

## Original Article

# Suitability of the Mediterranean-style diet in the modern world

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Epidemiological studies as well as randomised dietary trials suggest that Mediterranean diet may be important in relation with the pathogenesis (and prevention) of coronary heart disease (CHD). For instance, a striking protective effect of an alpha-linolenic acid (ALA)-rich Mediterranean diet was reported in the Lyon Diet Heart Study with a 50 to 70 % reduction of the risk of recurrence after 4 years of follow-up in CHD patients. According to our current knowledge, dietary ALA should represent about 0.6 to 1 % of total daily energy or about 2g per day in patients following a Mediterranean diet, whereas the average intake in linoleic acid should not exceed 7g per day. Supplementation with very long chain omega-3 fatty acids (about 1g per day) in patients following a Mediterranean type of diet was shown to decrease the risk of cardiac death by 30% and of sudden cardiac death by 45% in the GISSI trial. Thus, in the context of a diet rich in oleic acid and poor in saturated and not high in omega-6 fatty acids (a dietary pattern characterizing the traditional Mediterranean diet), even a small dose of very long chain omega-3 fatty acids (one gram under the form of capsules) might be very protective. These data underline the importance of the accompanying diet in any dietary strategy using fatty acid complements.

**Key Words:** Alpha-linolenic acid, linoleic acid, omega-3 fatty acids, omega-6 fatty acids, coronary heart disease, acute myocardial infarction, Mediterranean diet

## Introduction

The concept of the Mediterranean diet originated from several observational studies in the 1950s, the main one being the Seven Countries Study initiated by Ancel Keys.<sup>1</sup> Taken as a whole, these studies showed that, despite a quite high fat intake (in variable amounts in the different Mediterranean countries, high in Greece, quite low in Tunisia, Morocco and in Spain and in Catalonia for instance), these populations had low to very low rates of CHD (and other vascular diseases), of most types of cancer and of inflammatory and degenerative diseases resulting in a long life expectancy. Because of the lack of striking differences in the traditional risk factors with other less protected populations (especially in terms of smoking, diabetes and high blood pressure), the Mediterranean dietary pattern was considered to be largely responsible for the good health observed in these regions.<sup>2</sup>

The main characteristics of the Mediterranean diet include an abundance of plant foods (vegetables, fresh and dried fruits, whole grain cereals, nuts and legumes); olive oil as the principal source of fat; fish, egg and poultry in low to moderate amounts; very low consumption of red meat, high fat dairy products (saturated fats) and vegetable polyunsaturated (including trans fatty acids) oils and margarines; moderate consumption of wine.<sup>2</sup> However, despite these strong data, it remained that causal relationships could be demonstrated only by conducting clinical trials.

We now have results of randomised trials supporting the theory that the Mediterranean diet is a very healthy diet.

## The Lyon Diet Heart Study

The Lyon Diet Heart Study is a secondary prevention trial designed to test the hypothesis that a Mediterranean ALA-rich diet may improve the prognosis of patients having survived a first acute myocardial infarction.<sup>3-6</sup> The design, methods and results of the trial have been reported.<sup>3-5</sup> A striking protective effect of that Mediterranean diet was reported with a 50 to 70% reduction of the risk of recurrence after 4 years of follow-up.<sup>3-6</sup> Briefly, as regards lipids, the experimental Mediterranean diet tested in the trial supplied less than 30% of energy from fats and less than 8% of energy from saturated fats. Regarding essential fatty acids, the intake of linoleic acid (LA), the main omega-6 fatty acid, was restricted to 4% of energy and the intake of ALA, the main omega-3 fatty acid, made up more than 0.6% of energy. In practical terms, the dietary instructions were

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Accepted 1 October 2004

detailed and customized to each patient<sup>3-6</sup> and can 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, which were 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 LA and mostly in ALA, the two essential fatty acids. Finally, the oils recommended for salad and food preparation were exclusively olive and canola (erucid acid-free rapeseed oil) oils. The scientific rationale for that "dietary fat strategy" has been discussed elsewhere.<sup>3-6</sup> Briefly, it was hypothesized that, because the lowest rates of cardiovascular diseases in the world were observed in populations either following a Mediterranean diet or a diet low in n-6 fatty acids but rich in n-3 fatty acids, the best strategy to reduce the rate of complications in patients with established CHD should be to adopt an n-3 fatty acid-rich Mediterranean diet. Two other major components of the traditional Mediterranean diet, in addition to a low n-6/n-3 fatty acid ratio, are low saturated fat intake and high oleic acid intake. Patients also had to meet these two major criteria of a healthy diet. Thus, to meet the criteria of a Mediterranean diet, patients had to drastically reduce the consumption of foods rich in saturated (essentially animal) fat. Among vegetable oils, only olive oil (despite its lack of ALA) and canola oil (despite its moderate amounts of LA) have a fatty acid composition in line with our strategy. Thus, the patients were advised to use both oils. Because of their high content in LA, soy-bean, sunflower and walnut oils should not be used daily for food preparation and salad dressing. Peanut oil is too rich in saturated fatty acids and LA and linseed oil is too rich in polyunsaturated fatty acids. In theory, the best option could be to vary the use of several oils. However, when considering the difficult conditions of everyday life for many of our patients and their families, we decided to try and simplify our advice and to recommend the exclusive use of olive and canola oils.

This strategy was quite well accepted by the French patients who, at the end of the trial, were actually following a diet whose characteristics were close to that we envisaged, in theory, as the golden standard cardioprotective diet, at least in terms of lipid nutrients.<sup>3-6</sup> This exclusive use of olive and canola oils (and of canola-oil based margarine instead of butter to spread on the bread) to prepare meals and salad was a major issue in that trial as it resulted in significant differences in the fatty acid composition of both circulating plasma lipids (essentially lipoproteins) and cell membrane phospholipids.<sup>3,7</sup> The main differences between groups in platelet phospholipid fatty acids were not seen at the level of individual fatty acids (ALA is almost undetectable in cell membranes) but for the entire family of each group. Significant differences were also seen for the ratio of omega-6 (n-6) to omega-3 (n-3) fatty acids.<sup>3,7</sup> When comparing the dietary fatty acids in the two groups, control patients did consume about 0.7g of ALA per day against about 1.8g in the experimental group, i.e. giving an LA to ALA ratio of about 10 to 1 in controls against about 4 to 1 in the experimental group. It is noteworthy that if the risk of recurrence in the experimental group was lower than in the control group, the risk in the control group was not

high compared with previous studies indicating that the 10 to 1 ratio was, in theory, not so bad. Because the Mediterranean diet tested in that trial was different from the control diet in many other aspects than the LA to ALA ratio (less saturated fat, more antioxidants from various sources, and probably more vitamins of the B group including folic acid, more vegetable proteins, and so on), the next question was to try and specify the exact role of ALA in the cardioprotection observed in the trial. Using multivariate analyses and adjustment for several confounders, we found that the plasma ALA levels measured two months after randomisation were significantly (and inversely) associated with the risk of recurrence, and in particular of fatal recurrence.<sup>6</sup> It could be said, however, that it is not ALA per se that was protective but the very long-chain n-3 fatty acids derived from ALA, eicosapentanoic and docosahexanoic acids (DHA), which were also increased in the plasma of experimental patients.<sup>3,7</sup> These very long-chain n-3 fatty acids have indeed been demonstrated to prevent ventricular fibrillation (VF) and sudden cardiac death (SCD) in animal experiments<sup>8,9</sup> and in human trials.<sup>10,11</sup> However, in the Lyon trial, these fatty acids were not significantly (borderline non significant with DHA) associated with a lower risk, which suggests that ALA was the main protective factor. Also, a specific antiarrhythmic effect of ALA itself was reported in the animal studies where it was tested.<sup>9</sup> It does not mean, however, that the benefits of ALA are not due, at least partly, to its conversion into very long chain n-3 fatty acids, and further studies are required to differentiate the individual effects of each n-3 fatty acid in the context of myocardial ischemia and ventricular arrhythmias.

In fact, the potential effects of ALA on cardiovascular diseases were discovered when it was reported that populations with a high proportion of ALA in plasma lipids (the Greek and Japanese cohorts of the Seven Countries Study, for instance) are apparently protected from cardiovascular diseases,<sup>12</sup> and when it was found that these low-risk populations consume foods rich in ALA.<sup>13,14</sup> Another important point to encourage the consumption of ALA and to reduce the intake of LA (with a significant decrease in the omega-6/omega-3 ratios) came from the observation that the lowest ex vivo platelet aggregation measured in humans in response to adenosine diphosphate, a possible indicator of the risk of acute CHD events, was recorded with a dietary LA to ALA ratio of about 4 to 1<sup>15,16</sup> i.e. much lower than that of the present Western diet and also lower than what many experts recommend at present. This pointed out the potential importance of ALA to prevent the thrombotic complications of CHD.<sup>17</sup>

Another randomised controlled trial in which the consumption of ALA was encouraged (patients of the experimental group were advised to eat more fruit, vegetables and nuts) reported a significant reduction of the risk of cardiac events in post-AMI patients.<sup>18</sup> In that trial, the main source of ALA was nuts. Earlier works suggested that eating nuts was associated with a diminished risk of CHD.<sup>19</sup> Potentially protective constituents of nuts include ALA, folates, magnesium, potassium, fibre, vitamin E, arginine and favourable lysine-to-arginine and methionine-to-arginine ratios.<sup>19</sup> One noteworthy point is that the

fatty acid composition of the lipids in walnuts (also called *English walnut* or *noix de Grenoble* or *Californian nut*) is apparently the same on both sides of the Atlantic as the concentrations of ALA, LA and oleic acid measured in our laboratory are respectively 13.4, 60.5 and 15.6% in the *noix de Grenoble* and 12.9, 62.5 and 13% in the *Californian nut*.

It is clear that beside their fatty acid composition, certain nuts may be important because they provide large amounts of arginine (even more than meat) with the major advantage that they are quite poor in methionine (the precursor of the vasculotoxic homocysteine) as compared with meat and fish. This is especially true for walnut, almond and hazelnut, which are habitually eaten in quite great amounts by Mediterranean populations. The last, but certainly not least, point is the relatively high content in folates of most nuts. Low serum folate levels<sup>21</sup> and high homocysteine levels<sup>22</sup> have been clearly associated with an increased risk of CHD. The results of recent trials, consistent with a decreased risk of CHD following homocysteine-lowering treatment with folic acid (plus vitamin B6 or plus Vitamin B6 and vitamin B12), suggest a causal relationship between low folic acid-high homocysteine and CHD.<sup>23,24</sup> Thus, although there is no room here to fully discuss each of these points, nuts (which are both rich in ALA and important in the Mediterranean diet) can obviously be included as part of a healthy diet. In contrast, and because walnut oil is too rich in LA and does not contain the other healthy nutrients present in the fruit nut itself, daily consumption of that oil is not advisable. There are different ways to obtain 1.8 to 2g of ALA per day in the diet, as in the Lyon Diet heart Study, without using ALA-containing capsules or fortified foods. The simplest (and easiest) way is to use canola oil for food preparation and salad dressing. Since 100g of canola oil provide about 8g of ALA, two small (US) tablespoons provide about 2g of ALA. The canola-oil based margarine, which contains about 5g of ALA per 100g of margarine, may be useful. Obtaining 2g of ALA requires 35g of margarine, i.e. about six teaspoons. Alternatively, one can have one tablespoon of canola oil (with salad, for instance) and 2 teaspoons of canola margarine with a piece of bread. Both canola oil and margarine can be used in association with olive oil (that does not contain ALA) for food preparation because of the large amounts of oleic acid and flavonoids it contains, and also because of its particular taste. Regarding nuts, to obtain 2g of ALA, about 6 medium size English walnuts (or *noix de Grenoble*) are needed. If one likes English walnuts enough to eat them every day (as Mediterranean people used to do), it is possible to use exclusively olive oil for food preparation. A good (and definitely tasty) way is to add the nuts to salad. One can also use ground linseeds (not linseed oil) with salad or other green leafy vegetables, knowing that 100g of ground linseeds provide about 23g of ALA. Thus, only one tablespoon of ground linseeds provides about 2g of ALA.

It was the merit of Simopoulos and colleagues to show that many green leafy vegetables (such as purslane) largely consumed around the Mediterranean basin are a major source of ALA for the Cretan population at that period.<sup>13</sup> The Cretan diet is also rich in antioxidants. This

is a major point since, because of its three double bonds, ALA is highly sensitive to oxidation and a high intake of ALA must be combined with a high intake of antioxidants to protect it from oxidation. The same authors also reported that eggs from range-fed Greek hens (which make a feast of purslane and other ALA-rich fresh green grass) are richer in ALA and other n-3 fatty acids than eggs bought in US supermarkets which are, in turn, usually rich in LA.<sup>14</sup> The large difference in the fatty acid composition of the two types of eggs was indeed also due to the fatty acid composition of the industrial feedstuff given to US hens. This suggested that egg yolk might have been a considerable source of ALA and other n-3 fatty acids for people living in the Mediterranean area. The point is important because this population usually does not consume large amounts of marine products rich in very long chain n-3 fatty acids. Eating purslane (or other equivalent ALA-rich leafy vegetables) to obtain large amounts of ALA is not very easy, in particular because purslane (or its equivalents) is not available in many areas. In addition, one big portion (100g of purslane) provides less than 0.4g of ALA. Thus, for most adults, purslane should be associated with either walnuts or a salad dressing containing canola oil. One advantage of eating green leafy vegetables (and not only purslane) is to increase the diversity of the diet, which is probably a major component of any healthy diet and one advantage of eating eggs from hens fed with ALA-rich grains is to increase the intake of DHA, the longest omega-3 fatty acid otherwise only found in fatty fish and fish oil.

#### **The Indo-Mediterranean Diet Trial**

A recent publication brings important new information about the Mediterranean diet and the importance of an adequate balance between n-6 and n-3 dietary fatty acids.<sup>24</sup> Singh and colleagues report the results of a randomised trial in secondary prevention of CHD conducted in South Asian people, a population known to be at a high risk of CHD not explained by conventional risk factors.<sup>24</sup> Most randomised patients were vegetarians and did not eat fish or foods providing them with very long chain n-3 fatty acids. The experimental group ate more fruits, vegetables, legumes, walnuts and almonds than did controls. Also, the experimental group had an increased intake of whole grains and mustard or soybean oil that are rich in ALA. The investigators calculated that the mean intake of ALA was twice as high in the experimental group (1.8g vs. 0.8g among controls).

The total cardiac endpoints were significantly fewer (39 vs. 76 events in controls) in the experimental group, but the difference between groups for total and cardiovascular mortality was non significant. Thus, the intervention was less effective than in the GISSI and the Lyon Trials. How can we explain such a difference when the ALA intake was comparable to that reported in the Lyon trial? A possible explanation is that the intake in n-6 fatty acids was too high. In fact, if we calculate the n-6/n-3 ratio before and after the intervention in the two groups of the Indo-Mediterranean trial,<sup>24</sup> we observe that it was 38 and 33 before the intervention and 9 (experimental) and 21 (control) after 2 years. Thus, the ratio was still rather high in the experimental group (as high as in the control

group of the Lyon trial), and this may explain why the patients did not get the maximum benefit from their experimental diet despite an increased intake in ALA. In the Lyon trial, that ratio was 4 in the experimental group and scientists recommend a ratio of 2 or even 1. The use of mustard and soybean oils in the experimental group of the Indo-Mediterranean trial probably explains that the n-6/n-3 ratio remained high, since soybean oil, in particular, is very rich in n-6 fatty acids, especially LA.

A possible lesson from that trial is therefore that it is important to decrease the intake in n-6 fatty acids when we try increasing the intake of n-3 fatty acids to design a dietary intervention aimed at preventing fatal manifestations of CHD, as it has been done in the Lyon trial. This hypothesis remains to be confirmed. Also to be confirmed the theory that a cardioprotective diet rich in n-3 fatty acids, can be rich in either ALA or very long chain n-3 fatty acids (EPA+DHA) or, alternatively, rich in either EPA or DHA. The underlying question is whether the different major n-3 fatty acids, ALA, EPA and DHA, individually have the same cardioprotective properties. Another major unsolved question is what is the actual effectiveness of the endogenous synthesis of DHA from EPA and ALA; and whether this synthesis occurs in the same way in the different organs in humans. Recent data suggest that in humans, if any synthesis of DHA occurs from EPA or ALA, it is in small amounts.<sup>3,7,25,26</sup> Our last and major question for future work is whether an increased intake of ALA and DHA (in the context of a Mediterranean diet) could be more protective than a simple increased intake in ALA, as we did in the Lyon trial.<sup>3,7</sup>

### **Fish oil and very long chain n-3 fatty acids in clinical trials**

The theory that eating fish, a major component of the traditional diet of several Mediterranean populations (in South Italy, Sicily, Catalonia and also Portugal, for instance) may protect against cardiac death is derived from the results of a secondary prevention trial, the Diet And Reinfarction Trial (DART), which showed a significant reduction in total and CV mortality (both by about 30%) in patients who consumed at least 2 servings of fatty fish per week.<sup>10</sup> The authors suggested that the protective effect of fish might be result from a preventive effect on ventricular fibrillation (VF), since no benefit was observed on the incidence of nonfatal AMI. The hypothesis was consistent with experimental evidence suggesting that the very long chain n-3 fatty acids, the dominant fatty acids in fish oil and fatty fish, have an important effect on the occurrence of VF in the setting of myocardial ischemia and reperfusion in various animal models, both in vivo and in vitro.<sup>8</sup> Recently, Billman and colleagues, using an elegant in vivo model of SCD in dogs, demonstrated a striking reduction of VF after intravenous administration of pure n-3 fatty acids, including the very long chain fatty acids present in fish oil and alpha-linolenic acid, their parent n-3 fatty acid occurring in some vegetable oils.<sup>9</sup> Another important aspect of the involvement of n-3 fatty acids in SCD concerns their role in the metabolism of eicosanoids. In competition with n-6 fatty acids, they are the precursors to a broad array of structurally diverse and potent bioactive lipids (including eico-

sanoids, prostaglandins and thromboxanes), which are thought to play a role in the occurrence of VF during myocardial ischemia and reperfusion.<sup>27,28</sup>

Other interesting clinical data show suppression (by more than 70%) of ventricular premature complexes in middle-aged patients with frequent ventricular extrasystoles randomly assigned to take either fish oil or placebo.<sup>29</sup> Also, survivors of AMI<sup>30</sup> and healthy men<sup>31</sup> receiving fish oil were shown to improve their measurements of heart rate variability, which suggests that there are other mechanisms by which n-3 fatty acids may be antiarrhythmic. In fact, parasympathetic cardiac tone is thought to provide protection against VF.<sup>32</sup>

Support for the hypothesis of a clinically significant antiarrhythmic effect of n-3 fatty acids in secondary prevention of CHD, as put forward in DART,<sup>10</sup> came from epidemiological studies.<sup>33</sup> In particular, in a large prospective study (more than 20 000 participants with a follow-up of 11 years), Albert *et al.*, the examined whether fish might have antiarrhythmic properties and prevent SCD.<sup>34</sup> They found that the risk of SCD was 50% lower for men who consumed fish at least once a week than for those who had fish less than once a month. Interestingly, the consumption of fish was not related to non-sudden cardiac death, which suggests that the main protective effect of fish (or very long chain n-3 fatty acids) is related to an effect on arrhythmia.

The GISSI-Prevenzione trial was aimed at helping in addressing the question of the health benefits of foods rich in very long chain n-3 fatty acids (and also in vitamin E) and their pharmacological substitutes.<sup>11</sup> Patients (n=11324) surviving a recent AMI (<3 months) were randomly assigned supplements of n-3 fatty acids (1g daily), vita-min E (300mg daily), both or none (control) for 3.5 years. The primary efficacy endpoint was the combination of death and non-fatal AMI and stroke. Secondary analyses included overall mortality, CV mortality and SCD. Treatment with n-3 fatty acids significantly lowered the risk of the primary endpoint (the relative risk decreased by 15%). Secondary analyses provided a clearer profile of the clinical effects of n-3 fatty acids. Overall mortality was reduced by 20% and CV mortality by 30%. However, it was the effect on SCD (45 % lower) that accounted for most of the benefits seen in the primary combined end-point and both overall and CV mortality. There was no difference across the treatment groups for nonfatal CV events, a result comparable to that of DART.<sup>10</sup>

A major point is that in that trial conducted in Italy, all patients were advised to follow a Mediterranean type of diet after their AMI. In their report, the GISSI investigators confirmed that the patients of both groups actually did so; for instance, more than 80% reported that they consumed olive oil every day.<sup>11</sup> Thus we can say that in GISSI, the prevention of SCD resulting from the consumption of 1g very long chain n-3 fatty acids was observed in patients following a Mediterranean diet as background diet. Whether they would have been protected in the same way with a non-Mediterranean diet is an open question. Another way of seeing the question is to ask whether or not the GISSI patients were relatively deficient in very long chain n-3 fatty acids. If they were, we

can understand that even a small dose of n-3 fatty acids was so effective. In fact, recent (unpublished) data from the European IMMIDIET Project suggest that the Italian population could be relatively deficient in n-3 fatty acids as compared with British (South of London) and Belgian (Flemish) populations, thus confirming the hypothesis that a low dose of n-3 fatty acids was so effective in GISSI because the tested population was probably relatively deficient in n-3 fatty acids. Thus, in the context of a diet rich in oleic acid and poor in saturated and n-6 fatty acids, even a small dose of n-3 fatty acids (under the form of capsules) might be very protective. It is not sure whether the results would have been similar in another context, for instance in populations with high intake in n-6 fatty acids. Finally, in a separate analysis, the investigators of GISSI analyzed in their cohort (pooling together the randomized groups) the effect of adhering closely or not to the main Mediterranean diet principles.<sup>35</sup> They found that compared with patients in the worst Mediterranean diet score, the risk of dying (from any cause) for those in the best score was reduced by 50% after adjustment for age, sex, smoking, concomitant drug therapy and randomized treatment<sup>35</sup>, a result very similar to that observed in the Lyon trial.

### Conclusions

A recent epidemiological study from Greece again confirmed that following a Mediterranean diet considerably reduces the risk of premature death.<sup>36</sup> Indeed, Trichopoulos and co-workers report, using a food-frequency questionnaire and a Mediterranean diet scale (higher score indicating a greater adherence to the Mediterranean diet) in more than 22,000 adults in Greece, a reduction in total, cardiac and cancer mortality with a high degree of adherence to the Mediterranean diet.<sup>36</sup> The data are in line with previous observational and trial data. As a matter of fact, several national and international expert Committees, notably in Europe and USA,<sup>37</sup> have decided to definitely adopt the Mediterranean diet as the reference diet for the prevention of cardiovascular diseases.

The next very important question will be to precisely understand how that dietary pattern protects against CHD, and also most cancers and inflammatory diseases. We already have some answers with the biological (and well described cardioprotective) effects of omega-3 fatty acids, natural antioxidants, ethanol, oleic acid and folic acid, all being basic nutrients of the Mediterranean diet. In addition, recent data indicate that this type of diet, taken as a whole rather than as an addition of individual protective nutrients, attenuates inflammation and coagulation process,<sup>38</sup> and that among patients with the metabolic syndrome, defined according to the NCEP ATP III criteria,<sup>39</sup> adoption of the Mediterranean diet was associated with a 35% reduction of the risk of CHD.<sup>40</sup> Thus, even in the context of a medical condition typical of the modern world in both developed (at least one in five people have a metabolic syndrome in USA in 2002) and developing countries, the Mediterranean diet pattern appears to be the prototype of a healthy diet. It should be emphasized to conclude that in most trials where the Mediterranean diet was advised to patients and their families, there were never major problems or difficulties to adopt the main prin-

ciples of that dietary pattern. In addition, as seen in the Lyon Diet Heart Study, after the end of the trial, most patients continue to adhere to these principles. They usually do not come back to their previous dietary habits. This is a major point to support the view that actually that dietary pattern is the ideal diet for people living in the Western industrialized world.

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