thematic review

Thematic review series: The Pathogenesis of Atherosclerosis

An interpretive history of the cholesterol controversy: part II: the early evidence linking hypercholesterolemia to coronary disease in humans[1]

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Abstract **The first in this series of historical reviews dealt with the pioneering animal model work of Anitschkow, implicating blood cholesterol in the pathogenesis of atherosclerosis, and the pivotally important work of Gofman, providing evidence that lipoprotein-bound cholesterol was a major factor in the human disease. This second installment reviews the early lines of evidence linking hypercholesterolemia in humans to the progression of atherosclerosis and the risk of coronary heart disease. The argument is made that by 1970, the evidence was already strong enough to justify intervention to lower blood cholesterol levels if all the available lines of evidence had been taken into account. Yet, it would be almost two decades before lowering blood cholesterol levels became a national public health goal. Some of the reasons the "cholesterol controversy" continued in the face of powerful evidence supporting intervention are discussed.**—Steinberg, D. **An interpretive history of the cholesterol controversy, part II: the early evidence linking hypercholesterolemia to coronary disease in humans.** ***J. Lipid Res.*** **2005.** 46: **179–190.**

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The first in this series of reviews of the history of the "cholesterol controversy" focused on two early developments (1). The first was the groundbreaking studies of Anitschkow and others, showing that inducing hypercholesterolemia in rabbits was sufficient to produce arterial lesions closely resembling those in the human disease. A major, and legitimate, criticism of Anitschkow's work initially was that the rabbit, a strict herbivore, could hardly be considered a suitable model for man, an omnivore. Indeed, dogs and cats, carnivores, did not develop atherosclerosis on cholesterol feeding, as Anitschkow had himself recognized and reported (2). However, this was not because their arteries were somehow immune but simply because, despite the large increase in dietary cholesterol, their blood cholesterol levels did not rise high enough. These species have very effective systems for converting dietary cholesterol to bile acids and excreting it. The demonstration that lesions could also be readily produced in guinea pigs (3, 4), goats (5), hens and parrots (2), and, ultimately, in almost every animal species, including nonhuman primates (6), went a long way toward rebutting this criticism. Nevertheless, the extrapolation from animals to humans, in the absence of supporting evidence, was not and could not be automatically accepted. Evidence in humans was needed.

The second focus in the preceding review was on the work of Gofman and coworkers (7), which revealed the complexity of the plasma lipoproteins in humans and also demonstrated, albeit with only relatively small numbers of subjects, good correlations between elevated concentrations of plasma lipoproteins and relative risk of clinical coronary heart disease (CHD). Much larger surveys involving hundreds or thousands of cases would be needed to define the association convincingly.

The findings of these two pioneers suggested a causal relationship but fell short of proving the case. In the present review, we describe the several additional lines of evidence that over the ensuing two decades increasingly strengthened the case. By the late 1960s, many clinicians and investigators were already convinced that hypercholesterolemia should not only be considered a causative factor but should also be considered a legitimate and important therapeutic target (8–13). However, others, including many respected clinical and basic scientists, still hotly denied that the evidence was adequate (14–16). Were these two groups looking at different data sets? I don't think so. However, I submit that many of the vocal

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opponents of the lipid hypothesis were simply unwilling to consider and to weigh all of the available relevant evidence, namely: *1*) the biochemical and experimental evidence; *2*) the clinical and genetic evidence; *3*) the epidemiologic evidence; and *4*) the then still-limited but consonant interventional trial evidence; whereas the "believers," on the other hand, did take into account these several different kinds of evidence, weighed them to the extent possible, and thus took a more global approach. In one sense, then, the nonbelievers and the believers were actually not looking at the same data sets.

The nonbelievers were quite influential, particularly in the UK (16, 17), and their vocal opposition played a significant role in blocking any serious medical or public health approaches to the problem in the UK for more than a decade. Only after the NIH-sponsored landmark Coronary Primary Prevention Trial, showing that CHD risk was indeed reduced by lowering blood cholesterol levels (18), and the subsequent 1984 NIH Consensus Conference on Lowering Blood Cholesterol (19) did correction of hypercholesterolemia become a serious therapeutic goal here and abroad. There were multiple reasons for the delay (20), but my thesis here is that the case was already clear enough by 1970 to justify a call for preventive programs.

THE CLINICAL AND GENETIC EVIDENCE

Probably the first hints that CHD might be linked to cholesterol or other lipids came from scattered anecdotal case reports of children with xanthomas, large deposits of lipids just beneath the skin or attached to tendon sheaths on the backs of the hands or at the ankles. These were benign but sometimes unacceptably disfiguring and the parents consulted their family physician or a dermatologist. A number of these children developed serious heart problems at a startlingly early age. In 1889, Lehzen and Knauss (21) reported the case of a child that had had xanthomatosis since age 3 and who died suddenly at age 11. Postmortem examination revealed extensive xanthomatous deposits in the aorta and other large arteries, including the coronary arteries. Her sister, age 9, also had cutaneous xanthomas. In retrospect, this was clearly a case of homozygous familial hypercholesterolemia. But of course such cases were extremely rare (currently estimated to be about one per million births). Much more common were xanthomas associated with liver disease or diabetes or, most common of all, xanthomas limited to the eyelids (xanthelasma), which are present in many healthy older people. Some pathologists, while recognizing the presence of lipids in such lesions, nevertheless maintained that they were basically benign connective tissue tumors, akin to fibromas or sarcomas. Others, noting the presence of hypercholesterolemia in some of these cases, concluded instead that they were not true tumors but rather the result of deposition of cholesterol from the blood. Pinkus and Pick (22) were the first to describe the occurrence of an increase in blood cholesterol and of doubly refractile lipids in the lesions, showing that the stored lipid was not triglyceride but rather cholesterol esters. They correctly inferred that the cholesterol was being deposited from the blood into the tendons and into the vascular wall. Anitschkow, while working in Aschoff's laboratory in Germany, presented experimental evidence that at subcutaneous sites of artificially induced inflammation, cholesterol-fed rabbits formed xanthoma-like lesions from cells of the reticuloendothelial system, newly described by Aschoff. Similar conclusions were reached about human xanthomas by several authors over the next two or three decades, but the evidence remained limited and anecdotal (23–26).

Siegfried J. Thannhauser, internationally known for his studies of the lipidoses and his classification of xanthomatous diseases, was well aware of the occasional concurrence of hypercholesterolemia and vascular disease with xanthomatosis. He recognized that these cases were quite distinct, and he designated a separate category for what he called "primary essential xanthomatosis of the hypercholesterolemic type." However, in his comprehensive 1938 review of xanthomatous diseases (27), he rejected the idea that the cholesterol esters in the lesions were deposited there from the blood. Instead, he concluded that primary essential xanthomatosis of the hypercholesterolemic type was analogous to Gaucher disease and Niemann-Pick disease, lipid storage diseases characterized, respectively, by accumulation of cerebrosides and sphingomyelin. His view at the time was that the accumulation of cholesterol was due to a local metabolic disturbance in the cells. In neither Gaucher disease nor Niemann-Pick disease is there an increase in the blood levels of the lipid being stored, indicating that the storage is probably not due to deposition at the involved sites. Seeking a unifying hypothesis, Thannhauser chose to consider the hypercholesterolemia in essential xanthomatosis as "an exception that proves the rule." Apparently, he assumed that the hypercholesterolemia was secondary to the release of cholesterol from the xanthomas into the blood rather than the driving force causing the lesions.

In 1939, Carl Müller (**Fig. 1**), a Norwegian professor of internal medicine, published a now-classic paper in which he reviewed the already significant literature on the concurrent familial expression of xanthomatosis, hypercholesterolemia, and heart disease and added observations on 76 cases from 17 Norwegian families (28). Consanguineous marriages in some isolated communities in Norway were at the time still fairly common, and he was able to gather a number of cases of florid familial hypercholesterolemia with the classical xanthomas of skin and tendons (**Figs. 2**, **3**). Müller summarized his views as follows:

> The reports I have presented confirm the previous observations on xanthomatosis as a cause of hereditary heart disease. They reveal further that the syndrome of cutaneous xanthomatosis, hypercholesterolemia and angina pectoris presents itself as a well defined clinical entity . . . . There can be hardly any doubt but that xanthomatous deposits in the coronary artery and consecutive myocardial ischemia are the cause of the angina pectoris.

Over the next twenty-five years, Müller's characterization of familial hypercholesterolemia was borne out by more

Fig. 1. Professor Carl Müller, 1886–1983. He presented his landmark paper, *Angina Pectoris in Hereditary Xanthomatosis* before the Nordic Congress for Internal Medicine in 1937 and published in the Archives of Internal Medicine in 1939. He was the first to pull together the evidence linking familial hypercholesterolemia to coronary artery disease.

extensive studies of larger cohorts especially by Wilkinson, Hand, and Fliegelman (29), by Adlersberg, Parets, and Boas (30), and by Khachadurian (31). Their work established familial hypercholesterolemia as a monogenic defect, implying that the arterial disease was secondary to the elevated blood cholesterol, i.e., that the pathogenesis was analogous to the pathogenesis in Anitschkow's cholesterol-fed rabbits. Gofman and his group showed that in patients with familial hypercholesterolemia, the cholesterol elevation was all in the LDL and IDL fractions (32).

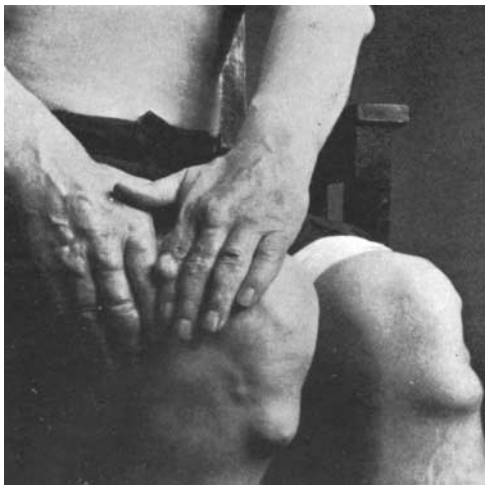

Fig. 2. Severe tendon xanthomas on the extensor tendons of the hands and in the pretibial area of Müller's Case 17, a 51-year-old man with angina pectoris. His blood cholesterol level was 435 mg/dl. The patient also had xanthomas on the eyelids, elbows, and heels (see Fig. 3). (Reprinted from ref. 28 with permission. Copyright © 1939. American Medical Association. All rights reserved.)

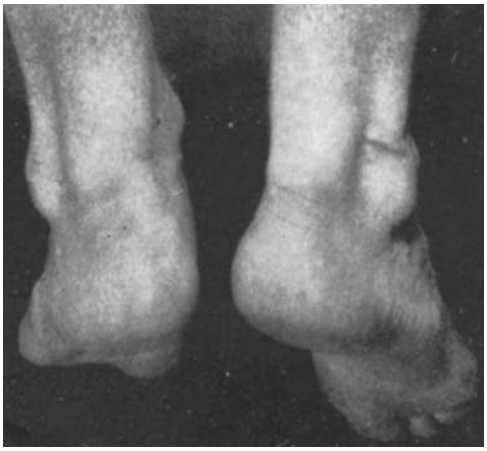

Fig. 3. Enormous thickening of the Achilles tendons due to deposition of cholesterol in Müller's Case 17. (See Legend to Fig. 2.) (Reprinted from ref. 28 with permission. Copyright © 1939. American Medical Association. All rights reserved.)

However, the nature of the gene involved remained unknown and there remained the possibility (although unlikely) that both the hypercholesterolemia and the arterial disease were determined by the single affected gene but by two independent pathways. The elegant studies of Michael S. Brown and Joseph L. Goldstein, beginning in the 1970s, dismissed that possibility (33). They identified the LDL receptor as the causative gene and demonstrated its critical role in determining blood levels of LDL. In a later section of this history, we will review in detail their Nobel Prize-winning work. Here we are concerned with the question of why the obvious and dramatic link between familial hypercholesterolemia and CHD, well established by the early 1960s, was not actively followed up at the time.

Is extrapolation from the extreme degrees of hypercholesterolemia in the familial disease justifiable?

It could be argued—and it was argued very vigorously by many—that the concentrations of blood cholesterol in patients with familial hypercholesterolemia are so extraordinarily high—300 to 400 mg/dl in heterozygotes and as much as 1,000 mg/dl in homozygotes—that it would make no sense to extrapolate to the general population. More moderate elevations of blood cholesterol might carry no risk. This was the same argument used to trivialize Anitschkow's findings in cholesterol-fed rabbits, and it is reminiscent of the arguments that surrounded the issue of whether there was a "threshold level" of radiation exposure that would carry no risk of genetic damage. In retrospect, the extrapolation made sense, but it would require more data on people with moderate elevations of blood cholesterol to make that clear.

How high is high? What does "normal" really mean? Today, when it is so obvious that hypercholesterolemia is critically important in atherogenesis, it is difficult to understand how so many skilled clinicians and researchers could have denied the cholesterol-heart attack connection. One important reason relates to how they defined "high" and where they drew the line between normal and abnormal.

In clinical medicine, the time-honored way of defining a high value for any blood component (e.g., blood glucose) is that it is any value higher than that found in 95% of the population (95th percentile value). So, if 95% of the people in United States have a blood glucose value less than 110 mg/dl, then any glucose value above that is considered abnormal, meriting further medical work-up, but anything below that is considered "normal." This arbitrary definition of normal versus abnormal works quite well for most of the measurements that are made in the clinic. However, suppose that a particular blood component is actually known to be causing tissue damage even at so-called normal levels ("normal" only in the sense that 95% of the population have levels below it). In that case, the cut-point between normal and abnormal would have to be redefined. Let's use a parable to illustrate.

An apocryphal tale. Before the importance of iodine in the diet was fully appreciated, there were many mountain villages in Switzerland where the diet contained insufficient iodine and consequently, enlargement of the thyroid gland was very common. The enlargement was due to high blood levels of thyroid-stimulating hormone (TSH). One day an American endocrinologist visited a hospital in such a village and looked over the medical charts. He was immediately struck by how many patients had high levels of TSH and asked his Swiss colleague, "What is the normal TSH level in this town?" The clinic director said, "Between 7 and 8." The American said that would be considered decidedly abnormal in the United States, where the upper limit of normal is about 5. The Swiss endocrinologist drew himself up and haughtily replied, " Surely we should know what is normal for our own population! Why if we used your American values for what is normal fully 25% of our citizens would be 'abnormal.' That would make no sense at all!" The American quietly told his colleague that from his walks around the town, he got the impression that almost one out of every four people he encountered had a goiter. Well, the Swiss doctors finally agreed to hold a "Consensus Conference on Lowering TSH Levels to Prevent Goiter." As a result, they initiated a "National Iodide Education Program" and started using iodized salt. The TSH values fell and the enlarged thyroid glands disappeared!

A true tale. When the classic 95th percentile yardstick was applied to blood cholesterol levels in the US in the 1940s, 95% of the population had values below 280, and so any value below 280 was considered "normal." Most heart attacks occur in people with cholesterol levels between 200 and 280, the risk being directly related to just how high the level is. So, if you consider as abnormal only those values above 280, most heart attacks will indeed be occurring in individuals with what are called "normal" blood cholesterol levels (200 to 280 mg/dl). It is understandable then that most clinicians concluded that having a high blood cholesterol level was irrelevant to most cases of atherosclerosis and heart attacks. One of our patients with familial hypercholesterolemia had had a routine Air Force physical in 1965 that turned up a blood cholesterol level of 380, but he was told not to be concerned! Fortunately, he was concerned and sought treatment. Today his cholesterol level, on treatment with a statin plus azetamibe, is less than 200, his LDL is about 80 and he is doing fine.

For a long time, physicians simply could not accept that a large fraction of the American public might have blood cholesterol levels within what was considered to be the normal range and yet be at a high risk for a heart attack. Like the Swiss endocrinologist in our parable above, American physicians simply could not handle the idea that a significant part of our population might be "abnormal." And yet we now know that that was exactly the case—20% or more of Americans with blood cholesterol levels that were once considered "normal" are actually working their way toward a heart attack. The results of the many recent trials with cholesterol-lowering drugs show that people in this category can sharply reduce their risk by using diet and drugs. Instead of 280, the desirable blood cholesterol level in this country, as recommended by the National Cholesterol Education Program, is now 200. Actually, there is now evidence that lowering the levels still further—so that the LDL cholesterol is between 70 and 100 mg/dl—will further reduce the risk.

Returning to the early postwar decades, there were then almost no data to turn to in order to decide whether risk was a continuous function of blood cholesterol level or whether there was a "threshold" level.

THE EPIDEMIOLOGIC EVIDENCE

The Seven Countries Study of Ancel Keys' group

By 1955, Ancel Keys, a pioneer in nutritional research at the University of Minnesota, was already convinced that blood cholesterol level was determined significantly by the amount and the nature of the fat in the diet (34). If blood cholesterol was a major determinant of CHD, then populations with fat-rich diets should have higher blood cholesterol levels and higher heart attack rates than other populations. He decided to launch an ambitious study in "geographic epidemiology" (35–37). Henry Blackburn, Keys' right-hand man throughout these studies, has written a fascinating "inside story" about the origins of the Seven Countries Study and the not-inconsiderable logistic problems that had to be overcome (38).

Keys and his colleagues selected for study seven countries that spanned the full range of blood cholesterol levels—from Japan, with the lowest, to Finland, with the highest. In each country (actually in several different communities in each country), blood samples were drawn for cholesterol measurement. The nature of the diet was determined by questionnaire (and in a subset of the population, by chemical analysis), and the CHD death rate was then correlated with these two variables. The average blood cholesterol in East Finland was over 260, whereas that in Japan was only a little over 160 mg/dl; the number of fatal heart attacks per 1,000 men over a 10-year period was about 70 in Finland and a little less than 5 in Japan. When coronary death rate was plotted against the blood cholesterol level for all seven countries, the data points fell roughly on the same straight line, strongly suggesting

that the population risk was roughly proportional to the blood cholesterol level over the range of values studied, as shown in **Fig. 4**. Keys' data also showed that the blood cholesterol levels were proportional to the saturated fat intake, as shown in **Fig. 5**. The contribution of saturated fats to the total daily calorie intake in Finnish men was over 20%, whereas that in Japanese men was about one-tenth of that—only about 2.5%. Again, the values for the other countries fell roughly along a single line. Taken together, the data showed that the population risk of fatal heart attacks is proportional to the blood cholesterol level, which is, in turn, proportional to the dietary intake of saturated fat. Little or no correlation was found between total fat intake and risk, implicating specifically saturated fat intake.

These findings had a major impact on the cholesterol controversy. What was shown was only correlational, but the correlation was strong, and strongly supported the lipid hypothesis. It did not necessarily establish dietary saturated fat or high blood cholesterol as causal. Conceivably, genetic differences or other differences in living habits might be the true explanation of the correlation. Keys and coworkers were well aware of this limitation, a limitation inherent in all epidemiological studies. Keys measured as many of the other possibly relevant factors as he could, including, of course, blood pressure, other dietary components, obesity, and many others. Even after taking these into account by appropriate statistical methods, the correlation with saturated fat intake was still significant.

The Japanese migration studies

How does one decide whether the differences in cholesterol levels and heart attack rates in different populations, like those studied by Keys, are really due to the differences

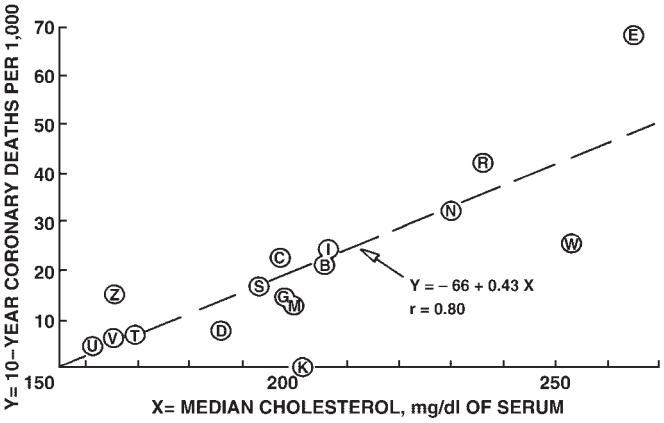

Fig. 4. Coronary death rate as a function of median serum cholesterol level in Keys' groundbreaking Seven Countries Study (37). Key to symbols: B: Belgrade, Yugoslavia; C: Crevalcore, Italy; D: Dalmatia, Yugoslavia; E: East Finland; G: Corfu, Greece; J: Ushibuka, Japan; K: Crete, Greece; M: Montegiorgio, Italy; N: Zutphen, Netherlands; R: Rome, Italy; S: Slavonia, Yugoslavia; T: Tanushimaru, Japan; U: USA; V: Velika Krsna, Yugoslavia; W: West Finland; Z: Zrenjanin, Yugoslavia. [Reprinted by permission of the publisher, from Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease by Ancel Keys (Ref. 37), pp. 122, 252. Cambridge, MA: Harvard University Press, Copyright © 1980 by the President and Fellows of Harvard College.]

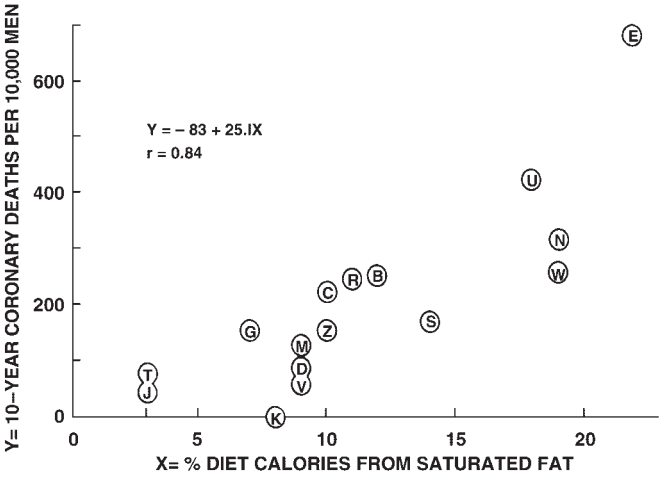

Fig. 5. Coronary death rate as a function of dietary saturated fat intake (percentage of daily calories from saturated fat). See legend to Fig. 4 for key to symbols. [Reprinted by permission of the publisher, from Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease by Ancel Keys (Ref. 37), pp. 122, 252. Cambridge, MA: Harvard University Press, Copyright © 1980 by the President and Fellows of Harvard College.]

in diet (or other environmental factors) and not due to differences in genetic makeup? A group of investigators in Hawaii hit on a clever approach to this question. Hawaii has a very large population of Japanese immigrants, and so does San Francisco. The investigators determined the blood cholesterol levels and the heart attack rates in the Hawaiian Japanese population and in the San Francisco Japanese population and compared them with the same measurements in native Japanese back on the island of Honshu (39). The results were striking. The Japanese who had moved to Hawaii had higher blood cholesterol levels and higher heart attack rates than the Japanese on Honshu who had not migrated. The difference was even more striking in those who had settled in San Francisco. Because the migrants studied had only been in their new environments for a few generations, there was no way their gene pools could have changed significantly. The rise in blood cholesterol levels and the accompanying increase in heart attack rates following migration must have been due to environmental factors, most likely changes in dietary habits. Certainly saturated fat intake was higher in Hawaii and San Francisco than on the island of Honshu!

The Framingham Heart Study

The Framingham Heart Study was carried out by the National Heart Institute in the small town of Framingham, Massachusetts, beginning in 1950 and continuing actively to this day (40, 41). The 28,000 residents of Framingham welcomed this community-based study with open arms. A large majority of those eligible agreed to participate. Measurements were made of most of the potentially relevant factors known at the time. These included blood cholesterol, blood pressure, smoking habits, obesity, diabetes, family history, and others. In later years, additional measurements were added as more was learned

about CHD. The initial cadre was followed with periodic exams for more than 24 years. The Framingham Project was initiated by Dr. Joseph Mountain, who turned it over to Dr. Thomas R. Dawber, and it was then continued under the guidance of Dr. William Kannel and Dr. William Castelli. It continues to this day, studying the offspring of the original cohort.

Without question the data gathered in the Framingham Project had more of an impact on CHD research than that from any other single epidemiologic study. It provided the first solid and unarguable evidence that individuals with higher blood cholesterol levels were more likely to experience a heart attack. It showed that this was also true for a number of other risk factors, such as high blood pressure and smoking. Moreover, the data showed that these risk factors were at least additive. Later studies identified additional risk factors, including diabetes, obesity, low HDL, lack of exercise, family history of CHD, and others.

What was needed was an intervention trial, a controlled experiment that would show that lowering cholesterol levels as a single variable could reduce coronary risk. However, safe and effective drug treatment for hypercholesterolemia was still some way down the road, and the effectiveness of manipulating dietary fat was only becoming clear in the late 1950s. Nevertheless, a few investigators decided to go ahead with testing the "diet-heart" hypothesis on a small scale, as discussed below.

DIETARY INTERVENTION TRIALS

There is a widespread notion that only after the statins were introduced did we have solid evidence that lowering cholesterol levels did indeed reduce risk. That is incorrect. By 1972, the importance of diet in determining serum cholesterol levels was well established, and the results of several dietary intervention trials were available. Three studies in particular—the Leren Oslo Study (42), the Wadsworth Veterans Administration Study (43), and the Finnish Mental Hospital Study (44, 45)—showed that diets rich in polyunsaturated fat could significantly lower serum cholesterol levels, and collectively provided very strong evidence for the lipid hypothesis, as pointed out in a recent historical perspective by Grundy (46). Before reviewing the data from these and other dietary intervention trials, it is instructive to reexamine the scientific basis for dietary treatment of hypercholesterolemia.

Background on the diet-blood cholesterol connection

Long before the relevance of blood cholesterol to heart disease was suspected, indeed just shortly after cholesterol was first characterized chemically and could be measured easily, a Dutch physician, C. D. de Langen, posted to the Dutch East Indies as a public health officer, demonstrated the role of diet in affecting blood cholesterol for the first time. He reported in 1916 that the blood cholesterol levels of the natives in Indonesia were considerably lower than those of the Dutch colonists (47). He speculated that this might be due in part to the very rich diet of the Dutch, compared with the much more spartan diet of the natives. The natives subsisted mainly on vegetables and rice, whereas the Dutch colonists enjoyed a rich butter, eggs, and meat diet. In 1922, he performed what is possibly the first reported controlled study of dietary effects on blood cholesterol (48). He put five Indonesian natives on a cholesterol-rich diet (rich in eggs and meat) and found that after 3 months, their blood cholesterol levels had increased by an average of 27%. He also reported that Indonesians who had migrated to Amsterdam had cholesterol levels just as high as those of their Dutch counterparts, presumably because they had adopted the dietary patterns of the host country. De Langen's work was published only in Dutch in a rather obscure Dutch journal and it is seldom cited, but he anticipated correctly the results of more extensive studies done 30 years later. Another opportunity missed.

The definitive demonstration that saturated fats tend to raise while polyunsaturated fats tend to lower blood cholesterol in humans came first from the laboratories of L. W. Kinsell in California and soon after from the laboratories of E. H. Ahrens, Jr., in New York (49, 50). These investigators carried out metabolic ward studies in hospitalized subjects under close surveillance. They did single-variable studies, i.e., they kept all the elements in the diet constant, except that a saturated fat was substituted for a polyunsaturated fat (or vice versa). The total fat content was not changed, and there was no change in body weight. Both Kinsell (51) and Ahrens (52) utilized the elegant tool of the liquid formula diet in their studies, i.e., the subjects took all their diet in the form of a "milk shake" of precisely known composition given orally several times daily. When the formula contained an unsaturated fat (corn oil, safflower oil), the blood cholesterol level fell from the level on an ad lib diet; when the unsaturated fat was replaced by a calorically equivalent amount of a saturated fat (butter, lard, coconut oil), changing nothing else in the formula, the blood cholesterol rose. Each subject served as his or her own control, so there was no confusion resulting from individual idiosyncrasies in response. The results were highly reproducible in any given individual. The magnitude of the effect varied from individual to individual, but on average, the cholesterol level was about 35 mg/dl lower on the unsaturated oil formula. Similar results were obtained by Beveridge, Connell, and Mayer (53), by Bronte-Stewart et al. (54), and by Keys, Anderson, and Grande (55), using different methods but all arriving at the same basic conclusion: substitution of polyunsaturated fats for saturated fats, other factors being held constant, including total fat intake, reduces blood cholesterol levels. Keys stated the case nicely in 1957: "It is clear that it is unnecessary to prescribe a diet extremely low in total fats to lower the serum cholesterol; exclusion of the saturated fats (in butterfat and meat fats) has the greatest effect, and this effect may be enhanced by substitution of such oils as corn oil and cottonseed oil"(56).

Another source of confusion has been the failure to take into account what the reference diet was—what was the patient switching from and what was he/she switching to. If the subjects studied are on a high-saturated fat diet

to start with, as most Americans are, they will show a nice drop when switched to a polyunsaturated fat-rich experimental diet. On the other hand, if they are already on a polyunsaturated fat-rich diet there may be little change. Also, there are many factors, including other dietary factors, that can affect blood cholesterol levels (e.g., caloric balance, plant sterol intake, the nature of the proteins). Changing diets to alter saturated fat intake may involve, intentionally or unintentionally, changes in some of these other factors as well, and may, therefore, modify the response. That is why cross-sectional studies within a fairly homogeneous population, e.g., the Framingham population, have sometimes failed to show a correlation between dietary fat patterns and serum cholesterol, as pointed out nicely by Jacobs, Anderson, and Blackburn (57).

It is very important to note that these studies did not directly address the question of whether the total fat content of the diet might be a determinant of blood cholesterol levels. Keys' original data had shown a positive correlation of total fat intake with CHD risk, but he stressed that this was largely due to the parallel accompanying increase in the intake of saturated fat. There continues to be controversy about the importance of total fat intake (58), largely due to misinterpretation of the points made above, but there is concurrence on the value of reducing the intake of saturated fats (59).

The cholesterol content of the diet also makes a difference, but the effect is usually less impressive than the effect of increasing the saturated fat content. As beautifully shown by Dr. William E. Connor and his colleagues, the effects of increasing the cholesterol content of the human diet beyond 300–400 mg per day are much smaller than the effects of adding even as little as 200 mg per day to a diet previously free of cholesterol (60). So, in order to lower blood cholesterol levels significantly, it is usually necessary to reduce the cholesterol content of the diet to 300 mg per day or less. If a subject starts off with a cholesterol intake of, say, 500 mg per day on his or her usual diet, adding more cholesterol (without changing anything else) will not increase blood cholesterol level very much. On the other hand, reducing the cholesterol intake to 100 or 200 mg per day can very significantly lower blood cholesterol. In some of the early metabolic studies, pure crystalline cholesterol was added to the diet. We know now that absorption of that cholesterol was very poor. Also, the impact of adding cholesterol to the diet is greater when the ratio of polyunsaturated to saturated fatty acids is low (61).

The results of three well-designed pre-1970 studies

The Paul Leren Oslo Study, 1966. Almost as soon as it was reported that diets rich in polyunsaturated fats and low in cholesterol would lower blood cholesterol levels, a young physician in Oslo, Dr. Paul Leren, started planning the "next step" study. In 1957, he ran a pilot study to see how much of a decrease in blood cholesterol level could be obtained by dietary means and whether it could be sustained. The key element of the diet was a sharp reduction in saturated fat and cholesterol intake and an increase in polyunsaturated fat intake. In fact, each subject had to consume a pint of soybean oil every week, adding it to salad dressing or using it in cooking or, if necessary, taking it neat! Leren bravely launched his watershed 5-year study with 412 myocardial infarction survivors, counting on their high level of motivation and intensive reinforcement from dietitians to keep them compliant, and they were. Sixty percent of the men were considered to be "Excellent" adherers and their blood cholesterol levels fell from an average starting value of 296 mg/dl to an average of 232 mg/dl during the course of the study—a drop of 21.6%. Adherence by the rest of the men was lower, so the mean drop in cholesterol for the group as a whole was 17.6% (**Fig. 6**).

The key finding was that 54 patients of the 206 in the control group (26%) had a second heart attack during the 5 years of the study, compared with only 34 of the 206 in the diet group (16%) (**Fig. 7**). The result was significant at the $P < 0.03$ level (42). A follow- up of the 412-man cohort at 11 years (62) showed a strikingly lower myocardial infarction mortality in the treated group ($P < 0.004$).

One criticism of Leren's study was that there was no difference in all-cause mortality, but the number of subjects that would have been needed in order to detect a statistically significant decrease in all-cause mortality would have been much larger.

Here was a carefully conducted study reported in 1966 with a statistically significant reduction in reinfarction rate. Why did it not receive the attention it deserved?

The Wadsworth Veterans Administration Hospital Study, 1969. The Wadsworth VA Hospital in Los Angeles includes a domiciliary facility where healthy but needy veterans can reside at no cost. They take almost all their meals in one of two dining halls on the premises. In the late 1950s, Seymour Dayton, Morton L. Pearce, and their coworkers saw this as an ideal setting for a test of the effects of unsaturated fat on atherosclerosis (43). All the men in the study were assigned to dining hall A or dining hall B. Dining hall A would continue to serve the usual diet, but dining hall B would serve a modified diet. The main difference was that in dining room B, vegetable oil, rich in polyunsaturated fat, would be substituted for about two-thirds of the animal fat. The total fat content of the two diets, however, would be kept the same, providing 40% of total calories. About 800 men, most of them in their 60s or 70s, were randomly assigned to one or the other dining room and followed for up to 8 years. To test whether the physicians examining the men and evaluating clinical outcomes knew to which group they had been assigned, the physicians were asked to fill out a questionnaire near the end of the study. The percentage of correct assignments was 49–54%, just what you would expect by chance alone.

An objective measure of adherence came from analysis of the fatty acids in adipose tissue biopsies. Samples were analyzed at the beginning of the study and again after 5 years. The shift to polyunsaturated fatty acids in the samples from most men in the experimental group was very close to that predicted if there was good adherence to the diet. Most of the men, but not all, were free of heart disease at the beginning of the study.

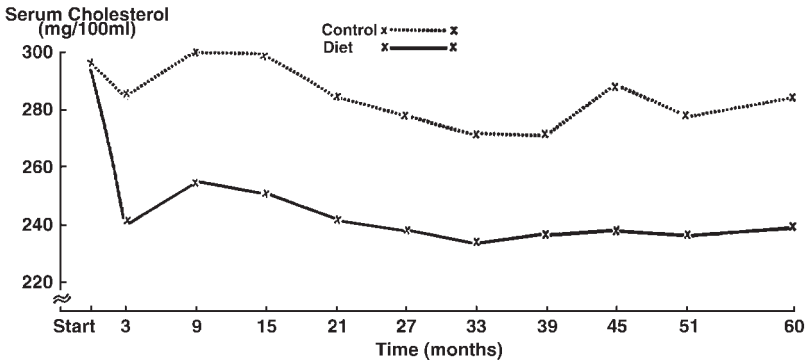

Fig. 6. Serum cholesterol levels in control and diet-treated groups in Paul Leren's pioneering 1966 study showing that cholesterol lowering by substitution of polyunsaturated fat for saturated fat reduces risk of myocardial infarction in men who had had a previous infarction. Note that the initial levels were close to 300 mg/dl in these men with advanced coronary heart disease (CHD). It fell promptly—by a little over 20%—on starting the experimental diet and remained well below that of the controls for the 5-year duration of the study. It was this unusually large drop in cholesterol level that enabled Leren to get a statistically significant 37% protective effect, even with only 206 men in each group (cf. Fig. 7). (From ref. 42.)

The blood cholesterol level in the men eating in dining room B fell promptly after the switch in diets and continued to be lower than that for the men eating in dining room A—mean difference 29.5 mg/dl, or 12.7%. Neither group showed any significant change in body weight.

The number of combined events (definite heart attack, fatal or nonfatal; stroke; or peripheral atherosclerosis requiring amputation) was reduced by 31% in the experimental group (48 versus 70), and that difference was statistically significant by the usual convention ($P < 0.05$). However, at the beginning of the study, the definition of "hard" end points had not included stroke or advanced peripheral arterial disease. If those were excluded, the difference in event rate was reduced to 18%, but that difference did not reach significance.

This study, along with the Finnish Mental Hospitals study discussed below, stands as one of the most important and persuasive studies of the prestatin era. It was a randomized trial of a very clever design, and it is difficult to fault the planning and execution. Yet if you evaluate it rigorously in the standard way—by asking if the end points as initially defined in the protocol showed a statistically significant effect—the answer is no. If you add stroke to the initially defined end points, then there is a significant effect ($P < 0.02$). At the time, it was thought that strokes did not necessarily follow the same pattern as myocardial infarction. Today, we know that aggressive lowering of cholesterol levels (with statins) does reduce stroke incidence as well. However, under the rules of the statistical game in 1969, that was not allowed. Result? This superbly done study got short shrift.

The Finnish Mental Hospitals Study, 1968. At almost the same time that the Wadsworth VA study was getting under way in Los Angeles, a group in Finland was planning a study using a very similar approach (44, 45). However, instead of two separate dining halls in a single institution, they would use two separate psychiatric hospitals, leaving the diet at one hospital (Hospital N) unchanged but introducing a polyunsaturated fat-rich diet at the other (Hospital K). The major diet changes were the use of "filled milk" (replace-

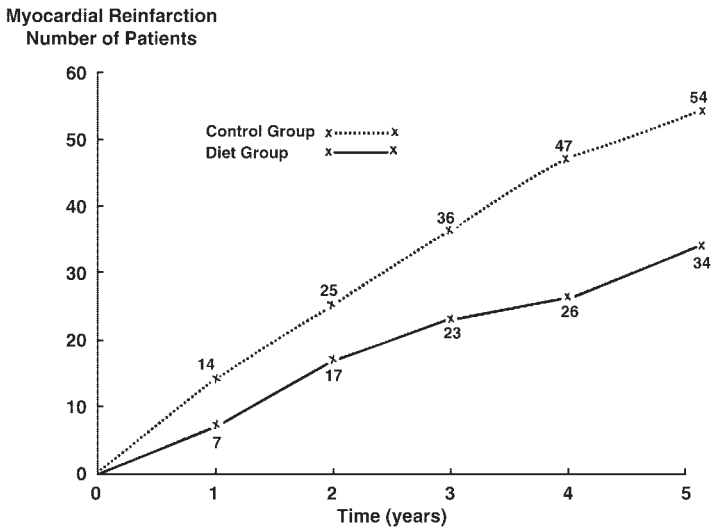

Fig. 7. Myocardial reinfarctions over the 5 years of Paul Leren's 1966 study of dietary cholesterol lowering to reduce CHD risk (cf. Fig. 6). (From ref. 42.)

ment of milk fat by soybean oil) and substitution of a special polyunsaturated fat-rich margarine for butter. The usual Finnish diet at the time was extraordinarily rich in saturated fat, and this was reflected in the blood cholesterol levels, averaging about 270 mg/dl at the beginning of the study.

The meaningfulness of this study was greatly enhanced by the use of a cross-over design. After 6 years, the diets at Hospitals N and K were switched. For the next 6 years, the patients in Hospital N now ate the polyunsaturated fat-rich diet, and the patients at Hospital K (probably with great sighs of relief) went back to their familiar fatty foods. This was a very large study, with almost 30,000 person-years of follow-up. Furthermore, the study was blinded, in the sense that the review of causes of death was carried out by physicians who did not know from which hospital the subjects came.

On the experimental diet, blood cholesterol levels were 12% to 18% lower than they were on the standard Finnish fare. For example, the level in the men in Hospital K, which continued on standard Finnish fare during the first 6 years, averaged 268 mg/dl; at Hospital N it was 217 mg/dl. As in the Wadsworth VA study, fat tissue biopsies were analyzed, and these verified adherence to the diet. Over 2,000 patients were involved, but not all were in the hospital for the full 12 years of the study.

There was a strikingly lower death rate from CHD on the experimental diet. Among the men, it was one-half or less than that on the standard Finnish diet and highly significant ($P < 0.001$). The results in women were in the same direction but reached statistical significance only among the women in Hospital N ($P < 0.001$).

Overview

These three studies, all statistically significant, followed over 3,600 subjects for 5 to 12 years. All three involved the reduction of serum cholesterol by substituting polyunsaturated vegetable fats for saturated animal fats, i.e., they reduced the relative and absolute daily intake of saturated fatty acids and cholesterol and simultaneously increased the daily intake of polyunsaturated fatty acids. They were NOT low-fat trials.

In all three studies, the drop in serum cholesterol was substantial with the use of this aggressively modified diet: 21.6% in Leren's Oslo study; 12.7% in the Wadsworth VA study; and 12% to 18% in the Finnish mental hospitals study.

These were not studies of low-fat diets. They were studies that lowered serum cholesterol by decreasing saturated fat intake and increasing polyunsaturated fat intake.

Additional pre-1970 studies

The Lester Morrison Study, 1955. Lester J. Morrison was a private practitioner of cardiology in Los Angeles. He was one of the few who took quite seriously the implications of the animal experiments of Anitschkow. He decided as early as 1946 that lowering blood cholesterol might be therapeutic and began what was probably the first study testing the possible benefit of cholesterol lowering (63, 64).

The design was very simple: every other patient referred to him after a heart attack was assigned a low-fat, low-cholesterol diet, whereas the alternate referrals were told to just continue their customary diet. There were only 50 patients in each group, mostly men, and the mean age was 61. The experimental diet was spartan—only about 25 g of total fat and only 50–70 mg of cholesterol daily—more rigorous even than the diet currently recommended by the American Heart Association. But these men were very highly motivated, having just recovered from a heart attack. The blood cholesterol level in Morrison's experimental group fell from 312 mg/dl to 220 mg/dl—almost a 30% change—reflecting their motivation! After 8 years of observation, 38 of the 50 patients in the control group had died but only 22 in the diet-treated group had died, a dramatic result indeed.

A major problem with this study is that like most dietary trials, it was, of necessity, not double-blinded. Both Dr. Morrison and the patients knew to which group they were assigned, making it not unlikely that the caregivers might (albeit subconsciously) lavish more TLC on those in the experimental group or pay more attention to their blood pressure and so on. In that connection, it is relevant that the patients on the experimental diet lost an average of 8 to 10 kg. The study group was small, and the report did not adequately compare the groups with regard to other risk factors, nor were the criteria for defining events described in sufficient detail.

Some investigators felt this was a "too-good-to-be-true" study. When the results of later dietary trials began to come in, reporting much more modest decreases in blood cholesterol level, that feeling was reinforced. In any case, Morrison's results were dismissed by most people in the field as a "fluke" (or worse).

In retrospect, Morrison's patients may have been more like those treated recently by Pritikin (65) or by Ornish (66) using an almost fat-free diet and prescribing intensive exercise and weight loss. On that regimen, patients do show remarkable drops in cholesterol levels and reductions in blood pressure, and some show actual regression of lesions, documented by coronary angiography.

The Anti-coronary Club Study, 1966. In 1957, the same year Leren started his study in Oslo, the Bureau of Nutrition of the New York City Department of Health began a very similar study to test whether a cholesterol-lowering diet would protect against heart attacks (67). They studied a group of 814 men free of CHD at the beginning of the study but at high risk because of elevated blood cholesterol. Most of them also had at least one additional risk factor—high blood pressure or obesity. As in Leren's study, the diet was low in total calories from fat and very low in saturated fat and cholesterol. A little over 800 men completed the study, which spanned 7 years. The cholesterol level in the experimental group fell by 13%, while that in the control group remained unchanged. During the first 2 years, there was no significant difference between the two groups in the rate of appearance of new CHD, but after an additional 2 to 3 years of follow-up, the difference was large (more than a 60% reduction in event rate) and statistically significant (P value, 0.01). Certainly a dramatic result—but the study was flawed.

First, the control group was recruited from a quite different population—men who had volunteered for examination at a cancer clinic. So baseline characteristics may have been different. Second, the "new cardiovascular events" included soft end points—angina pectoris and electrocardiographic changes not necessarily diagnostic of CHD. Third, the total number of subjects studied was small, and the number of events was unexpectedly small—8 out of 814 in the experimental group and 12 out of 463 in the controls. Still, the difference was statistically significant at the $P < 0.01$ level.

Here we have a study with a number of important weaknesses. Even though the end result was statistically significant, if this had been the only study available, few researchers would have been persuaded. Again, however, the result was positive and statistically significant, deserving some consideration in assessing the lipid hypothesis.

The Bierenbaum St. Vincent's Hospital Study, 1967. Bierenbaum et al. (68) studied 100 young men (aged 20 to 50) with prior myocardial infarction on fat-modified diets for 5 years They actually used two different diets, but the cholesterol lowering was equal on the two, and so the data were merged. Total serum cholesterol decreased by 9%. The controls were a cohort of myocardial infarction survivors chosen to match the experimental group in relevant baseline characteristics, i.e., this was case-control study, not a randomized trial. Over the 5-year study period, the recurrence rate for infarctions in the experimental group was 4.4% and in the controls 7.1%, a statistically significant 38% reduction ($P < 0.01$).

Again, a limited study with serious flaws but statistically significant and supportive of the lipid hypothesis.

The British Medical Research Council Study, 1968. This study, carried out in four cooperating London hospitals, involved about 400 men who had had their first heart attack very recently. They were randomly assigned either to a control group or to an experimental group instructed to cut back on saturated fats and to consume three ounces of soya bean oil daily. The end point was the first major relapse, defined as a definite second heart attack, fatal or nonfatal. Other nonfatal relapses were rapidly worsening angina or heart failure due to a new heart attack. Half the men were in the trial for four years or more, the other half for less (69).

Serum cholesterol fell by 33% initially, but by the fifth year was only 12% below the baseline value. There was no statistically significant difference in event rates in the two groups, although there were more events in the control group (74 vs. 62; nonsignificant).

The major problem with this study is the small sample size. In their Discussion, the authors point out that with the limited number of patients enrolled (400), they would have detected a significant result only if there had been a 50% decrease in event rates in the experimental group. To be sure of detecting with confidence even a 25% reduction of all relapses, they would have had to enroll four times as many patients, i.e., 1,600 instead of 400. So the "negative" result here does not prove that the diet is without effect; it only says that the effect, if any, is less than a 50% reduction in event rates. This is a major problem with most of the early studies. The Medical Research Council investigators point out that their study design and the characteristics of their patients were very similar to those in the Leren study in Oslo (70). In fact, when they combined the results of the two studies, there was a 31% reduction in nonfatal myocardial infarctions.

Overview of the pre-1970s diet intervention studies

Looking over the studies described above, what can one reasonably conclude? Yes, some of the studies, particularly the Leren study, the Wadsworth VA study, and the Finnish Mental Hospital study, were positive and statistically significant. Others, although showing a trend toward protection, did not reach statistical significance. The British Medical Research Council Study showed no benefit at all. However, the numbers of subjects in that study and the degree of cholesterol lowering were such that they would have demonstrated statistically significant benefit only if the diet had reduced risk by fully 50%! Even the positive studies involved rather small numbers of subjects, and not all of them satisfied the statisticians' conventional criteria for significance. Taking an overview of all the trials, one would say that the case was strong but by no means airtight. Was the evidence strong enough that physicians should have started recommending dietary changes to their patients? Strong enough to justify a national program to get people to change their diets?

Most practicing physicians were less than impressed. This attitude reflected, in part, their weighing of the evidence but may also have reflected their pessimistic feeling that getting people to change their diets radically was a vain hope.

Others felt that the data were indeed sufficient. They based their conclusion not only on the formal clinical trial data but also on the wealth of data from studies in animal models of atherosclerosis, epidemiologic studies, and extrapolation from experiences with patients having extraordinarily high cholesterol levels resulting from inherited abnormalities. They contended that the overall information available more than justified a recommendation that at least patients at high risk of heart attack (e.g., because of extremely high cholesterol levels or already-expressed coronary artery disease) should be urged to lower their cholesterol levels by dietary means.

The American Heart Association (AHA) went on record as early as 1961 to recommend reducing dietary fat to no more than 25–35% of total calories, reducing total calorie intake, and substituting polyunsaturated fats for saturated fats (12). They guardedly said, "Those people who have had one or more atherosclerotic heart attacks or strokes *may* [emphasis added] reduce the possibility of recurrences by such a change in diet." Time has proven how right they were, but at the time, the evidence was slim. The AHA dietary guidelines were faulted, in that direct evidence had not been shown that lowering total fat intake would reduce CHD risk. What has been lost sight of is that at the time, most Americans took in huge amounts of saturated fat, and reducing total fat intake would have, in al-

most every case, also reduced saturated fat intake and would therefore have lowered serum cholesterol. Adding polyunsaturated fat to replace saturated fat would have had an even greater effect, as shown by the careful studies of Kinsell et al. (49) and of Ahrens (50).

A possible catechism for 1970 "believers"

At the outset, we suggested that the "believers" and the "nonbelievers" as of 1970 were really not looking at the same data sets. The "nonbelievers" were largely confining themselves to the intervention trial data per se. The "believers," if they had had a catechism, might have recited it something like this: *1*) Cholesterol derived from plasma lipoproteins is a consistent and striking feature of atherosclerotic lesions, *2*) People with dramatically high blood cholesterol levels, as in familial hypercholesterolemia, have dramatically premature CHD (28–32), *3*) People with even relatively modest elevations of blood cholesterol levels are at significantly higher risk. This is true across a wide spectrum of blood cholesterol levels and holds on comparison of populations from different countries (37) and also within populations (40, 41), *4*) Blood cholesterol level is increased when dietary saturated fat intake is increased, as shown by carefully controlled metabolic ward studies (49, 50). Moreover, populations with dietary habits that include a high saturated fat intake have higher blood cholesterol levels and a higher CHD incidence than populations with lower saturated fat intake (37), *5*) The wide differences in blood cholesterol levels and CHD risk between populations of different countries are due largely to environmental factors (probably diet) rather than genetic factors, as shown, for example, by the Japanese migration studies (39), *6*) Dietary intervention to lower blood cholesterol by decreasing saturated fat intake in favor of polyunsaturated fat intake reduces blood cholesterol levels and decreases risk of CHD and other atherosclerotic complications (42–45, 62–70). *7*) We should be treating hypercholesterolemia.

THE WARS CONTINUE

In 1969, the Chairman of the Council on Arteriosclerosis of the American Heart Association said, "It is now good medical practice to treat—and I use the word advisedly—people who have definite hyperlipoproteinemia. In short, we have come . . . to the point where we are probably preventing a disease that was considered to be an inevitable accompaniment of aging not very long ago" (13). It would be another 15 years before this point of view would prevail.

There were definitely other points of view. Sir John McMichael, a "Dean" of British cardiology, took the gloves off in an editorial essay (17) ominously titled "Fats and Atheroma: An Inquest." He summarized his evaluation of the data available in 1979 in this way: "All well-controlled trials of cholesterol-reducing diets and drugs have failed to reduce CHD mortality and morbidity." Elsewhere, he lamented that "some of our profession are stretching so much speculative and insecure evidence to support a dietetic theory no longer [held] tenable by informed medical scientists."

On this side of the Atlantic, George V. Mann, a physician and nutrition expert at Vanderbilt University, dismissed the evidence from the dietary trials as totally unconvincing. In a New England Journal of Medicine review (14) entitled "Diet-Heart: End of an Era," he suggested that "the dietary dogma was a money-maker for segments of the food industry, a fund-raiser for the Heart Association, and busy work for thousands of fat chemists" and, perhaps plaintively, that "to be a dissenter was to be unfunded because the peer-review system rewards conformity and excludes criticism."

The cholesterol wars continued apace.

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