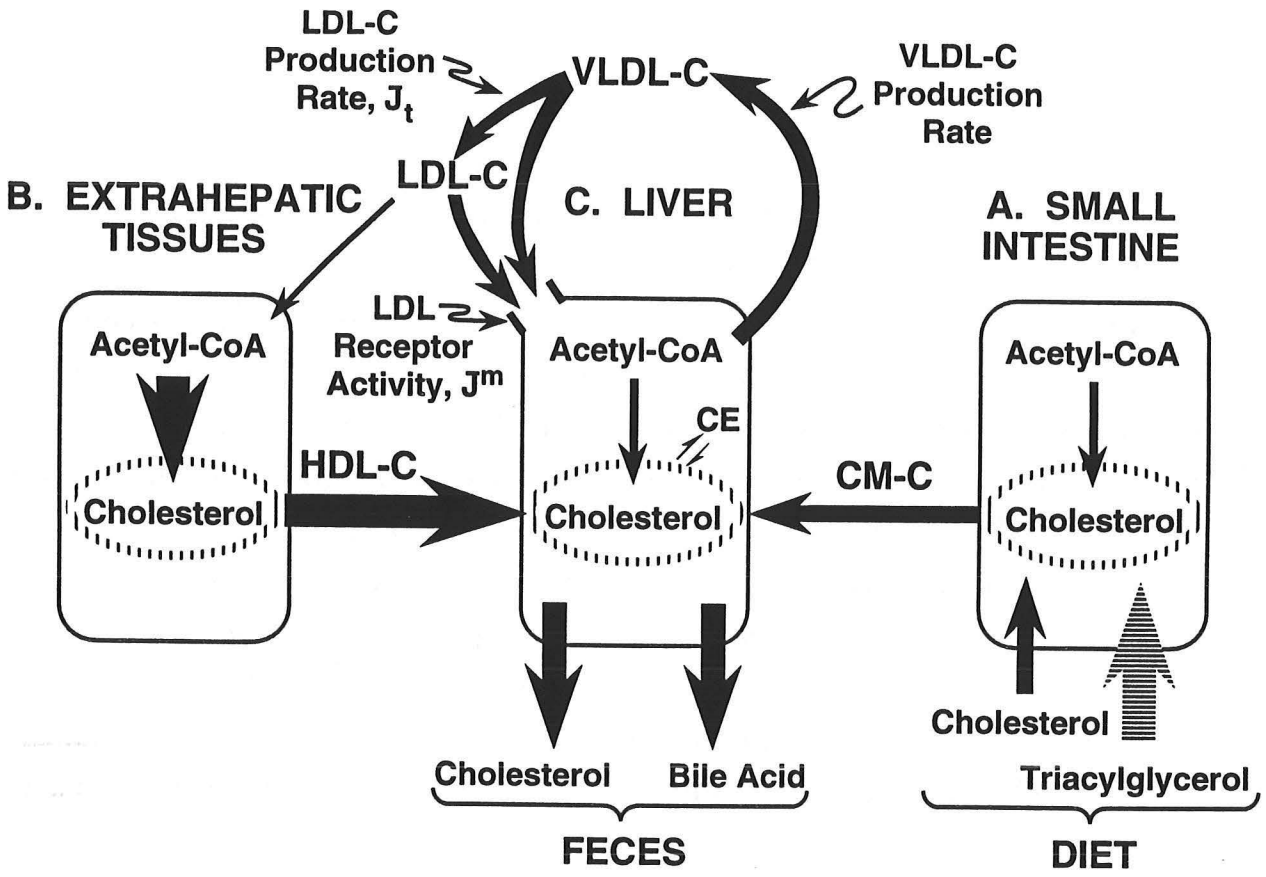


Figure 15

which describes the fraction of the LDL-C pool removed from the plasma each hour or day. Thus, the rate at which LDL-C is removed from the plasma can be expressed three different ways, i.e., the absolute rate of LDL-C transport (mg/d 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.

Just as the rate of cholesterol synthesis in the whole animal varies with body weight, there is also a relationship between animal size and the fractional catabolic rate of LDL-C. Small animals such as the mouse, hamster and 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, 1 kg of a human will clear

only about 0.6-0.7 ml of plasma per hr of its LDL-C content while 1 kg of these small animals will clear 6-7 ml/hr. It should be noted that the same relationship between body weight and fractional LDL turnover exists in animals that are omnivorous, carnivorous and herbivorous.

The next question of importance is which tissues in the body account for these rates of receptor-dependent LDL-C uptake observed in the whole animal. With the development of radio-labeled markers for LDL that are retained in the different organs and short-term steady-state infusion techniques using homologous and derivitized LDL-C, it has become possible to determine the absolute rates of LDL uptake in every organ in the live animal. **Figure 16** summarizes the available data of this type. The general profile of LDL transport is similar in all species. As is apparent, about 70% of LDL-C clearance takes place in the liver each day. A similar figure appears to be appropriate in the case of humans. The small intestine and all of the remaining extrahepatic tissues

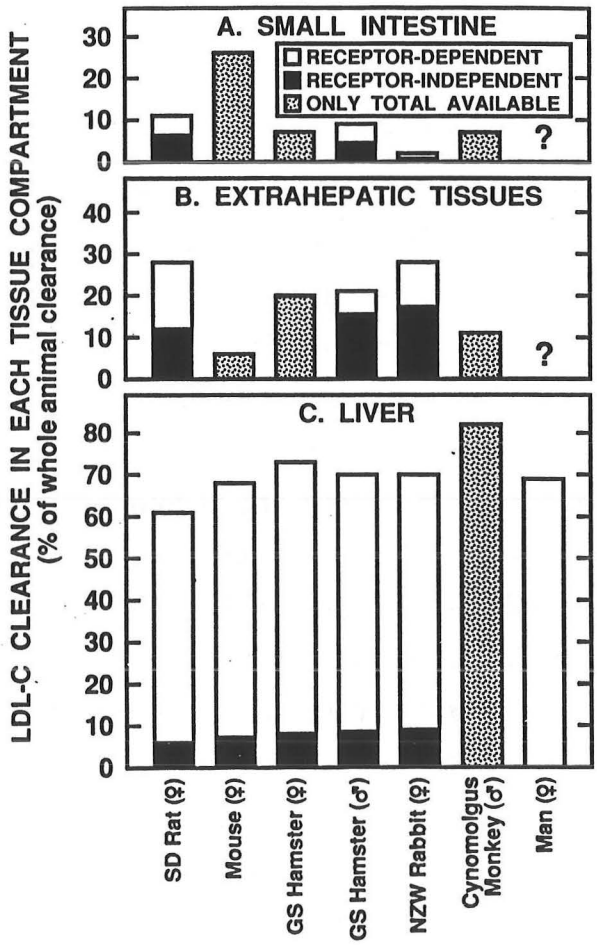


Figure 16

together account for the degradation of only about 30% of LDL-C. Furthermore, most of the LDL-receptor activity that can be detected in vivo is present in the liver. Thus, for example, in the primate, including humans, approximately 90% of such receptor-mediated LDL degradation can be identified in the liver. The relatively small amount of LDL-C that is cleared by the remaining extrahepatic tissues takes place primarily by a mechanism that is receptor independent. Thus, any genetic or environmental factor that reduces receptor-dependent LDL-C transport into the liver will necessarily be associated with a rise in the steady-state plasma LDL-C concentration.

In virtually all experimental animals, including several different species of primates, the plasma concentration of LDL-C varies from about 20-70 mg/dl under conditions where the animals are eating diets that are low in both cholesterol and triacylglycerol. Insofar as data are available, this appears to be true in several groups of humans similarly maintained on diets where most calories come from protein and carbohydrate. The abnormally high plasma cholesterol values seen in virtually all Americans is the result of environmental factors (dietary lipids) superimposed upon a number of genetic polymorphisms. As shown in **Figure 15** the steady-state concentration of LDL-C is primarily determined by the rate at which LDL-C is produced (the LDL-C production rate) and the amount of LDL receptor activity (LDL-R) that is manifest in the liver. Both of these rates appear to be influenced by the small pools of unesterified and esterified cholesterol that exist within the liver cell. Under circumstances where the diet contains small amounts of cholesterol there is a net increase in sterol delivery to the liver. This results in an expansion of both the unesterified and esterified pools of sterol which, in turn, is associated with partial suppression of LDL-R activity and an increase in the outflow of cholesteryl esters from the liver as VLDL-C. The net effect of these two events is to raise the steady-state concentration of LDL-C in the plasma a small amount. This increase in the steady-state LDL-C concentration is essentially a linear function of the amount of cholesterol fed in the diet each day. In human populations, this load of dietary cholesterol varies between about 1 and 10 mg/day per kg body weight. In Americans, it equals 3-6 mg/day per kg.

In the presence of small amounts of dietary cholesterol, the amount of dietary triacylglycerol also profoundly affects these kinetic events. The addition of

oleic acid on top of the dietary sterol load reduces the concentration of unesterified cholesterol in the regulatory pool and markedly expands the pool of cholesteryl esters. As a result, there is an increase in outflow of cholesterol from the liver as VLDL-C and, at the same time, upregulation of LDL receptor activity. The net effect of these two opposing events is to raise the circulating plasma LDL-C level. Other specific fatty acids have different effects. Certain long-chain saturated fatty acids, for example, actually suppress LDL receptor activity and further elevate the circulating LDL-C concentration. Thus, in addition to the effects of dietary cholesterol, dietary triacylglycerol also markedly elevates the plasma cholesterol concentration and the magnitude of this effect is dependent upon both the types of fatty acids in the triacylglycerol and the absolute amount of dietary triacylglycerol eaten each day. In the majority of human populations around the world, the daily intake of triacylglycerol is low and commonly in the range of 10-20% of total calories. In Western populations triacylglycerol commonly accounts for 35-50% of the caloric intake. If populations such as the Tarahumara Indians are placed on Western diets, their plasma cholesterol levels promptly rise from their traditional low values to the levels seen in the United States. Thus, there is little doubt that the environmental factors of small amounts of dietary cholesterol coupled with large amounts of dietary triacylglycerol account for the high plasma cholesterol levels that are seen in approximately 85% of Americans.

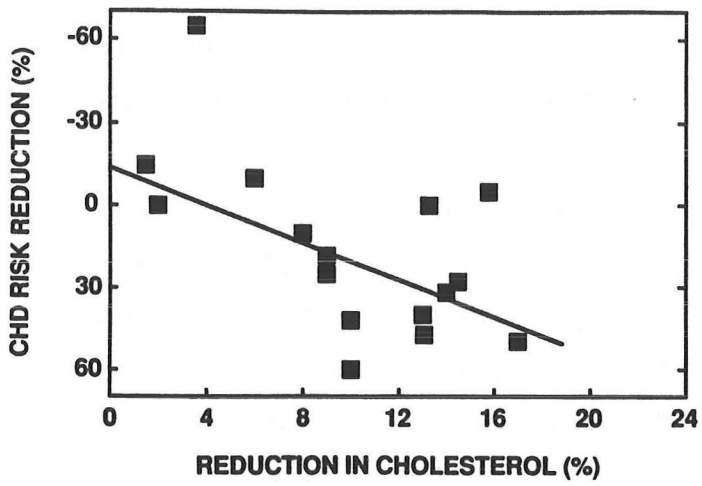
In addition, individuals may possess specific genetic polymorphisms in key enzymes or transporters that account for the variable response of the plasma LDL-C concentration to a standardized diet. For example, if one has a single animo acid substitution in the apoB or E proteins, one will have an altered binding coefficient of the LDL particle for the LDL receptor that will change the steady-state LDL-C concentration achieved at any dietary cholesterol and triacylglycerol input. Alternatively, individuals may have different rates of bile acid synthesis or cholesterol absorption which will also modify their specific response to dietary lipids. Nevertheless, it is clearly the superimposition of the large loads of dietary lipid in the American diet on top of these various genetic polymorphisms that account for the marked shift in the plasma cholesterol concentrations to very high and abnormal values.

VII. DOES LOWERING THE PLASMA CHOLESTEROL CONCENTRATION DECREASE THE INCIDENCE OF CORONARY ARTERY DISEASE?--THE OLD EVIDENCE

The recognition that there was a clear association between clinical heart disease and the total (and LDL) cholesterol levels led to a number of intervention trials that were carried out over the past 20-25 years in an attempt to reduce the incidence of heart disease by reducing plasma cholesterol concentrations. Unfortunately, nearly all of these studies were carried out in an era when it was difficult to lower the plasma LDL-C concentration more than 10%. Furthermore, some of the treatments were potentially toxic. As a result, the results of many of these trials were equivocal and many clinicians were unconvinced of the beneficial effects of lowering LDL-C levels.

Twenty-one of these trials are summarized in **Table I.** Some of these involved primary prevention of heart disease while others were devoted to

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



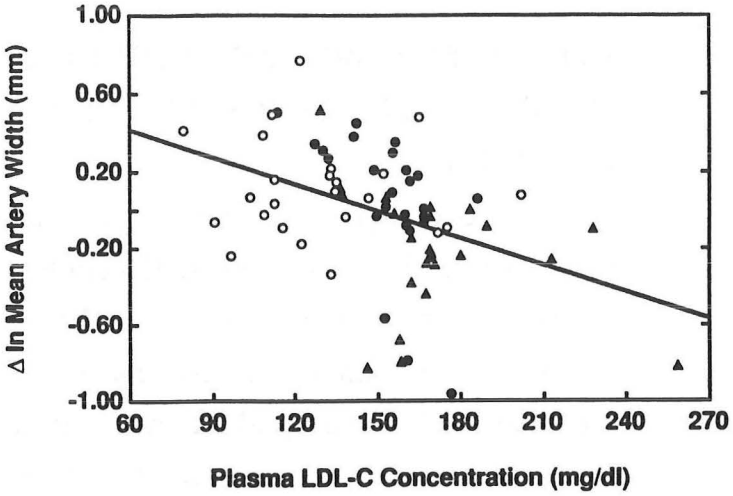


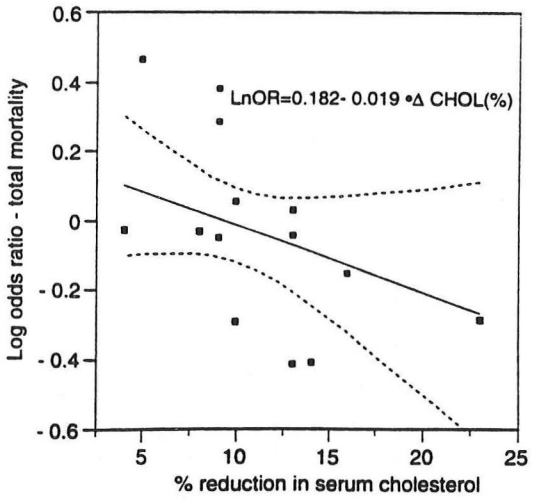
Figure 17 **Figure 18**

secondary prevention. These studies involved a variety of genetically different populations, both men and women, and utilized various endpoints. Many of these studies were combined and subject to careful analysis by Dr. Ingar Holme. **Figure 17**, for example, shows the decrease in coronary heart disease risk as a function of the percent reduction in circulating plasma cholesterol concentrations. As is apparent in the great majority of these studies, the reduction in plasma cholesterol levels was only in the range of 8-15%. Nevertheless, in these 16 trials the risk of CHD decreased 2.5% for each 1% reduction in the plasma cholesterol level.

That this reduction in CHD risk was associated with a change in the morphology of the coronary artery is suggested by the data shown in **Figure 18**. As is apparent, mean artery width in these patients actually decreased in the control group (▲), remained unchanged in those subjects treated with diet alone (●) and increased in the group more aggressively treated with drugs (O).

As shown in **Figure 19**, there was apparently also a small reduction in total mortality in these various studies. Thus, even though the treatment regimens available in this era were not good enough to lower the plasma cholesterol concentrations in Americans or Europeans to those much lower levels seen in the majority of humans, there was, nevertheless, a strong indication that the risk of cardiovascular disease and, possibly, all cause mortality could be reduced by aggressively lowering the circulating cholesterol concentration. However, because these effects were relatively small and because questions were raised

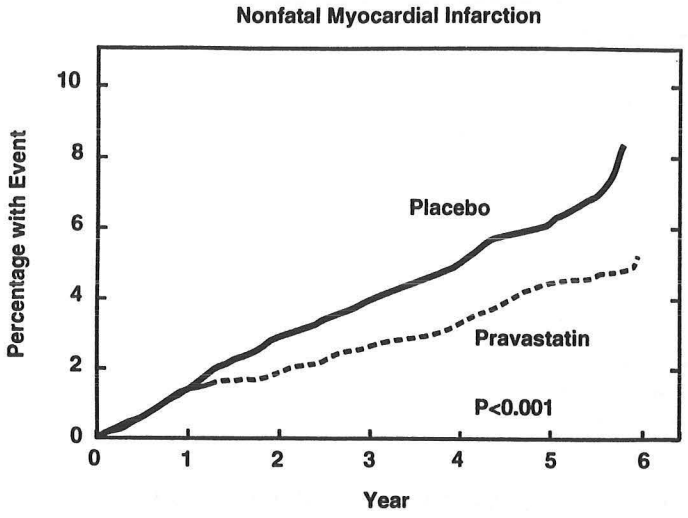
Figure 19



about all cause mortality rates and the incidence of cancer in some of the treated groups, the results of all of these studies remained somewhat controversial.

VIII. DOES LOWERING THE PLASMA CHOLESTEROL CONCENTRATION DECREASE THE INCIDENCE OF CORONARY ARTERY DISEASE?--THE NEW EVIDENCE

Even though the effects of lowering the plasma cholesterol levels on coronary heart disease have been extensively studied, because of the very small decreases in the plasma cholesterol levels that were achieved in most of these investigations, the results were fairly equivocal. This problem was further complicated by the fact that some of the agents that were utilized in these studies had toxicity that may have contributed to higher rates of all cause mortality. Much more definitive studies became possible once the HMG CoA reductase inhibitors became available. These agents clearly had three major advantages over prior therapy: 1) they were capable of lowering the plasma LDL-C levels to a significantly greater degree; 2) they could be taken as one or two tablets per day so that there was much better patient acceptance; and 3) they were relatively free of side effects. Utilizing these new statins, three major studies were initiated approximately 6-7 years ago, and the results of these studies have just become available in the last year. The general scheme of these studies was to select a group of patients with specific characteristics and, in a random manner, treat the individuals either with a placebo or statin. All studies lasted approximately five years, and the results have been reported in a form shown in **Figures 20** and **21**. In **Figure 20**, for example, the percentage of individuals that developed a



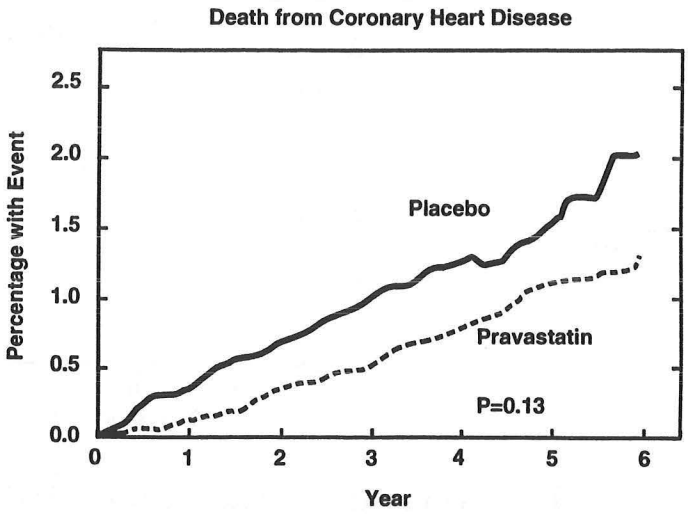
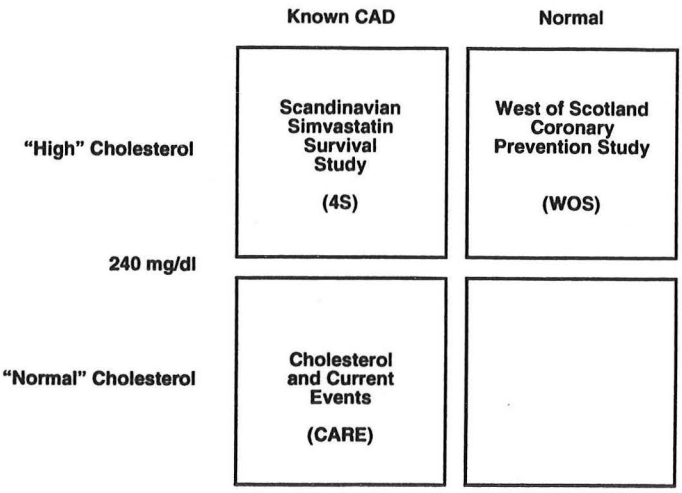


Figure 20 **Figure 21**

myocardial infarction event clearly was reduced in those individuals treated with the drug. These curves appeared to be continuously separating throughout the period of observation. In a similar manner, death from coronary heart disease was also clearly lower in the treated than in the placebo groups (**Figure 21**). In these studies all cause mortality rates also were lower in the individuals treated with the statins. While this method of presentation is useful from a statistical point of view, from the detailed raw data presented in each of these studies it is also possible to calculate absolute event and mortality rates in these different experimental groups and to present these values relative to the older, classical studies on the relationship of plasma cholesterol levels to the incidence of coronary artery disease.

Before beginning a detailed discussion of these studies, it is useful to consider the problems being examined in terms of two variables. As illustrated diagrammatically in **Figure 22**, the patient groups that were examined had either "high" plasma TC levels (>240 mg/dl) or "normal" values (< 240 mg/dl) and had either no known coronary artery disease or did have such clinical disorders (primarily manifest as a prior MI or angina). As is apparent in **Figure 22**, the studies that have just become available deal with three of these four possible groups of patients: 1) normal individuals with "high" TC levels, 2) patients with known coronary artery disease and "high" TC levels and 3) patients with known CAD and "normal" cholesterol concentrations. In each study the raw data have

Figure 22



been recalculated so as to give event and death rates expressed as cases per 1000 patients per 10 years. These rates can be compared directly to the rates established earlier in the MRFIT studies (**Figure 11**).

West of Scotland Study: This study asks whether lowering the plasma cholesterol concentration in individuals without known coronary artery disease lowers the risk of developing fatal or non fatal myocardial infarction. As summarized in **Figure 23**, the individuals entered into this study were 45 to 64 years of age and had mean TC concentrations of 272 mg/dl and LDL-C concentrations of 192 mg/dl. Thus, all of these individuals would be classified as having "high" TC and LDL-C concentrations. The patients were treated with a statin for 4.9 years and had a very modest reduction in TC (20%) and LDL-C (26%) concentrations. This very modest reduction in plasma cholesterol concentrations was, however, associated with a 30% reduction in coronary events and a 27% reduction in coronary deaths. From these raw data, event rates have also been calculated (in parentheses) and in the control group the number of deaths equalled 32 cases per 1000 patients per 10 years. Of interest is the fact that this cardiovascular death rate can be superimposed upon the MRFIT curves as seen in **Figure 24**. Thus, these patients from Western Scotland appeared to be comparable to the Americans that were entered into the MRFIT study. As also shown in **Figure 24**, however, the treated group did not attain the anticipated reduction in death rates after 4.9 years. This finding suggests that approximately 8-9 years of treatment would be required to fully reduce the anticipated rate of

West of Scotland (~55 y.o., ~4.9 yr R_X)

	Cholesterol (mg/dl)		Patients And Events (Rates)		
	TC	LDL-C	Total	CV Events	CV Deaths
Control	272	192	3293	248 (154)	52 (32)
	↓ 20%	↓ 26%		↓ 30%	↓ 27%
Statin	218	142	3302	174 (107)	38 (23)

Figure 23

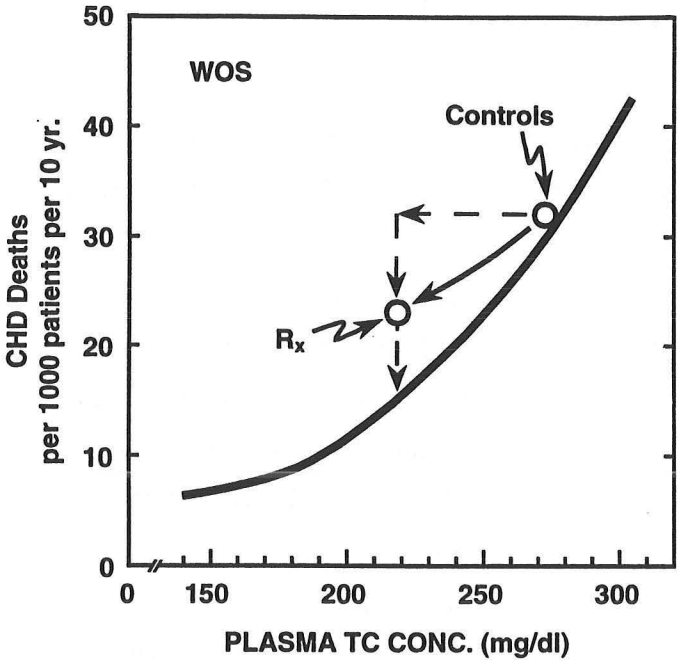


Figure 24

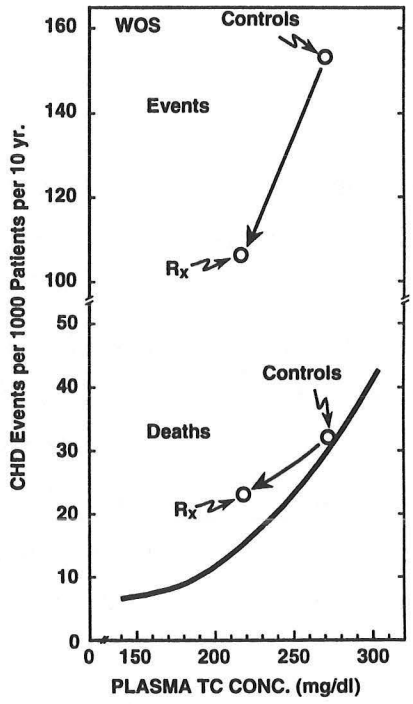
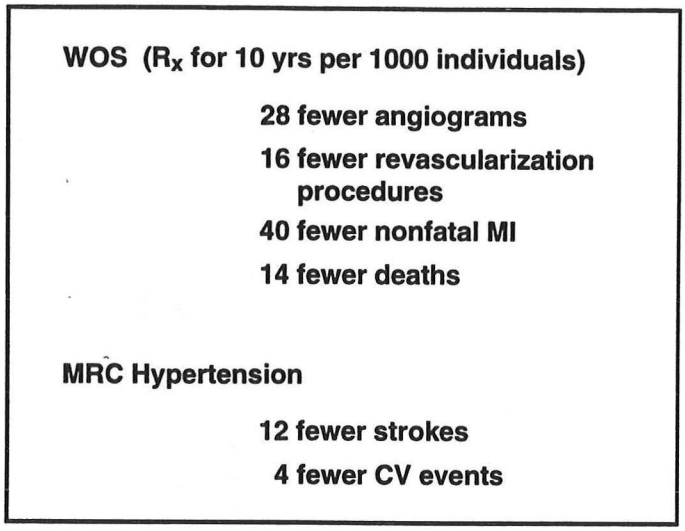


Figure 25

death. **Figure 25** plots the absolute CHD event rates and compares these data to the death rates and the MRFIT curve. Another point of importance with respect to these data is that in the control patients there was one death per 4.8 CHD events while in the treated group there was one death per 4.7 CHD events. Thus, while treatment with the statins dramatically reduced both the event and death rates, the ratio of death/event did not change. Subgroup analysis revealed essentially the same favorable response in the patients with other risk factors and the total, all cause mortality rates were clearly reduced by treatment with the statin. This study, therefore, clearly demonstrated that in patients with "high" cholesterol levels and no prior myocardial ischemia, very modest reductions in the TC and

LDL-C resulted in significant reductions in both coronary events and coronary death rates. It is likely, based on the data in **Figure 24** that these results

Figure 26



grossly underestimated the effects of lowering the cholesterol levels in this type of patient. Finally, **Figure 26** shows the calculated reductions in a variety of surgical procedures and deaths in this study. These results are contrasted with the favorable results that would be anticipated in treating a similar group of patients for 10 years for mild hypertension. There were no adverse effects of these drugs in this study.

Scandinavian Sinvastatin Survival Study: This study is comparable to the West of Scotland Study in that the patients were considered to have a "high" plasma cholesterol concentration but in contrast to the Scotland study, these individuals all had manifest coronary artery disease (an old infarct or angina). As summarized in Figure 27, these individuals had TC and LDL-C levels comparable to those in the West of Scotland Study. Treatment with the statin reduced the TC level 25% and the LDL-C concentration 35%. This resulted in a reduction in coronary events of 30% and coronary deaths of 41%. The absolute CHD deaths calculated from these data are shown in **Figure 28** relative to the MRFIT curve. As is apparent, the death rates were much higher in these individuals that had prior ischemic heart disease. Nevertheless, there was a dramatic reduction in these death rates when the TC concentration was reduced

4S (CAD, ~60 y.o., 5.4 yr. R_x)

	Cholesterol (mg/dl)		Patients And Events (Rates)		
	TC	LDL-C	Total	CV Events	CV Deaths
Control	261	188	2223	502 (417)	189 (157)
	↓ 25%	↓ 35%		↓ 30%	↓ 41%
Statin	196	122	2221	353 (294)	111 (92)

Figure 27

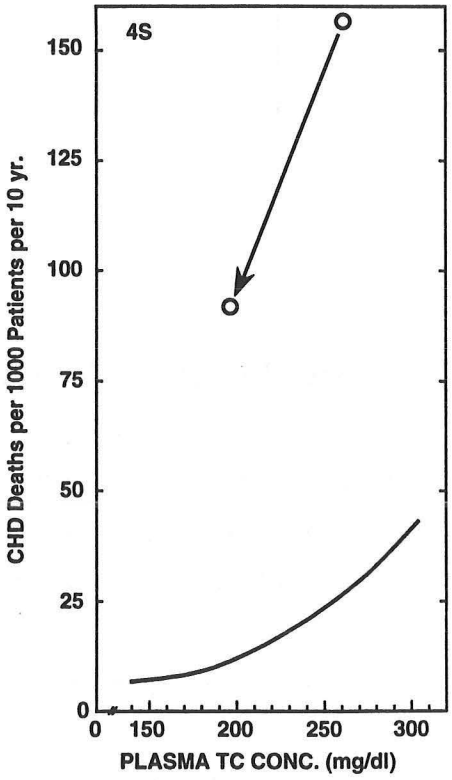


Figure 28

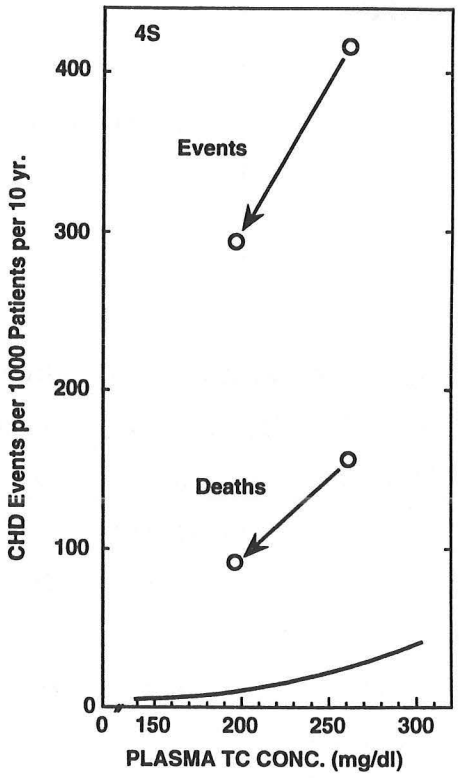


Figure 29

to 196 mg/dl. As in the West of Scotland Study, the ratio of death rates to event rates apparently did not change with treatment. There was one death per 2.7 events in the control patients and one death per 3.2 events in the treated group. Thus, the death rate was higher in this group of patients with prior ischemic heart disease as compared to those patients in the West of Scotland Study; however, treatment with the statin did not lead to a change in the ratio of deaths to events. A similar beneficial effect of treatment with the statin was seen in various subgroups. Thus, as in the West of Scotland Study, very modest reductions in

these "high" levels of plasma cholesterol resulted in dramatic reductions in new coronary events and cardiovascular death rates.

Figure 30

Treatment for 5.4 years in 100 patients averts...
23 acute hospitalizations for cardiovascular disease
231 days in hospital for cardiovascular disease
16 acute hospitalizations for coronary hears disease
104 days in hospital for coronary heart disease
6 coronary revascularizations
76 days in hospital for coronary revascularizations

Cost per life-year saved =

(Drug costs-Savings in hospital costs)/Life-years saved

Figure 31

Country	Estimated cost per life-year saved (US $)
Norway	4900
Sweden	6400
Germany	5900
Italy	4700
Portugal	6400
Australia	4500
New Zealand	6800

From the results of these studies, economists in England have calculated the costs of preventing recurrent coronary artery disease in this group of patients. As seen in **Figure 30**, treatment for 5.4 years with a statin in 100 patients averted a large number of cardiovascular procedures and hospitalizations. Based upon

these considerations and the cost of treatment of all patients in the experimental group, the estimated cost per life-year saved equals approximately $5,000-6,000 (US dollars) in the various countries. The cost per life-year saved for the treatment for mild hypertension is approximately 4 times higher than this value and for hemodialysis is 4-10 times higher. Thus, there is little question that aggressive treatment of the plasma cholesterol level in individuals with prior myocardial diseases is very beneficial both with respect to prevention of subsequent myocardial infarction and death. This treatment is also clearly cost effective when compared to other medical procedures.

Cholesterol and Current Events Study: It should be emphasized that while this study is complete, the results have not yet been published. The data shown in **Figure 32** have been gathered from press releases from the meeting and from an oral presentation that took place at the American College of Cardiology in March,

Figure 32

CARE (CAD, ~50 y.o., 5 yr. R_x)

	Cholesterol (mg/dl)		Patients And Events (Rates)		
	TC	LDL-C	Total	CV Events	CV Deaths
Control	209	139	~2080	269 (258)	116 (112)
	↓ 20%	↓ 30%		↓ 24%	↓ 19%
Statin	167	98	~2079	206 (198)	97 (93)

1996. Like the Scandanavian study, this study was designed to determine if patients with manifest coronary artery disease would be benefited by lowering their plasma cholesterol level. In contrast to the 4S study, however, all of these individuals had "normal" plasma cholesterol concentrations. The age of the patients varied widely but apparently averaged about 50 years old. The individuals were treated with statin for approximately 5 years. As is apparent in **Figure 32** these individuals had TC concentrations of only 209 mg/dl and this was reduced to 167 mg/dl by treatment with a statin. This decrease resulted in a 24% reduction in cardiovascular events and a 19% reduction in CV deaths.

Summary of These Three Studies: Taken together these three studies have provided unequivocal evidence that lowering the plasma TC concentration

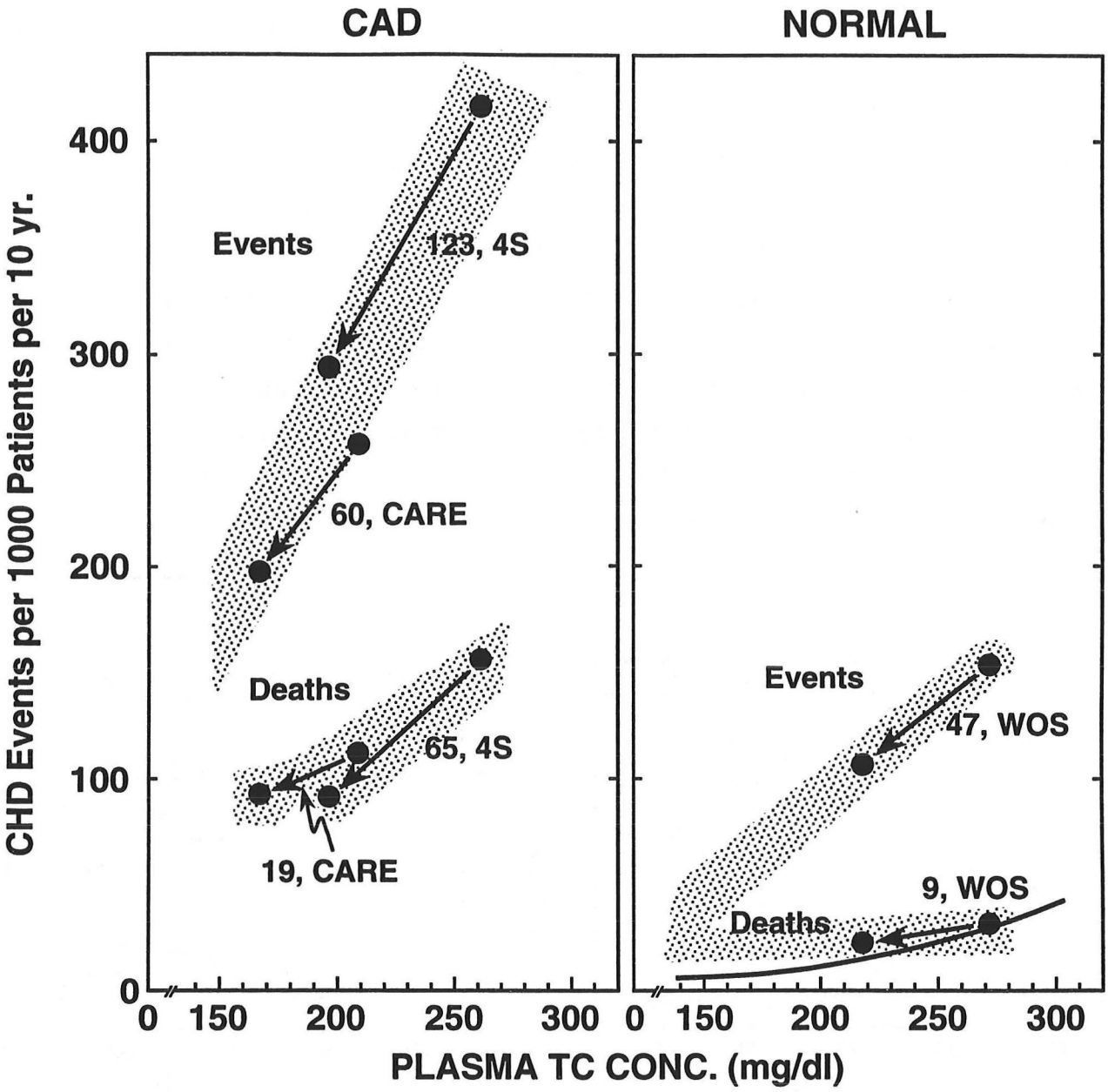


Figure 33

dramatically lowers the incidence of coronary artery events and death due to cardiovascular disease in both normal subjects and in patients who have already had manifest coronary artery disease. The results of these three studies are summarized in **Figure 33**. As shown in the left panel, in patients who have already had a prior myocardial infarction or angina, decreasing the TC concentration from approximately 270 mg/dl to 150 mg/dl decreases the incidence

of new CHD events by over 200 cases per 1,000 patients per 10 years. Similarly there is a significant reduction in deaths due to recurrent cardiovascular disease. It should be emphasized that these reductions in events and deaths appear to be essentially a linear function of the plasma cholesterol between 150 and 270 mg/dl. Clearly, reduction in the plasma cholesterol concentration is indicated in any patient who has already manifested ischemic heart disease.

Similar dramatic reductions in event and death rates are seen in individuals who have not yet had ischemic heart disease, as shown in the right panel of **Figure 33**. While a study comparable to the CARE study has not yet been done in normal individuals, these results suggest that reduction of the TC concentration to about 150 mg/dl would essentially eliminate CHD events and deaths within this normal population.

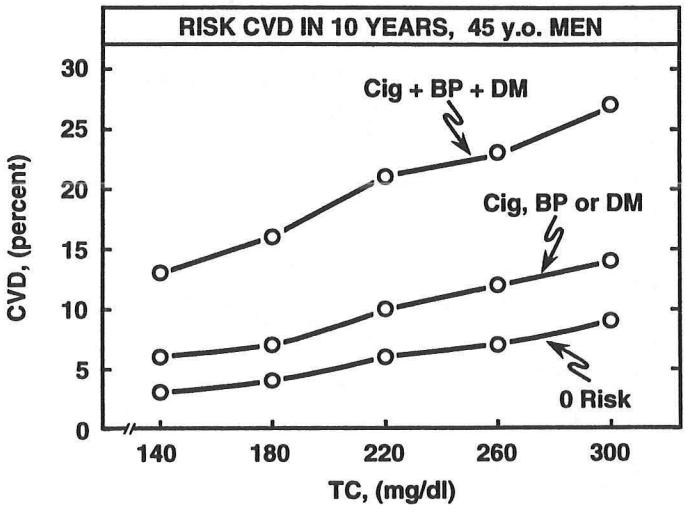


Figure 34

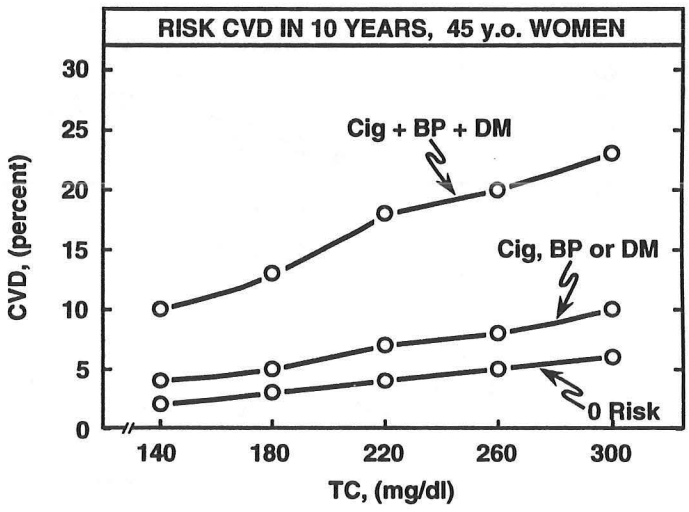


Figure 35

However, it is well recognized that within this group of normal subjects who have not yet manifested ischemic heart disease, there is variation in the relative risk. These relative risks are illustrated in **Figures 34** and **35** in the case of a 45 year old man or woman. These figures show the percentage of the individuals that will develop a coronary artery event in 10 years. The three curves show these relative risks in individuals without specific superimposed risk

factors, in those that have one risk factor such as cigarette smoking, a moderately elevated blood pressure or diabetes, and those individuals that have multiple risk factors including smoking, an elevated blood pressure and diabetes. As is apparent, in every case, the risk of developing CVD is essentially a linear function of the plasma TC concentration between 140 and 300 mg/dl.

IX. CONCLUSIONS

It is clear that these new studies, combined with the extensive epidemiological data of the past 30 years, support the concept that it is the steady-state plasma total cholesterol concentration (the concentration of LDL-C and other apoB-containing lipoproteins) that primarily dictates the incidence of cardiovascular disease in different populations and in the individual patient. Furthermore, it is now clear that lowering these levels of circulating cholesterol dramatically reduces the incidence of such heart disease. This new class of agents that partially inhibit HMG CoA reductase are far more effective in lowering the plasma cholesterol concentration than were the older agents. The statins are well tolerated by patients, have few side effects, and have been demonstrated to reduce mortality rates, both all cause and cardiovascular. Clearly the use of these agents should be significantly expanded to include essentially all patients with manifest ischemic heart disease and normal individuals who have a significantly increased risk of cardiovascular disease.

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