1.1.1 ***Fats***

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1.1.1.1 Total fat

Total fat includes all dietary lipids—both invisible fat from animal and vegetable sources as well as visible fat from vegetable oils, butter and margarine.

Both a too high and a too low fat intake may have health implications. Two main arguments have been used to justify a recommendation of a moderate total fat intake: a high fat diet may increase risk of coronary heart disease (CHD) and a high fat diet may promote overweight. How strong is the evidence for such associations? In the seven countries study a positive correlation was found between mortality from CHD in middle-aged men and the percentage of calories from fat in their diet.[1]

The correlation was weak, however, and explained by the increase in saturated fat as total fat increases; the correlation with saturated fat was much higher than with total fat.[1, 2]

It is noteworthy that the men of Crete with the highest fat content in the diet also had the lowest mortality from CHD despite having the highest body weights. The fatty acid composition of this diet differed from the others with its high content of oleic acid derived from olive oil. These early observations suggest that the quality of the dietary fat is more important than the quantity of total dietary fat in determining the risk of cardiovascular disease. The fat composition of western diets with their high proportion of animal products is such that almost any increase in total fat will result in an increase in saturated fat, dietary cholesterol and energy density. This is one reason why IOM 2002[3] and The Dietary Guidelines for Americans 2005 (DGA 2005)[4] included a recommendation on total fat which they set as acceptable up to a level of 35% of energy (E%) from fat. The arguments were clearly set out in the Dietary Guidelines as follows: "A high intake of fat (greater than 35 percent of calories) generally increases saturated fat intake and makes it more difficult to avoid consuming excess calories. A low intake of fats and oils (less than 20 percent of calories) increases the risk of inadequate intakes of vitamin E and of essential fatty acids and may contribute to unfavourable changes in high-density lipoprotein (HDL) blood cholesterol and triglycerides."

A high fat diet has a high energy density, and physiological and metabolic studies on diets of different energy density fed *ad libitum* to adults show that the dietary energy density rather than the fat content *per se* is the factor conducive to weight gain through what has been termed "passive over-consumption." This unconscious over-consumption is much more likely if an individual is relatively inactive. This implies that when populations become progressively more inactive they need to consume a higher quality diet lower in energy density to avoid weight gain. This is why the WHO analyses on diet

and physical activity in relation to chronic diseases[5] focused on energy density and physical inactivity as the two key features selectively determining societal weight gain. The report then emphasised the quality of the dietary fat as the principal issue to be considered when dealing with coronary heart disease. A low fat diet may also be favourable in relation to blood pressure. In an eight-month intervention study with 30 healthy young individuals it was shown that switching from an habitual Danish high fat diet (37 E% fat, 14 E% saturated fat, 45 E% carbohydrates) to a low fat diet (30 E% fat, 8 E% saturated fat, 58 E% high fibre carbohydrates) resulted in a significant reduction in total cholesterol, no change in triglycerides and in the men, a significant reduction in systolic blood pressure.[6] Meticulous metabolic studies have also shown that increases in total fat increase blood pressure when body weight, salt, fruit and vegetable intakes are held constant (DASH trials) but the increased risk of coronary heart disease induced by higher blood pressures is dominated by the impact of specific dietary fats on blood lipids and the process of atherosclerosis. Thus, there are populations with high blood pressure and a major problem of strokes but the development of coronary artery disease depends on increasing dietary saturated and *trans* fatty acid intakes with increases in the blood concentrations of total and low density cholesterol. Nevertheless, greater intakes of total fat (whether or not the fat is of a saturated kind) will increase the blood pressure and appreciably increase the risk of strokes.

The issue of total fat and its overall effect has for a long time been a controversial issue, in part because the reasons why children and adults gain excess weight are complex and relate to physical inactivity and several different aspects of dietary quality. The amount of foods eaten depends on a marked variety of social, economic and physiological factors only some of which link to dietary quality.[7] There are dietary factors which either promote or limit the likelihood of the individual eating an energy intake which is in excess of their lower energy needs when they are physical inactive. The dominant dietary factor is the overall energy density of the diet and the additional likelihood of drinking energy-containing drinks.[5] A high fat diet is of high energy density[8] and therefore likely to promote overweight because of "passive over-consumption." The DGA 2005 also cite evidence that low fat diets tend to be hypo caloric compared to high fat diets and therefore might decrease the risk of obesity. They use this as an argument for the upper limit of 35 E% from fat. This level of fat intake is lower than many Americans consume but very high when related to the traditional diets of low and middle income countries where cardiovascular disease was unusual until comparatively recently.

A large number of studies on the relationship between fat intake and weight change have been published, both clinical and observational, but the results are conflicting. From two recent extensive reviews the conclusion was drawn that there is insufficient evidence for an association between total fat intake and body weight.[9,10] The fact that body weight is increasing in most European countries in spite of a decreasing trend in the fat content of the diet suggests that factors other than dietary fat *per se* are important determinants for the obesity epidemic. From the available literature one must conclude that in order to maintain or lose weight, calorie adjustment and physical activity are very important, and the calorie adjustment is influenced by the proportion of fat in the diet. However, to limit

the likelihood of having a high energy density diet when one is relatively inactive requires the replacement of dietary fat with unrefined carbohydrates which, in general, have a high fibre content and are therefore bulkier. This is why the IOM and the American Dietary Guidelines 2005 advocate a dietary fat intake which can vary from 20 to 35% of energy depending on both the degree of physical activity and the other dietary components contributing to the energy density of the diet.

The IOM 2002 set a lower acceptable level of 20 E% from fat.[3] This was based on unfavourable effects of a low fat/high carbohydrate diet on blood lipids, i.e. increased triglycerides (TG) and lowering of HDL-cholesterol, increased total cholesterol/HDL-cholesterol ratio, and on the risk of insufficient intakes of polyunsaturated fatty acids (PUFA) and fat soluble vitamins.[3] In the DGA 2005 a modelling system based on dietary intakes in the US suggested, however, that 25 E% from fat was required to meet the desired level of PUFA given the nature of the fats and oils in the US.[11,4] A question that remains is to what extent increased physical activity and avoidance of overweight might counteract any unfavourable effects on blood lipids of a low fat/high refined carbohydrate diet.

The importance of considering the type of carbohydrate in association with changes in fat intake is fundamental. Studies with refined carbohydrate have suggested that there is no benefit in replacing saturated fat with carbohydrate, but these analyses relate to carbohydrates with a low fibre content, where the starch has been refined. The diets also often contain substantial amounts of free sugars. Thus, metabolic studies using refined carbohydrate diets have failed to show improvements in blood lipid levels, and some prospective studies assessing the impact of carbohydrates on cardiovascular disease have suggested that they may not be beneficial. This may not be the case, however, when fat is replaced by carbohydrates high in fibre.[12] Also, the remarkable reduction in cardiovascular death rates in Finland, with a marked fall in both total and saturated fat intakes and an increase in carbohydrate intakes, suggests that the *type* of carbohydrate may be of major public health importance. Finnish diets are recognised as having one of the highest non-starch polysaccharide contents in Europe and North America.[13]

Conclusion on total fat

The above data suggest that a population mean of 30 E% from fat is a reasonable interim target. The figure from the EHN 2002 report should thus be maintained. This is also in line with the recent European Guidelines on Cardiovascular Disease Prevention (ESC Guidelines).[14] European diets are high in animal products and a long-term goal to reduce saturated fat below the current recommendation will necessarily require further reductions in total fat. Reduction of total fat to 20 to 25 E% would thus be a reasonable long term goal and is in keeping with the IOM analyses provided that the quality of the fat is appropriate.

1.1.1.2 Saturated fat

Saturated fatty acids (SAFA) increase plasma total- and LDL-cholesterol which in turn are strongly related to the risk of CHD. The ESC Guidelines state that "There are strong, consistent, and graded relationships between saturated fat intake, blood cholesterol levels and mass occurrence of cardiovascular disease (CVD). The relationships are accepted as causal."[14] The LDL particle is considered the pathogenic factor for the development of atherosclerosis, the pathological basis of CHD. The rationale for the original recommendation to limit intake of saturated fat to 10% of energy was that at this level of intake mean total cholesterol will be around 5.2 mmol/L (200 mg/dL). In the 1970s and early 1980s an adult population mean of 5.2 mmol/L (200 mg/dL) or lower would contribute to a major reduction in CHD as a major public health problem.[15,16] However, based on both animal experiments and human epidemiological and physiological studies, it is now recognised that lower LDL cholesterol levels would be advantageous in limiting atherosclerosis. Thus, a total cholesterol of 3.88 mmol/l (150 mg/dL) or LDL-cholesterol of 2.58 mmol/L (100 mg/dL) would be desirable.[17]

It thus appears that there is no lower limit of cholesterol where the risk of CHD disappears. In the seven countries study CHD mortality was very low in the Japanese and Greek populations.[1] Mean total cholesterol was of the order of 3.6 – 4.4 mmol/L (140-170 mg/dL) and 5.2 mmol/L (200 mg/dL), and saturated fat intake below 5 E% and 7-8 E% respectively. Coronary heart disease has been shown to be almost non-existent in rural China when mean cholesterol levels are of the order of 3.5 mmol/L (135 mg/dL), with total fat intakes about 15%E, and saturated fat intakes extremely low. This has raised the question of whether the goal for total cholesterol in the population should be set lower than 5.17 mmol/L (200 mg/dL). Based on the Chinese studies it has been suggested that in order to reduce CHD mortality to a minimum level the population mean should be reduced to 3.88 mmol/L (150 mg/dL).[18] This would probably require the amount of SAFA to be reduced to a level of around 5%E.[19] In the latest report from the American Heart Association the recommended level of SAFA intake is set at 7 E%[20] and the 2010 Dietary Guidelines for Americans[21] also recommends limiting saturated fat intakes to less than 7% of energy. A similar recommendation has been made for developing countries in Asia with total fat intake set at 21 E%, SAFA at 7 E%, MUFA at 7 E% and PUFA at 7 E%.[22] So far, no other expert groups have recommended such a low level of saturated fat intake.

The strong relationship between saturated fat intake and total- and LDL-cholesterol is based on a very large number of metabolic experiments reviewed in several papers and reports.[3,4,23] Increasing saturated fat also increases the ratio between LDL-cholesterol and HDL-cholesterol, a strong predictor of CHD risk. It should be mentioned, however, that even though HDL-cholesterol is a strong metabolic indicator of a reduced risk of CHD, there is so far no direct evidence that increasing HDL-cholesterol by drugs or diet lowers the risk of CHD.[24]

There is, as stated above, no lower limit for total cholesterol levels below which risk reduces no further. It therefore becomes a matter of judgment what the blood overall goal for total cholesterol level should be for the population. The DGA 2005 recommendations are based on the individuals' LDL-cholesterol level. For those with levels below 3.36 mmol/L (130 mg/dL) less than 10 E% from saturated fat is recommended and for those with elevated LDL-cholesterol (≥ 130 mg/dL) less than 7 E% from saturated fat is recommended.[4] This is consistent with the evidence-based recommendation for individuals made by the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults,[25] and the Committee's meta-analysis of trials and a review of 33 more recent controlled trials on saturated fat intake and health.

In the more recent AHA Scientific Statement on Diet and Lifestyle Recommendations Revision 2006 a limitation on saturated fat intake to less than 7 E% is advocated for all individuals above two years of age.[26] No clear argument is given, however, for the 7 E% limit. It is simply stated: "In view of the positive relationship among dietary saturated fat, LDL cholesterol, and CVD risk, and current US intakes, the AHA now recommends a population-wide goal of <7% of energy."

As part of the Joint FAO/WHO Expert consultation on fat and fatty acids in human nutrition, Elmadfa and Kornsteiner reviewed the reports above and much of the background material.[27] They concluded that the recommendation should be for an intake not exceeding 10 E%, but this is based on a recognition that saturated fatty acid intakes in Europe are on average often appreciably above 10%. The final conclusion of the Expert Consultation is that dietary intakes of SAFAs should not exceed 10 E%.[28]

Recent dietary surveys in European countries have shown intake of saturated fat varying between 8.8 and 14.6 E% (after excluding Romania as an outlier with 26.3 E%).[29] In Scandinavian countries, for example, there has been a remarkable decline in CHD. In Finland and Norway in particular, a reduction in serum total cholesterol explains a large part of the decline and this has happened with a reduction in mean saturated fat intake from about 20% to about 12-13 E%.[30,31] The mean cholesterol levels in the European populations are still high, however. The MONICA study indicated a total blood cholesterol value near 5.8 mmol/L in the early 90s[32] while more recent data from a smaller selection of eight countries gave a mean of 5.3 mmol/L.[33] A further reduction is thus desirable. Whether the goal for saturated fat reduction should extend below 10 E% becomes as stated above, a matter of judgement. From the correlations between saturated fat intake and serum cholesterol given in the IOM 2002 it can be calculated that to reduce total blood cholesterol from 5.5 mmol/L (assumed to be near the population mean for the European population) to the proposed desirable level of 5.17 mmol/L (200 mg/dL) saturated fat intake has to be reduced by about 5 E%. This would favour a recommendation for a population mean closer to 8 E% than to 10 E%. However, the ESC Guidelines propose as one of the objectives of CVD prevention to achieve a total cholesterol level of < 5 mmol/L (190 mg/dL)[34] which implies an even lower saturated fatty acid intake.

Changing one nutrient will inevitably result in changes in other contributors to the diet's energy content and it is therefore necessary to investigate the effect of exchanging saturated fat for other nutrients. The question is what should replace SAFA? In metabolic experiments replacing SAFA by carbohydrates reduces total and LDL cholesterol.[35,36] In individuals with dyslipidemia and the metabolic syndrome, however, carbohydrates may actually result in increased triglycerides (VLDL) and lower HDL cholesterol which could, in theory, increase CVD risk. Large prospective studies in Western populations have in fact shown increased risk when replacing SAFA by carbohydrate (see below).[37] This may appear a paradox since in many parts of the world, e.g. in China and in Japan during the time of the Seven Country studies, the population were consuming <15% total fat and well below 5% saturated fat at a time when CHD was considered a rare medical condition.[38] The reason for this discrepancy may be that these populations are characterised by consuming higher fibre, complex carbohydrates and having considerably higher energy expenditures and lower BMIs compared to affluent populations [39]

Replacing saturated fat by PUFA in Western societies is the simplest way of reducing serum cholesterol on a population scale.[40,41] Prospective studies also show that replacing saturated fat by PUFA lowers the risk of CHD mortality.[42] Studies suggest that replacing SAFA by MUFA is less efficient for cholesterol reduction[40,41] and epidemiological studies show that replacing SAFA by MUFA does not alter the risk.[43] There are, however, concerns that the older studies included in these analyses relate to a period when monounsaturated fatty acids in the diets featured in the studies were from industrial sources—rather than olive oil—and therefore accompanied by *trans* fatty acids which may have influenced the findings.

Considering the strong relationship between saturated fat intake and total cholesterol and risk of CHD one would expect an association between saturated fat intake and mortality from CHD. Such a relationship has not been consistently found, however. The recent extensive meta-analyses of a total of 28 cohort and 16 randomised control studies by Skeaff and Miller yielded disappointing results.[44] No association with intake of saturated fat was found. The most clear cut result was the increase in risk of CHD death and events with intake of *trans* fatty acids. Intake of very long-chain n-3 PUFA (VLCn-3 PUFA) was associated with decreased risk of CHD mortality and events. The results with PUFA intake were somewhat contradictory in that an increase in risk of CHD **death** was seen at the highest category of dietary PUFA while a 5% increase in PUFA intake was associated with a significant reduction in CHD events.[44] The authors discuss the shortcomings of this kind of study, in particular measurement errors, regression dilution bias, confounding etc.

A more recent meta-analysis of such studies also failed to find consistent associations between intake of saturated fat and risk of CHD.[45] This does not mean, however, that no such association exists. One problem with these studies is the adjustment for confounders, as seriously criticised in an editorial relating to this meta-analysis.[46] A major error in that analysis is the adjustment for serum cholesterol in six of the 16 studies that included CHD as the endpoint. SAFAs strongly affect blood cholesterol levels which

means that adjustment for the cholesterol level removes the biomarker of the mechanism for generating CVD. This approach will, therefore, bias the results of the meta-analyses towards finding no association between dietary saturated fat intake and CVD. The marked individual variation in cholesterol responses to saturated fat intakes is well recognised to have a genetic origin. This variation also means that, in cohort studies within a single population with a relatively narrow range of SAFA intakes, it will be difficult if not impossible,[47] to discern the link between SAFAs and heart disease. Keys' multi-country studies allowed a much greater range of saturated fat intakes to be related to coronary heart disease and demonstrated the very clear relationship with blood cholesterol levels, a finding which was subsequently repeatedly confirmed.

Another main problem with studies on the effects of diet on health outcomes is the difficulty of obtaining reliable data for food intake. Most often food frequency questionnaires are used. With this method misclassification is considerable and it has been questioned whether it is at all reliable.[48,49] Any misclassification or random errors in the measurements will result in reduced association eventually to zero compared to any true association. Attempts to overcome this difficulty by selecting a biomarker for particular fat intakes—e.g. C17:0 as an index of dairy fat intake—are subject to substantial errors and do not necessarily provide an appropriate guide to dietary saturated fat intakes.[50]

An additional problem arises when attempts are made to relate animal food intakes to CVD, because different animal feeding systems will alter both the total amount of fat in the milk or carcass, and its fatty acid composition.

The recent meta-analysis[45] which failed to show a link between SAFA and CVD was also unable to study the effect of substituting one nutrient for another. This was the main focus of another recent pooled analysis of 11 cohort studies selected based on strict quality criteria, in particular the quality of dietary assessment.[37] Among the 344,696 persons included 5,249 coronary events and 2,155 coronary deaths occurred during a follow up of 4-10 years. The main finding was a significant decreased risk of CHD death and events when 5 E% of SAFA was replaced by PUFA in the diet. Replacement of SAFA by refined carbohydrate increased the risk slightly while no effect on the prevailing risk was observed when SAFA was replaced by MUFA.[37] In an editorial these results were discussed in relation to observations of earlier clinical trials with high P/S diets and the effects of PUFA on LDL-cholesterol and the ratio of total- to HDL-cholesterol and the conclusion was drawn that the studies suggest towards a risk reduction of PUFA substitution for SAFA of similar magnitude.[51] It seems reasonable, therefore, to conclude that there remains strong evidence that PUFA lowers risk of CHD when replacing SAFA in the diet. Interestingly, the findings of a recent large Danish prospective cohort study suggested that replacing SAFA with carbohydrates with high glycemic index (GI) was associated with increased risk of myocardial infarction while replacing SAFA with carbohydrates with low GI (i.e. fibre-rich) was associated with reduced risk.[52] This supports the contention that replacing SAFA with complex carbohydrates lowers the risk of coronary heart disease.

Conclusion on SAFA

Based on the above, a population mean of 8 to 10 E% (i.e. 9 E%) from SAFA keeps the cholesterol level at a level of < 5 mmol/L and this is compatible as an interim target for inducing a lower CVD risk in Europe. A longer term goal would be to reduce the risk further by reducing SAFA intake as much as possible, e.g below 7 E%. A reduction below a SAFA intake of 7 E% would, in practice, require an appreciable reduction in animal and vegetable SAFA fat intakes in many European diets.

1.1.1.3 Polyunsaturated fatty acids (PUFA)

This section summarises the latest evidence on polyunsaturated fatty acids, starting with linoleic acid (LA, 18:2 n-6), followed by alpha-linolenic acid (ALA, 18:3 n-3) and then very long chain n-3 fatty acids.

1.1.1.3.1 Linoleic acid

Linoleic acid and alphalinolenic acid cannot be formed in the body and are thus essential fatty acids. The estimated minimal daily requirement of LA to avoid overt clinical symptoms is probably of the order of 1 to 2% of energy intake.[9] Because of the low conversion of ALA to EPA a small amount of EPA (and DHA?) may also be required for optimal health. LA and ALA lower total- and LDL-cholesterol and already in the 1960s and 70s clinical trials indicated that increased intake of PUFA lowered risk of CHD. Based on reviews of the totality of studies performed since then[9,37,53,54] it is safe to conclude that there is now convincing evidence that increased intake of PUFA decreases the risk of CHD. The risk reduction is particularly important when PUFA are replacing SAFA in the diet and the reduction is seen over a wide range of PUFA intake [37, 53]

Based on the effects on total- and LDL-cholesterol and the results from observational and intervention studies a range from 6 E% to 11E% of PUFA has been proposed by FAO, following the FAO/WHO Expert Consultation in 2008, as acceptable for CHD prevention.[28] The AHA Science Advisory 2009 advocates an n-6 PUFA intake of at least 5% to 10%.[53] Adding about 1 E% as ALA to the proposed n-6 PUFA target then makes these two sets of recommendations essentially identical. As to the lower range it is notable that the reduction in CHD mortality in most European countries has been associated with an increase in PUFA intake.[30,31,55] Currently the PUFA intake is around 5 to 7% of energy in most European countries.[29] In the AHA Science Advisory 2009 report it is argued that there is little evidence to support a harmful effect of a omega-6 PUFA intake of up to 10% of energy and that even higher intakes may be safe and even beneficial.[53] In support of this conclusion are several studies including one from Israel where 25% of the population consumes >12% of energy as LA and where an inverse

association was found between the concentration of LA in adipose tissue biopsies and the incidence of acute myocardial infarction.[56]

Recent analyses have been interpreted as suggesting that intake of LA is so beneficial that the upper recommended limit of 10E% linoleic acid should be reconsidered and increased.[53,57,58] The prospective studies cited above,[59] together with a re-evaluation of eight earlier randomised controlled trials (RCTs), are presented as support for such a view.[57] The aim of these trials was to test if a reduction in serum cholesterol by diet would lower the risk of CHD. At that time a low SAFA/high PUFA diet was known as the most efficient way of reducing serum cholesterol. PUFA intake in the trials varied between eight and 20 E%. Seven of these eight trials showed a reduction in CHD events. Based on the reported intake of PUFA the reduction in risk was estimated to be 13% for each 5 E% increase in PUFA. However, the use of the results of these RCTs as an argument for a high consumption of n-6 PUFA was recently strongly opposed.[60] The basis for the different perspective was that some of the trials had weak designs and should have been excluded (e.g. the two Helsinki mental hospital trials), whereas other trials with an unfavourable outcome should have been included, particularly those that used a high corn oil diet (which is very low in ALA). When dividing the trials into those that included both LA and ALA, rather than LA alone, then a reduction in risk was observed when an appreciable amount of ALA was included in the PUFA whereas those trials that used corn oil (containing LA but almost no ALA) showed an increase in risk.[60] Furthermore, in all the trials PUFA substituted not only SAFA but probably also an appreciable amount of *trans* fatty acids (TFA), so the lowering of risk may to a large extent be explained by a reduced intake of TFA rather than increase in PUFA.

It should be added that the aim of all these trials was to test if a reduction of serum cholesterol by diet would reduce the risk of CHD and they were not designed to test optimal intakes of any PUFAs. Unfortunately, the meta-analyses[57,60] of these different trials do not include data for effects on serum cholesterol and how this related to analysed changes in CHD risk.

We may conclude that at the present time there is insufficient evidence to advocate particularly high intake of linoleic acid and that care should be taken to ensure that PUFA in the diet includes ALA and the very long chain n-3 fatty acids in addition to LA. Given the potential, although much debated, concern for the proposed inflammatory and prooxidative properties of n-6 PUFA[61,62] their inhibitory effects on the metabolic conversion of the n-3 series of fatty acids to important biologically active leucotrenes, a prudent policy seems sensible. This cautious approach is also reasonable because no population has yet been exposed to life long very high intakes of PUFA. For these reasons one should not propose on the basis of current evidence unnecessarily large increases in n-6 PUFA intakes.

<u>Conclusion on PUFA</u>

The evidence is in keeping with the FAO/WHO Expert Consultation recommendation that the intake of PUFA (LA plus ALA) should be between six and 11% of energy based on a total fat content of 30 E%. A longer-term goal, which takes account of lower total fat and saturated fatty intakes, would require a PUFA intake of between five and eight per cent of energy, based on a proportionate reduction in PUFA intakes.

1.1.1.4 Alphalinolenic acid (ALA)

Alphalinolenic acid (ALA) is an essential fatty acid and an intake of 0.5 to 1 E% will cover the minimum requirement.[9] ALA reduces total- and LDL-cholesterol in a manner similar to that of LA. It may therefore be inferred that it also reduces the risk of CHD when replacing saturated fat. Epidemiological studies have, however, given conflicting results. While some have demonstrated a beneficial association between ALA intake and risk of CHD[63] a review of prospective cohort and randomised trials could not demonstrate a significant association between ALA intake and risk of CHD events or CHD deaths.[44]

The n-6 and n-3 fatty acids use the same series of enzymes for their metabolism—in fact they compete for them. In-vitro research suggests that the important Δ6 desaturase enzyme prefers alphalinolenic acid to linoleic acid, followed by oleic acid.[64]

In view of the essential biological need for ALA, its cholesterol lowering effect and to allow for competition between the metabolic pathways of the n-3 and n-6 fatty acids in generating important metabolites of major importance for cell regulation, it is recommended that ALA makes up at least 1 to 2%E so that over the range of total PUFA intake the ratio of n-6 to n-3 does not exceed five. However, it should be noted that there is no consensus in the literature about any optimal level or the usefulness of such a ratio, and for planning purposes a ratio of n-6 to n-3 PUFA between three and nine has been proposed to be adequate.[65]

1.1.1.5 Very long-chain n-3 fatty acids (VLCn-3 PUFA)

From several recent reviews one can draw the conclusion that there is convincing evidence that intake of VLCn-3 PUFA in the form of fish or as supplements of EPA and DHA reduces the risk of CHD death and sudden cardiac death and possibly CHD events in patients with heart disease.[27,44,66,67,68] A pooled analysis of both prospective cohort studies and randomised clinical studies in individuals with pre-existing CHD demonstrated a 36% lower risk of CHD death at an estimated intake of 250 mg/d of EPA + DHA with hardly any further decrease above this level.[67] It should be noted, however, that when related to fish consumption one to two servings of (oily) fish per week, i.e. approximately 250 to 500 mg per day (mg/d) was associated with near maximal reduction in risk.[69] After having reviewed the available quantitative evidence a recent symposium came to the following conclusion: “Consumption of 250 mg DHA and EPA per day, from either dietary or supplement sources, should be part of management for primary

prevention of CHD death and after a coronary event to reduce risk of CHD death. Given the uncertainty of this estimated target intake and no evidence for harm at higher intakes, a target of 250–500 mg/d EPA plus DHA is reasonable. Differences based on relative amounts of EPA versus DHA, if any, still need to be determined".[70]

Conclusion on VLCn-3 PUFA

There is convincing evidence that intake of VLCn-3 PUFA reduces the risk of CHD. The current state of knowledge supports a recommendation of a minimum intake of 250 mg/d of VLCn-3 PUFA as a population goal for the prevention of CHD. This is equivalent to two portions of fish per week.

1.1.1.6 Monounsaturated fatty acids (MUFA)

Oleic acid (18:1 n-9) is the most abundant fatty acid in food and in the organism. Meat and dairy fat are the most abundant sources in western diets. Vegetable oils like olive oil and rape seed oil are also important sources. Olive oil may be a dominating source of oleic acid in olive growing Mediterranean countries. Small amounts of 16:1 and 18:1 n-7 are also present in small amounts in foods. However, very long chain monoenes like erucic acid (22:1n-9) in rape seed oil and mustard oil and cetoleic acid (22:1 n-11) in marine oils have been suspected of having cardiotoxic effects in animals. In view of these concerns, the rape seed oil now on the market as canola oil is practically devoid of erucic acid.

There is no physiological need for oleic acid since it can be rapidly formed by desaturation of stearic acid by an omnipresent steaoryl-CoA desaturase.

Metabolic experiments have shown that oleic acid when replacing carbohydrates in the diet has practically no effect on total cholesterol or LDL-cholesterol but increases slightly HDL cholesterol and decreases triglycerides.[36,71] When replacing SAFA and *trans* fatty acids LDL-cholesterol is reduced while HDL-cholesterol remains unchanged.[36,72] A diet high in oleic acid appears to have a more favourable effect on HDL-cholesterol and triglycerides than a diet high in complex carbohydrates.[73] Prospective cohort studies have given more mixed results. This is understandable since the results might be confounded by changes in the intake of saturated fatty acids where the main sources are meat and milk fat and by different polyunsaturated fat intakes where the source of MUFAs is mainly vegetable oils. In the most recent meta-analysis no association with risk of CHD death was found when SAFA were replaced by MUFA.[37]

Conclusion on Mono-unsaturated fatty acids

Since there is no physiological need for oleic acid and the risk reduction linked to MUFA *per se* is, if present, rather modest then any recommendation has to be set on the basis of simply providing the fat which accounts for the difference between total fat and the sum

of SAFA, PUFA and TFA intakes. A distribution of <10 E% SAFA, 6-11 E% PUFA and <1 E% TFA and a total of 30 E% would leave 8 to 13 E% for MUFA. In most European countries the intake of MUFA is around 12-13 E% (Greece is an exception with 22 E%) when based on food availability data[29] while around 11 E% when based on surveys.[74] A longer-term goal for MUFA intakes, taking into account the reductions in total, saturated and *trans* fatty intakes, is between 7.5 and 9.5 E%.

1.1.1.7 *Trans* fatty acids (TFA)

Trans fatty acids (TFA) are formed by bio-hydrogenation in the rumen or industrially by partial hydrogenation of vegetable and marine oils. During heat treatment of fats and oils by processes such as deodorisation, cooking, frying etc a small amount of *trans* bonds may be formed.[75] These processes can affect the chain length, number of double bonds and the unusual position of *cis* bonds and the insertion of *trans* double bonds means that the resulting fats can have an almost uncountable number of isomers with a number of unnatural *cis*-isomers and a wide array of, often unknown, metabolic effects.[76] Any specific health effects of these different isomers are, therefore, also unknown. Because of their effect on total cholesterol, TFAs were for long considered similar to saturated fatty acids, while analytically they were traditionally included in the monounsaturated fatty acids category. This, in practice, meant that there was an overestimation of the content of monounsaturated fatty acids in the diet. This situation changed with the publication of Mensink and Katan who demonstrated that TFAs increased LDL-cholesterol but also decreased HDL cholesterol with a magnitude of effect on a weight-to-weight basis which implied they could be more harmful than SAFA.[73] Since then a large number of publications have appeared demonstrating that in fact the intake of *trans* fatty acids is associated with an increased risk of CHD and this risk may be two to three times higher than that of an equivalent intake of SAFA.[77,78] Replacement of TFA by any other fatty acids may thus be potentially favourable. It should be noted that the comparison between TFA and SAFA is done with a mixture of SAFA in an ordinary diet i.e. including fatty acids that may not be associated with CHD risk. No attempt has been made to compare with palmitic acid, an important cholesterol increasing fatty acid in the diet. The widespread replacement of *trans* fat by palm oil with increasing intake of palmitic acid is thus of limited benefit.[79]

A WHO Scientific Update on *Trans* Fatty Acids was presented at a meeting in Geneva in 2007 and extensive scientific reviews of the participating experts have recently been published.[80] The conclusions from this Update are quite clear: there is convincing evidence that the intake of *trans* fatty acids is associated with an increased risk of CHD. TFA produced by partial hydrogenation should be considered as an industrial food additive, having no demonstrable health benefits and posing a clear risks to human health. The evidence on the effects of TFA and disease outcomes strongly supports the need to remove partially hydrogenated vegetable oil from the human food supply.

Ruminant TFA and industrially produced TFA have similar metabolic effects.[81,82] The ruminant TFAs cannot, however, be removed from the diet unless one avoids all ruminant-sourced fats. Their intake is low in most populations, generally below 0.5 E%[82] and therefore probably not of any significant health concern. Small amounts of TFA also result from refining of oils, particularly during deodorisation. A realistic upper limit of total intake of TFA has been set at 1 E% by WHO.[5] Removal of all partially hydrogenated fat from the food chain, selecting optimal conditions for refining of oils and some reduction in ruminant meat and dairy fat makes it possible to set an upper limit of 0.5 E%.

Conclusion on *trans* fatty acids

There is convincing evidence that intake of *trans* fatty acids is associated with increased risk of CHD and more so than the intake of SAFA. The intake should be limited to <0.5 E%.

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