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Dietary fats and cardiovascular health: a summary of the scientific evidence and current debate

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ABSTRACT

This narrative review summarises the main studies of the role of the different fatty acids in coronary heart disease (CHD) and cardiovascular disease (CVD) risk and the current scientific debate on dietary recommendations. Reduction and substitution of the saturated fatty acids (SFAs) with the polyunsaturated fatty acids (PUFAs) are still the main dietary recommendation to prevent CHD and CVD. In the last few years, however, the strength of the scientific evidence underlying this dietary advice has been questioned. Recent investigations reappraise the previously declared deleterious role of the SFAs and reduce the positive role of PUFAs, mainly the omega-6, whereas the role of monounsaturated fatty acids (MUFAs) remains unclear. In contrast, the negative effects of trans fatty acids (TFAs) seem stronger than previously thought. Finally, criticisms have emerged from a dietary recommendation approach focussed on individual components rather than on wide food items and eating habits.

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Introduction

Fatty acids are the most abundant components of almost all lipids, and in the human body range from 15% (in men) to 18–20% (in women), with important roles at every level of cell life (German and Dillard 2010). They are classified mainly on the basis of the length of the carbon chain and the degree of saturation, which affects important properties, such as chemical stability and melting temperature (Fahy et al. 2011). Fats rich in saturated fatty acids (SFAs), e.g. animal fats are solid at room temperature, while fats rich in monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs), e.g. vegetable oils are liquid at room temperature.

Hydrogenation is a chemical process which – typically in the presence of a catalyst, such as nickel or platinum – involves the addition of pairs of hydrogen atoms to a molecule. Fat hydrogenation has been extensively developed in order to reduce the number of double bonds (i.e. boost the degree of saturation) of vegetable and marine oil MUFAs and PUFAs, to increase their hardness and chemical stability, and to create solid fats more suitable for certain applications in the food industry (e.g. spreadable fats like margarine).

The use of hydrogenated fats rose from the 1960s to the 1980s to replace animal fats, which at that time were considered unhealthy, with vegetable oils (Lichtenstein 2014). However, hydrogenation leads to the formation of trans fatty acids (TFAs), which contain one or more double bonds in the trans conformation (carbon atoms on the opposite side of the double bond). Naturally, occurring MUFAs and PUFAs are in cis conformation (carbon atoms on the same side as the double bond) with the exception of some fats produced through a natural process of anaerobic bacterial fermentation in the rumen, which leads to small amounts of naturally present TFAs in milk and other dairy products.

Fatty acids are molecules with a wide spectrum of biological activity besides their main function, which is to provide energy. They are important for energy storage, they have a structural function, as the essential constituents of all cell membranes, they are the precursors for the synthesis of hormones and bile salts, and they permit the absorption of fat-soluble vitamins (e.g. vitamin A). They also account for half of the energy in human milk and are required for normal growth and physical activity in infants; it has been suggested that low fat intake in early life could

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Figure 1. The hierarchy of clinical evidence given by the study designs to establish a cause and effect relationship. Modified from Rosner (2012).

increase the susceptibility to overweight and leptin resistance at later ages (Rolland-Cachera et al. 2013).

Despite their vital functions, in the last 50 years recommendations have been put forward to reduce total fat consumption, and to use the healthier unsaturated fatty acids instead of the unhealthy SFAs. This became the main dietary recommendation to prevent coronary heart disease (CHD) and cardiovascular diseases (CVD) (Aranceta and Pérez-Rodrigo 2012). In the last few years, however, some authors (Chowdhury et al. 2014; Harcombe et al. 2015; Ramsden et al. 2016) have questioned the soundness and strength of the scientific evidence underlying the current and previous dietary advice.

This narrative review summarises the evidence on the role of the different fatty acids in CHD and CVD risk and the scientific debate on current dietary recommendations, focussing on the study designs in the upper part of the hierarchy of the clinical evidence (Rosner 2012) (Figure 1).

Saturated fatty acids

The link between SFAs and the risk of CHD and CVD was postulated in the 1950s from the studies of the American physiologist Ancel Keys. The Seven Countries Study (Keys et al. 1984; Keys et al. 1986; Menotti et al. 1989; Kronenberg et al. 1999) investigated whether there were real differences in the incidence of CHD among different populations and whether certain characteristics, including dietary habits, could explain the differences. The study enrolled

>12,000 men, aged between 49 and 50 years, from seven countries: Italy, United States, Finland, Yugoslavia, Greece, Japan, and The Netherlands. There were large differences between countries in CHD and all-cause mortality. Serum cholesterol was related to different outcomes in different cohorts. SFAs, as a percentage of calories were deemed the most powerful lifestyle predictors of heart diseases, with MUFAs, PUFAs and carbohydrates having possible protective roles (Menotti et al. 1989). The Keys equation showed that cholesterol levels could be predicted from saturated fat intake. These studies led to the “lipid theory” which stated that a diet rich in SFAs caused an increase in serum cholesterol, which would deposit in the arterial wall, accelerating the progression of atherosclerosis, and increasing the risk of CVD and CHD events.

Although the Ancel Keys studies were criticised by researchers at the time (Yerushalmy and Hilleboe 1957), they had great influence on subsequent nutritional research efforts and food policies (Lamarche and Couture 2014). In the USA, dietary recommendations to reduce overall fat consumption to 30% of total energy intake and SFAs to 10% were introduced in 1977. Since the 1980s, the reduction of total fats, particularly saturated ones, has been the main target of dietary recommendations in order to lower morbidity and mortality related to CVD (Aranceta and Pérez-Rodrigo 2012). The European Food Safety Authority (EFSA) proposed that SFA intake should be “as low as possible”, while the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) set recommended intakes at 20–35% for total fat, 10% for SFAs, up to 15–20% for MUFAs and 6–11% for PUFAs, in relation to the total energy intake (FAO/WHO 2010). Although these recommendations focussed on the reduction of serum cholesterol, most of them did not include limits for dietary cholesterol intake, since its neutral role in CHD/CVD risk (Fernandez and Calle 2010).

Subsequent to the Seven Countries Study, large cohort studies investigated the effects of dietary fats on health outcomes (Kushi et al. 1985; Ascherio et al. 1996; Hu et al. 1997; Gillman et al. 1997; Mozaffarian et al. 2004) and not all actually supported Keys’ lipid theory. Gillman and co-workers, for instance, even found an inverse association between dietary SFAs and ischaemic stroke in a cohort of 832 men free of cardiovascular disease at baseline (Gillman et al. 1997). The Women’s Health Initiative Randomized Controlled Dietary Modification study, involving 48,835 post-menopausal women following a total dietary and saturated fats reduction, failed to show any

significant reduction in the risk of CVD, after 8.1 years of follow-up (Howard et al. 2006). In the Multi-Ethnic Study of Atherosclerosis, the association of SFA consumption from different foods and the incidence of cardiovascular events was investigated in a population of 5209 people aged 45–84 years at baseline. After adjustment for various confounding factors, the results indicated that the association between SFAs and health depended on food-specific fatty acids or other nutrient constituents in foods that contained SFAs. Indeed SFAs in dairy products resulted in a lower associated risk of CVD, whereas SFAs from meat were associated with a higher risk (de Oliveira Otto et al. 2012).

Siri-Tarino and co-workers published a meta-analysis of prospective cohort studies evaluating the association of saturated fats with CVD (Siri-Tarino et al. 2010). They identified 21 studies, involving 347,747 subjects for a follow-up of 5–23 years. They found no association between fat intake and risk of CVD. Another systematic review and meta-analysis of randomised controlled trials (RCTs) found benefits when PUFAs were substituted for SFAs (Mozaffarian et al. 2010). The same group (Micha and Mozaffarian 2010) also reviewed the evidence from RCTs of lipid and non-lipid risk factors. In comparison with carbohydrates, the total-/high-density lipoprotein (HDL) cholesterol ratio was not significantly affected by myristic or palmitic acid, non-significantly reduced by stearic acid, and significantly lowered by lauric acid.

Evidence for the effects of SFA consumption on other risk predictors (e.g. vascular function, insulin resistance and diabetes) was mixed, with many studies finding no clear effects. The study stressed that the health benefit of reducing saturated fats depended on the replacement nutrients. Replacement with PUFAs for 5% of energy reduced heart disease risk by 10%. Replacement with MUFAs had uncertain effects and replacement with carbohydrates did not give any benefit. The authors concluded that the policies aimed at prioritising saturated fat reduction without considering the replacement nutrients had little or no effect on cardiovascular risk.

An intervention review of The Cochrane Collaboration (Hooper et al. 2015) assessed the effect of reducing dietary SFAs and replacing them with PUFAs, MUFAs, carbohydrates and proteins on mortality or cardiovascular morbidity. Fifteen RCTs, comprising about 59,000 participants showed a significant reduction in risk of cardiovascular events when SFAs were replaced with PUFAs but no effect when they were replaced with carbohydrates and proteins. Some recent extensive systematic reviews and meta-analyses have analysed the relations between dietary SFAs and

health outcomes. The first (Chowdhury et al. 2014) reviewed the links between dietary, circulating, and supplement fatty acids and coronary risk. The authors found essentially null associations between dietary and circulating SFAs and risk of coronary events. Circulating SFAs only partially reflect the corresponding dietary intake since they combine the consumption of SFAs through the diet and the metabolism with endogenous *de novo* synthesis. The only significant results with SFAs were an inverse relation between circulating margaric acid (a minor SFA in milk and dairy fat) and coronary risk.

The second study (Harcombe et al. 2015) reviewed the evidence from RCTs available at the time of issuing the dietary guidelines on reduction of total fat and SFA intake in the USA in 1977 and in the UK in 1983. The authors found no difference for all-cause mortality and a non-significant difference in spite of reductions in serum cholesterol levels.

The third study reviewed the evidence from observational studies of the association between the intake of dietary SFAs and risk of mortality for all causes, CVD, CHD, ischaemic stroke and type 2 diabetes (de Souza et al. 2015). There were no associations with any of these health endpoints.

Ramsden et al. (2016) published the unpublished findings from the Minnesota Coronary Experiment. This double-blind RCT was conducted between 1968 and 1973 to test whether replacing saturated fat with vegetable oil rich in linoleic acid reduced CHD risk. No benefit was seen for the intervention group in the full randomised cohort or for any prespecified subgroup, despite significant reductions in serum cholesterol. In the same paper, the authors also updated a systematic review and meta-analysis of RCTs assessing the replacement of SFAs with vegetable oils rich in linoleic acid on CHD mortality: the results showed no evidence of benefit.

Another study published in the same journal and in the same period (Zong et al. 2016) reported the results of a prospective, longitudinal cohort study, involving the cohort of 73,147 women (Nurses' Health Study) and 42,635 men (Health Professionals Study) who were free of major chronic diseases at baseline. Higher dietary intakes of major SFAs were associated with an increase in the risk of CHD. There was an advantage in terms of CHD risk when 1% of the energy from SFAs was replaced with PUFAs, MUFAs, whole-grain carbohydrates and plant proteins.

Finally, Dehghan and colleagues published the results of the Prospective Urban Rural Epidemiology (PURE) a large cohort study on the association of fats and carbohydrates with CVD and mortality

(Dehghan et al. 2017). The study enrolled >135,000 subjects aged 35–70 years in 18 countries, with a median follow-up of 7.4 years. High intake of SFAs was associated with lower risk of total mortality and stroke and not associated with major CVD outcomes.

In conclusion, some large prospective cohort studies found direct associations between SFA intake and CHD/CVD events, but others – and most of the RCTs – did not. Moreover, the most recent meta-analyses failed to find significant effects and reappraised the generally assumed deleterious role of the SFAs on cardiovascular health. It is important to bear in mind, however, that both observational studies and RCTs have intrinsic methodological problems in the nutritional field.

Omega-3 polyunsaturated fatty acids

Interest in PUFAs can be traced back to the 1970s and to the epidemiological studies by Bang and Dyerberg (1972), Bang et al. (1980) and Bang and Dyerberg (1987). The two researchers noticed that among the Inuit (former Eskimo) population, living in their country of origin (Greenland), death from ischaemic heart diseases constituted only 3.5% of all deaths, despite a lifespan of >60 years. Subsequently, the researchers analysed the serum lipid profiles in an Inuit sample population (Bang and Dyerberg 1972), in order to seek an explanation. In comparison to the Danish population control samples, they found lower levels of total cholesterol, triacylglycerols, low-density lipoprotein (LDL) cholesterol and very-low-density lipoproteins and higher levels of HDL cholesterol. One of the more marked differences was that the PUFAs of the omega-6 family were replaced by the omega-3 family: in particular, arachidonic acid was replaced by eicosapentaenoic acid (EPA). This was attributed to a different diet and not to genetics, since Inuit living in Denmark had lipid profiles similar to the Danish population. Examination of the composition of Inuit food later on (Bang et al. 1980) showed that seal and fish were the main items and the marked differences between Inuit and Danish food were related to PUFAs, and to the ratio of PUFAs to SFAs. In addition, the Inuit population had a higher intake of long-chain omega-3 PUFAs – EPA, docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) – than the Danes (13.1 in comparison to 0.8% of the total fatty acid intake). The researchers concluded that the rarity of ischaemic heart disease among the Greenland Inuits could be partly explained by the antithrombotic effect of long-chained PUFAs, especially EPA, prevalent in diets rich in marine oils.

Since then, over the years a large number of observational and experimental studies (mostly clinical trials) and meta-analyses have been reported, making the omega-3 PUFAs the nutrients most investigated in relation to cardiovascular outcomes. Most of the findings indicated that consumption of fish oil significantly reduced mortality from cardiac causes (Mozaffarian and Rimm 2006; León et al. 2008).

To date, the *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico* (GISSI) study (Tavazzi et al. 2008) was the first, and one of the most important, large-scale clinical trials assessing the effect of omega-3 PUFAs on morbidity and mortality in a large population of patients with symptomatic heart failure of any cause. It was a randomised, double-blind, placebo-controlled, multicentre study, involving 7046 patients randomly allocated to treatment with 1 g daily of omega-3-PUFAs or placebo, in addition to secondary prevention drugs. Primary endpoints were time to death or admission to hospital for cardiovascular reasons. Long-term use of omega-3 PUFAs (median follow-up 3.9 years) reduced the primary endpoints by about 10%.

The JELIS trial, conducted in Japan on 18,645 patients, tested the effect of EPA on major coronary events. Hypercholesterolemic patients were randomly assigned to receive 1.8 g of EPA daily with a statin, or a statin only, with a 5-year follow-up. At a mean follow-up of 4.6 years, there was a significant decrease in major coronary events, in unstable angina and non-fatal coronary events. Serum LDL cholesterol decreased but was not a significant factor in the reduction of risk for coronary events (Yokoyama et al. 2007).

However, not all clinical trials confirmed the reduction in cardiovascular outcomes after omega-3 PUFA intake. For instance, the diet and reinfarction trial (DART-2) study, involving patients with stable angina, gave opposite results, with an increase in mortality from cardiovascular and sudden death risk among subjects advised to eat oily fish (Burr et al. 2003). In a multicentre, double-blind, placebo-controlled trial on 4837 patients who had had a myocardial infarction and were receiving state-of-the-art therapy, Kromhout et al. (2010) assessed the effect of low doses of marine omega-3 PUFAs (EPA and DHA) and the plant omega-3 alpha-linolenic acid. Low supplementation with EPA, DHA or alpha-linolenic acid did not significantly reduce outcomes in these patients. Among the different reasons offered to explain the discrepancy from the results of the GISSI and other trials, were differences between patient populations and the improvement in cardioprotective drug treatment,

which made it harder to prove any beneficial effect of low doses of EPA–DHA.

In a systematic review and meta-analysis conducted in 2012 on 20 RCTs, comprising 68,680 patients, evaluating the effect of omega-3 PUFA supplementation in adults, Rizos et al. (2012) concluded that supplementation of omega-3 PUFAs was not associated with a lower risk of all-cause mortality, sudden death, myocardial infarction or stroke.

A later double-blind clinical trial enrolled a cohort of patients with multiple cardiovascular risk factors or atherosclerotic vascular disease but not myocardial infarction (Risk and Prevention Study Collaborative Group et al. 2013). The patients were assigned to omega-3 PUFAs (1 g per day) or placebo (olive oil). The study found no difference between the two groups in the proportion of patients who died from any of the other identified causes; there was no difference in the number of hospital admissions for cardiovascular causes but there were significantly fewer admissions for heart failure among patients who received omega-3 fatty acids compared to those assigned placebo. The authors concluded that omega-3 fatty acids gave no significant benefit in reducing the risk of death from cardiovascular causes or hospital admission for cardiovascular causes.

A subsequent comprehensive meta-analysis of both cohort and intervention studies (Chowdhury et al. 2014) reviewed the relation between dietary, circulating, and supplement fatty acids and coronary risk and found a significant inverse association for coronary outcomes in cohort studies with dietary long-chain omega-3 PUFAs. Coronary events were also inversely related with circulating long-chain omega-3 PUFAs. RCTs on long-chain omega-3 PUFA supplements, however, failed to find any significant reduction. Finally, a more recent comprehensive quantitative assessment of the relation between these fatty acids and CHD risk (Alexander et al. 2017) confirmed these results. The authors found a significant reduction of coronary risk in prospective cohort studies but not in RCTs. However, results of subgroup analysis showed a reduction of CHD risk among higher-risk population.

In summary, since the 1970s PUFAs have been considered healthy for the cardiovascular system. Worldwide dietary recommendations, besides discouraging SFA intake, suggested increasing the consumption of omega-3 PUFAs. The beneficial effects of these fats were supported by case-control and cohort studies (Siscovick et al. 1995; Albert et al. 1998; Albert et al. 2002; Albert et al. 2005), and by basic research showing that these fatty acids reduce cardiac arrhythmias (Kang and Leaf 1996; Billman et al. 1999) and stabilise

atherosclerotic plaques (Thies et al. 2003), two mechanisms that could explain the reduction of cardiovascular risk. However, the most recent meta-analyses of RCTs did not confirm that omega-3 PUFA supplementation is associated with a reduced risk of CHD events.

Omega-6 polyunsaturated fatty acids

Most of the evidence for a protective role of omega-6 PUFAs comes from cohort studies where PUFAs included both omega-3 and omega-6 fatty acids but primarily linoleic acid (Jakobsen et al. 2009). However, a more recent meta-analysis specifically considering total omega-6 fatty acids failed to find any significant decrease in coronary events (Chowdhury et al. 2014).

Only a few RCTs on cardiovascular risk have investigated the effect of omega-6 specific PUFAs without a substantial contribution of omega-3 PUFAs (Rose et al. 1965; Woodhill et al. 1978; Frantz et al. 1989). No benefits of these interventions have been shown, but rather an increase of risk has been seen. The double-blind trial on 80 patients with ischaemic heart disease by Rose et al. (1965), for instance, showed that patients receiving corn oil (a high omega-6 PUFA oil) fared worse than those in the control groups. The authors concluded that under the circumstances of that trial, corn oil cannot be recommended in the treatment of ischaemic heart disease.

Ramsden et al. (2013) published an analysis of data recovered from the Sidney Diet Heart Study, a single-blind RCT for secondary prevention conducted in 1966–1973 on 458 men with a recent coronary event (Woodhill et al. 1978). The intervention involved the replacement of dietary saturated fats with omega-6 PUFAs (linoleic acid). There were significant increases in the intervention group for mortality for all causes, CHD and CVD. The authors also updated a previous systematic review and meta-analysis of the effects of PUFA interventions on risk of CHD, finding no evidence of benefits. They suggested that the differences between the results from different trials were mainly due to the heterogeneity of the individual PUFAs under investigation. Trials using vegetable fats as sources of PUFAs and as a consequence mainly linoleic acid, an omega-6 PUFA showed a tendency to an increased risk of mortality for CHD, whereas trials using omega-3 PUFAs showed benefits in terms of cardiovascular risk. Thus trials whose interventions provided mixed omega-3 and omega-6 PUFAs showed benefits in terms of cardiovascular risk due to the

contribution of the omega-3 PUFA (Ramsden et al. 2013).

Finally, a recent meta-analysis on the effect of replacing SFAs with omega-6 PUFAs (Hamley 2017) used a novel approach, which was to identify the trials with substantial confounding variables, so to classify them as adequately or inadequately controlled, and then to do separate subgroup analysis for each category. Pooling the results of only the adequately controlled trials, indicated no effect for major and total CHD events and for total mortality, whereas the pooled results including all trials showed a significant reduction in major CHD events but not in total CHD events or total mortality. The authors concluded that it was unlikely that replacing SFAs with omega-6 PUFAs would reduce CHD events and mortality.

In summary, there is little evidence for a protective role of omega-6 PUFAs of vegetable origin. The few prospective cohort studies and RCT with linoleic acid (the most abundant PUFA in the diet) failed to find any significant trend towards lower risk of CVD and CHD.

Monounsaturated fatty acids

The general consensus about the favourable effect of the Mediterranean diet for CHD prevention has suggested that a diet rich in olive oil – and as consequence in oleic acid, the main dietary MUFA – could help to explain the low rate of cardiovascular mortality found in southern European Mediterranean countries, in comparison with other westernised countries (Covas et al. 2009). Compared to PUFAs, there are far fewer studies on the effect of MUFAs on cardiovascular risk and no RCTs. The primary prevention of cardiovascular disease with a Mediterranean diet (PREMED) randomised trial (Estruch et al. 2013), however, although not designed to specifically assess the effect of MUFAs, tested a diet supplemented with extra-virgin olive oil – therefore, with oleic acid – in a population at high cardiovascular risk. Among these subjects, the intervention diet reduced the incidence of major CVD events.

Clinical trials have been conducted on intermediate biomarkers, such as blood lipids or blood pressure, mainly when MUFAs were substituted for SFAs (Mensink et al. 2003). Most of the studies, but not all (Fattore et al. 2014) showed improvements in these risk factors (Gillingham et al. 2011). The OmniHeart randomized trial, for instance, a large cross-over feeding study on 164 adults with prehypertension or stage 1 hypertension, found that diets predominately rich in MUFAs lowered blood pressure and improved the

blood lipid profile (Appel et al. 2005). A systematic review and meta-analysis of 12 long-term randomised dietary intervention trials found that in comparison to low-MUFA, high-MUFA diets significantly improved fat mass and blood pressure (Schwingshackl et al. 2011).

The effects of MUFAs on CVD and CHD were discordant in different observational studies. A pooled analysis of 11 American and European cohort studies (Jakobsen et al. 2009), on the association of energy intake from MUFAs and risk of CHD, failed to find any significant effect. The Kuopio Ischemic Heart Disease Risk Factor Study, involving 1981 men free of CHD at baseline, found that MUFA intake was associated with increased CHD risk (Virtanen et al. 2014). A larger prospective cohort study on 7038 participants at high risk of CVD from the PREMEDI trial showed, on the contrary, that the intake of MUFAs was inversely associated with CVD risk and all-causes of deaths (Guasch-Ferré et al. 2015). Results were similar in the two large prospective cohorts from the Nurses' Health Study and Health Professional Follow-up Study, including respectively 83,349 women and 42,884 men, free from cardiovascular disease. This study showed that dietary intake of MUFAs was associated with lower total mortality and, primarily in women, with lower CVD mortality (Wang et al. 2016). The large PURE study (Dehghan et al. 2017), including 18 countries with a median follow-up of 7.4 years, showed that the percentage of energy from MUFAs was inversely related to total mortality but not to CVD events or CVD mortality. Finally, the meta-analyses by Chowdhury et al. (2014) of prospective cohort studies on dietary and circulating MUFAs did not find any significant inverse relation between MUFA and coronary outcomes.

Trans fatty acids

Artificial TFAs are trans-isomers of unsaturated fatty acids, produced by hydrogenation of marine or vegetable oils, in order to create the semi-solid fats more suitable for certain applications in the food industry. These fats were produced mainly from the 1960s to the 1980s by many food manufacturers in order to replace animal fats which, as mentioned above, were considered unhealthy, since they contained SFAs and consequently raised total- and LDL-cholesterol and increased the CHD and CVD risk.

The first evidence of a negative role of TFAs came from nutritional intervention studies (Vergoesen 1972; Mensink and Katan 1990) measuring biomarkers of cardiovascular risk. Diets containing TFAs, in

comparison with the *cis* isomers, raised total and LDL-cholesterol, like SFAs, but also lowered HDL-cholesterol and increased triacylglycerols, thus inducing a more unfavourable lipid profile than SFAs.

A later meta-analysis of nutritional clinical trials on the effects of palm oil on blood lipid biomarkers (Fattore et al. 2014) confirmed these results, showing that consumption of TFAs induced a more adverse blood lipid profile than the other main dietary fats.

TFAs seem to increase the plasma activity of the cholesteryl ester transfer protein, which transfers cholesteryl esters from HDL to lipoproteins of lower density, thus contributing to the rise of LDL-cholesterol and the fall in HDL-cholesterol (van Tol et al. 1995). However, the detrimental effects of TFAs seem to be greater than those predicted by changes in lipoprotein concentrations and probably involve the promotion of an inflammatory process (Wallace and Mozaffarian 2009; Brouwer et al. 2013). Han et al. (2002) showed that in individuals with moderately elevated cholesterol levels diets high in hydrogenated fats adversely affected the inflammatory process in atherosclerosis by increasing the production of inflammatory cytokines, with no adverse effects on cellular immunity.

To our knowledge, no clinical trials have been done on TFAs and CVD outcomes – as is obvious since the unfavourable lipid profile in terms of cardiovascular risk would make it unethical to test these fats in patients. Evidence for an adverse effect of TFAs on cardiovascular risk mainly comes from observational studies. Prospective cohort studies, for instance, found a positive relation between TFA intake at low levels and coronary outcomes. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen et al. 1997) examined 21,930 male smokers aged 50–69 years, initially free of CVD. At 6.1 years of follow-up, there was a significant relationship between the intake of TFAs and risk of coronary death (RR 1.39, 95% CI 1.09–1.78). Results were similar in the Zutphen Elderly Study (Oomen et al. 2001), which investigated a Dutch population with a fairly high intake of TFAs. After 10 years of follow-up, the risk of CHD was positively related to the intake of TFAs. In the Nurses' Health Study (Willett et al. 1993; Hu et al. 1997), a cohort involving 85,095 women without diagnosed CHD, stroke, diabetes, or hypercholesterolaemia, the dietary intake of TFAs was directly related to CHD risk (RR 1.50, 95% CI 1.12–2.00), after adjustment for age and total energy intake.

A meta-analysis of four large prospective studies found that an average intake of about 2% of total energy of TFAs was associated with a 23% increase in

the risk of CHD (Mozaffarian et al. 2006). These results were confirmed by a more recent meta-analysis (Chowdhury et al. 2014) of five prospective studies involving 155,270 subjects and 4662 coronary outcomes: the total dietary TFA intake was positively related with coronary disease risk, whereas the association with circulating TFAs was not significant. However, these results are weakened by the paucity of the data.

Most of the studies mentioned referred to TFAs from hydrogenation. For the naturally occurring TFAs in the rumen, the evidence from epidemiological studies is less clear, showing either no association or an inverse association with CVD (Gebauer et al. 2011). Dietary intervention studies on biomarkers of CVD suggest that at low doses, such as those normally attainable in the diet, these natural fatty acids did not cause an unfavourable lipid profile, whereas at higher doses the effect could be similar to that of hydrogenated TFAs. A systematic review and meta-analysis of prospective cohort studies specifically designed to evaluate the effect of natural rumen dietary TFAs showed a slight protective effect of these fats on CHD risk (Bendsen et al. 2011). The authors suggested that this might be because these fats are usually consumed together with dairy products, which may be heart-protective.

A later recent meta-analysis of observational studies, such as prospective cohort, case-control, nested case-control or case-cohort studies (de Souza et al. 2015) found no significant association between naturally occurring TFAs and coronary events, whereas this association was significant with hydrogenated TFAs. This might reflect the sources of TFAs, since their isomer composition is different or to consumption levels, which are generally lower for naturally occurring TFAs. In the meta-analysis by de Souza et al. (2015), the intake of industrially produced trans fats was on average about 2.5-times that of ruminant trans fats, providing more statistical power for the detection of associations.

Gayet-Boyer et al. (2014) made a systematic review and meta-analysis to investigate this issue. They included 13 randomised nutritional trials on ruminant TFAs and blood lipid biomarkers of CHD/CVD and found no relation between ruminant TFA intake up to 4–19% of total energy dietary intake and the biomarkers, indicating that TFAs of natural origin have no unfavourable effects on lipid profiles at the usual levels of intake.

Current dietary guidelines recommend an intake of <1% of total energy or “as low as possible” (EFSA Panel on Dietetic Products Nutrition and Allergies

2010); however, the adverse effects for artificial TFAs are seen at a very low intake (i.e. 1–3% of total energy, corresponding to 20–60 kcal for a person consuming 2000 kcal per day) (Mozaffarian et al. 2006), so the dietary guidelines may not be protective enough for the general population. In 2003, the US Food and Drug Administration (FDA) required the TFA content to be declared on the nutrition label of foods and dietary supplements, and in 2015 revoked the generally recognised as safe (GRAS) status, giving manufacturers 3 years to phase out TFAs from processed foods. These initiatives were accompanied by a substantial reduction in artificial TFA intake. Representative national data on plasma TFA concentrations in American adults showed a decline of >50% from 1999–2000 to 2009–2010 (Vesper et al. 2017). A retrospective observational pre-post study of residents in the New York State counties, where artificial TFAs were restricted starting from 2007, showed a reduction of hospital admissions for myocardial infarction and stroke in comparison to counties without restrictions, beyond temporal trends (Brandt et al. 2017).

Since 2008, the European Parliament has recommended banning artificial TFAs in Europe and recently the WHO called for a complete ban on artificial TFAs throughout Europe as part of a new action plan on diet and health. Some countries (Denmark, Austria, Switzerland, Iceland, Hungary, Norway and Latvia) introduced legal ban on sales but most of them rely on food producers to voluntarily reduce TFAs. Stender et al. (2016) monitored the changes in the occurrence of TFAs in biscuits, cakes, and wafers in six countries in South-Eastern Europe (Slovenia, Croatia, Serbia, Bosnia and Herzegovina, Montenegro and the Former Yugoslav Republic of Macedonia) from 2012 to 2014, and showed that, at least in these countries, there are still large amounts of these TFAs in popular foods and voluntary reduction seems ineffective, since some population subgroups still consume TFAs in amounts that increase their risk of CHD (Stender et al. 2014).

Conclusions

Currently, the soundness and strength of the scientific evidence underpinning current and previous dietary advice on dietary fat intake for total fats and SFAs has been questioned (Mozaffarian 2011; Willett 2011). One of the main issues is that the evidence of the relation between SFAs and CHD, and CVD risk was mainly based on observational or animal studies, and not on RCTs (Harcombe et al. 2015), which are the

only designs that can detect a cause and effect relationship. Observational cohort studies have the advantage of investigating long-term exposures than those feasible in RCTs, but can hardly control some confounders, such as the healthy consumer or misreporting bias, which can distort or even reverse the causal relation. Other limitations too emerged in studies that initially found a positive relation between SFA intake and CVD. For example in several original studies, as well as in dietary recommendations, SFAs were considered as one group, assuming that the individual SFAs in the diet all had the same effect on serum cholesterol. This assumption is no longer appropriate, since different studies have shown that the main dietary SFAs have different effects on lipid profile (Mensink and Katan 1992; Clarke et al. 1997; Mensink et al. 2003; Fattore et al. 2014).

Another criticism was that it was not clear whether the intervention studies reducing SFAs were controlled for the energy balance (Mensink et al. 2003), so the advantage from the reduction of SFAs could well arise from lower caloric intake. Adjustment for energy intake is a key issue in nutritional studies in order to assess the real effects of individual macronutrients beyond just their energy content.

A crucial issue is that results of clinical trials that did not confirm the “lipid theory” were not published. As discussed by Ramsden et al. (2016), different reasons explain the lack of publication of the results of the Minnesota Coronary Experiment, e.g. the authors’ concern that they might have made some mistakes which would have biased the results or the difficulty of publishing results disagreeing so much with prevailing beliefs. This “non-publication” certainly influenced public health choices since it affected the underlying scientific evidence.

Another aspect emerging from these studies was that total serum cholesterol or LDL-cholesterol, which have long been considered the main risk factors related to SFA intake, failed to predict CVD or CHD mortality, since they decreased in the group with the higher rate of mortality. In recent years, advances in nutritional sciences have produced a more complex picture and other biomarkers related to blood cholesterol have suggested valid or at least better risk predictors (McQueen et al. 2008). A recent cross-sectional analysis of dietary nutrients on blood lipids and blood pressure in low-, middle- and high-income countries suggested the ApoB-to ApoA1 ratio as the best biomarker of the effect of nutrients on cardiovascular risk (Mente et al. 2017).

Finally, another important criticism is that different fats never occur alone in diet but always co-exist in

several foodstuffs and this is presumably one of the reasons for the many discordant results from the observational studies. To investigate the health effects of individual food ingredients that never occur on their own is important in basic research to discover bioactive molecules. However, the effects of such ingredients when they are in food may be different from when they are isolated, for instance, because of differences in doses or interactions with other food components. Thus dietary recommendations based on the effects of single pure ingredients should be replaced with a more realistic approach considering the foods where these ingredients occur and eating habits. Dietary recommendations that do not take the overall diet into account can be misleading for people, who may be led to make inappropriate changes in their dietary habits and may be open to manipulation from the food industry and/or campaign groups.

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