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## APPENDIX-F

### Dietary Advice in Prevention and Management of Ischemic Heart Disease

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The new evidence, presented earlier in this monograph, has identified medical radiation as a very important cause of Ischemic Heart Disease (Chapters 40, 41, 64, 65). That finding is fully consistent with the Lipid Hypothesis of Atherogenesis. Indeed, our Unified Model of Atherogenesis and Acute IHD Events (Chapter 45) proposes that medical radiation and atherogenic serum lipoproteins BOTH are necessary co-actors in most cases of fatal IHD in the United States. This monograph has identified some practical ways to reduce exposure to medical radiation (Chapter 1), and so it should also identify some practical (dietary) ways to reduce exposure to atherogenic serum lipoproteins. That is the purpose of Appendix-F.

#### ● Part 1. Open Exasperation in the Literature over Dietary Advice

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"Ninety years of research should permit the scientific community to speak with a consistent voice about diet and the prevention of coronary disease," wrote Dr. William E. Connor and Sonja L. Connor (M.S., R.D.) in a recent "Clinical Debate" in the New England Journal of Medicine (Connor 1997, p.566). The topic was "Should a Low-Fat, High-Carbohydrate Diet Be Recommended for Everyone?" The Connors' apparent exasperation is over the LACK of consensus.

#### The Carbohydrate Effect: Elevated Serum Triglyceride

Elsewhere (not in that particular "Clinical Debate"), the so-called "triglyceride" issue is an intimate part of the debate over LowFat HighCarbo Diets. Such diets very commonly --- although not in every case --- ELEVATE blood levels of the triglyceride-rich Std Sf 20-400 segment of the serum lipoproteins (Chapter 44, Part 4). Not surprisingly, total serum triglyceride also is often found to be elevated by such diets (Knopp 1997).

Elevation of Std Sf 20-400 serum lipoproteins has serious negative implications for health, if some or much of that lipoprotein spectrum is atherogenic. In our opinion, the evidence was already strong 40 years ago that the Std Sf 20-100 lipoproteins and some portion of the Std Sf 100-400 lipoprotein spectrum ARE independently atherogenic, and now additional evidence exists (some of it is mentioned in Part 9).

Here, as promised at the outset of Chapter 44, Part 4, we present some compelling experimental evidence (from consenting humans), on the effects of dietary changes upon serum lipoprotein levels. It is particularly compelling evidence because (a) only ONE dietary variable was evaluated per experiment, and (b) the experiments were not encumbered by the simultaneous administration of a variety of pharmaceutical preparations which could render results uninterpretable.

The evidence in Parts 3, 4, and 5 was first presented in reports by Nichols, Dobbin + Gofman in the journal Geriatrics (Nichols 1957) and by Gofman in the American Journal of Cardiology (Gofman 1958).

The findings and statements issued in 1958 are still highly germane to the dietary debates in 1998. Moreover, the evidence argues strongly for making measurements TODAY which are capable of revealing whether or not certain dietary advice results in a harmful conversion of non-atherogenic lipoproteins into atherogenic varieties (Parts 6 + 8).

#### The Omega-3 Fatty Acids: Cardio-Protective Effects

"Heart healthy" dietary advice today is also intimately involved with the issue of a possible protective effect from ingestion of omega-3 (n-3) fatty acids, listed in Part 7. In Parts 8 + 10, we will consider some of the recent, exciting work on this issue by Connor, Harris, DeLorgeril, Renaud, and others.

#### The Weight-Gain Effect: Elevated Triglyceride-Rich Serum Lipoproteins

Lastly (Part 12), we offer evidence which leads us to wonder if much of the unhealthy increase, in atherogenic serum lipoproteins with advancing age in the USA, could be eliminated if we would just maintain the lower weights we had at around age 20.

### ● Part 2. Dietary Management of Blood Lipoproteins: Goals and Pitfalls

Goals and pitfalls, of dietary management of blood lipoproteins, have hardly changed in some 40 years. The paper "Diet in the Prevention and Treatment of Myocardial Infarction" (Gofman 1958) presented (a) the discussion which follows below, and (b) the experimental evidence which is shown in Parts 3 and 4 (based on Nichols 1957). What follows is taken from Gofman 1958 (pp.271-273). We have subdivided paragraphs and added some subtitles here.

#### 2a. No Single Dietary Regime Is Beneficial for Everyone; Potential Harm

"Historically, the earliest interest in blood lipids in relationship to coronary disease centered around the blood cholesterol level, and certainly such interest provided an important stepping stone to our present position of knowledge." And:

"However, today, attention to the blood cholesterol alone provides only the most naive approach to the problem of clinical management of myocardial infarction. Indeed, if preventive and therapeutic measures are considered only in the light of the blood cholesterol level, serious errors of management will eventuate and many patients will be denied effective therapy." And:

"Cholesterol is only one of several chemical lipid constituents of the blood, some of the other major chemicals being triglyceride, fatty acids, and phospholipids. None of these chemical entities has an existence in the blood stream as such, but all are instead building blocks of a series of very large lipid-protein complexes designated as lipoproteins. Cholesterol is a constituent of essentially all the lipoproteins of blood, being quite abundant in some but at very low abundance in others. Similarly, triglyceride or phospholipid is to be found in essentially all the lipoproteins, but at differing abundance in the various lipoprotein classes." And:

"Thus, if cholesterol represents only 5 per cent of the composition of one class of lipoproteins whereas it represents 30 per cent of the composition of some other class of lipoproteins, it becomes evident that one milligram of cholesterol measured in the blood could mean the presence of 20 mg of the first class of lipoproteins but it could mean only 3 1/3 mg of the second class of lipoproteins. Similar considerations apply to the measurements of phospholipids and of triglycerides. The study of the blood lipoproteins in relationship to coronary arteriosclerosis and myocardial infarction has demonstrated that the desired information is the actual level of certain of the lipoprotein classes that circulate in the blood (Gofman 1950-b)." And:

"In addition it has been shown that the various lipoprotein classes involved in coronary disease have differing responses to dietary measures being considered for preventive and therapeutic purposes, indeed differing to the extent that a particular diet may depress the blood level of one important lipoprotein class while at the same time it elevates the level of one of the other important lipoprotein classes (Nichols 1957). This carries with it the implication that a particular dietary regimen designed

for a patient with an elevation of one lipoprotein class may in fact be quite harmful for a patient whose lipoprotein elevation is largely in some other class."

#### 2b. Targeting the Specific Lipoprotein Classes Which Are Elevated

"The most extensive detailed characterization of the lipoproteins as they actually circulate in the blood is available through the technique of ultracentrifugation (DeLalla 1954-a). Indeed, at present no other technique reveals the lipoprotein differences that are so crucial in the design of preventive and therapeutic regimens for myocardial infarction ... Four lipoprotein classes have been identified to be associated with coronary intimal arteriosclerosis and to have predictive implications for the development of myocardial infarction. These are the Standard Sf 0-12, Standard Sf 12-20, Standard Sf 20-100, and Standard Sf 100-400 lipoprotein classes ... All four of these lipoprotein classes are involved in the development of coronary heart disease." And:

"However, the extent to which one of these lipoprotein classes is elevated in a particular person, as compared with the extent to which the other three classes are elevated, is quite different from individual to individual. Thus marked elevation of the Std Sf 0-12 lipoproteins may exist in an individual with moderate or even LOW levels of Std Sf 12-20, Std Sf 20-100, and Std Sf 100-400 lipoproteins. In this individual, the marked elevation of Std Sf 0-12 lipoproteins is the lipid factor predisposing to myocardial infarction and hence preventive or therapeutic efforts would be centered around the modification of the blood level of this particular class of lipoproteins. In some other patient the efforts would be directed against elevation of some other class of lipoproteins."

#### 2c. Patients with Xanthoma: Visible Evidence of Response to Targeted Measures

"The general philosophy underlying such an approach to coronary disease is exceedingly simple and direct. If high levels of certain lipoproteins increase the risk of coronary disease, prevention would appear to lie in the direction of lowering the blood levels of such lipoproteins. There is ample experimental evidence in humans [with their consent] to support this approach to the problem." And:

"Thus in cases of xanthoma tuberosum and more recently in xanthoma tendinosum, each associated with extreme specific lipoprotein-class elevation, we have been able to demonstrate consistently that dietary and pharmacologic measures which lower the levels of the involved classes of lipoproteins result in diminution in size of xanthomatous lesions or in their complete disappearance. Further, in such individuals the development of new lesions is inhibited when the lipoprotein levels are maintained at lowered values. It cannot be proved that the intimal arteriosclerotic lesions will behave precisely as does the xanthomatous lesion, but there is every reason to consider that the same type of processes will occur, although at perhaps a different rate and to a greater or lesser extent."

#### 2d. Not Speculative: Lipoprotein Classes Respond Differently to the Same Therapy

"If the factors which control the levels of the various classes of lipoproteins were identical and if the responses to therapeutic measures were identical, it would be of little moment which particular classes of lipoproteins contributed to the coronary disease risk in a patient, since the management would be the same for all types of lipoprotein derangement. SUCH, unfortunately, IS DISTINCTLY NOT THE CASE." [Emphasis is like that in the original]. And:

"Our studies have proven very conclusively that measures which modify the Std Sf 0-12 lipoproteins favorably may have absolutely no effect on the Std Sf 100-400 lipoproteins or may even have a deleterious effect of raising the level of such lipoproteins. Conversely, factors which may favorably modify elevated levels of the Std Sf 20-400 lipoproteins may have no effect whatever on elevated levels of Std Sf 0-12 or Std Sf 12-20 lipoproteins."

#### 2e. Clinical Implications: What Measurements the Physician Must Obtain

"The implication of these facts for the prevention and management of acute myocardial infarction is extremely important to the clinician. In order to manage the problem intelligently in a particular patient, the physician must know which of the lipoproteins are elevated if he is to advise preventive or therapeutic measures. Without such knowledge, a dietary or pharmacologic approach is

'hit or miss'; it may fail to provide certain patients with the correct measures, and it may result in the advice of positively harmful measures in other cases." And:

"As will be pointed out later in this paper, the measurement of the serum cholesterol level will not serve as a substitute for a lipoprotein analysis, for it will fail to reveal which [atherogenic] lipoprotein classes are predominantly elevated and hence in need of correction." Part 5a and Box 3 of this Appendix provide a real-world example.

## **2f. "We All Eat Too Much Fat" --- A Dangerous Over-Generalization**

"Several years ago, when the author and other workers proposed dietary measures for the management of lipoprotein elevations in the effort to prevent and treat myocardial infarction, there existed considerable skepticism ... about the possible efficacy of diet in lowering lipoprotein levels. Today the pendulum has swung so far in the other direction that the problem no longer resides in skepticism concerning dietary measures, but rather in the irrational blanket-use of dietary measures in all persons irrespective of the indications in a particular individual. Thus we are today bombarded by generalizations such as 'we all eat too much fat' or 'we all eat too much animal fat'." And:

"There is some element of truth in such statements, but on the other hand there is a considerable element of falsehood in them. Action based upon these generalizations may do almost as much medical harm as good, and in individual cases we can be certain that more harm than good will result. That the medical profession and the public have become receptive to the concept, that perhaps coronary disease can be modified by dietary means, is welcome. It is a corollary to this that valid and sane advice be provided as to how to put such dietary measures into intelligent use."

## **● Part 3. Experimental Dietary Evidence: Vegetable-Fat vs. Animal-Fat (Box 1)**

Nichols, Dobbin, and Gofman (1957) evaluated the effect of both the quantity and origin (vegetable vs. animal) of dietary fats upon the blood level of all four classes of serum lipoproteins involved in coronary disease, namely the Std Sf 0-12, Sf 12-20, Sf 20-100, and Sf 100-400 classes. As noted in Part 1, these studies gave careful attention to evaluation of one dietary variable at a time, and the studies were not encumbered by the simultaneous administration of a variety of pharmaceutical preparations which could render results uninterpretable.

### **3a. Conduct of the Dietary Experiments**

The effects of three different diets were evaluated in five males, ranging in age from 20 to 49 years. The same men followed each of the three diets (Nichols 1957, p.9, Table 5). Here, for brevity, we shall call these diets the Vegetable-Fat Diet (duration = 10.5 weeks), Animal-Fat Diet (duration = 11 weeks), and HighCarbo-LowFat Diet (total duration = 24 weeks).

Breakfast was standard throughout all the dietary periods and was eaten at home by the subjects. The average breakfast consisted of fruit juice, toast or cereal, and skim milk or coffee. Such breakfasts contained on the average about 13 grams of protein, 2 grams of fat, 54 grams of carbohydrate, and negligible quantities of cholesterol. Lunch and dinner were served at the Cowell Memorial Hospital of the University of California (Berkeley) under the supervision of Chief Dietitian, Mrs. Virginia Dobbin.

Boxes 1 and 2, adapted from Gofman 1958, present the composition of all three diets, including the breakfast. The diets were designed so that the total daily intake of calories was very nearly the same in all three regimes.

During the study, lipoprotein levels were determined at weekly intervals on all subjects by the ultracentrifugal technique (DeLalla 1954-a). It became apparent in these studies that, with respect to the dietary alterations of lipoprotein levels, the Std Sf 0-12 and Std Sf 12-20 classes of lipoproteins behaved similarly. Hence their measurements were combined as the Std Sf 0-20 class. The Std Sf 20-100 and Std Sf 100-400 classes behaved similarly to each other, but very differently from the lipoproteins of the Std Sf 0-20 class. The results for the Std Sf 20-100 and Std Sf 100-400 lipoproteins are presented together as the Std Sf 20-400 class.

Boxes 1 and 2 show the mean values of these two lipoprotein classes, individually for the five participants and for the five participants combined.

### **3b. Box 1: Results & Clinical Implications --- Veg-Fat vs. Animal-Fat**

Box 1 shows that, at equal daily intake of fat and unequal daily intake of cholesterol:

- 1. The cholesterol-rich Std Sf 0-20 lipoprotein levels were consistently and highly significantly elevated during the Animal-Fat Diet, compared with the Veg-Fat Diet. Conversely, the Std Sf 0-20 lipoprotein levels were very much lower in all five participants during the Veg-Fat Diet than during the Animal-Fat Diet.
- 2. The triglyceride-rich Std Sf 20-400 lipoprotein levels showed no consistent trend and no significant difference in mean level, when the two dietary periods are compared.

At the time, we wrote: "Why there should exist this remarkable difference in behavior of the two lipoprotein classes, with respect to the origin of dietary fat, is not at all clear from present evidence. The fact of the existence of the difference is however solidly established, and as such has important bearing on the problem of prevention and treatment of myocardial infarction" (Gofman 1958, pp.274-275) And (p.275):

"It should be clear that where a patient has an elevation of the Std Sf 0-20 lipoproteins, one can expect to accomplish a striking reduction in lipoprotein levels by a shift from the ingestion of animal fat to the use of vegetable oil (unhydrogenated cottonseed oil in these studies), even without any restriction upon the TOTAL fat intake. However, if a patient is already low in the Std Sf 0-20 lipoproteins but high in the Std Sf 20-400 lipoproteins, essentially nothing would be accomplished by such a dietary shift. This would be one illustration of the folly of such a nonscientific blanket generalization as 'too much animal fat is the problem'."

### **3c. A Puzzle: What Explains Such a Remarkable Result?**

The remarkable difference --- with respect to effect on the serum levels of Std Sf 0-20 lipoproteins --- between a diet high in fat of vegetable origin and one high in fat of animal origin, posed several questions, including (Gofman 1958, p.275):

- (1) Is the different effect caused by some noxious substance, present in animal fat, which raises the Std Sf 0-20 lipoprotein levels?
- (2) Is the different effect caused by some substance, present in the vegetable fat, which lowers the Std Sf 0-20 lipoprotein levels?
- (3) If there is something noxious in animal fat, could its effect be overcome by the addition of vegetable oil to the diet without removal of the animal fat from the diet?

In seeking answers to questions (1) and (2), we undertook the comparison described in Part 4.

### **● Part 4. More Evidence: Vegetable-Fat Diet vs. HighCarbo-LowFat Diet (Box 2)**

We reasoned that, if vegetable fat provides a positively beneficial substance which lowers the Std Sf 0-20 lipoprotein levels, then Std Sf 0-20 lipoprotein levels would RISE on a diet from which we removed that kind of fat, since the hypothetical protective substance would be absent. So we removed most of the vegetable fat from the Veg-Fat Diet and replaced it mainly by carbohydrate. This is the diet called HighCarbo-LowFat in Box 2, and it was followed by the same five participants who had followed the Veg-Fat and Animal-Fat Diets (Box 1).

We anticipated, also, that if the animal fat contains a possible noxious substance which raises the Std Sf 0-20 lipoprotein levels, then there would be little difference in levels of the Std Sf 0-20 lipoproteins in a comparison of the Veg-Fat and HighCarbo-LowFat diets, because animal fat would be virtually absent in both diets --- as shown in the "Composition" section of Box 2.

#### **4a. Results: Std Sf 0-20 Lipoprotein Levels on the HighCarbo-LowFat Diet**

The Std Sf 0-20 lipoprotein levels were not shifted in a consistent direction, nor was there any significant change in their mean levels when the Veg-Fat and HighCarbo-LowFat Diets are compared (Box 2). These results provided no basis for believing that vegetable fat contains any positively beneficial agent capable of actively lowering the Std Sf 0-20 lipoprotein levels. The results seemed to suggest that the elevation of Std Sf 0-20 lipoprotein levels observed on the Animal-Fat Diet (Box 1) was due to the presence in such fats of a factor or factors capable of elevating them.

Our suspicions were not limited to animal-fats. We cited the work of Ahrens 1955 for the extension of suspicion to saturated and hydrogenated fats of any origin, and we wrote (Gofman 1958, p.278): "It appears at present that the natural fats of animal origin such as dairy fat, meat fat, and egg fat are least favorable with respect to content of the noxious agents which affect the blood lipids [Std Sf 0-20], that saturated vegetable oils, either naturally occurring or produced by hydrogenation, are unfavorable but not to the extent of animal fats, and that the unsaturated oils, while not BENEFICIAL with respect to maintaining low blood lipid levels [Std Sf 0-20], are at least neutral in this regard."

#### **4b. Results: Std Sf 20-400 Lipoprotein Levels on the HighCarbo-LowFat Diet**

The Std Sf 20-400 lipoprotein levels were consistently and highly significantly elevated during the HighCarbo-LowFat Diet, compared with the Veg-Fat Diet (Box 2). We had not anticipated such a strong effect.

The "Carbohydrate Effect," as it came to be called in many circles, is the unwelcome effect of high carbohydrate intake on RAISING the levels of the atherogenic triglyceride-rich Std Sf 20-400 lipoprotein levels.

#### **4c. Knopp 1997: The "Unwanted" Carbohydrate Effect**

By now, there are many references in the literature to the fact that high carbohydrate intake can elevate serum levels of total triglyceride --- a measurement which reflects elevation in levels of the triglyceride-rich lipoproteins, without revealing which part of the Sf 20-40,000 segment is the source.

Here, we cite only a recent example (Knopp 1997) which recognizes elevated serum triglyceride (hypertriglyceridemia) to be an "unwanted" effect. Knopp and co-workers studied the effect of 4 fat-restricted diets (followed for 1 year) on two groups of men having high serum levels of LDL-cholesterol. One group (HC for Hyper-Cholesterolemic) had only the LDL-cholesterol elevated, pre-diet. The other group (CHL for Combined Hyperlipidemic) ALSO had serum total triglyceride elevated, pre-diet. One of their concluding comments is this (Knopp 1997, p.1514):

"Among the unwanted effects of an aggressively low-fat diet were the plasma TG [triglyceride] increases of 22% and 39% on diets 3 and 4 in HC subjects, even after 1 year. This elevation was confirmed when TG levels were examined among subjects with intakes of carbohydrate consistently higher than 60% (Retzlaff 1995). These findings indicate that hypertriglyceridemia is induced by an aggressively fat-restricted, high-carbohydrate diet and that the elevation persists longterm. The failure of plasma palmitic acid content to fall, despite progressive dietary saturated fat restriction, suggests that fatty acid synthesis may increase as fat intake is restricted and carbohydrate intake is increased. This mechanism may explain both the induction of hypertriglyceridemia and the lack of further LDL-C lowering with extreme dietary fat restriction." (Note: The mean LDC-cholesterol reductions on the 4 diets were modest --- ranging from 2.8% to 13.4%; Knopp, p.1509). In 1998, Hegsted cites a report (Antonis 1961) that the duration of the carbohydrate effect on plasma triglyceride is "temporary" (Hegsted 1998, p.918). In response, Hu et al cite evidence (Mensink 1992, + Knopp 1997) that the effect is NOT "transient" (Hu 1998, p.919).

#### **• Part 5. Infarct Case + Xanthoma Case: Responses to LOW-Carbo Diets (Boxes 3 + 4)**

Boxes 3 and 4 illustrate --- forcefully --- how the Carbohydrate Effect can be clinically applied, in reverse, to LOWER the blood levels of the atherogenic Std Sf 20-400 lipoproteins in persons who need such therapy.

**5a. Infarct Survivor with Very-High Sf 20-400 & Below-Avg. Sf 0-20 Levels**

There exist many persons in whom the hazard of premature atherosclerosis and myocardial infarction is causally related to a marked elevation of Std Sf 20-100 lipoproteins, or to Std Sf 100-400 lipoproteins, or to a combination of these two classes. We do not consider it reasonable to re-prove these facts. In such individuals, the Std Sf 0-20 lipoproteins may be quite low, and hence contribute little to the coronary disease risk.

Box 3 presents such a case: A 65-year-old male survivor of a Myocardial Infarction, studied well beyond the acute phase of his infarct. On his usual diet, before he followed a very LOW-Carbohydrate diet, his blood levels of the Std Sf 0-20 lipoproteins were BELOW the average of his day, and his 20-400 levels were FAR ABOVE the average for his day. These comparisons can be made by looking at average levels in Box 5.

In such cases, the problem of management necessarily resides in efforts to reduce the elevated Std Sf 20-400 levels. An obvious application of the findings reported in Part 4 would be to recommend a LOW-CARBOHYDRATE diet. The diet advised for this case contained only 100 g of carbohydrate per day, and the diet used vegetable oil to replace calories lost from carbohydrate. No appreciable change in body-weight occurred during this controlled diet (duration = 60 days).

Box 3 presents the favorable response: A massive fall occurred in the plasma Std Sf 20-400 lipoprotein levels, without a significant increase in the Std Sf 0-20 lipoprotein levels. Notably, the total serum cholesterol measurement did not change during the diet. Thus, the cholesterol measurement revealed nothing about the major improvement in what we consider to be major plasma atherogens.

**5b. A Case of Xanthoma Tuberosum with Exceedingly High Sf 20-400 Levels**

This same principle of carbohydrate restriction has been applied successfully in several types of extreme derangement of lipoprotein levels. For example:

Box 4 presents the lipoprotein and cholesterol measurements of a patient with Xanthoma Tuberosum, pre-diet and during 36 days of an extremely LOW-carbohydrate diet. Pre-diet, her Std Sf 20-400 levels were exceedingly high (Box 5 provides a comparison with "normals" of that time). After five weeks on the controlled diet, her mean levels of Std Sf 20-400 lipoproteins were dramatically down (with no dramatic rise in Std Sf 0-20 levels) --- although, to be sure, there was quite a lot of room for improvement beyond what was achieved. In fact, this patient's xanthomata underwent considerable regression during further periods of carbohydrate restriction on a home diet. And she was not an exception (Gofman 1959, p.211).

**● Part 6. Could a Declining Level of Total Serum Triglyceride Be Unhealthy?**

We have worried a lot that certain preventive or therapeutic regimes might transform non-atherogenic lipoprotein molecules into atherogenic ones, without recognition (Chapter 44, Part 3a).

Specifically, we have worried that some pharmaceutical and dietary regimes which reduce hypertriglyceridemia --- as measured by total serum triglyceride (TG) --- might actually increase the atherogenic Std Sf 20-400 lipoproteins. We think patients deserve evidence that this is NOT happening. In our own work, we had such assurance because we measured the responses to therapy of the Std Sf 0-12, 12-20, 20-100, and 100-400 lipoproteins in their native state, and we saw levels of the Sf 20-400 lipoproteins DECLINE --- for instance, during LowCarbo-High(Veg)Fat diets.

How might it be possible to INCREASE the triglyceride-rich Sf 20-400 lipoproteins while simultaneously REDUCING the total serum triglyceride? Hydrolysis of triglyceride transforms it into something else (fatty acids), and thus TG hydrolysis results in a lower level of total serum triglyceride. Suppose that a therapeutic regime promotes TG hydrolysis. Suppose that the lower TOTAL serum triglyceride conceals an unhealthy redistribution of residual post-hydrolysis triglyceride --- i.e., conceals a lower frequency of the Sf 400-40,000 lipoproteins and a HIGHER frequency (concentration) of the atherogenic Sf 20-400 lipoproteins.

This would represent a serious increase in atherogenic risk --- and no warning would be provided by the chemical measurement which shows a reduced amount of total serum triglyceride. By itself, the chemical triglyceride analysis does not provide the crucial information. If proper studies are done, either by ultracentrifugal analysis or by some equivalent alternative, we can really KNOW the answer --- an answer which we are obligated to know.

Today, there exists some potentially good news on this very issue, within the elegant work of William S. Harris and colleagues on omega-3 fatty acids and levels of blood lipoproteins (Part 8b). Before describing it, we must set forth a few terms (Part 7).

#### ● Part 7. The Healthful-Oils Hypothesis: Nomenclature and Sources

A great deal of evidence has been published indicating that the distinction, between fats of animal vs. plant origin, is not adequate to separate harmful dietary fats from healthful ones. Certain "marine oils" from fatty fish (clearly an animal source) and from some other marine animals may be very healthful, while coconut oil (a plant source) has a composition very similar to beef-fat and butter-fat (animal sources). Soon after the lipoprotein spectrum was revealed, the specific fatty-acid composition of dietary fats was studied at many labs, including Donner (Freeman 1952 + 1953). Ongoing research today indicates that anti-atherogenic benefits may depend upon EXACTLY which kinds of fatty acids are in the dietary fat. Before discussing evidence which may be of great benefit to persons trying to avoid IHD, Part 7 defines certain terms and abbreviations.

##### 7a. Fatty Acids, Triglycerides, and Esters

● **FATTY ACID.** Fatty acids have a methyl group at one end, a carbon-carbon chain of variable length, and a carboxyl terminus (COOH --- which makes them "carboxylic" acids).

● **TRIGLYCERIDE.** Triglyceride (sometimes called "neutral fat" or just "fat") is any molecule formed when the 3-carbon alcohol (glycerol) combines with 3 fatty acids. The combination of glycerol with only 1 fatty acid yields a monoglyceride; with 2 fatty acids, a diglyceride.

● **ESTER, ETHYL ESTER, and ESTERIFIED.** Esters are organic compounds which are formed by the splitting out of water between an alcohol (e.g., glycerol, ethanol, cholesterol) and a carboxylic acid. Triglyceride is a fatty acid glyceryl ester. When the alcohol is ethanol, the combination is an "ethyl ester." The "cholesterol esters" are a prominent feature in Appendix-E, Boxes 1 + 2. When an alcohol and carboxylic acid combine, the alcohol is "esterified."

##### 7b. Major Long-Chain Fatty Acids of Dietary Importance

Because fatty acids differ in the length of their carbon-carbon chains and in the number of double-bonds in that chain, a notation is commonly used, to indicate "what's what." For example, 12:0 denotes the fatty acid which has 12 carbons in its chain and zero double-bonds in that chain: Lauric Acid.

By definition, saturated fatty acids have no double-bonds in the carbon-carbon chain, so the entry after the colon is ZERO for all of the saturated fatty acids.

By contrast, the UNsaturated fatty acids have at least one double-bond in the carbon-carbon chain. The position of a double-bond in the carbon chain is denoted either as its omega-position or its n-position. Thus, omega-3 is the same as n-3. When a chain has multiple double-bonds, usually the position only of the FIRST double-bond is named.

#### ● SATURATED FATTY ACIDS.

Lauric Acid	12:0
Myristic Acid	14:0
Palmitic Acid	16:0
Stearic Acid	18:0

#### ● MONO-UNSATURATED FATTY ACIDS.

Oleic Acid	18:1 n-9	One double-bond, at the 9 position in the chain.
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● **POLY-UNSATURATED FATTY ACIDS.**

Linoleic Acid	18:2 n-6	Two double bonds, one at n-6 and one at n-9.
$\alpha$ -Linolenic Acid	18:3 n-3	Three double bonds: n-3, n-6, n-9.
EicosaPentaenoic Acid (also referred to as EPA).	20:5 n-3	Eicosa signals the 20; Penta, the 5. Five double bonds: n-3, n-6, n-9, n-12, n-15.
DocosaHexaenoic Acid (also referred to as DHA).	22:6 n-3	Docosa signals the 22; Hexa, the 6. Six double bonds: n-3, n-6, n-9, n-12, n-15, n-18.

Some Rich Dietary Sources of the UnSaturated Fatty Acids

- Oleic Acid (n-9): Olive oil.
- Linoleic Acid (n-6): Certain oils, especially safflower, sunflower, walnut, soybean, and corn.
- Alpha-Linolenic Acid (n-3): Certain oils, especially flaxseed oil, walnut oil, canola (rapeseed) oil.
- EPA and DHA (both n-3): Certain fish or "marine oils," including salmon, tuna, herring, mackerel. However, the body can and does produce these fatty acids from their precursor: Alpha-Linolenic Acid.
- The dietary examples above are much expanded in books such as Simopoulos 1998, and others.

● **Part 8. Encouraging Evidence about the n-3 Marine Oils: The 1989 Review by W.S. Harris**

For about two decades, William S. Harris, William E. Connor, D. Roger Illingworth, B.E. Phillipson, and co-workers have been leading figures in the effort to elucidate the effects of dietary omega-3 fatty acids on lipid metabolism and on management of serum lipoprotein levels, especially with respect to Ischemic Heart Disease. Harris provided a superb review of the "fish oil" evidence in his 1989 paper "Fish Oils and Plasma Lipid and Lipoprotein Metabolism in Humans: A Critical Review" (Journal of Lipid Research). Harris begins (Harris 1989, p.785):

"The purpose of this report is to review the effects of the long-chain, n-3 fatty acids (also referred to as omega-3 fatty acids) found in fish oils on human plasma lipids and lipoproteins." His magnificent Table 1 lists the results from 68 dietary experiments which increased the intake of fish oils, and reported upon the observed changes in (a) total serum cholesterol, (b) total serum triglyceride, (c) LDL cholesterol, and (d) HDL cholesterol. Altogether, there were 928 participants, including 596 persons whose pre-diet plasma levels were considered "normal," + 37 "Type II-a patients" (hypercholesterolemia), + 194 "Type II-b patients" (combined hyperlipidemia), + 101 patients with isolated hypertriglyceridemia (Types IV and V).

8a. Overall Results on the Harris Meta-Analysis

Harris' Figure 1 (p.790) summarizes the results of the meta-analysis, in percent change. All four types of participants experienced large decreases in total serum triglyceride, and small increases in HDL cholesterol. Changes in total cholesterol and LDL cholesterol were negligible, with one exception: The patients with isolated hypertriglyceridemia (who experienced a 52% decrease in total serum triglyceride) experienced a 30% increase in LDL cholesterol (mostly in the Type-IV patients).

Also notable, in terms of fish oil in the potential prevention of IHD, are the responses specifically of the participants with pre-diet "normal" measurements. They "responded to n-3 fatty acids with essentially no change in total or LDL cholesterol, a 25% decrease in triglyceride levels, and a slight rise in HDL-C (+3%)" (Harris 1989, p.787).

We note that these results are echoed in the 1995 review paper by Martijn B. Katan and colleagues, who cite work by Harris and others about EPA and DHA: "These very-long-chain (n-3) polyunsaturates do not share the LDL-lowering effect of linoleic acid (18:2 n-6, n-9). On the contrary, several studies have shown that fish oils raise LDL and apoprotein B, and similar results

have been reported for fatty fish. Fish oil and fatty fish do, however, have favorable effects on serum triglycerides and very-low-density lipoprotein (VLDL), which can be reduced by intake of a few grams of fish oil per day. Whether this effect is responsible for the lower incidence of Coronary Heart Disease, observed in fish-eating populations in epidemiologic studies, remains uncertain; fish oils also can modulate many other physiological processes, including blood platelet function" (Katan 1995, p.1371-S). Katan and colleagues also discuss the "carbohydrate effect".

The parts of the 1989 Harris paper, discussed below, relate (a) to our worry that falling levels of total serum triglyceride might conceal an unrecognized increase in blood levels of the atherogenic Std Sf 20-400 lipoproteins, and (b) to the growing recognition that such lipoproteins are atherogenic. We recommend also all parts of the Harris paper NOT discussed below, including "Fish Oils and Lipoprotein Metabolism," "Fish versus Fish Oil Supplements," "Linolenic Acid versus Fish Oils," and "Fish Oils in the Treatment of Hyperlipidemia."

#### **8b. Does Extra Fish Oil Increase the Atherogenic Std Sf 20-400 Lipoproteins?**

Harris 1989 uses the following terminology at page 794:

- VLDL-1 refers to large lipoproteins in the Sf 100-400 range.
- VLDL-2 refers to smaller lipoproteins in the Sf 20-60 range.

Harris, citing Sullivan 1986, describes the response of four patients (Type-IV) with isolated hypertriglyceridemia. They took a 15 gram dose of MaxEPA daily for 2 weeks. MaxEPA is described (in Simopoulos 1998, p.353) as concentrated fish-body oils having (17.8 grams EPA + 11.6 grams DHA) per 100 grams of total oil. The result of the Sullivan experiment is possibly worrisome. From Harris 1989, p.793-794):

"The distribution of light and heavy LDL particles, the percent apolipoprotein B, and the cholesterol to protein ratios were not different before and after fish oil in the Type IV patients. However, the size distribution of VLDL changed significantly, with a decrease in the large particles (Sf 100-400) and an increase in the small particles (Sf 20-60) with fish oil supplementation. The average diameter of the VLDL particles decreased by 22%."

Although the findings are worrisome, it is a real pleasure to see use of an appropriate technique (ultracentrifugation) to identify the VLDLs under investigation. From the limited evidence Harris gives us on the Sullivan experiments, we wonder whether there was a net increase in atherogenic risk, as a result of some decrease in Sf 100-400 and some increase in the Sf 20-60 VLDLs. These data leave open the possibility of decrease overall, no change overall, or increase overall in the atherogenic impact of the remaining mix of VLDLs.

In addition, Harris provides relevant evidence from his own laboratory, and it is NOT ambiguous on this important issue. He reports (Harris 1989, p.794): "In a preliminary study from our laboratory (Inagaki 1988), five Type IV patients were given 6 grams of n-3 fatty acid ethyl esters daily for 4 weeks. Before and after the supplements, the plasma lipoproteins were separated by gel filtration chromatography according to Rudel, Marzetta, and Johnson (Rudel 1986) and analyzed for compositional changes (Table 2). There appeared to be greater reduction in the large VLDL-1 particles than in the small ones (VLDL-2 and IDL) after the fish oil treatment. LDL and HDL cholesterol and protein contents both increased."

These results indicate that serum levels decreased for BOTH groups of VLDL (Sf 20-60 and Sf 100-400). This study (small though it is) provides encouragement that fish oil supplements may be able to lower the IHD risk from both groups of VLDL at the same time, without causing a HIDDEN increase in atherogenic species --- hidden by a reduced "total triglyceride" measurement which might conceal a net INCREASE in levels of Std Sf 20-400 lipoproteins (Part 6).

Of course, we do not dismiss the non-hidden increase in LDL cholesterol in the patients who had Type IV hypertriglyceridemia. We note, however, that the LDL cholesterol rise was absent or small in the other three types of participants described in Part 8a. Nonetheless, there are many other types of persons whose responses we do not know. Meanwhile, we consider the "fish oil" frontier to be an extremely important area in efforts to help prevent IHD.

### ● Part 9. Why Reduce Serum Triglyceride Levels? Harris's "Good Reasons"

In Harris's section on "Fish Oils in the Treatment of Hyperlipidemia," he asks (Harris 1989, p.801):

"Since elevated triglyceride levels are not generally regarded as independent risk factors for CHD, why be concerned about them? There are several good reasons why VLDL in hypertriglyceridemic patients should be reduced."

#### 9a. The "Good Reasons" Listed by Harris (Harris 1989, p.801)

- "Data from the Framingham Heart study have shown that 90% of hypertriglyceridemic individuals are, in fact, at increased risk for CHD; the exception being the 10% of patients with low total to HDL cholesterol ratios (Castelli 1986)." And (p.801):
- "In women over age 50, triglyceride levels are independently correlated with CHD risk and are even better predictors than LDL-C levels (Reardon 1985)." And (p.801):
- "Carlson, Bottiger and Ahfeldt in the Stockholm Prospective Study found that serum triglyceride levels were independent predictors of subsequent myocardial infarction in both sexes (Carlson 1979)." And (p.801):
- "Gemfibrozil was recently shown to lower coronary risk in the Helsinki Heart Study (Frick 1987). This drug lowered VLDL levels by 40%, raised HDL-C levels by 8%, and lowered LDL-C by only about 9%. Since gemfibrozil and fish oils appear to produce similar changes in VLDL and HDL, one might expect that n-3 fatty acids may be anti-atherogenic as well."

#### A Recommendation from Harris for Any Patient at Increased Risk for CHD

Several paragraphs later, Harris concludes the section (p.801): "Finally, a case could be made for providing low levels of n-3 fatty acids (0.3 to 1 gram/day, or one or two capsules/day) to any patient at increased risk for CHD, especially if that patient cannot or will not increase fish intake. This level of supplementation would provide as much n-3 fatty acid as consuming three 100-g servings of salmon per week, and would surpass the n-3 fatty acid intake associated with reduced coronary events in the study of Kromhout et al (Kromhout 1985). Although this low dose may have little, if any, measurable effect on plasma lipid levels, it may, in the long run, slow the progression of atherosclerosis, and the potential risk to the patient from this intake is essentially nil."

#### 9b. "The Triglyceride Issue: A View from Framingham" (Castelli 1986)

Directly from William Castelli's 1986 paper, we provide some details not mentioned by Harris, above.

Castelli presents two bar graphs showing "CHD Risk According to Lipid Level on Entry into the Framingham Study; Men Aged 30-62" (Castelli 1986, p.434, Figure 4). These graphs show lipoprotein level versus CHD morbidity ratio, separately for the cholesterol-rich Sf 0-20 lipoproteins and for the triglyceride-rich Sf 20-400 lipoproteins. Each graph displays an almost perfect positive relationship between rising level of serum lipoprotein and morbidity ratio. Castelli states (p.434):

"Figure 4, which shows lipid levels in men on entry into the Framingham Study, indicates that the higher the VLDL or triglyceride level, measured as Sf 20-400, the higher the subsequent CHD rate. This is the univariate association of triglyceride levels with the risk of CHD." He continues (p.435):

"Early in the study, however, it was observed that if one adjusted the relationship of the Sf 20-400 by the concomitant cholesterol level, the impact of triglyceride on risk disappeared. It was then concluded that triglycerides were not an independent risk factor, at least in men. In women the story is quite different ..." In women, the triglyceride levels "were better than LDL in predicting subsequent CHD." Returning to the men, Castelli states (p.435):

"More recent analyses by Abbott et al (Abbott 1984) ... indicated that triglycerides do play an independent role in CHD risk in men over the age of 50, and that this role has been masked previously by the association of triglycerides with other risk factors (e.g., HDL) that are themselves related to CHD. When one untangles such relationships, triglycerides emerge as an important risk for CHD in men (Table 2)." Our Appendix-G presents some striking graphs and data on the inverse relationship between levels of EACH class of serum lipoproteins (Std Sf 0-12, 12-20, 20-100, 100-400) and levels of HDL. Castelli concludes (pp.436-437):

"The final view from Framingham is that individuals who have high triglyceride levels should be considered at high risk for CHD. The only exceptions to this judgment are those with a very low total/HDL cholesterol ratio. Since only about 10% of these high-triglyceride patients will have a low total/HDL cholesterol ratio (<3.5), 9 of 10 patients with high triglyceride levels are at a high risk for premature CHD, even though almost half of these patients will have a total cholesterol less than 250 mg/dl."

#### ● Part 10. A Beneficial "Mediterranean Diet": The Lyon Diet Heart Study

With respect to reducing mortality rates from Ischemic Heart Disease, we are impressed by results reported in the Lyon Diet Heart Study, conducted by Michel de Lorgeril, Serge Renaud, and co-workers. The relationship of their results with the Lipid Hypothesis, and with our Unified Model of Atherogenesis and IHD Death, will be discussed too (Part 11). Papers presenting the Lyon Diet Heart Study and its results include:

- DeLorgeril et al, 1994 in the Lancet 343: 1454-1459, "Mediterranean Alpha-Linolenic-Acid-Rich Diet in Secondary Prevention of Coronary Heart Disease."
- Renaud et al, 1995 in the American Journal of Clinical Nutrition 61 (suppl): 1360S-1367S, "Cretan Mediterranean Diet for Prevention of Coronary Heart Disease."
- DeLorgeril et al, 1996 in the Journal of the American College of Cardiology 28: 1103-1108, "Effect of a Mediterranean Type of Diet on the Rate of Cardiovascular Complications in Patients with Coronary Artery Disease; Insights into the Cardio-protective Effect of Certain Nutriment." See also DeLorgeril 1997 and 1998 in our Reference List.
- DeLorgeril et al, 1999 in the journal Circulation 99: 779-785, "... Final Report of the Lyon Diet Heart Study." Commentary: Leaf 1999 in our Reference List.

#### 10a. A Comparison of the Test-Diet and the Control-Diet

The purpose of the Lyon Diet Heart Study was "to test in France the hypothesis that a Mediterranean diet, especially the one consumed on the island of Crete in the early 1960s, could have more of a protective effect against Coronary Heart Disease than the prudent diet that is often recommended for coronary patients" (Renaud 1995, p.1360S; see also pp.1364-65S).

In the Lyon Diet Heart Study, "the control patients received no dietary advice apart from that of hospital dietitians or attending physicians. Patients in the experimental group were advised by the research cardiologist and dietitian, during a one-hour-long session, to adopt a Mediterranean-type diet: More bread, more root vegetables and green vegetables, more fish, less meat (beef, lamb, and pork to be replaced by poultry), no day without fruit, and butter and cream to be replaced with margarine supplied by the study ... The oils recommended for salads and food preparation were rapeseed [canola] and olive oils exclusively. Moderate alcohol consumption in the form of wine was allowed at meals. At each subsequent visit of the experimental patients, a dietary survey and further counseling were done by the research dietitian. Diet evaluation comprised a 24-hour recall and a frequency questionnaire" (DeLorgeril 1994, p.1455). The margarine supplied, free of cost, was based on rapeseed (canola) oil. It had a composition very similar to olive oil (DeLorgeril 1994, p.1455), and contained only 6% trans fatty acids (Renaud 1995, p.1365S).

A comparison of the average control-diet and average test-diet, after 1 to 4 years of follow-up, is provided in DeLorgeril 1994 (p.1456, Table 5) in terms of daily intake (g/day) of various food-types. Omitting the standard errors and p-values, we list the mean values in g/day from Table 5, with the control diet listed first and then the experimental diet:

Bread 145, 167. Cereals 99.4, 94.0. Legumes 9.9, 19.9. Vegetables 288, 316. Fruits 203, 251. Delicatessen (ham, sausage, offal) 13.4, 6.4. Meat 60.4, 40.8. Poultry 52.8, 57.8. Cheese 35.0, 32.2. Butter + cream 16.6, 2.8. Margarine 5.1, 19.0. Oil 16.5, 15.7. Fish 39.5, 46.5. DeLorgeril 1994 (p.1454) reports:

"The experimental group consumed significantly less lipids, saturated fat, cholesterol, and linoleic acid but more oleic and alpha-linolenic acids confirmed by measurements in plasma. Serum lipids, blood pressure, and body mass index remained similar in the 2 groups. In the experimental group, plasma levels of albumin, vitamin E, and vitamin C were increased, and granulocyte count decreased." And (p.1457):

"After 52 weeks, there were higher [plasma] concentrations of oleic, alpha-linolenic, and eicosaPentaenoic [EPA] acids and reduced concentrations of stearic, linoleic, and arachidonic acids in the experimental group (Table 4). The increase in eicosaPentaenoic acids was probably related to alpha-linolenic acid intake since intake of fish was not significantly increased (Table 5)."

#### 10b. Who Was Enrolled, and What Were the Clinical Outcomes?

The Lyon Diet Heart Study was a prospective, randomized, single-blinded, multi-clinic, secondary prevention trial which enrolled (between March 1988 and March 1992) mostly male patients under age 70 who had survived a first myocardial infarction six or fewer months beforehand. After randomization, the control and experimental groups were quite well matched in infarction history and treatment, age (mean = 53.5 years), and "main cardiovascular risk factors" (DeLorgeril 1994, Tables 2 + 6). At the outset, there were 303 patients in the control group and 302 in the experimental group.

"After the randomization visit, patients of both groups were scheduled to be seen 2 months later and then annually at the Research Unit. These visits did not replace their regular visits to the attending physicians, who were responsible for all aspects of treatment, including use of medication and of invasive diagnostic and therapeutic procedures" (DeLorgeril 1996, p.1104). Mean follow-up time was nearly the same: 27.1 months (594 person-years) in the control group and 26.9 months (606 person-years) in the experimental group (DeLorgeril 1994, p.1456, and p.1457, Table 6).

The primary endpoints evaluated were cardiovascular death and nonfatal acute myocardial infarction. Six major secondary endpoints and ten minor secondary endpoints also were evaluated (DeLorgeril 1996, p.1105, Table 2).

With respect to the primary endpoints, there were 33 in the control group, and 8 in the experimental group. With respect to the secondary endpoints, there were 37 in the control group and 6 in the study group. With respect to the minor secondary endpoints, there were 84 in the control group and 58 in the experimental group (DeLorgeril 1996, p.1105, Table 2). These are striking differences.

We also wish to mention that, in this study, use of aspirin (about 250 mg/day) was the only pharmaceutical which showed a significant and inverse relationship with the primary endpoints (DeLorgeril 1996, p.1105).

#### 10c. DeLorgeril and Colleagues Ask: Are the Benefits "Plausible"?

Although one can not rule out the possibility, in such studies, that the result was "built-in at the start" (i.e., that pure bad luck distributed more extremely sick patients into the control group than into the experimental group), it would be irresponsible to dismiss beneficial results merely because such a possibility exists. More studies either will or will not confirm this very promising work.

DeLorgeril and co-workers themselves address several questions about possible bias in the study. For example, with respect to possibly different TREATMENT (other than diet) within the two groups, they note that "no difference between the two groups in the use of medication was detectable" (DeLorgeril 1996, p.1106).

According to Renaud (as quoted in Jones 1996, p.63), "The experiment had to be stopped. It would have been unethical to continue" because too many of the patients on the regular diet were dying compared with those on the Mediterranean Diet. "You cannot continue. You are watching them die."

Convinced that the benefits of the Mediterranean Diet are real, DeLorgeril, Renaud, and colleagues want to know WHICH elements of the diet explain those benefits (DeLorgeril 1996, p.1107):

"To evaluate the plausibility of the results of the trial, it is important to try to identify which biologic factors, modified by the Mediterranean Diet, may have been cardio-protective. Two major biologic factors were modified by the intervention: 1) anti-oxidant vitamins, alpha-tocopherol and ascorbic acid, which were increased in the plasma of the study [experimental] patients; 2) the plasma fatty acid profile, with a noticeable increase in omega-3 fatty acids and a decrease in omega-6 fatty acids in the study group. Other factors such as the anti-oxidant flavonoids and minerals, arginine, glutamine and methionine and vitamins of the B-group including folic acid probably played important roles but were not measured in the study."

DeLorgeril and co-workers point out that their results are consistent with other sets of clinical observations linked to diets which reduce the ratio of n-6 to n-3 fatty acids to the neighborhood of about 5:1 (see especially Renaud 1995, pp.1365-66 S; also DeLorgeril 1996, p.1103). They discuss:

1) The lower mortality rate from Coronary Heart Disease in southern Europe compared with northern Europe (Keys 1984), and particularly lower CHD mortality in Crete, where there is a relatively low ratio of n-6 to n-3 fatty acids in serum cholesterol esters (Sandker 1993); 2) the very low MortRates from CHD in the Japanese of Kohama Island, where plasma levels are high in the n-3 alpha-linolenic acid (Kardinal 1993); 3) the reduced rate of death and myocardial re-infarction in the DART Study which had an increased intake of fish and n-3 fish oil (Burr 1989); 4) "results comparable to the Lyon study" in the diet-study reported by Singh et al (Singh 1992-a + 1992-b).

DeLorgeril, Renaud and co-workers also point out that there are several lines of other evidence which may explain WHY the Mediterranean Diet would have the striking benefits which it appears to have. Part 11, below, describes some of them.

#### ● Part 11. How the Lyon Study May Relate to the Lipid Hypothesis and Our "Unified Model"

The Final Report of the Lyon Diet Heart Study states: "The protective effect of the Mediterranean dietary pattern was maintained up to 4 years after the first infarction, confirming previous intermediate analyses. Major traditional risk factors, such as high blood cholesterol and blood pressure, were shown to be independent and joint predictors of recurrence, indicating that the Mediterranean dietary pattern did not alter, at least qualitatively, the usual relationships between major risk factors and recurrence" (DeLorgeril 1999, p.779).

The Lyon Diet Heart Study did not measure serum lipoprotein levels. It measured total serum cholesterol, LDL-cholesterol, HDL cholesterol, and total serum triglyceride in control patients and in experimental patients, at the outset of the study, at week 8, at week 52 in about 80% of the initial participants, and at week 104 in about 57% of the initial participants (DeLorgeril 1994, p.1457, Table 6).

The two groups were well matched in the mean measurements at the outset, and they REMAINED alike. Such measurements can not reveal whether changes occurred in the lipoprotein pattern. However, if one considers "total triglyceride" (TG), it is clear that the appreciable decreases observed during diets supplemented by EPA and DHA fish oils (Harris review, in Part 8, above) did NOT occur in the Lyon Diet Heart Study. Is this an instance of conflicting observations? Probably not. Rather, the results may reflect an effect of dosage. In the Lyon Study, the amount of ingested fish oil was almost certainly far lower than in the diets described by Harris in Part 8.

If the benefits observed in the Lyon Diet Heart Study are real, and if we suppose that they occur without reducing blood-levels of the atherogenic lipoproteins, then what is their explanation?

#### 11a. Four of the Explanations Proposed by DeLorgeril, Renaud, and Colleagues

In lucid and well-documented discussions, DeLorgeril 1994, Renaud 1995, and DeLorgeril 1996 present lines of evidence that cardio-protective benefits of the Mediterranean-Type Diet could arise from four different routes.

1) ANTI-INFLAMMATORY EFFECT. DeLorgeril 1996 (p.1107) reports that "Recent studies in humans (Rapp 1991, + Felton 1994) have shown a direct influence of dietary fatty acids on the fatty acid composition of arterial lesions. Rapp et al reported the incorporation of dietary omega-3 fatty acids in obstructive arterial lesions within some days after starting supplementation ... Omega-3 fatty acids may have an anti-inflammatory and stabilizing effect on the lipid-rich lesions because they have been shown in various animal models [two references] and humans [four references] to interfere with the many secretory and pro-inflammatory properties of leukocytes ... Thus, loading plaque with omega-3 fatty acids, as occurs in patients with high intake and high plasma levels of omega-3 fatty acids (Rapp 1991, + Felton 1994), can induce local anti-inflammatory activity." Reduction in certain aspects of the inflammatory response (Chapter 44, Part 7b) may indeed help to make the lipid-pools of plaques less thrombogenic --- when such plaques have not been prevented in the first place.

2) ANTI-OXIDANT EFFECT. DeLorgeril and Renaud cite observations that there are lower rates of CHD in persons with high intake of the anti-oxidant Vitamin E (for instance, Rimm 1993, + Stampfer 1993) and high intake of oleic acid, which is "remarkably resistant to oxidation" compared with some other fatty acids (DeLorgeril 1996, p.1107, citing 2 references, and citing Witztum 1991 for the view that "oxidized lipids are also thought to play a major role in arterial complications by stimulating macrophages, injuring endothelial cells and promoting leukocyte coagulant activity and platelet reactivity"). Both Vitamin-E and oleic acid intake were elevated in the Lyon Diet Heart Study.

3) ANTI-THROMBOTIC EFFECT. Renaud 1995 (p.1365S) states that the intake of 18:3 n-3 [Alpha-Linolenic Acid] is associated with inhibitory effects on the clotting activity of platelets, on their response to thrombin [2 references], and on the regulation of arachidonic acid metabolism (Budowski 1985)," and that "n-3 fatty acids give rise to a different family of prostanoids than do n-6 fatty acids, with major consequences for thrombogenesis (Knapp 1986)." In addition, he cites two studies which report that a high intake of 18:2 n-6 [Linoleic Acid] reduces the conversion of 18:3 n-3 (Alpha-Linolenic Acid) to the longer-chain n-3 fatty acids, EPA and DHA. DeLorgeril 1996 (p.1107) states that the Lyon Diet produced a ratio of arachidonic acid to eicosapentanoic acid [EPA] in the plasma which "was also extremely favorable for obtaining an anti-thrombotic effect through an improved balance in the generation of prostacyclin and thromboxane [2 references]."

4) ANTI-ARRHYTHMIC EFFECT. DeLorgeril 1994 (p.1459) ends the discussion section as follows: "The fact that no sudden death occurred in the experimental group [of the Lyon Diet Heart Study] against 8 in the control group, suggests a possible additional anti-arrhythmic effect, consistent with observations in man (Burr 1989, + Riemersma 1989) and animals (McLennan 1993) indicating that n-3 fatty acids, especially alpha-linolenic acid, markedly reduced the incidence of lethal arrhythmias."

#### 11b. How Do These Possible Mechanisms Relate to the Lipid Hypothesis?

The four possible mechanisms described above, for explaining the cardio-protective effects of the Mediterranean-Type Diet, are fully consistent with the Lipid Hypothesis and with our Unified Model of Atherogenesis and Acute IHD Events (Chapter 45). The Unified model proposes that atherosclerotic plaques in the coronary arteries and most acute IHD events occur due to the interaction of atherogenic blood lipoproteins and atherogenic mutations in those arteries.

When people have any atherosclerotic plaques in their coronary arteries, we would expect such persons to fare better with respect to outcome if they have help from anti-inflammatory, anti-thrombotic, anti-arrhythmic, and anti-oxidative agents than if they lack such help (Chapter 46, Part 5). Levels of these helpers may well be increased by the Mediterranean-Type Diet. We think that the contributions by DeLorgeril, Renaud, and their colleagues look very promising indeed --- with one possible caveat: A 1999 multi-variate analysis (incorporating adjustments for over a dozen variables) reports "an increased risk of breast cancer associated with omega-3 fat from fish" (Holmes 1999, p.919; Table 2 at p.916 shows MultiVariate Relative Risk = 1.09).

#### ● Part 12. Effects of Weight-Changes on the Atherogenic Lipoproteins

So far, Appendix-F has described effects from manipulating the composition of diets, while maintaining calorie-intakes at levels which do not alter weight. Now we must mention the observed effects of weight-changes on the blood levels of atherogenic lipoproteins, for no chapter about dietary prevention and management of Ischemic Heart Disease should fail to mention this important aspect of diet.

Box 5 shows that, between the ages of about 20 to 60 years in both males and females (USA), the serum levels of the cholesterol-rich Std Sf 0-20 lipoproteins and the triglyceride-rich Std Sf 20-400 lipoproteins rise. During these years, Americans are typically gaining weight.

#### 12a. At Equal Age, Do Average Lipoprotein Levels Rise with Weight?

If we hold age constant, do we see a difference in average levels of the atherogenic lipoproteins as weight increases?

In order to explore this question, we examine 834 males, all in the age-range of 30-39 years. The data below come from the Livermore Lipoprotein Study (Appendix-E, Part 12-c). We sort the 834 men by their relative weight, divide them into six groups of ascending weight from 0.86 to 1.37, and calculate for each group the mean serum levels of the Std Sf 0-20, 20-400, and 0-400 lipoproteins. On their habitual U.S. diet, these men --- all within a fairly narrow age-band --- experience major increases of Std Sf 20-400 serum lipoproteins with increase in relative weight.

Number of Subjects	Relative weight (Means)	LDL + IDL Std Sf 0-20	VLDL Std Sf 20-400	Total Std Sf 0-400
97	0.86	382	105	487
219	0.95	397	122	519
249	1.05	407	140	547
168	1.14	422	165	587
75	1.23	416	191	607
26	1.37	425	203	627

#### 12b. Do Average Lipoprotein Levels Decrease with Weight-Loss?

Below are data from the study of 28 women who participated in a weight-reduction program under medical guidance (Nichols 1957, p.11). The diet prescribed was a 1,000 calorie diet, low in animal fat, and necessarily low in carbohydrate. Over a 2-month period, the women lost an average of 14 pounds each. The changes in serum lipoproteins levels are tabulated below (all concentrations are in mg/dl).

	LDL + IDL Std Sf 0-20	VLDL Std Sf 20-400	Combination Std Sf 0-400	Weight (pounds)
Initial Values	465	158	623	212
Final Values	387	99	486	198
Change in Values	-78	-59	-137	-14

For the Std Sf 0-400 combination, the change is -9.8 mg/dl per pound of weight-loss in 2 months.

The data presented here show that a 1,000 calorie weight-reduction diet resulted in appreciable falls both in the Std Sf 0-20 lipoproteins and the Std Sf 20-400 lipoproteins. The magnitude of the effects observed is such that the lowering of Std Sf 0-20 lipoprotein levels could be attributed to the reduced animal-fat intake of the 1,000 calorie diet, and that the lowering of the Std Sf 20-400 lipoprotein levels could be attributed to the reduced carbohydrate intake of this diet. Thus, the major effects of weight reduction upon blood lipids in these women could be assigned to mechanisms known to operate in the absence of weight reduction.

#### 12c. How Helpful Would It Be, to Maintain Our Age-20 Weights?

These are only two brief illustrations from a vast literature which shows that a relationship exists between serum lipoprotein levels and weight or weight-change. Appendix-I presents additional evidence for U.S. adults on the habitual American diet of the 1950s, namely:

- Positive caloric balance (weight-gain) is associated with RISING levels of Std Sf 0-400 lipoproteins.



- Negative caloric balance (weight-loss) is associated with FALLING levels of Std Sf 0-400 lipoproteins.

- Caloric equilibrium (stable weight) is associated with STABLE levels of Std Sf 0-400 lipoproteins. This observation is fully consistent with the observations that, at caloric equilibrium, certain changes in the COMPOSITION of the diet will change lipoprotein profiles (for instance, Boxes 1 + 2).

On the average, caloric equilibrium at a higher weight yields higher Std Sf 0-400 levels than caloric equilibrium at a lower weight (Part 12a). Thus, once a period of weight-gain is over, one continues indefinitely to "pay the price" of having gained the weight.

Such observations, combined with the tendency in the USA to gain weight as age advances from 20 toward 60, suggests that weight-gain may explain nearly all of the observed RISE in the average levels of atherogenic Std Sf 0-400 lipoproteins during the adult years (Box 5). There is one highly probable exception: The small percent of the population having Std Sf 0-100 (Type-II) hyperlipoproteinemias. These disorders are hereditarily controlled and are not determined by the same forces affecting the population at large.

Although the typical American diet and average serum lipoprotein levels may have changed since the 1950s, we doubt very much that those changes have repealed the weight-effect. We expect that, even on truly heart-healthy diets, the lipoprotein profile becomes less favorable if caloric balance is positive.

Indeed, we have been wondering for about 20 years (Gofman 1978) if a large share of the unhealthy increase in atherogenic serum lipoproteins, which occurs in the USA with advancing age, could be prevented if we would just maintain the lower weights we had at around age 20.

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### Box 1 of Appendix-F

Comparison: Impact of Vegetable-Fat Diet vs. Animal-Fat Diet on Lipoprotein Levels.  
This box is adapted from Nichols 1957 and Gofman 1958, pp.274-275.

- The same five males (ages 20-49) followed the three diets described in Boxes 1 + 2.
- Ultracentrifugal measurement of serum lipoprotein levels was done weekly during the diet-periods, and the means are presented below. Measurements are mg/100 ml serum.

#### • Sf 0-20: Mean Measurements for the Cholesterol-Rich Std Sf 0-20 Lipoproteins:

Individual Case	Vegetable-Fat Diet Duration = 10.5 weeks	Animal-Fat Diet Duration = 11 weeks
No.1	337	448
No.2	386	563
No.3	346	571
No.4	292	373
No.5	352	430
Grand Mean	343	477

#### • Sf 20-400: Mean Measurements for the Triglyceride-Rich Std Sf 20-400 Lipoproteins:

Individual Case	Vegetable-Fat Diet	Animal-Fat Diet
No.1	277	251
No.2	236	241
No.3	157	201
No.4	48	57
No.5	208	193
Grand Mean	185	189

#### • Composition of the IsoCaloric Vegetable-Fat and Animal-Fat Diets:

- For diet-composition, entries below do not match the tabulated entries in Gofman 1958 because the entries here include the standard breakfast, whereas breakfast grams were only in the text of Gofman 1958 (p.274). Our error-check (by calories) yields just trivial discrepancies.

	Veg-Fat Diet: Daily Intake = ~ 2,360 calories		Animal-Fat Diet: Daily Intake = ~ 2,345 calories	
Calories				
from fat	~ 40%	944 cal.	~ 40%	945 cal.
from protein	~ 16%	378 cal.	~ 16%	375 cal.
from carbo.	~ 44%	1038 cal.	~ 44%	1032 cal.
.....				
Vegetable Fat	87 g	783 cal.	9 g	81 cal.
Animal Fat	15 g	135 cal.	96 g	864 cal.
Protein	91 g	364 cal.	91 g	364 cal.
Carbohydrate	254 g	1016 cal.	254 g	1016 cal.

- The Vegetable-Fat Diet is the same in Boxes 1 and 2. In the Vegetable-Fat Diet, unhydrogenated cottonseed oil (Wesson) was the main source of the vegetable fat. The small amount of fat of animal origin was almost wholly meat fat. Cholesterol intake/day was about 0.5 g.

- In the Animal-Fat Diet, the distribution of animal fat was 30 g from meat, 23 g from dairy products, 37 g from egg origin. Cholesterol intake/day was about 2.2 g, almost wholly from 7 egg-yolks per day.

Related text = Part 3.

### Box 2 of Appendix-F

Comparison: Impact of Veg-Fat Diet vs. HighCarbo-LowFat Diet on Lipoprotein Levels.

This box is adapted from Nichols 1957 and Gofman 1958, pp.275-276.

- The same five males (ages 20-49) followed the three diets described in Boxes 1 + 2.
- Ultracentrifugal measurement of serum lipoprotein levels was done weekly during the diet-periods, and the means are presented below. Measurements are mg/100 ml serum.

• Sf 0-20: Mean Measurements for the Cholesterol-Rich Std Sf 0-20 Lipoproteins:

Individual Case	Vegetable-Fat Diet Duration = 10.5 weeks	HighCarbo-LowFat Diet Duration = 24 weeks
No.1	337	391
No.2	386	395
No.3	346	358
No.4	292	292
No.5	352	317
Grand Mean	343	351

• Sf 20-400: Mean Measurements for the Triglyceride-Rich Std Sf 20-400 Lipoproteins:

Individual Case	Vegetable-Fat Diet	HighCarbo-LowFat Diet
No.1	277	477
No.2	236	310
No.3	157	234
No.4	48	89
No.5	208	215
Grand Mean	185	265

• Composition of the IsoCaloric Vegetable-Fat and HighCarbo-LowFat Diets:

• For diet-composition, entries below do not match the tabulated entries in Gofman 1958 because the entries here include the standard breakfast, whereas breakfast grams/calories were only in the text of Gofman 1958 (p.274). Our error-check (by calories) yields only trivial discrepancies.

	Veg-Fat Diet: Daily Intake = ~ 2,360 calories		HighCarbo LowFat Diet: = ~ 2,370 calories/day	
Calories				
from fat	~ 40%	944 cal.	~ 8%	190 cal.
from protein	~ 16%	378 cal.	~ 18%	427 cal.
from carbo.	~ 44%	1038 cal.	~ 74%	1754 cal.
.....				
Vegetable Fat	87 g	783 cal.	10 g	90 cal.
Animal Fat	15 g	135 cal.	10 g	90 cal.
Protein	91 g	364 cal.	108 g	436 cal.
Carbohydrate	254 g	1016 cal.	439 g	1756 cal.

• The Vegetable-Fat Diet is the same in Boxes 1 and 2. In the Vegetable-Fat Diet, unhydrogenated cottonseed oil (Wesson) was the main source of the vegetable fat. The small amount of fat of animal origin was almost wholly meat fat. Cholesterol intake/day was about 0.5 g.

• The High-Carbohydrate Low-Fat Diet differs from the Vegetable-Fat Diet mainly in the substitution of carbohydrate for vegetable fat. The number of calories/day are virtually the same in both diets.

Related text = Part 4.

**Box 3 of Appendix-F**

**Infarct Case: Effect of a LOW-Carbohydrate HighFat Diet on Lipoprotein Levels.**

This box is adapted from Gofman 1958 (p.279).

- The patient was a 65-year-old male survivor of a past Myocardial Infarction.
- The post-infarct low-carbohydrate diet (only 100 g/day of carbohydrate) did not cause appreciable weight-change during the 60-day period of observation and measurement (August 20-Oct.19).

● The tabulation below reveals the very different kind of information conveyed by the Std Sf 20-400 measurement, which fell to about HALF of its pre-diet level, and the total cholesterol measurement, which did not change with the diet. The total cholesterol measurement provided no hint of the dramatic decrease which occurred in the level of the atherogenic triglyceride-rich Std Sf 20-400 lipoproteins. The mean level of cholesterol-rich Std Sf 0-20 lipoproteins rose by a factor of 1.08 --- (308/286).

- Composition of the LowCarbo-HighFat diet was as follows:

Carbohydrate .....	100 g/day
Animal fat .....	60 g/day
Vegetable oil (unhydrogenated cottonseed oil) .....	80 g/day
Protein .....	91 g/day
Total calories .....	2,024 cal/day

<u>Pre-diet levels (on the patient's usual home diet)</u>			
Date	Std Sf 0-20 (mg/100 ml)	Std Sf 20-400 (mg/100 ml)	Cholesterol (mg/100 ml)
7/18	347	482	---
8/11	226	585	186
Mean Values (pre-diet)	286	533	

<u>Values on low-carbohydrate diet (100 g carbohydrate daily, started 8/20)</u>			
8/31	320	225	209
9/7	281	150	170
9/14	279	245	172
9/21	297	253	190
9/28	271	230	166
10/5	399	245	206
10/12	297	237	186
10/19	321	234	186
<u>Mean values during the 60 days of low-carbohydrate diet</u>			
	308	227	186

Related text = Part 5.

**Box 4 of Appendix-F**

**Xanthoma Case: Effect of a LOW-Carbohydrate HighFat Diet on Xanthomata & Lipoprotein Levels.**  
This box is adapted from Gofman 1958 (p.280).

- The patient was a retired female school teacher with long-standing Xanthoma Tuberosum --- a disorder typically associated with extremely high serum levels of the Std Sf 20-400 lipoproteins.
- From January 24-March 1, the patient followed a very LOW-carbohydrate diet (details are below), during which the second set of measurements was made.
- By comparison with mean measurements on the patient's usual home diet, mean measurements during the 36-days on the LowCarbo HighFat Diet showed that Std Sf 0-20 lipoprotein levels rose by a factor of 1.07, that Std Sf 20-400 lipoprotein levels fell by a factor of 0.52, and that serum cholesterol fell by a factor of 0.85.
- Subsequent to the 36-day period, the patient underwent further periods of carbohydrate restriction on a home diet, and her xanthomata underwent considerable regression.
- Composition of the LowCarbo-HighFat Diet was as follows:

Carbohydrate .....	100 g/day
Animal fat .....	100 g/day
Vegetable oil (unhydrogenated cottonseed oil) .....	20 g/day
Protein .....	75 g/day
Total calories .....	1,780 cal/day

<u>Pre-diet levels (on the patient's usual home diet)</u>			
Date	Std Sf 0-20 (mg/100 ml)	Std Sf 20-400 (mg/100 ml)	Cholesterol (mg/100 ml)
1/5	361	915	408
1/24	411	767	418
Mean Values (pre-diet)	386	841	413

<u>Values on low-carbohydrate diet (100 g carbohydrate daily, started 1/24)</u>			
2/1	512	452	388
2/8	404	401	333
2/15	392	334	320
2/21	352	465	342
3/1	400	550	383
<u>Mean values during the 36 days of low-carbohydrate diet</u>			
	412	440	353

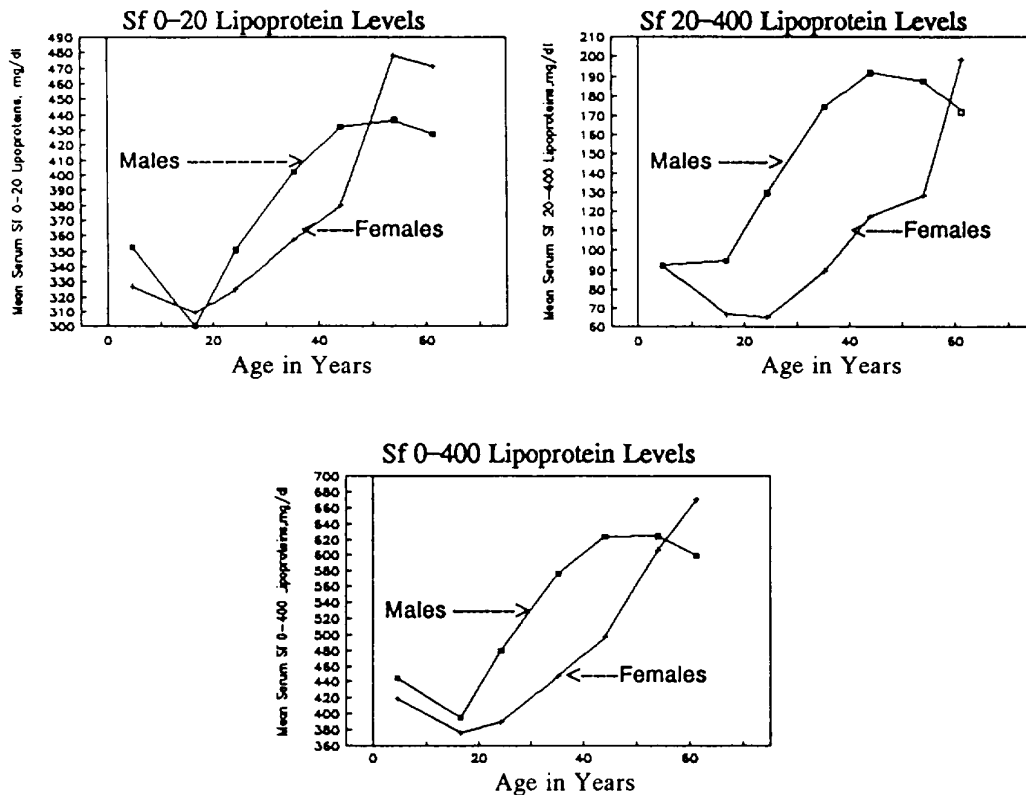
Related text = Part 5.

### Box 5 of Appendix-F

Changes in Std Sf 0-20 and 20-400 Serum Lipoprotein Levels by Gender, with Rise in Age.

● The plots demonstrate the rise in mean serum lipoprotein levels beyond about 20 years of age until --- especially for males --- the rising slope flattens and even declines a bit. The rise after age 20 coincides with the tendency (USA) to gain weight during adulthood. The text provides data on the effect of weight-change on serum lipoprotein levels.

● The scale of values is different for the three plots. All measurements, made by the Donner Lab, come from Glazier 1954 and are listed below the graphs. From age 30 upward, the population sample consisted of clinically healthy entrants into the Framingham Heart Study, and below age 30, samples are from clinically healthy children and persons of the U.C. Berkeley campus (details in Appendix-E, Part 3).



Mean Lipoprotein Concentrations (mg/dl) in Clinically Healthy Persons as a Function of Age:

Mean Age (Years)	Persons:	Std Sf 0-20	Std Sf 20-400	Std Sf 0-400
<b>Males</b>				
4.3	9	352.4	92.3	444.7
16.2	29	300.4	94.7	395.1
25.0	75	350.6	129.5	480.1
35.1	358	402.1	174.5	576.6
44.2	313	432.1	191.8	623.9
54.1	228	436.6	187.7	624.3
61.1	43	427.4	172.0	599.4
<b>Females</b>				
5.0	6	326.9	92.0	418.9
16.9	32	309.1	66.5	375.6
23.5	86	325.0	65.0	390.0
35.2	452	357.5	89.4	446.9
44.0	399	379.7	117.4	497.1
53.9	269	478.2	128.4	606.6
61.5	43	471.3	198.7	670.0

Related text = Parts 5a + 12.