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Modified Cretan Mediterranean Diet in the Prevention of Coronary Heart Disease and Cancer: An Update

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Prospective studies of the epidemiology of coronary heart disease (CHD) and cancer have shown that mortality from these diseases differs greatly among populations and that at least some of the differences are associated with differences in dietary habits [1, 2]. Mediterranean populations, for instance, are protected from CHD and certain cancers, and the particular composition of the Mediterranean (Med) diet has been put forth to explain this [3–5]. However, epidemiological studies only provide associations between the risk factors and clinical endpoints, not causal relationships. Several confounding factors may play a part in the associations. The economic situation and the presence or absence of extended social support systems have been proposed to explain the low prevalence of CHD in some Mediterranean countries. Although an effect of these factors cannot be totally excluded, data from Albania, where a low CHD rate was associated until recently with economic misery and modest health services, do not agree with this possibility [6, 7].

Randomized trials are the only way to make sure that a given dietary pattern results in a significant cardioprotective and/or anticancer effect.

Some dietary trials in primary or secondary prevention of CHD have reported an impressive reduction of CHD risk, especially in terms of mortality [8–10]. In contrast, other dietary trials specifically aimed at reducing blood cholesterol failed to significantly improve the prognosis of the dieters [11]. The successful trials tested dietary patterns characterized by a low intake of total, saturated and omega-6 polyunsaturated fats [8, 9] and an increased intake of omega-3 fatty acids [8–10, 12]. Their aim, with the exception of one assay [8], was not to primarily reduce blood cholesterol. Two of these trials [8, 9] also

included a high intake of fresh fruits and vegetables, legumes and cereals containing large amounts of fiber, antioxidants, minerals, vegetable proteins and vitamins of the B group. The credibility of these trials was considerably reinforced by a number of studies showing major cardioprotective effects of most of these foods and nutrients [13–21], with a particular emphasis on omega-3 fatty acids [10–12, 16, 17] and on folates for their role in hyperhomocysteinemia and in the arginine-nitric oxide-BH4 pathway [17–21].

The Lyon Diet Heart Study was a randomized single-blind secondary prevention trial aimed at testing whether an experimental modified Cretan diet (Exp) may reduce the risk of recurrence after a first myocardial infarction (MI). A significant reduction of the rates of cardiovascular complications was reported [9, 22, 23], and no major bias was detected in the trial [23, 24]. In addition, the trial suggested that patients following the cardioprotective Exp diet may also be protected from cancer [25]. Although further trials are warranted to confirm the cancer data, those obtained from the Lyon trial are in line with several studies [26], in particular prospective studies with heavy emphasis on the role of selenium (of which the various typical Med diets contain large amounts) in the reduction of the incidence of, and mortality from, carcinomas at several sites [27]. Olive oil (both oleic acid and phenolic compounds) and the vegetable components of the Med diet also might be important [28–30], whereas the effects of omega-3 fatty acids from either plant or marine sources are still controversial [31–36].

Recent Epidemiological Studies about Mediterranean Diets and Coronary Heart Disease

Since the publication of Volume 87 of *World Review in Nutrition and Dietetics* about Med Diets in 2000 [37], prospective cohort and case-control studies about Med diets and CHD have been reported. One randomized trial, the ‘Indo-Mediterranean diet trial’, also reported protective effects but raised concerns about some ethical and scientific aspects [38]. For that reason, and even though the article was not withdrawn by the publisher and the data not retracted by the authors, we prefer to wait some time before further discussing this potentially important report.

Regarding the epidemiological studies, and whatever the country where the studies were conducted and the general context of each study (international comparison or monocentric analysis, primary or secondary prevention), the data are impressive [39–45]. None of these studies suggests that the Med diet is not highly protective against CHD and not associated with longer survival. One problem with these studies, however, is the way to define, and then to quantify, the traditional Med diet. In general, the technique used by the investigators

was to calculate a Med score from individual data obtained through an extensive, validated food-frequency questionnaire. It is clear that this technique does not fully reflect the complexity of a dietary pattern such as the traditional Med diet. However, the combination of Med foods in a given person or patient can actually be summed up as a score, which can provide some information if there is a statistically significant correlation with clinical endpoints. Another weakness of these studies was the lack of biomarkers, for instance blood antioxidants and vitamin B, or plasma or red cell fatty acids. This, in fact, raises the major question of whether one should define a diet in terms of foods or in terms of nutrients. We think, as shown in our own studies [22–25] and discussed below, that the preferred technique is probably to use both foods and nutrients to define the kind of Med diet that we are exploring, and then to quantify the dietary habits of individuals or populations. In spite of these technical limitations and differences in the way of evaluating Med scores, investigators report that adherence to a Med diet style is associated with a reduction in total mortality. The effect on mortality was seen in both primary and secondary prevention of CHD and also was attributable to a reduced risk of cancer death [40, 41, 43–45]. This emphasized the importance of dietary habits in the prevention of CHD and cancer, and confirmed beyond any doubt both the results of the Lyon Diet Heart Study and the relevance of the Med diet.

Summary of the Lyon Diet Heart Study Results

The design and methods of the trial have been reported [9, 22–24]. Briefly, statistical analyses were done on the intention-to-treat principle. Event-free survival for MI, cardiovascular death (CD) and three composite outcomes (CO) were estimated using the Kaplan-Meier method. The Cox proportional hazard model was used to calculate the risk ratios and to quantify the associations between each traditional risk factor and the different COs, namely MI plus CD (CO 1) or MI plus CD plus major secondary events (CO 2) or the precedents plus minor events requiring hospital admission (CO 3). In the Exp group, CO 1 was reduced (14 events against 44, $p > 0.0001$), as were CO 2 (27 events against 90, $p > 0.0001$) and CO 3 (95 events against 180, $p > 0.0002$). Adjusted risk ratios ranged from 0.28 to 0.53 [23].

In CHD patients, adopting a slightly modified Cretan diet resulted in a 50–70% decrease in the risk of recurrence.

Among the traditional risk factors, total blood cholesterol (1 mmol/l being associated with an increase in risk by 18–28%), systolic blood pressure (1 mm Hg being associated with an increase in risk by 1–2%), leukocyte count (adjusted

risk ratios ranging from 1.64 to 2.86 at counts >9 giga/l), female gender (adjusted risk ratios: 0.27–0.46) and aspirin use (adjusted risk ratios: 0.59–0.82) were each significantly and independently associated with recurrence. The data indicate that the Exp diet did not alter, at least qualitatively, the usual relationships between major risk factors of CHD (e.g. elevated cholesterol and blood pressure) and recurrence. This is a very important point for the physicians in charge of the patients, since it means that pharmacological treatment (with aspirin, blood pressure- and cholesterol-lowering drugs) and dietary prevention are not mutually exclusive but have additional and independent beneficial effects, although further trials combining the two approaches are warranted.

Finally, the fact that the two groups of the Lyon trial were similar in terms of traditional risk factors during the study [9, 23] should not be interpreted as meaning that the Exp diet did not have any effect on blood lipids or blood pressure in patients with established CHD. In fact, the vast majority of the patients of both groups were taking blood pressure-lowering drugs (prescribed by their attending physicians) as part of a systematic secondary prevention treatment, which resulted in low blood pressure levels in both groups and probably masked any blood pressure-lowering effect of the Exp diet. It has been shown that a diet including high intakes of vegetables and reduced intakes of meat and high-fat dairy products (a diet quite similar to the traditional Med diets) could indeed reduce blood pressure [46], and data from investigators in Italy found that such diets, also in the Med context, actually lower blood pressure [47]. Thus, the cardioprotective effect of the Med diet may be also due to an unidentified effect on factors regulating blood pressure and presumably involved in the pathogenesis of CHD.

The same reasoning applies for blood lipids. One third of each group of patients in the Lyon trial was taking cholesterol-lowering drugs, with a trend towards more patients being treated in the Western group during the trial [24]. Thus, a small effect of the Exp diet on blood cholesterol may have been masked by the drug therapy. In fact, any lipid-lowering effect of a Med diet should be evaluated by comparing patients following a Med diet with patients following a typical Western diet, including high intakes of saturated fats and dietary cholesterol. Thus, the evaluation of the true effect of any type of Med diet on cholesterol level in CHD patients with various dyslipidemias warrants further investigations and trials specifically designed for that purpose.

The Experimental Diet Tested in the Lyon Trial

The theoretical principles of the Exp Med diet (a modified Cretan diet) tested in the trial have been described [9, 11, 22–24]. The Seven Countries

Study showed that mortality from CHD is lower in Crete than in the other Southern Mediterranean countries [1] where the amount of fat in the diet (about 30% of total energy) is considerably lower than in Crete (about 40%). In these other Mediterranean countries (Italy and Spain for instance), people do not eat as many wild plants, walnuts and legumes as the people in Crete and their intake of alpha-linolenic acid (ALA) is lower. What is so special about the Cretan diet is the much higher ALA content and lower linoleic acid content, with higher amounts of fruits and less meat, along with the otherwise traditional components of the Med diet.

Briefly, in terms of lipids, the Exp diet would supply about 30% of energy from fats and less than 10% of energy from saturated fats. Regarding the essential fatty acids, the intake of 18:2 ω 6 (linoleic acid, LA) should be restricted to 4% of energy and the intake of 18:3 ω 3 (ALA) should compose more than 0.6% of energy. In practical terms, the dietary instructions were detailed and customized to each patient [9] and could be summarized as: more bread, more cereals, more legumes and beans, more fresh vegetables and fruits, more fish, less meat (beef, lamb, pork) and delicatessen food, which are to be replaced by poultry; no more butter and cream, to be replaced by an experimental, canola oil-based margarine. This margarine was chemically comparable with olive oil but slightly enriched in 18:2 ω 6 and mostly in 18:3 ω 3, the two essential fatty acids. The concentrations of trans fatty acids were around 5% in the first part of the trial (first two years) and about 0% thereafter. Finally, the oils recommended for salad and food preparation were exclusively olive and canola (erucid acid-free rapeseed oil) oils. What the patients of the Exp and Western diet groups actually ate during the trial has been reported [9, 23]. Patients of the Western group followed a prudent diet, with about 34% of energy as total fats, 12% saturated fats and a mean of 312 mg/day of dietary cholesterol. These patients were assuredly not following a typical Western diet, rather a prudent diet not too different from the US National Cholesterol Education Program (NCEP) step I. In contrast, patients of the Exp group were close to step II of the NCEP recommended for survivors of a previous infarct (except for the omega-3 and omega-6 fatty acids), with 30% as total fats, 7–8% saturated fats and 200 mg/day of cholesterol. Intake of the main food groups has been reported [9, 23] and is summarized in table 1. The main points concerned the consumption of bread, fresh fruits and vegetables, meat and delicatessen food, oils, and butter (and cream) versus the canola oil-based margarine. The exclusive use of olive and canola oils and of canola-oil based margarine to prepare meals and salad is a major issue in that study since it resulted in major differences in the fatty acid composition of both circulating plasma lipids (essentially lipoproteins) and membrane cell phospholipids (tables 2–4), which in turn were probably associated with critical effects on the major mechanisms involved in the occurrence of the cardiovascular complications, as discussed below.

Table 1. Summary of the intake of the main food groups recorded at the last visit of the patients in the Lyon trial: results in g/day, mean \pm SD

	Western	Exp	p
Bread	140 (58)	171 (86)	<u>0.004</u>
Other cereals	69.7 (116)	97.0 (132)	0.11
Vegetables (without potatoes)	340 (203)	427 (222)	<u>0.004</u>
Fruits	214 (201)	271 (218)	<u>0.05</u>
Potatoes	82.2 (123)	66.6 (129)	0.38
Delicatessen foods	21 (42)	3.0 (14)	<u>0.0001</u>
Meat	53.4 (70)	35.1 (62)	<u>0.04</u>
Poultry and lean meat	57.1 (75)	48.1 (62)	0.30
Fish	32.8 (68)	47.9 (72)	0.12
Whole milk	19.8 (83)	3.10 (37)	0.09
Skimmed milk and light milk	147 (202)	118 (178)	0.30
Cheese	36.3 (30)	29.7 (30)	0.12
Butter and cream	12.4 (13)	2.60 (7)	<u>0.0001</u>
Margarine	7.9 (10)	17.4 (15)	<u>0.0001</u>
Oil	15.5 (13)	18.9 (12)	0.05

Table 2. Plasma fatty acid differences (Diff) in patients following either an experimental modified diet of Crete (Exp) or a prudent Western (West) type of diet

Ratio	After 2 months			After 1 year		
	Exp (n = 236)	West (n = 247)	Diff %	Exp (n = 141)	West (n = 139)	Diff %
18:1/18:2	0.809	0.658	+23	0.799	0.668	+20
18:2/18:3	42	76	-45	44	79	-44
20:4/20:5	6.8	9.1	-25	6.2	9.1	-32

All differences are statistically significant ($p < 0.05$).

18:1 is oleic acid (18:1 omega-9); 18:2 is linoleic acid (18:2 omega-6); 18:3 is alpha-linolenic acid (18:3 omega-3); 20:4 is arachidonic acid (20:4 omega-6); 20:5 is eicosapentaenoic acid (20:5 omega-3).

Finally, it must be mentioned that the main antioxidant vitamins, alpha-tocopherol and ascorbic acid, were significantly higher in the plasma in the Exp group than in the Western group [9], which stresses the potent antioxidant effect of the Exp diet. This is not surprising because beyond the obvious effects of the Exp diet on the fatty acid composition of lipids (which is by itself an antioxidant

Table 3. Platelet phospholipids fatty acids

	Exp	West	p
<i>Saturated fatty acids</i>			
14:0	0.28 ± 0.01	0.31 ± 0.01	<0.05
16:0	14.70 ± 0.25	14.39 ± 0.17	NS
18:0	15.81 ± 0.17	16.35 ± 0.14	0.01
Sum	30.79 ± 0.28	31.05 ± 0.21	NS
<i>Unsaturated fatty acids</i>			
<i>Omega-9 family</i>			
18:1 ω 9	12.56 ± 0.32	11.64 ± 0.14	0.01
20:1 ω 9	0.88 ± 0.04	0.74 ± 0.03	0.01
20:3 ω 9	0.16 ± 0.01	0.15 ± 0.01	NS
Sum	13.50 ± 0.35	12.43 ± 0.15	<u>0.01</u>
<i>Omega-6 family</i>			
18:2 ω 6	5.09 ± 0.16	5.46 ± 0.17	NS
20:3 ω 6	1.22 ± 0.03	1.31 ± 0.05	NS
20:4 ω 6	25.89 ± 0.33	26.45 ± 0.28	NS
22:4 ω 6	2.72 ± 0.09	3.02 ± 0.10	<0.05
Sum	34.93 ± 0.42	36.26 ± 0.29	<u>0.01</u>
<i>Omega-3 family</i>			
20:5 ω 3	0.57 ± 0.04	0.48 ± 0.04	NS
22:5 ω 3	2.11 ± 0.08	1.94 ± 0.06	NS
22:6 ω 3	2.59 ± 0.10	2.38 ± 0.10	NS
Sum	5.27 ± 0.17	4.81 ± 0.16	<u>0.05</u>

Results are mean ± SEM.

NS = Nonsignificant.

Table 4. Comparisons of the different families of platelet phospholipids fatty acids

Ratios	Exp	West	p
Omega-6/omega-9	2.70 ± 0.12	2.95 ± 0.05	0.05
Omega-6/omega-3	6.92 ± 0.27	7.88 ± 0.29	<u><0.05</u>
Omega-6/omega-9 + omega-3	1.90 ± 0.06	2.13 ± 0.04	<u><0.005</u>
Saturated/omega-6	0.88 ± 0.01	0.86 ± 0.01	0.13
Saturated/omega-9 + omega-3	1.68 ± 0.05	1.82 ± 0.03	<u>0.01</u>
Saturated/unsaturated	0.57 ± 0.01	0.58 ± 0.01	0.90

intervention as it modifies the main substrate of oxidation), this diet actually provided large amounts of various antioxidant compounds including vitamins, trace elements and polyphenols, including flavonoids [48]. The Exp diet is a non-strict vegetarian diet, low in saturated and polyunsaturated fat and high in oleic acid, omega-3 fatty acids, fiber, vitamins of the B group and various antioxidants.

Coronary Heart Disease Is an Inflammatory Disease

Most investigators now agree that atherosclerosis and CHD are chronic inflammatory diseases [49]. Proinflammatory factors (free radicals caused by cigarette smoking, hyperhomocysteinemia, diabetes, peroxidized lipids, hypertension, elevated and modified blood lipids) contribute to injuries of the vascular endothelium resulting in alterations of its antiatherosclerotic and antithrombotic properties. This is thought to be a major step in the initiation and formation of arterial fibrostenotic lesions [49]. From a clinical point of view, however, an essential distinction should be made between unstable, lipid-rich and leukocyte-rich lesions and stable, lipid-poor and acellular fibrotic lesions, because the propensity of these two types of lesion to rupture into the lumen of the artery, whatever the degree of stenosis and lumen obstruction, is totally different. We hypothesized the role of inflammation and leukocytes in the onset of acute CHD events many years ago [50], and this was confirmed later [51–54]. One of the main mechanisms underlying the sudden onset of acute syndromes, including unstable angina MI and sudden death, is the erosion or rupture of an atherosclerotic lesion [51, 52], triggering thrombotic complications and considerably increasing the risk of ventricular arrhythmias [53, 54]. Leukocytes have been also implicated in the occurrence of ventricular arrhythmias in the clinical and experimental settings [55, 56], and they contribute to myocardial damage during both ischemia and reperfusion [55].

Clinical and pathological evidence showed the importance of inflammatory cells and immune mediators in the occurrence of acute CHD events [49, 57, 58] and prospective epidemiological studies showed a strong and consistent association between acute CHD and systemic markers of inflammation [59, 60].

Any theory to explain the efficiency of the Cretan diet to reduce the rate of CHD should take into account the importance of inflammation in CHD.

The next question is whether the Med diet might be associated with a lower prevalence of inflammatory syndromes. At least three studies testing some forms of traditional Med diet, including 2 randomized trials, recently showed that the adoption of a Med diet reduces inflammatory markers such as serum

C-reactive protein, interleukins, TNF- α , P-selectin, fibrinogen or white blood cell counts [61–64]. Interestingly, two of them were also associated with an improvement of endothelial function, a marker of the risk of CHD complications such as plaque erosion or rupture [61, 63] as discussed below.

Role of Inflammation and Oxidation in Plaque Rupture and Progression of Coronary Heart Disease

Another critical question is why macrophages and activated lymphocytes [49] are present in atherosclerotic lesions, and how they get there, with the associated issues of local inflammation, plaque rupture and attendant acute CHD complications. In 1989, Steinberg et al. [65] hypothesized that lipoprotein oxidation causes accelerated atherogenesis. Elevated plasma levels of low-density lipoproteins (LDL) are a risk factor of CHD, and the reduction of blood LDL levels resulted in less CHD in many trials, although the effect on mortality was either small or non-significant in most recent statin trials conducted in patients with optimal drug treatment [66–70]. However, the mechanism(s) behind the effect of high LDL levels is not fully understood. The notion that LDL oxidation is a key characteristic of unstable lesions is supported by many reports [49]. Two processes have been proposed. First, when LDL particles become trapped in the arterial wall, they are progressively oxidized and internalized by macrophages, which results in the formation of the typical atherosclerotic foam cells. Oxidized LDL is chemotactic for other immune and inflammatory cells and up-regulates the expression of monocyte and endothelial cell genes involved in the inflammatory reaction [49, 65, 71, 72]. The inflammatory response itself can have a profound effect on LDL [48], thus creating a vicious circle of LDL oxidation, inflammation and further LDL oxidation.

Second, oxidized LDL circulates in the plasma for a period sufficiently long to enter and accumulate in the arterial intima, suggesting that the entry of oxidized lipoproteins within the intima may be another mechanism of lesion inflammation, in particular in patients without hyperlipidemia [73–75]. Elevated plasma levels of oxidized LDL are associated with CHD, and the plasma level of malondialdehyde-modified LDL is higher in patients with unstable CHD syndromes (usually associated with plaque rupture) than in patients with clinically stable CHD [76]. In the accelerated form of CHD typical of post-transplantation patients, higher levels of lipid peroxidation [77–79] and of oxidized LDL [80] were found compared with the stable form of CHD in non-transplanted patients. Reactive oxygen metabolites and oxidants influence thrombus formation [81], and platelet reactivity is significantly higher in transplanted patients than in non-transplanted CHD patients [82]. However, platelet

phospholipid fatty acids (which partly determine platelet function) are different from those of non-transplanted CHD patients [82], which may explain, at least partly, the high platelet reactivity of transplanted patients. Since the effects of reactive oxygen metabolites on platelet function are quite complex [83], further studies are warranted to conclusively answer the question of the relations between lipid peroxidation and platelet function in CHD.

Another question is whether antioxidant intervention might alter the risk of CHD. There are different types of antioxidant treatment, including either a global dietary approach (aimed both at reducing oxidant stress and at increasing antioxidant defenses, with the Med diet as a good example) or a pharmacological approach using capsules of various antioxidants. The second approach has been extremely disappointing.

With respect to antioxidant vitamins and CHD, randomized trials have failed to fulfill the promises of observational epidemiological studies, which were also supported by a body of evidence from basic research suggesting plausible biological mechanisms [84].

This does not mean that oxidized lipids do not have a role in CHD complications. However, there is clearly a missing link in the current theory, and further research is needed before we can recommend systematic antioxidant supplementation for the prevention of CHD.

Lipids and Inflammation in Coronary Heart Disease

The oxidized LDL theory is not inconsistent with the well-established lipid-lowering treatment of CHD, as there is a positive correlation between plasma LDL levels and lipid peroxidation markers [78, 85]. Low LDL levels actually result in reduced amounts of LDL available for oxidative modification. The lowering of LDL can be achieved by drugs or by reducing saturated fats in the diet. Reduction of the oxidative susceptibility of LDL was reported when dietary fat was replaced with carbohydrates [86]. This is rather in line with the first approach (global dietary approach) described above and different from the pharmacological antioxidant approach.

Pharmacological/quantitative (lowering of cholesterol) and nutritional/qualitative (increased antioxidant defenses, reduction of oxidative stress) approaches of the prevention of CHD are not mutually exclusive but additive and complementary.

An alternative way to reduce LDL concentrations is to replace saturated fats with polyunsaturated fats. However, diets high in polyunsaturated fatty acids increase the polyunsaturated fatty acid content of LDL particles render them more susceptible to oxidation [87] and increase oxidative stress, which would argue against the use of such diets. As a matter of fact, in the secondary

prevention of CHD, such diets failed to improve the prognosis of the patients [11]. In this context, the Exp diet tested in the Lyon trial, which included both low saturated and polyunsaturated fat intakes, appears to be the best choice. However, diets rich in oleic acid increase the resistance of LDL to oxidation independently of the antioxidant content [88, 89] and result in leukocyte inhibition [90, 91]. Thus, oleic acid-rich diets decrease the proinflammatory properties of oxidized LDL. Constituents of olive oil other than oleic acid may also inhibit LDL oxidation [92]. Various components of the Med diets may also affect LDL oxidation. For instance, alpha-tocopherol or vitamin C or a diet combining reduced fat, low-fat dairy products and high intakes of fruits and vegetables were shown to favorably affect LDL oxidation itself and/or the cellular consequences of LDL oxidation [93–96]. Finally, a significant correlation was found between certain dietary fatty acids and the fatty acid composition of human atherosclerotic plaques [97, 98], suggesting that dietary fatty acids are rapidly incorporated into the plaques. This implies a direct influence of dietary fatty acids on plaque formation and the process of plaque rupture. It is conceivable that fatty acids that stimulate LDL oxidation (omega-6 fatty acids) induce plaque rupture, while those which inhibit LDL oxidation (oleic acid) or leukocyte function (omega-3 fatty acids) [99] help stabilize the dangerous lesions.

Thus, any dietary pattern combining high intakes of natural antioxidants, low intakes of saturated fatty acids, high intakes of oleic acid, low intakes of omega-6 fatty acids and high intakes of omega-3 fatty acids would logically result in a highly cardioprotective effect. Such a dietary pattern has now been tested in a randomized trial [22–25], and the results of the ‘Lyon Diet Heart Study’ were confirmed by several epidemiological studies [39–45].

Thus, the concept of Med diet with its multiple approaches (including the notion that the main targets of oxidative stress within the LDL are the polyunsaturated fatty acids, while monounsaturated fatty acids are resistant to oxidation) leads to reconcile a body of inconsistent data. Large doses of non-natural antioxidant vitamins (as they were tested in randomized trials) in the absence of consideration for the target of oxidation might be pro-oxidant in certain patients or certain conditions, and this may explain the failure to prevent CHD complications in these trials. We believe that any antioxidant intervention must be included in a more global dietary approach such as the Med diet.

Fatty Acids and the Experimental Modified Cretan Diet

Tables 1–4 summarize dietary, plasma and cell fatty acid data from the patients of the Lyon trial. They provide a scientific rationale to explain, at least partly, the clinical results of the trial. In table 2, for instance, the differences between groups in the plasma 18:1/18:2 ratio could be considered an indicator

of lipid oxidizability as fatty acids are transported by the lipoproteins and are the main determinants of LDL oxidizability. However, while circulating blood lipids are crucial in the development of CHD, cell membrane lipids are also important because they are also affected by oxidant stress. The cell membrane consists of a bilayer primarily composed of phospholipids and cholesterol. Embedded in this essential matrix are also a variety of proteins, such as receptors, transporters and enzymes, performing important cellular functions. The requirement for a dynamic state of lipids in the bilayer is one of the fundamental features of the fluid-mosaic model of membrane structure [100]. Any factors modifying cell membrane fatty acid or the nature and/or content of the proteins must also influence the physical state of the bilayer and, as a consequence, alter cellular function [101]. Any consistent variation in the dietary intake of fats will be reflected in the composition of structural lipids within cell membrane, as shown in tables 3 and 4 of this study. Significant differences were observed for individual saturated fatty acids and for the 3 main families of unsaturated fatty acids (table 3). The exact physiological and pathophysiological significance of these differences is not yet clearly identified. However, the data suggest that cell membrane lipids in the Exp group were less oxidizable than those in the Western group (table 4). Further studies are warranted to examine the molecular and cellular consequences of these variations in the fatty acid composition on cellular function in the various types of cells involved in CHD (platelets, endothelial cells, leukocytes and smooth muscle cells). However, this reasoning includes only theoretical explanations based on the quantitative or qualitative (oxidized LDL theory) aspects of the lipoprotein and lipid paradigm. In fact, the dramatic cardioprotective effect resulting from the adoption of the Exp Med diet may be explained through the modification of other metabolic pathways that we will briefly discuss in the next sections.

Omega-3 Fatty Acids in the Med Diets and in Coronary Heart Disease

Sandker et al. [102] reported high concentrations of omega-3 fatty acids, in particular ALA, in the plasma of the Greek population, and Simopoulos and co-workers [103, 104] confirmed that ALA is a major component of the Cretan diet. There is now a large body of evidence indicating that dietary ALA is a major protective factor against CHD, including fatal complications of CHD [105–109]. Thus, there is no doubt that a high intake of ALA is a major feature of the Cretan Med diet, as also confirmed in the Lyon Diet Heart Study [22–25]. On the other hand, consumption of fish once or twice a week is clearly associated with a significant reduction in CHD mortality [110–115]. This effect