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Trans Fatty Acids and Human Health

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1. Introduction

According to various studies, fats of animal and vegetable origins satisfy 22% to 42% of the daily energy demands of human beings (Srinivasan et al., 2006; Wagner et al., 2008; Willet, 2006). Some fats, and especially those that are hydrogenated, contain *trans* fatty acids (TFAs), i.e. unsaturated fatty acids with at least one double bond in a *trans* configuration (Craig-Schmidt, 2006). This *trans*-double-bond configuration results in a greater bond angle than for the *cis* configuration, thus producing a more extended fatty-acid carbon chain that is more similar to that of the saturated fatty acids (SFAs), rather than to that of the *cis*-unsaturated double-bond-containing fatty acids (Fig. 1) (Moss, 2006; Oomen et al., 2001).

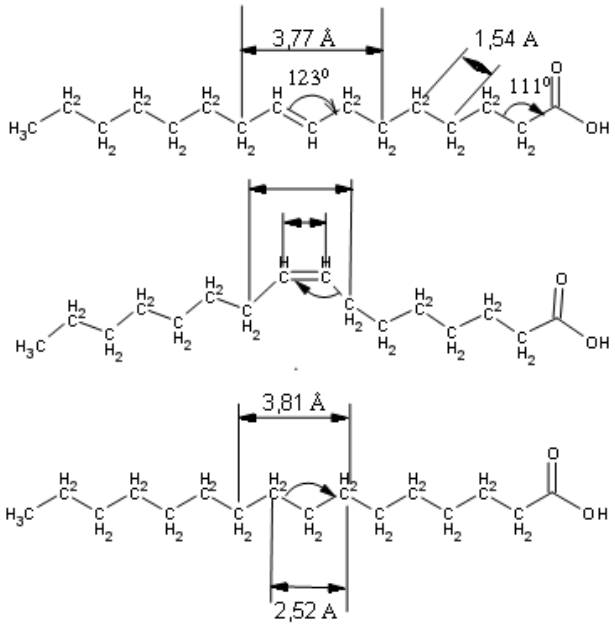

Fig. 1. Structure of different isomers of C16 (Willett, 2006)

Fat is a thus major source of energy for the body, and it also aids in the absorption of vitamins A, D, E and K, and of the carotenoids. Both animal-derived and plant-derived food products contain fat, and when eaten in moderation, fat is important for correct growth and development, and for the maintenance of good health. As a food ingredient, fat provides taste, consistency and stability, and helps us to feel 'full'. In addition, parents should be aware that fats are an especially important source of calories and nutrients for infants and toddlers (up to 2 years of age), who have the highest energy needs per unit body weight of any age group.

However, SFAs and TFAs raise low-density lipoprotein (LDL; or 'bad') cholesterol levels in the blood, thereby increasing the risk of heart disease. Indeed, prospective epidemiological studies and case-control studies support a major role for TFAs in the risk of cardiovascular disease, and therefore dietary cholesterol can also contribute to heart disease (see below). Unsaturated fats, which can be mono-unsaturated or polyunsaturated, do not raise LDL cholesterol and are beneficial to health when consumed in moderation.

Hydrogenated oils tend to have a higher TFA content than oils that do not contain hydrogenated fats. In the partially hydrogenated soybean oil, which is the major source of TFAs worldwide, the main isomer is *trans*-10 C18:1. In the European countries with the highest TFA intake (The Netherlands and Norway), consumption of partially hydrogenated fish oils was common until the mid-1990s, after which they largely disappeared from the dietary fat intake. These partially hydrogenated fish oils included a variety of very-long-chain TFAs. Recent findings from Asian countries (India and Iran) have indicated a very high intake of TFAs from partially hydrogenated soybean oil (4% of energy). Thus, TFAs appear to be a particular problem in developing countries where soybean oil is used.

Formation of these *trans* double bonds thus impacts on the physical properties of a fatty acid. Fatty acids that contain a *trans* double bond have the potential for closer packing and alignment of their acyl chains, which will result in decreased molecular mobility (Willett, 2006). Therefore, the oil fluidity will be reduced when compared to that of fatty acids that contain a *cis* double bond. Partial hydrogenation of unsaturated oils results in the isomerisation of some of the remaining double bonds and the migration of others, producing an increase in the TFA content and a hardening of the fat. It has been shown that foods that contain hydrogenated oils tend to have a higher TFA content than those that do not contain hydrogenated oils (Moss, 2006; Oomen et al., 2001). Nevertheless, the hydrogenation of oils, such as corn oil, can result in both *cis* and *trans* double bonds, which are generally located anywhere between carbon 4 and carbon 16 of the fatty acids. One of the major TFAs is elaidic acid (*trans*-9 C18:1), although during hydrogenation of polyunsaturated fatty acids (PUFAs), small amounts of several other TFAs are produced, including: *trans*-9,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *cis*-9,*cis*-12,*trans*-15 C18:3; and *cis*-5,*cis*-8,*cis*-11,*cis*-14,*trans*-17 C20:5 (Craig-Schmidt, 2006; Wagner et al., 2008). Conversely, one way to produce 'zero' levels of TFAs is through the *trans*-esterification reaction between vegetable oils and solid fatty acids, like C8:0, C12:0, C14:0 and C16:0.

Correlations between high intake of industrially produced TFAs (IP-TFAs) and increased risk of coronary heart disease (CHD) have been reported (Stender et al., 2006; Tarrago-Trani

et al., 2006), and lowering the intake of TFAs can also reduce the incidence of CHD (Willett, 2006). Estimates based on changes in plasma concentrations of LDL and high-density lipoprotein (HDL) indicate around a 4% reduction in CHD incidence, while based on epidemiological associations, when TFA intake is lowered by 2% (5 g/day), the estimates indicate a >20% reduction in CHD incidence (Katan, 2006; Moss, 2006). In The Netherlands, a major reduction in the TFA content of retail foods was achieved in the 1990s through the efforts of the industry and with minimal government intervention. Society pressure is also now helping to reduce the TFA content of 'fast foods'. This illustrates the feasibility of reducing TFAs in fast foods without increasing the saturated fats, with the daily intake kept as low as possible, to minimise the health risks (Stender et al., 2006).

Comparison of the different recommendations for macronutrients in some European countries, for the World Health Organisation/ Food and Agriculture Organisation of the United Nations (WHO/FAO), and in the USA and Canada, are given in Table 1. Most of the recommendations are the same, or are in similar ranges. The recommendations for protein, however, are expressed differently, either as grams per day or grams per kilogram per day, and usually without any indication of a representative weight at each age to allow conversion of one to the other. The Joint FAO/WHO/United Nations University (UNU) Expert Consultation of 1985 (WHO, 1985) defined the protein requirement of an individual as "the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in persons maintaining an energy balance at modest levels of physical activity". The human body can synthesise both SFAs and mono-unsaturated fatty acids (MUFAs) from acetate, whereas PUFAs (in both the n–6 linoleic acid and n–3 linolenic acid series) are required in the diet, and they are therefore known as essential fatty acids. These essential fatty acids are important for various cell-membrane functions, such as fluidity, permeability, activity of membrane-bound enzymes and receptors, and signal transduction. Linoleic and linolenic acids can be elongated and desaturated in the body, and transformed into biologically active substances, like prostaglandins, prostacyclins and leukotrienes. These substances participate in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions, and many other functions (Nordic Nutrition Recommendations, 2004). The DACH Reference Values for Nutrient Supply (DACH, 2000) for total fat intake in adults (not more than 30% of the energy intake) are related to light work, heavy muscle work (not more than 35% of energy intake) and extremely heavy work (not more than 40% of energy intake). SFAs should not exceed 10% of energy intake. PUFAs should provide about 7%, and up to 10% if SFAs provide more than 10% of energy intake. MUFAs should constitute the rest. TFAs should contribute not more than 1% of the daily energy. The ratio of n–6 linoleic acid to n–3 linolenic acid should be about 5:1 (WHO/FAO, 2002). These fatty acids compete for the metabolic enzymes, and it is therefore important to maintain a balance between them (Nordic Nutrition Recommendations, 2004). The Nordic Nutrition Recommendations indicate the limiting of the intake of SFAs plus TFAs to about 10% of the daily energy and the total fat intake to 30% of the daily energy (25%-30%) (Filip et al., 2010). The recommendations for carbohydrate intake are from 50%of the daily energy in the DACH (2000) reference values, to 55% (50%-60%) in the Nordic Nutrition Recommendations (2004), 55%-75% by WHO/FAO, and 45%-65% in the USA/ Canada recommendations, as detailed in Table 1.

Component	NNR (2004)	DACH (2000)	WHO/FAO (2002)	Euro Diet (2000)	USA/Canada AMDR (2002)
Total energy from fat (%)	30 (25-35)	30	15-30	<30	20-35
SFAs (%)	≤10	10	<10	<10	Minimise
PUFAs (%)	5 (10)	7-10	6-10	-	-
n-6 FAs (%)	4 (9)	2.5	5-8	4-8	5-10 (linoleic)
n-3 FAs (%)	1	0.5	1-2	2 (linolenic)	0.6-1.2
TFAs (%)	Included in SFAs	1	<1	<2	Minimise
MUFAs (%)	10-15	The rest of the total			-
Total energy from carbohydrates (%)	55 (50-60)	50	55-75	>55	45-65
Energy from sugars (%)	<10	30	<10		<25
Fibre (g/day)	25-35 (3 g/MJ) (12.5 g/1000 kcal)				25-38 (14 g/1000 kcal)
Energy from proteins (%)	15 (10-20)	8-10	10-15	-	10-35
Cholesterol (mg/day)	300		<300		Minimise
Salt (sodium) (g/day)	5–6 (2.3-2.7)		<5 (2)		

NNR, Nordic Nutrition Recommendations; DACH, Austria–Germany–Switzerland Reference Values for Nutrient Supply; WHO, World Health Organisation; FAO, Food and Agriculture Organisation of the United Nations; AMDR; acceptable macronutrient distribution; FAs, fatty acids; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; TFA, *trans* fatty acids; MUFAs, mono-unsaturated fatty acids.

Table 1. Comparison of reference daily intakes for adults according to different recommendations around the World (Pavlovic et al., 2007)

As indicated above, prospective epidemiological studies and case-control studies using adipose-tissue analyses have confirmed a major role for TFAs in the risk of CHD. The magnitude of the association with CHD is considerably stronger than for SFAs, and it is stronger than that predicted for the effects of TFAs on LDL and HDL cholesterol (Katan, 2006; Tarrago-Trani et al., 2006). In this context, it needs to be considered that data for the Russian Federation show that every year 1,005 people per 100,000 of the population between 25 and 64 years of age die because of circulatory system diseases (WHO, 2008). As a consequence influence of TFAs on CHD, in 2003, the United States FDA issued a ruling that required food manufacturers to list the TFAs in the nutritional facts labels of all packaged food products (FDA, 2003), with the food industry being given until 1 January, 2006 to comply. Along with these growing health concerns about TFAs, this mandate led to marked changes in the fat and oil industries, with newer technologies developed to reduce the TFA contents of fats and oils used in the manufacture of food products. Conversely, given the labelling mandate and these technological advances, it is possible that food products traditionally considered to be sources of TFAs are now much lower in, or indeed do not

contain, TFAs (Borra et al., 2007). Then in late 2006, New York City became the first major city in the United States to pass a regulation limiting IP-TFAs in restaurants. This has served as a model for others to follow, with these regulations including: a maximum level per serving size of 0.5 g TFAs; a distinction between frying and baking, with a phased-in implementation; a help centre to assist restaurants to make the switch to more healthy options; and plans to evaluate the regulation and its impact on CHD (Borra et al., 2007).

Accurate quantification of C18:1 TFAs in food products is thus an important issue, with policies recently implemented in different countries to limit their consumption and their occurrence in food products because of their relationship with CHD (Carriquiry et al., 2008; Chen et al., 2007).

2. History

Margarine was invented in 1869 by Hippolyte Mège Mouriès, a French food research chemist, in response to a request by Napoleon III for a wholesome alternative to butter. It is not entirely clear whether the primary aim was the betterment of the working classes or the economics of the food supply to the French army. In the laboratory, Mège Mouriès solidified purified fat, after which the resulting substance was pressed in a thin cloth, which formed stearine and discharged oil. This oil formed the basis of the butter substitute. For the new product, Mège Mouriès used margaric acid, a fatty-acid component isolated in 1813 by the Frenchman Michel Eugène Chevreuil. While analysing the fatty acids that are the building blocks of fats, he singled out this one and named it margaric acid, because of the lustrous pearly drops that reminded him of the Greek word for pearls, i.e. margarites (Chen et al., 2008; Craig-Schmidt, 2006).

In 1871, Mège Mouriès sold this know-how to the Dutch firm Jurgens, which is now part of Unilever. In the early days, margarine contained two types of fat: a large proportion of animal fat and a small proportion of vegetable fat. As time passed, the small vegetable-fat element increased, through two specific stages in the process. First, by improving the process of refining vegetable oils, use could be made of a greater variety of liquid oils and a higher proportion of solid vegetable fats. Secondly, through the development of processes for turning liquid oils into solid fats on a commercial scale, use could be made of larger quantities of liquid vegetable oils (Filip, 2010).

During the early years of this period, in the late 1800s, TFA intake from partially hydrogenated vegetable oils was minimal. Indeed, it was not until the late 1800s that the process of partial hydrogenation of oils was invented in Europe. These partially hydrogenated oils apparently entered the United States food supply by 1920. Although the rate of increase before 1950 is not completely clear, by 1950 the amount of IP-TFAs in the food supply was quite substantial. Partly because of economic effects during World War II, margarine production rose rapidly as a replacement for butter (Chen et al., 2007). Then during the 1960s, margarine became viewed as a healthy alternative to butter because of its absence of cholesterol and its low content of SFAs. Thus, consumption increased further, and so margarine, which was heavily hydrogenated at that time, became widespread in the food supply and was the major source of IP-TFAs. This phenomenon is illustrated in Figure 2. The total TFAs consumption was approximately 2% to 3% of the food energy. Since then, the sources of TFAs have changed, from mainly margarine to mainly deep-fried fast foods and commercially baked products, although per capita, the intake has remained roughly the same (Willett, 2006).

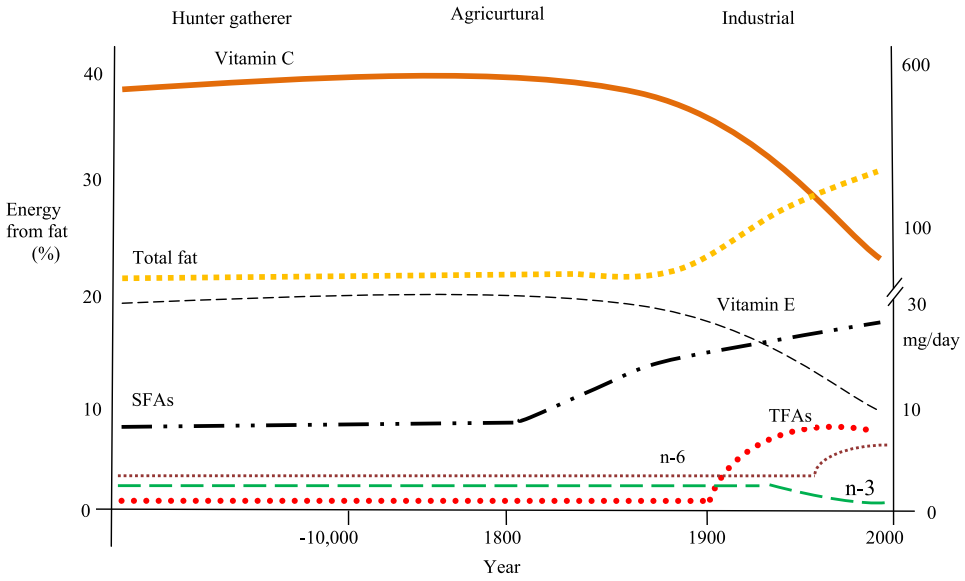

Fig. 2. Relative food energy supplied by the different fatty acids, and the predicted changes for the food industry and fat hydrogenation (Simopoulos, 2004)

After World War II, the process of making hydrogenated and hardened fats from cheaper sources of vegetable oils was widely adopted. Margarines were developed and marketed as alternatives to butter, and vegetable shortening increasingly replaced animal fats in cooking (Albers et al., 2008). However, as early as 1975, at what is now the University of Glamorgan in South Wales, a group of scientists led by Leo Thomas suspected that deaths from CHD were connected with this eating of partially hydrogenated fats. It is now generally accepted that TFAs are actually worse for health than the SFAs that they were designed to replace (Blake, 2009).

3. Studies of *trans* fatty acids

Increases in 'civilization diseases' in the developed world led scientists to investigate why this was happening. While there is enough food that is also cheaper and more accessible than ever before in the developed world, we are witnessing more and more overweight and obese populations. Modern populations have worse nutrition habits than ever before, except for some specific small social groups e.g. through religion, ecology and ethnic aspects (WHO, 2008). Obesity is a severe health issue that is characterised by fat accumulation and defined by means of the body mass index (BMI), as body weight [kg]/ (height [m])^2. According to this index, different obesity levels have been described, ranging from overweight (BMI, 25.0-29.9), through obese (BMI, 30.0-40.0) to the most detrimental stage, morbid obesity (BMI, ≥40) (Garaulet et al., 2011). The relevance of this classification is that as the BMI increases, the morbidity and mortality risks also increase (Bray, 2003). Furthermore, regional fat accumulation is an important factor in the development of obesity-related alterations. It has been suggested that excess visceral fat is more detrimental than excess

subcutaneous fat, because visceral deposits release free fatty acids directly into the portal vein (Bray, 2003). The fatty acid pattern carried to the portal circulation is of great importance, because different fatty acids show distinct atherogenicities, depending on the chain length and degree of unsaturation. Here, SFAs have been associated with increased cardio-metabolic risk, while n-3 and n-9 unsaturated fatty acids have been proposed as protective agents against these alterations (Garaulet et al., 2011).

3.1 Studies in animals

In milk fat, TFAs are produced by anaerobic fermentation of PUFAs in the rumen of lactating cows (Destaillats et al., 2007; Fournier et al., 2006). This fermentation process is called biohydrogenation, and it results in TFAs that can be further metabolised in the mammary gland. Accurate estimations of fatty-acid compositions are vital not only for the definition of the nutrient composition of foods, but also to accurately determine treatment effects that can alter the fatty-acid composition of the foods (Ascherio, 2002; Burdge et al., 2005; Kummerow et al., 2004; Murrieta et al., 2003; Triantafillou et al., 2003).

There is a considerable overlap of TFA isomers in fats of ruminant origin and in partially hydrogenated vegetable oils, as they have many isomers in common. However, there are considerable differences in the amounts of individual TFAs in these sources. While there is evidence of unfavourable effects of TFAs from hydrogenated vegetable oils on LDL and other risk factors for atherosclerosis, at present it is not certain which of the component(s) of the TFAs created by chemical hydrogenation are responsible for these a negative metabolic effects (Ascherio, 2002). Prospective studies addressing the effects of TFA intake on CHD risk, where estimates of TFA intake were based on dietary protocols, have mostly been carried out in populations with a relatively low intake of dairy or ruminant TFAs (Pfeuffer & Schrezenmeir, 2006). Nevertheless, the biggest effects of fatty-acid composition and the nutritive quality of foods of animal origin, like meat and milk products, depend on the feed quality and the health of the animals.

3.2 Studies in humans

These TFA-containing fats can be incorporated into both foetal and adult tissues, although the transfer rate through the placenta continues to be a contradictory subject. In preterm infants and healthy term babies, the *trans* isomers have been inversely correlated with infant birth mass (Koletzko & Müller, 1990). Maternal milk reflects precisely the mother's daily dietary intake of TFAs, with presence of 2% to 5% total TFAs in human milk. The levels of linoleic acid in human milk are increased by a high *trans* diet, although long-chain polyunsaturated TFAs remain mostly unaffected (Koletzko, 1992; Koletzko & Desci, 1994). Alterations in the maternal dietary intake of PUFAs cause similar changes in the PUFA content of their milk. Several investigations have shown that supplementation of the consumed fat with fish oils increases the amounts of C20:5n-3 and C22:6n-3 in the milk and in the maternal and infant erythrocyte lipids. Likewise, infant tissues incorporate the TFAs from the maternal milk, increasing the levels of linoleic acid and decreasing arachidonic acid and docosahexaenoic acid. This suggests an inhibitory effect of TFAs on the liver n-6 fatty-acid-desaturase activity (Jensen et al., 1992).

As opposed to blood and liver, the brain appears to be protected from TFA accumulation in experimental animals, although no data have yet been reported for newborn humans (Larqué, 2001). A significant interaction between diet and pregnancy was shown for the activities of Δ6-desaturase and glucose 6-phosphatase in liver microsomes: dietary TFAs decreased the activities of both of these enzymes, although only in pregnant rats (Larqué et al., 2000; Larqué & Zamora, 2000; Larqué et al., 2003). In Spain, TFAs in human milk were investigated by Boatella et al. (Boatella et al., 1993), and they showed that the average content of TFAs in 38 samples was 0.98% of the milk fatty acids. This value is lower than that for human milk from other developed countries, where consumption of hydrogenated fats is higher. In a study by Chen et al. (Chen et al., 1995) on TFAs in human milk in Canada, the mean total TFA content was 7.19% (±3.03%) of the total milk fatty acids, with a range from 0.10% to 17.15%.

The compelling data linking dietary TFAs to increased risk of CHD have originated from large, prospective, population-based studies, which included from 667 to 80,082 men and women across different age groups who were monitored for six to 20 years. This link has also been seen in controlled feeding trials (Oomen et al., 2001). Among these studies, there are: the United States Health Professional's follow-up study; the Finnish alpha-tocopherol, β-carotene Cancer Prevention Study; the United States nurse's health study (with 14-year and 20-year follow-up) (Willett, 2006); and the Dutch Zutphen elderly study (Oomen et al., 2001). These studies are consistent in their finding of a strong positive association between TFA intake and the risk of CHD. Interestingly, a weaker correlation between SFA intake and the risk of CHD also has been reported (Willettt, 2006).

The Zutphen elderly study included 667 men from 64 to 84 years of age who were free of CHD at baseline (Oomen et al., 2001). Dietary surveys were used to establish the food consumption patterns of the participants. Information on risk factors and diet were obtained in 1985, 1990 and 1995. After a 10-year follow-up, from 1985-1995, there were 98 cases of fatal or non-fatal CHD. The findings showed that over this period, the mean TFA intake decreased from 4.3% to 1.9% of the food energy. After adjustments for age, BMI, smoking and dietary covariates, TFA intake at baseline was positively associated with 10-year risk of CHD. Thus, a high intake of TFAs, which included all types of isomers, contributed to the risk of CHD. A substantial decrease in TFA intake, which was mainly due to the lowering of the TFA content in edible fats in the Dutch industry, therefore had a large impact on public health (Craig-Schmidt, 2006; Larqué et al., 2001).

In multiple and rigorous randomised trials, the intake of TFAs has been consistently shown to have adverse effects on blood lipids, and most notably on the LDL/HDL cholesterol ratio, which is a strong marker of cardiovascular risk. When a mixture of TFA isomers obtained by partial hydrogenation of vegetable oils is used to replace oleic acid, there is a dose-dependent increase in the LDL/HDL ratio. The relationship between the levels of TFAs as the percentage of energy and the increase in the LDL/HDL ratio appears to be approximately linear, with no evidence of a threshold at low levels of TFA intake, and with a slope that is twice as steep as that observed by replacing oleic acid with a SFA (Borra et al., 2007; Mensink & Nestel, 2009). Studies comparing animal and vegetable TFAs have shown similar effects on the total/HDL cholesterol ratio. The effects of TFAs on lipoproteins from both sources appeared at doses exceeding 2% of energy (Mensink & Nestel, 2009). The average impact of TFA-induced changes in the LDL/HDL ratio corresponds to tens of

thousands of premature deaths in the United States alone (Mensink & Nestel, 2009). Although dramatic, this effect is substantially smaller than the increase in cardiovascular mortality associated with TFA intake in epidemiological studies, suggesting that other mechanisms are likely to contribute to the toxicity of TFAs (Ascherio, 2006). Thus, although there is accumulating evidence linking inflammatory proteins and other biomarkers to CHD, lipid concentrations in the blood remain one of the strongest and most consistent predictors of risk. Therefore, the LDL/HDL cholesterol ratio is probably the best marker to date for estimating the effects of TFAs on plasma lipids, which are most likely relevant to CHD incidence and mortality (Larqué & Zamora, 2001).

Further rigorous randomised trials to establish the effects of hydrogenated fats and TFA intake on individual lipoprotein classes started in 1990, when a report from The Netherlands suggested that a diet enriched in elaidic acid (*trans*-9 C18:1) increases the total and LDL cholesterol concentrations and decreased HDL cholesterol concentrations, compared to a diet enriched in oleic acid. In contrast, enrichment of the diet with SFAs increases LDL cholesterol, but has no effect on HDL cholesterol, thus resulting in a smaller adverse change than in the case of elaidic acid (Mensink & Katan, 1993; Mensink & Nestel, 2009).

3.3 Studies of antioxidant effects

In one study (Filip et al., 2011), the effects of natural antioxidants on formation of TFAs during heat treatment of sunflower oil was investigated. The data from the fatty acid analyses are summarized in Table 2. Here, the non-treated control sunflower oil had a 7.5% palmitic acid content, with 4.5% stearic acid, 25.0% oleic acid, and 60.5% linoleic acid, as is usual for the common (not high in oleic acid) sunflower oils; these data compare well with those of other studies (Sánchez-Gimeno et al., 2008; Bansal, Zhou, Tan, Neo, & Lo, 2009). This sunflower oil was purchased directly from a supplier of oils that are used mainly by small food enterprises (Zvijezda d.d., Zagreb, Croatia). The natural antioxidant extract of rosemary (*Rosmarinus officinalis* L.) that was added to this sunflower oil (SOR) was purchased directly from Vitiva d.d., Markovci, Slovenia (INOLENS4®; Product N° 301770; Batch N°. LAB. 09-779004), and had a carnosic acid content of 4.30%. Similarly, the lutein added to this sunflower oil (SOL) was from pelargonium (2.2% mixture), as obtained from Etol, d.o.o., Celje, Slovenia (NovaSoL® Lutein; Aquanova AG, Birkenweg 8-10, Germany).

The initial levels of the total TFAs in the samples was 0.91% (±0.01%). This compares with the range from 0.15% to 6.03% reported by Bansal et al. (2009) for TFAs in refined oils (soybean, corn, sunflower, high oleic sunflower, low erucic rapeseed and high erucic rapeseed oils). The aim in this study with the sunflower oil was to evaluate the effects of heat on this TFA composition of the oil when subjected to treatment representative of deep-fat frying (185 ±5°C). Since sunflower oil is in common use for deep-fat frying, it is particularly important to know what species and levels of TFA isomers appear during such heat treatment (Filip et al., 2011; Martin et al., 2007).

In this study, we focussed mainly on these effects of heat on the TFAs with 18 carbon atoms, which were the most represented. Prior to the treatment, the content of *trans* C 18:1, t-9 was 0.67% (±0.08%). At the end of the heat treatment (120 h at 185 ±5°C), in the control sunflower oil the *trans* C 18:1, t-9 increased to 1.12% (±0.14%), in SOR, to 0.99% (±0.04%), and in SOL, to 0.91% (±0.01%). Within each treatment, these increases were significantly different from the

Component	Time (h)					
	0	24	48	72	96	120
Sunflower oil (control)						
SFAs (%)	12.43 ±0.13^{b}	12.54 ±0.12^{b}	12.77 ±0.58^{b}	14.02 ±0.49^{ab}	14.62 ±2.63^{b}	14.76 ±0.35^{a}
MUFAs (%)	26.13 ±0.68^{d}	28.56 ±1.22^{c}	29.40 ±1.06^{cb}	29.84 ±0.85^{abc}	31.18 ±0.96^{ab}	31.59 ±0.35^{a}
PUFAs (%)	61.44 ±0.75^{a}	58.50 ±1.23^{b}	57.83 ±1.33^{b}	56.14 ±1.15^{bc}	56.20 ±3.01^{bc}	53.64 ±2.08^{c}
n6 PUFAs (%)	61.13 ±0.76^{a}	58.20 ±1.23^{b}	57.82 ±1.34^{b}	55.64 ±1.17^{bc}	55.66 ±3.03^{bc}	53.05 ±2.08^{c}
n3 PUFAs (%)	0.31 ±0.02^{b}	0.30 ±0.00^{b}	0.31 ±0.02^{b}	0.34 ±0.01^{a}	0.35 ±0.02^{a}	0.36 ±0.01^{a}
n6/n3	199.93 ±15.84^{a}	192.92 ±4.71^{a}	185.41 ±11.56^{a}	163.68 ±7.73^{b}	159.78 ±16.01^{b}	149.37 ±6.99^{b}
TFAs (%)	0.91 ±0.03^{d}	0.99 ±0.06^{d}	1.25 ±0.07^{c}	1.46 ±0.15^{b}	1.56 ±0.09^{b}	1.71 ±0.07^{a}
Sunflower oil with rosemary extract (SOR; 1.0g/kg oil)						
SFAs (%)	12.39 ±0.57c	12.70 ±0.48c	12.74 ±0.41c	13.80 ±0.29^{b}	14.02 ±0.70^{ab}	14.68 ±0.61^{a}
MUFAs (%)	25.72 ±1.92bc	25.37 ±1.67c	28.73 ±0.81ab	25.69 ±3.46^{bc}	28.87 ±0.40^{ab}	30.25 ±2.24^{a}
PUFAs (%)	61.88 ±1.67a	61.93 ±1.43a	58.53 ±0.57bc	60.51 ±3.33^{ab}	57.11 ±0.55^{cd}	55.07 ±1.71^{d}
n6 PUFAs (%)	61.58 ±1.66a	61.62 ±1.43a	58.13 ±0.56bc	60.07 ±3.32^{ab}	56.56 ±0.57^{cd}	54.45 ±1.72^{d}
n3 PUFAs (%)	0.31 ±0.02b	0.31 ±0.01b	0.31 ±0.01b	0.33 ±0.01^{a}	0.33 ±0.01^{a}	0.35 ±0.01^{a}
n6/n3	201.53 ±10.73a	198.56 ±6.72a	186.36 ±5.67b	179.91 ±9.26^{bc}	169.54 ±7.89^{c}	154.65 ±3.96^{d}
TFAs (%)	0.91 ±0.09d	0.82 ±0.02d	1.02 ±0.05c	1.24 ±0.20^{c}	1.35 ±0.10^{b}	1.55 ±0.16^{a}
Sunflower oil with lutein (SOL; 0.1g/kg oil)						
SFAs (%)	12.51 ±0.72^{c}	12.36 ±0.35^{c}	13.06 ±0.36^{bc}	13.21 ±0.65^{bc}	13.91 ±0.54^{ab}	14.84 ±0.96^{a}
MUFAs (%)	26.25 ±2.60^{b}	25.05 ±0.64^{b}	28.22 ±2.49^{b}	27.55 ±2.79^{b}	28.07 ±0.85^{b}	32.79 ±1.63^{a}
PUFAs (%)	61.24 ±2.82^{ab}	62.59 ±0.72^{a}	58.72 ±2.47^{b}	59.24 ±3.15^{b}	58.02 ±1.37^{b}	52.37 ±0.74^{c}
n6 PUFAs (%)	60.93 ±2.83^{ab}	62.29 ±0.72^{a}	58.31 ±2.48^{bc}	58.80 ±3.15^{bc}	57.51 ±1.40^{c}	51.80 ±0.73^{d}
n3 PUFAs (%)	0.31 ±0.02^{b}	0.31 ±0.01^{b}	0.32 ±0.01^{ab}	0.33 ±0.02^{ab}	0.33 ±0.02^{ab}	0.35 ±0.03^{a}
n6/n3	199.10 ±16.69^{ab}	203.99 ±5.54^{a}	180.24 ±9.67^{bc}	177.99 ±19.87^{c}	174.65 ±12.92^{c}	149.07 ±10.26^{d}
TFAs (%)	0.91 ±0.06^{c}	0.84 ±0.03^{c}	1.01 ±0.02^{b}	1.23 ±0.16^{b}	1.28 ±0.11^{b}	1.43 ±0.04^{a}

SFAs, saturated fatty acids; MUFAs, mono-unsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, trans fatty acids; $^{a, b, c, d}$ Values followed by a different letter are significantly different along each row according to the Duncan test ($P < 0.05$);

Table 2. Effect of cooking heat (185 ±5°C) on the fatty acids composition of sunflower oil, with the addition of the natural antioxidants of a rosemary extract (SOR) and of lutein (SOL) (Filip et al., 2011)

start to the end of the treatment (P <0.001), and also the decreases in *trans* C 18:1, t-9 production with the addition of rosemary oil and lutein were statistically significant in comparison with the control (SOR vs. sunflower oil: 0.32% vs. 0.45%; SOL vs. sunflower oil: 0.24% vs. 0.45%; P <0.001 for both). These data are consistent with an earlier report where there were reductions in *trans*-isomerisation and polar compounds in model oils when α-tocopherol (1%) was added as an antioxidant (Tsuzuki et al., 2008).

When the content of the total TFAs is expressed as the sum of the unsaturated FAs with at least one *trans* double bond, these increased significantly from the initial control sunflower oil of 0.91% (±0.03%), to 1.71% (±0.07%) at 120 h, with significantly lower increases for SOR and SOL, to 1.55% (±0.16%) and 1.43% (±0.04%), respectively (Table 2). Indeed, these differences among treatments were statistically significant (P <0.001) at each step of the heat treatment (24, 48, 72, 96, 120 h). These data relating particularly to the increases in TFAs are comparable to those of Gamel et al. (1999), where they looked at the effects of phenol extracts on TFA formation during frying. A linear relationship between the amounts of elaidic acid and the number of frying cycles has also been reported (Bansal et al., 2009).

According to the nutritional recommendations of the various health authorities, the content of SFAs should not exceed 30% in dietary fats. Sunflower oil thus fits into this recommendation, even though its content in the control sunflower oil increased from 12.43% (±0.13%) to 14.76% (±0.35%), and in the SOR and SOL to 14.68% (±0.61%) and 14.84% (±0.96%), respectively (Table 2).

The initial PUFA:SFA ratio here was 4.94 (±0.10), and after the full time of the heat exposure for the control sunflower oil, this was significantly decreased to 3.64 (±0.14) (P ≤0.05). Meanwhile, , for the SOR and SOL at 120 h of heat treatment, the PUFA:SFA ratio decreased to 3.75 (±0.09; P <0.001) and 3.54 (±0.18; P <0.001). As higher PUFA/SFA ratios are more nutritionally appropriate, these data confirm that the heat treatments of this sunflower oil also worsened this nutritional factor.

4. *Trans* fatty acids and legislation

Governments are increasingly recognising that the risks to consumers from the increased consumption of TFAs cannot be ignored. In 2003, Denmark became the first country to introduce laws to control the sale of foods containing TFAs. This started with the publication of a study in The Lancet by Willett in 1993. Then the Danish Nutrition Council, which was established in 1992, was the driving force behind the campaign that convinced Danish politicians that IP-TFAs can be removed from foods without any effects on their taste, price or availability. The Nutrition Council argued that as no positive health effects of IP-TFAs had ever been reported, then just the suspicion that a high intake has harmful effects on health justified the ban (Astrup, 2006; Mjøs, 2003). The Danish success story might be interesting for other countries, where this unnecessary health hazard could also be eliminated from the foods.

Then in January 2006, it became law in the United States that the contents of TFAs have to be specifically listed on food labels. There is a complication to this, however, because there were two reasons why the consumers might not see a TFA content on the label of a food product. First, although products entering interstate commerce on or after 1 January, 2006, had to be labelled, the FDA realized that it would take some time for food products to move

through the distribution chain to a store shelf. Then, foods that contain less than 0.5 g TFAs per serving can be labelled as being free from TFAs. Furthermore, in Europe, the declaring of TFAs on food labels is still not obligatory in many countries. At the same time, these regulations only applied to food that was labelled; food sold in restaurants and canteens was not covered by this law (FDA, 2003; Moss, 2006; Stender et al., 2006). Thus many still feel that foods that contain more than 4 g/100 g SFAs and TFAs together should not be claimed to be healthy food. Indeed, Danish law prohibits the sale of foods that contain more than 2 g TFAs per 100 g of fat, excluding food that naturally contains more TFAs (Filip et al., 2010). Denmark decided to impose this maximum level of IP-TFAs as labelling was deemed insufficient to protect consumers, and especially for risk groups like children and adults with a high intake of fast foods (Garchés & Mancha, 1993; Leth et al., 2006).

Then, in December 2006, the Board of Health of New York City banned many TFAs from restaurants in the city, prompting similar moves in Philadelphia, Montgomery County in Maryland, and the Boston suburb of Brooklyn. The first phase of the regulation applies to oils, shortening and margarine, used in cooking and as spreads, and for recipes that contain more than 0.5 g TFA per serving. Since 1 July, 2007, New York City officials have also called for restaurants to clearly display calorie counts next to their menu items, in a bid to increase consumer awareness of the nutritional content of their food. By 1 July, 2008, the ban had been extended to include TFAs used in baked goods, including bread and cakes, in prepared foods, salad dressings and oils used for deep frying, and in dough and cake batter. Similar bans are being proposed in Chicago and in the state of Illinois; other cities may follow suit, most likely in California (Albers et al., 2008; Blake, 2009).

The American Heart Association recommends a healthy dietary pattern and lifestyle to combat heart disease, limiting TFA consumption to less than 1% (or approximately 2 g on a 2,000-calorie diet), and saturated fat consumption to less than 7% of the total daily calories (Borra et al., 2007). This is consistent with the TFA recommendations made by the American Dietetic Association and the Dietitians of Canada (ADA, 2007).

The benefits of adding TFAs on food Nutrition Facts labels in the United States means that consumers now know the levels of SFAs, TFAs and cholesterol in the foods that they choose to eat. This enables them to make heart-healthy food choices, to help them to reduce their risk of CHD. This labelling is also of particular interest to those concerned about high blood cholesterol. However, to gain the full benefit of this system, all of the consumers need be aware of the risk posed by consuming too high levels of SFAs, TFAs and cholesterol.

At the same time, about half of the convenience products on the Austrian market that have been tested contained less than 1% TFAs, and one third less than 5% (Wagner et al., 2008). However, almost 5% of the products tested contained more than 20% TFAs. A similar level was seen for fast food products, with the highest TFA levels of 8.9%, while the total TFAs of household fats were significantly lower (1.45% ±1.99%) than fats for industrial use (7.83% ±10.0%; $P < 0.001$). Compared to investigations in Austria (and Germany) around 10 years ago, the TFA contents of foods have decreased significantly. About half of the investigated products contained less than 1% of TFAs or total fatty acids, although very high levels of TFAs (>15%) are still detected, and an intake of more than 5 g TFA per portion is possible, which has been shown to significantly increase the risk of CHD (Oomen et al., 2001; Wagner et al., 2008; Wilett, 2006).

5. Analytical methods for *trans* fatty acid determination

The fatty acid composition of food is usually determined using gas-liquid chromatography of the corresponding fatty acid methyl esters (FAMEs) (Baggio et al., 2005; Bondia-Pons et al., 2004; Chen et al., 1999; Ratnayake, 1995; Ulberth & Henninger; 1992). Usually, the FAMEs can be conveniently prepared by heating lipids with a large excess of either acid-catalysed or base-catalysed reagents. However, most of the analytical methods are time consuming and impractical for the processing of large numbers of samples, because the lipids have to be extracted prior to preparation of the FAMEs. For this reason, some procedures have been developed that can be used to prepare FAMEs directly from fresh tissue (Park & Goins, 1994; Garchés & Mancha, 1993).

6. Consumption of *trans* fatty acids

Vaccenic acid (*trans*-11 C18:1) accounts for over 60% of the natural TFAs, whereas with IP-TFAs, a broad mixture of TFAs is produced, with elaidic acid (*trans*-9 C18:1) as the main product (Oomen et al., 2001). In recent years, new technologies have been developed to reduce the TFA content in fats and oils used in the manufacture of food products. As indicated above, the content of TFAs in Danish food has been monitored for the last 30 years. In margarine and shortening, the TFA content has steadily declined, from about 10 g per 100 g of margarine in the 1970s, to practically no TFAs in margarine in 1999, to efficiently reduce the health risk related to TFAs.

In North America, the daily TFA intake has been estimated using food frequency questionnaires, and it was found to be 3-4 g per person (ADA, 2007), while by extrapolation of human milk data, it was said to be greater than 10 g per person (Chardigny et al., 1995). The data also show that the levels of TFAs can vary considerably among foods within any specific category, reflecting the differences in the fats and oils used in the manufacturing or preparation processes. For example, the range of TFAs in 17 brands of crackers was from 23% to 51% of the total fatty acids, which represents differences of 1 g to 13 g TFAs per 100 g of crackers. These data thus show that the wide variability in the TFA content of different foods can result in large errors in the estimation of the TFA intake of individuals, and potentially, of groups (Innis, 2006).

TFA consumption in European countries varies considerably. The diet in northern European countries traditionally contains more TFAs than that in the Mediterranean countries, where olive oil is commonly used. The diet in France has always been relatively low in TFAs, because France has traditionally used predominantly ruminant fats, as compared to hydrogenated vegetable oils. A more recent decrease in dietary TFAs has been seen due to the modification of commercial fats and changes in consumer choice (Larqué et al., 2001). In the TRANSFAIR study (Poppel et al., 1998), which was based on a market basket analysis of diets across 14 European countries, the mean daily intake of TFAs in European countries ranged from the lowest in Greece (1.4 g TFA per day) to the highest in Iceland (5.4 g TFA per day) (Fig. 3).

The lover daily intake of TFAs was recorded in Greece where 1.4 g of TFAs are consumed per day what represent 0.6 % of daily energy intake. The highest daily intake of TFAs was recorded in Iceland where 5.4 g of TFAs are consumed per day what represent 2.0 % of daily energy intake. As shown by researches (Innis et al., 1999; Leth et al., 2006; Poppel et al., 1998) the lowest TFA intake is more often in countries with Mediterranean type of nutrition habits (Mediterranean diet).

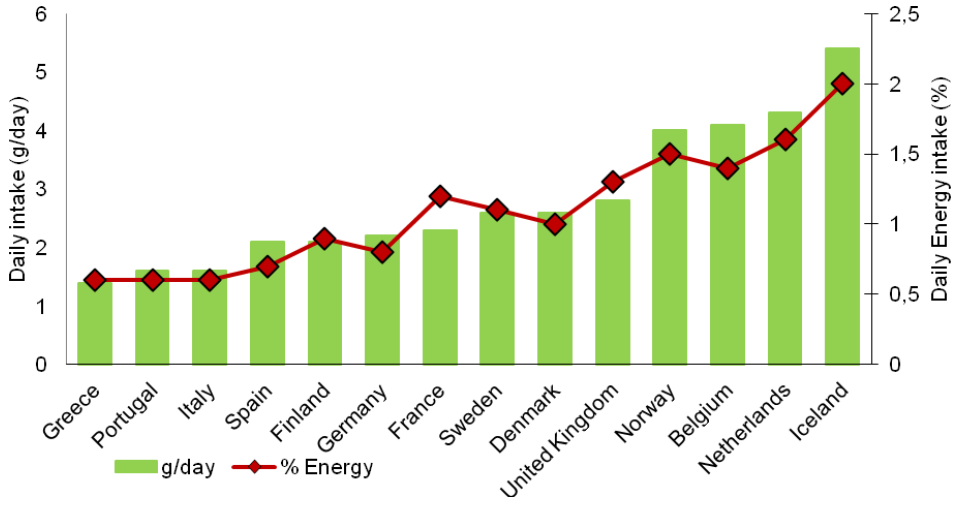

Fig. 3. Mean daily intake of TFAs across the European countries (Innis et al., 1999; Leth et al., 2006; Poppel et al., 1998)

6.1 Dairy products and *trans* fatty acids

Milk fat is also the most abundant source of conjugated linoleic acids (CLAs), which are a group of geometrical and positional isomers of linoleic acid (LA *cis*-9,*cis*-12 C18:2). The major isomer of the CLAs in milk fat is *cis*-9, *trans*-11, and it represents 80 g to 90 g per 100 g of the total CLAs (Chardigny et al., 1995; Ledoux et al., 2005; Seçkim et al., 2005). Some of these fatty acids have biological, physiological and nutritional properties that are very interesting for consumer health, as especially seen for butyric acid and CLAs (Pandya & Ghodke, 2007). The CLAs are synthesised in ruminants both from dietary linoleic acid (*cis*-9,*cis*-12 C18:2) in the rumen by the microbial flora, and from vaccenic acid (*trans*-11 C18:1) in the mammary glands during *de-novo* synthesis (Bauman & Griinari, 2001).

6.2 Industrially produced fat and *trans* fatty acids

Brát and Pokorný (Brát & Pokorný, 2000) investigated a series of 20 margarines, nine cooking fats, and butter that were available on the Czech market. They used the American Oil Chemistry Society standard analysis methods, with capillary gas chromatography. The margarines contained 15.2% to 54.1% cooking fats, and 16.5% to 59.1% SFAs, which was less than the butter. The content of linoleic acid varied between 3.7% and 52.4% in the margarines; small amounts of linolenic acid were present in most samples, while oleic acid prevailed in the cooking fats. Monoenoic TFAs were present only in trace amounts in 10 samples, and *trans*-polyenoic acids were present only in small amounts. Most cooking fats had a high content of TFAs. They summarised these data by indicating that the number of *trans*-free margarines had rapidly increased over a few years.

More recently, Cenčič-Kodba (Cenčič-Kodba, 2007) examined 13 margarines and fatty food samples in Slovenia, which were selected according to the frequency of use among the

population group in the community. All of the fried food and bakery food samples included in this study contained TFAs, the levels of which varied from less than 0.5% to 6.8%. The highest TFA content in the margarines was 5.2%, with 0.3% as the lowest, and a mean margarine TFA content of 2.3%. The main TFAs were the *trans* isomers of mono-unsaturated octadecenoic acid (C18:1).

Similarly, the findings of Larqué et al. (Larqué et al., 2003) suggest that Spanish margarines have moved to becoming products with a potentially healthier distribution of fatty acids. Even so, the great variability shown in the fatty-acid compositions of margarines and the poor labelling continue to highlight the importance of greater consumer information to avoid detrimental changes to the traditional Mediterranean diet in Spain.

7. Conclusions and future trends

It can be concluded at present that the reduction of TFAs in the food supply is a complex issue that has involved, and still involves, interdependent and interrelated stakeholders. Any further actions taken to reduce TFAs need to be carefully considered, regarding both the intended and unintended consequences related to nutrition and public health. As shown above, the WHO (WHO/FAO, 2002) has already included TFA levels in their recommended daily food intake (Table 1). Many different options of alternative oils and fats can now be used to replace TFAs, as many of these are already available, while others are still being developed. However, decisions on which alternatives to use are complicated and often time consuming, and they involve considerations of health effects, food availability, quality and taste, research and development investments, supply-chain management, operational modifications, consumer acceptance, and cost (Borra et al., 2007; FDA, 2003).

As industry responses are now well underway following the policy actions over the past few years, it is possible to take a present-day 'snapshot' of industry activities that provide preliminary answers to these considerations. The first results of most of the anti-*trans* fat campaigns can be seen as modifications that have been made to the fatty-acid compositions of industrial fats. In these fats, there are significantly higher levels of SFAs and possibly a higher index of atherogenicity. Several major food companies have announced efforts to remove TFAs from their leading brands over the past two decades, starting with Unilever in the 1990s, and then more recently with Nestlé in 2002, Kraft in 2003, Campbell's in 2004 (for Goldfish crackers), Kellogg's in 2005, and Frito-Lay in 2006 (for chips). It is of note that the earliest announcements came from European firms, where the use of partially hydrogenated soy was not as common as it was in the United States, and thus this reformulation process has not been as onerous.

The announcements over the last three years or so have reflected the attention brought to this issue through lawsuits and debates about nutritional labelling regulations. Many companies even chose to implement the disclosure of these *trans*-fat contents earlier than the January 1, 2006, deadline, particularly when they were able to advertise 'zero' *trans* fats on their products (Crisco, 2008).

One aspect for producing such zero TFAs lies in the transesterification reactions between vegetable oils and the SFAs of C8:0, C12:0, C14:0 and C16:0. These reactions can be catalysed by an immobilised sn-1,3 specific *Rhizomucor miehei* lipase. When considering a TFA-free or

low TFA fat that is suitable for use as a confectionery fat, a non-hydrogenated vegetable fat composed of an inter-esterified fat can be used: this can be obtained by subjecting a blend of at least one fat rich in lauric acid and at least one fat without lauric acid to inter-esterification (Farmani et al., 2007).

For all of the products introduced in 2005 and 2006 that have claimed to contain no *trans* fats, the most commonly used oil ingredients have been canola, sunflower and soybean oils. Palm oil, which is high in saturated fat, also appears among the commonly used ingredients, but not as an alternative to reducing TFAs. Eleven percent of food producers in the United States still use partially hydrogenated oils as ingredient, because the regulations allow 0.5 g per serving of *trans* fats in products that claim to contain 'no *trans* fat', while the use of small amounts of partially hydrogenated oils has facilitated the reformulation of some products (Unnevehr & Jagmanaite, 2008).

Between 2006 and 2007, consumer awareness of *trans* fats increased and attained levels similar to those for saturated fats. This increased awareness has been associated with improved self-reporting behaviour in consumer shopping for groceries (Eckel et al., 2009). However, food labels and food claims that accompany packed foods are still largely incomprehensible for consumers, and therefore they appear to be of very little use at present. Moreover, in Europe, consumers still cannot identify the content of TFAs in the labelling of food products, particularly as the only legislation that restricts the content of TFAs in Europe is in Denmark.

At the same time, we have to be aware that indicators are showing that the world population is still increasing and is expected to reach nearly 8.9 thousand million (8,900,000,000) by the year 2050 (UN, 2004). Knowing of some of the problems that are associated with this increasing population, we are now combating the need that will arise for more and more potential food products to be used for biofuels (Fink & Medved, 2011). Thus, in the future, it will become increasingly difficult to assure food security and food safety, as well as the nutritional quality of food. Indeed, it is the nutritional quality of food and its distribution all over the World that are the main factors that will have a huge impact on human health. In this way, human health is more than just of personal value, as it is also part of the welfare of the whole of our society.

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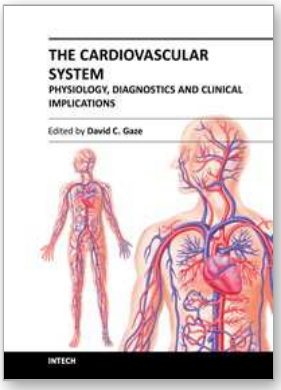

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The cardiovascular system includes the heart located centrally in the thorax and the vessels of the body which carry blood. The cardiovascular (or circulatory) system supplies oxygen from inspired air, via the lungs to the tissues around the body. It is also responsible for the removal of the waste product, carbon dioxide via air expired from the lungs. The cardiovascular system also transports nutrients such as electrolytes, amino acids, enzymes, hormones which are integral to cellular respiration, metabolism and immunity. This book is not meant to be an all encompassing text on cardiovascular physiology and pathology rather a selection of chapters from experts in the field who describe recent advances in basic and clinical sciences. As such, the text is divided into three main sections: Cardiovascular Physiology, Cardiovascular Diagnostics and lastly, Clinical Impact of Cardiovascular Physiology and Pathophysiology.

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