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Trans fatty acid isomers in human health and in the food industry

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ABSTRACT

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. These fatty acids occur naturally in dairy and other natural fats and in some plants. However, industrial hydrogenation of vegetable or marine oils is largely the main source of *trans* fatty acids in our diet. The metabolic effect of *trans* isomers are today a matter of controversy generating diverse extreme positions in light of biochemical, nutritional, and epidemiological studies. *Trans* fatty acids also have been implicated in the etiology of various metabolic and functional disorders, but the main concern about its health effects arose because the structural similarity of these isomers to saturated fatty acids, the lack of specific metabolic functions, and its competition with essential fatty acids. The ingestion of *trans* fatty acids increases low density lipoprotein (LDL) to a degree similar to that of saturated fats, but it also reduces high density lipoproteins (HDL), therefore *trans* isomers are considered more atherogenic than saturated fatty acids. *Trans* isomers increase lipoprotein(a), a non-dietary-related risk of atherogenesis, to levels higher than the corresponding chain-length saturated fatty acid. There is little evidence that *trans* fatty acids are related to cancer risk at any of the major cancer sites. Considerable improvement has been obtained with respect to the metabolic effect of *trans* fatty acids due the development of analytical procedures to evaluate the different isomers in both biological and food samples. The oleochemical food industries have developed several strategies to reduce the *trans* content of hydrogenated oils, and now margarine and other hydrogenated-derived products containing low *trans* or virtually zero *trans* are available and can be obtained in the retail market. The present review provides an outline of the present status of *trans* fatty acids including origin, analytical procedures, estimated ingestion, metabolic effects, efforts to reduce *trans* isomers in our diet, and considerations for future prospects on *trans* isomers.

KEY WORDS: *trans* fatty acid isomers, positional and geometric isomerization of fatty acids, health effects of *trans* isomers, hydrogenation and *trans* fatty acids, conjugated linoleic acid.

1-INTRODUCTION

1-A Basic structural background

Wherever there is a double bond in a fatty acid, there is a possibility for the formation of both positional and/or geometric isomers. Under conditions of partial hydrogenation (see below), a double bond may change from a *cis* to a *trans* configuration (geometric isomerization) or move to other positions in the carbon chain (positional isomerization) (Dutton, 1979). Both types of isomerization may frequently occur in the same fatty acid molecule. The two hydrogen atoms at a *cis* double bond are on the same side of the carbon chain, a situation that produces a bend in the carbon chain. The two hydrogen atoms at a *trans* double bond are diagonally opposite each other and thereby straighten the carbon chain. The *trans* structure of a fatty acid increases its melting point when compared to the *cis* isomer and approaches that of the corresponding saturated form. Therefore, the isomer may be regarded as an intermediate between an original *cis* unsaturated fatty acid and a more completely saturated fatty acid. *Trans* fatty acids (TFAs) are unsaturated fatty acids with at least one double bond in the *trans* configuration. The most common TFAs are monounsaturated, but various diunsaturated *cis*, *trans* and trans, *cis* isomers, and even

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triunsaturated *cis*, *trans* isomers may occur formed from the processing of oils with complex fatty acid composition, such as marine oils (Almendingen *et al.*, 1995).

1-B Historical background

In 1993, a report published in Lancet by Willett *et al.* took the American public and the rest of the western world by surprise. An extensive study of more than 85,000 nurses concluded that women who ate four or more teaspoons of margarine a day had more heart attacks than women who rarely ate margarine. The main goal of this controversial study was the correlation of dietary vegetable oil-based TFA intake with coronary heart disease. The results of the publication received wide media coverage, and consumers who had switched from butter to margarine as part of a "heart healthy diet," became confused and even angry. The results also started biochemical, toxicological and epidemiological research aiming to elucidate the real nutritional and health impact of *trans* fatty acids. Controversy has arisen about the potential health hazards of TFAs in the western diet. Extreme positions range from calling for the elimination of TFAs from the diet by avoiding specific foods, to removing them from processed foods and/or including their amounts on food labels, to the denial of any adverse effects from *trans* isomers, or to the need to modify food processing and production, food labels, health claims, or individual intakes.

2- NOMENCLATURE FOR *TRANS* FATTY ACIDS

According to official International Union of Pure and Applied Chemistry (IUPAC) nomenclature, the position of double bonds in fatty acids is counted from the carboxyl end of the molecule. Thus the vaccenic acid, the major *trans* isomer in milk (Hay & Morrison, 1970), is *trans*-11 or *trans*-Δ11-octadecenoic acid. Biochemists and physiologists find it more advantageous to use the n- ("n minus") terminus, which counts from the methyl end of the molecule, so that numbers remain the same when the carbon atoms are added to or removed from the carboxyl end during metabolism and when double bonds are inserted or removed. The addition of the n- designation to the formulas for TFAs underscores the relationship between *cis* fatty acids and the *trans* isomers formed from them. Thus, vaccenic acid, which arises in the rumen of the cow through bio-hydrogenation of linoleic acid, is designated as *trans*-C18:1 Δ11 according to IUPAC nomenclature, and as *trans*-C18:1 n-7 according to the n-system. Linoleic acid must be *cis*, *cis* C18:2 n-6, n-9 according to this latter nomenclature.

3- CHEMICAL AND PHYSICAL PROPERTIES OF *TRANS* FATTY ACIDS

In a *trans* double bond in an unsaturated fatty acid the two hydrogen atoms bound to the carbon atom that form the double bond are located on opposite sides of the carbon chain. In contrast to the more typical *cis* isomeric configuration, the double bond angle of the TFAs is smaller and the acyl chain is more linear, resulting in a more rigid and straight molecule with a higher melting point. In the *cis* configuration, the hydrogen atoms are on the same side of the carbon chain, resulting in a "kink" in the acyl chain and a more flexible molecule. The spatial structure of TFAs is between that of saturated fatty acids and *cis* unsaturated fatty acids. As a result, oleic acid or *cis* C18:1 n-9 melts at 13°C ; its *trans* isomer elaidic acid (*trans* C18:1 n-9) melts at 44°C; and stearic acid (C18:0), which is straight and saturated, melts at 72°C. Therefore, the number, geometry, and position of double bonds in fatty acids affect the melting behavior of the fats of which they form part. Triglycerides high in saturated fatty acids will easily stack in a crystal lattice and are therefore solid at room temperature. Bends in the triglyceride molecule that hinder crystal formation introduced by *cis* unsaturated double bonds explain why oils are liquid at room temperature or at even lower.

4- NATURAL SOURCES OF *TRANS* FATTY ACIDS

TFAs occur naturally in dairy and other natural fats and in some plants. It has long been known that the rumen and feces of animals and humans contain relatively high amounts of TFAs. Apparently hydrogenation of multiple unsaturated fatty acids from plant material by the ruminal and intestinal bacterial flora is responsible for this occurrence (Kepler *et al.*, 1966). It is well documented that ruminal bacteria can reduce different unsaturated fatty acids, such as linoleic and linolenic acid, to yield largely stearic acid and some *trans* acyl derivatives (Mackie *et al.*, 1991). TFAs arise in the first stomach of ruminants as intermediates of the hydrogenation of dietary unsaturated fatty acids during bacterial fermentation. The first step in this biohydrogenation is the isomerization of linoleic acid to mainly *cis*, *trans* C18:2 n-9, n-11 catalyzed by the anaerobic bacterium *Butyrivibrio fibrisolvens* (Kepler *et al.*,1966). These intermediates are then hydrogenated to form a mixture of mainly *trans* vaccenic acid and elaidic acid (*trans* C18:1 n-9). The physiological reasons for these fatty acid transformations in the anaerobic environment are still unknown. It has been reported that the polyunsaturated fatty acids are more toxic to the anaerobic bacteria than are the product of hydrogenation. Therefore, one function of bacterial fatty acid transformations may be to destroy growth-inhibiting substances (Verhulst *et al.*, 1986). Another suggestion is that this process enables the strictly anaerobic bacteria dispose of the excess of reducing power (Mackie *et al.*, 1991). This bacterial activity is also responsible for the occurrence of TFAs in cow's milk and milk products such as butter (Hay & Morrison, 1970). Depending on the diet, milk fat has 2-9% total TFA isomers (Parodi, 1976). These isomers are also found in the body fat of ruminants such as cattle and sheep, with concentrations ranging from 4% to 11% (similar to butter fat). The major TFAs in milk, butter, and beef fat are *trans* 18:1 n-11 (vaccenic acid). TFAs are also found in goats, deer, and marsupials. In addition to the consumption of artificial-derived fats as hydrogenated vegetable or marine oils, uptake of ruminal bacterial transformation products leads to the presence of TFAs in humans.

In contrast to the TFAs formed by rumen bacteria, the membrane constituents of aerobic bacteria are synthesized by a direct isomerization of the complementary *cis* configuration of the double bond without a shift of the position (Keweloh & Heipieper, 1996). Anaerobes always shift the position of the double bond during fatty acid conversion (Seltzer, 1972). This system of isomerization is located in the cytoplasmic membrane. The conversion of *cis* unsaturated fatty acids to *trans* changes the membrane fluidity in response to environmental stimuli, particularly where growth is inhibited due the presence of high concentrations of toxic substances. Under these conditions, lipid synthesis also stops so that cells are not able to modify their membrane fluidity by any other mechanism. The quantity of TFAs in bacteria can often be greater than that of *cis* unsaturated fatty acids. Similar to other fatty acids in bacteria, the relative amounts of TFAs depend on the physiological condition of the cells such as growth rate, medium composition, and environmental factors.

The chloroplast membranes of higher plants and algae also contain some fatty acids with a double bond with of *trans* configuration (Lamberto & Ackman, 1994). TFAs can be detected in minor amounts in marine animals (fish, shellfish and mammals). These occurrences may be caused by the input and incorporation of fatty acids of microbial or plant origin into membrane lipids.

5- THE DEVELOPMENT OF HYDROGENATED FATS

The types of edible fats harvested, prepared, and sold as fats (the so-called "visible fats") have been influenced by changes in the relative cost of rendering fat from different sources, in the need for fats with specific qualities, and in the growing interest in the healthfulness of the population. In the early 1900's, the primary fat available for

commercial use was lard, which was rendered easily and inexpensively from pork fat. It had a good shelf life and excellent shortening properties, although without the benefits of modern refrigeration, it became semi-solid at warm temperatures. Lard is also high in saturated fat, and growing health concerns about the dangers of excess dietary saturated fat stimulated efforts to find an alternative source of fat.

Pressing seeds and vegetables could produce liquid fat, but this oil lacked the stability and shortening properties of lard. The hydrogenation process was therefore developed to modify liquid vegetable oils so these fats would be suitable substitutes for lard (Patterson, 1996). The first hydrogenated products were a blend of totally hydrogenated cottonseed oil and refined liquid cottonseed oil. This created a product that had the consistency of lard but which was less likely to liquefy at warmer temperatures. The technique of partial hydrogenation was developed in the 1930's and complemented the development of a high-yield solvent extraction method to render fats from vegetables and seeds. In the process of partial hydrogenation, using pressure, temperature, and a metal catalyst (most frequently nickel), hydrogen gas is bubbled through liquid vegetable oil. Under these conditions, the double bonds on monounsaturated and polyunsaturated fatty acids in the liquid oil are subjected to structural modification. Three different modifications can occur: (1) A double bond can be changed to a single bond, e.g., changing a 2-polyunsaturated fatty acid into a monounsaturated fatty acid or a monounsaturated fatty acid into a saturated fatty acid. (2) The location of the double bond can be moved up or down the fatty acid chain, and/or (3) the configuration of the double bond can be changed to either *cis* o *trans*. Highly polyunsaturated fatty acids are the most susceptible to the process of hydrogenation as they contain more double bonds than other fatty acids. Because hydrogenation raises the melting point of the fat, the usual product is a fat that is a solid at 25°C, although there is a wide variation in physical properties, depending on the starting fat and the degree of hydrogenation.

The hydrogenation process is controlled by selecting the appropriate temperature, pressure, duration, catalyst, and source of fat or fats to achieve a product of the desired composition and function. Partial hydrogenation of fats and oils results in a mixture of fatty acids: polyunsaturated (including *trans* isomers), monounsaturated (also including *trans* isomers), and saturated fatty acids. As the degree of hydrogenation increases, the proportion of polyunsaturates decrease, monounsaturates and TFAs increase, and saturates increase only slightly. If hydrogenation is taken to completion, this process results in a fully-hydrogenated fat that contains neither *cis* nor TFAs, but which is made up completely of saturated fatty acids, primarily stearic acid. The basic Food and Drug Administration (FDA, USA) definition of a hydrogenated fat is one that is solid at room temperature. Such fats typically contain 15-25% *trans* fatty acids. Partially hydrogenated oils are defined by the FDA as liquid at room temperature and are lower in *trans* fatty acids. Many vegetable fats and oils consumed in United States, Europe, and some countries of Latin America have been slightly hydrogenated or moderately hydrogenated. Fats such as margarine frequently represent mixtures of fats that are hydrogenated (slightly or moderately) and fats that have not been processed. The types of fats added to a product and their processing methods must be described on the composition label.

6- QUANTIFICATION OF *TRANS* FATTY ACIDS IN BIOLOGICAL SAMPLES OR IN FOODS

The two major assay methods for TFAs are infrared absorption spectroscopy and capillary gas-liquid chromatography. Infrared spectroscopy is the classical method routinely used to determine TFAs in foods over the last two decades. Generally infrared spectroscopy measurements are carried out using TFA methyl ester-derivatives. A major problem is that samples analyzed by infrared spectroscopy as methyl esters produce *trans* levels that are 1.5%-3.0% lower for *trans*

values from 1 to 15% (Firestone & LaBouliere, 1965). The Association of Official Analytical Chemists (Official Methods of Analysis, 1994) therefore proposed correction factors to compensate for the lower absorption of methyl esters.

Gas-liquid chromatography is now the most popular technique. It is widely available and allows identification of individual fatty acids when the suitable standards are available, whereas infrared absorption spectroscopy does not. However, in complex mixtures of isomeric fatty acids such as those present in some foods containing partially hydrogenated vegetable or fish oil, all fatty acid isomers are rarely resolved by gas chromatography. As a result, *cis* and *trans* isomers may overlap, and results may be biased. Pre-separation of *cis* and *trans* isomers by argentation chromatography may solve the problem but is extremely laborious (Ulberth & Henninger, 1992) The problem is even further pronounced by the multitude of isomers of C20:1, C20:2, C22:2, and C22:3 unsaturated fatty acids that can be formed in hydrogenated and partially hydrogenated fish oil (Morgado *et al.*, 1998). Gas chromatography is therefore unsuitable for routine assessment of the TFA content of foods containing partially hydrogenated fish oil, but can be applied successfully to biological samples (*e.g.*: tissue samples) due to the restricted variety of *trans* isomers frequently present in such samples.

Infrared absorption spectroscopy is less sensitive then gas chromatography (although sensitivity may be increased with Fourier transforming equipment) and does not distinguish individual TFAs or detect positional *cis* isomers. This method is nonetheless invaluable as a check on results obtained by gas chromatography. In general, the routine assay of TFAs is more complicated than that of other fatty acids, and figures in food tables and consumption figures for TFAs may be unreliable.

7- CONSUMPTION OF *TRANS* FATTY ACIDS

TFA ingestion has been estimated from dietary questionnaires or recall data, analysis of adipose tissue or milk fat data, or food content analysis data. The basis of TFA intake calculation commonly varies, resulting in varying intake levels in the literature. Another reason for differences are individual and cultural eating habits. Different analytical techniques such as gas-chromatography determination and infrared absorption spectroscopy measurements are also responsible for widely varying intake estimations.

Edible oils, such as refined or unrefined olive, sunflower, safflower, rapeseed, soybean, peanut oil or coconut fat, contain only negligible amounts of TFAs. Refined oils have higher TFA contents than unrefined oils. The amount of TFAs in refined oils is influenced by the duration and temperature of refining. Refined and bleached soybean oil treated with temperatures between 160°C and 240°C was found to contain *trans* 18:3 isomers (O'Keefe *et al.*, 1984). Rapeseed oil heated at 275°C for 3 hours contained approximately 4% *trans* 18:3 isomers (Denecke, 1995). During lower deodorization conditions, *e.g.*: 220°C for 3 hours, the isomerization rate of rapeseed oil was approximately 25% lower. One reason for the increased isomerization rate at 275°C might be the more intense oil mixing resulting from the use of steam, which rules out local overheating with its strong influence on *trans* isomerization (Deneke, 1995).

Historically, the consumption of TFAs has increased steadily since the 1920's, in parallel with the increasing commercial production of margarine and shortenings. TFA consumption within a given population will differ greatly depending on lifestyle and socioeconomic status. The estimated average consumption in developed countries is approximately 7-8 g per capita per day, or 6% of the total fatty acid intake (Report of expert panel, 1995). However, such average intake figures mask the wide range of isomeric fat intakes that actually occur. Although the average absolute intake of *trans* isomers may have declined slightly in recent years due to changes in the extent of hardening of edible oils and a move toward the consumption of softer, less

hydrogenated margarine, average intake is predicted to remain at a relatively constant 7-8 g per capita per day over the next few years. The recent replacement of tallow (3-5% *trans*) by hydrogenated vegetable oil (30% *trans*) for frying in the fast food industry can result in products (*e.g.*: french fried potatoes) with 24%-35% of the total fatty acids in the *trans* form. This suggests that there may be marked increases in the intake of TFA isomers in the present fast-food generation, and average intakes within the population could rise rather than remaining relatively constant. It is difficult to ascertain the absolute intake of isomeric fatty acids in population groups using food availability data based on the figures, and more detailed studies are needed to assess intakes in population groups. The metabolic aspects of isomeric fatty acids relate primarily to *trans* positional isomers of mono- and dienoic acids with the *cis* isomeric having been largely neglected, despite the fact that they can represent 20% or more of the total *cis* fatty acids and have distinct metabolic effects.

8- METABOLISM OF *TRANS* FATTY ACIDS

Dietary TFAs are readily absorbed and incorporated into most mammalian tissues, including human tissues, at concentrations that apparently reflect their content in the diet. The extent of incorporation of individual *cis* and *trans* isomers into tissue lipids is different for specific tissues and between neutral and phospholipids in the same tissues. The brain and placenta apparently discriminate most readily against isomer inclusion compared with heart, liver, and adipose tissue, and the isomer content of phospholipids is generally lower than that of triglycerols (Ohlrogge *et al.*, 1981). The level of dietary polyunsaturated or essential fatty acids will also influence *trans* isomer incorporation into tissue lipids. Inadequate intakes allow greater isomer incorporation (Beare-Rogers, 1988).

Previous research reported a marked discrimination against placental transfer of TFAs to fetal tissues (Ohlrogge *et al.*, 1982). More recent observations, however, clearly show a significant incorporation of *trans* isomers into umbilical cord blood lipids (Koletzko, 1991), although at lower levels than in maternal blood, thus indicating a degree of discrimination against these isomers in the placenta. Low levels of *trans* isomers in premature fetal brain compared with other tissues is also indicative of discrimination by this organ. Breast-fed infants in industrialized countries have a greater intake of TFAs through their mother's milk (2-5% of total milk fatty acids) than those in less developed countries (<1% of total milk fatty acids) which reflects the maternal consumption of these fatty acids (Koletzko, 1991). The high content of TFAs in human milk, even in the upper socioeconomic groups in industrialized countries, reflects their widespread occurrence in human foods (Koletzko *et al.*, 1988). A reduction in the content of these isomers in human milk within days of lowering their intake indicates that they are readily mobilized and catabolized or excreted.

9- BIOLOGICAL EFFECTS OF *TRANS* FATTY ACIDS

9-A TFAs and the physical behavior of biological membranes

The physical properties of biological membranes, which can occur either in a highly-ordered or a fluid crystalline phase state, are determined by the composition of the membrane lipids and fatty acids (Cevc, 1991). The cellular barrier is simultaneously the matrix for enzymes, whose activity depends on its fluidity conditions (Carruthers & Melchior, 1983). On the other hand, some environmental factors, such as the temperature and organic solvents, have a strong influence on membrane fluidity, and thus on the physiological properties of a membrane. Microorganisms, however, can adapt to changes of their membrane fluidity; they react to externally dictated changes in their environment by modifying their membrane to maintain a constant degree of fluidity. This mechanism is called "homeoviscosic

adaptation" (Suutari & Laakso, 1994). Eukaryotic cells mainly regulate the fluidity of their membranes by changes in the cholesterol content. Bacteria, which do not generally posses steroids, must rely on other mechanisms. Most bacteria regulate the fluidity of their membranes by varying the degree of saturation of the membranes' fatty acids (Keweloh *et al.*, 1991).

In membranes with a high content of saturated fatty acids, the acyl chains of the fatty acids produce the optimal hydrophobic interaction with each other, which leads to a highly-packed, rigid membrane. Due to the free rotation around C-C bonds, saturated acyl chains can occupy an all-*trans* conformation, which allows the chain to be maximally extended. This conformation is preferred if the fluidity of a membrane is low. The temperature for the transition from the gel to the liquid-crystal membrane phase rises with an increasing ratio of saturated toward unsaturated fatty acids. Contrary to this, the double bond of *cis* unsaturated fatty acids possesses a non-mobile bend with an angle of 30° in the acyl chain. Due to this bend, the highly ordered package of the acyl chains is disturbed, which leads to a lower phase transition temperature of these membranes (Cronan & Gelman, 1975). The transition temperature of a phosphatidylethanol amine bilayer decreases by about 42°C if only one saturated acyl chain per lipid molecule is replaced by a *cis* unsaturated one (Okuyama *et al.*, 1991). The *trans* configuration has a completely different effect on the fluidity of the phospholipid bilayer than the *cis* one (Cevc, 1991). TFAs can take up a long extended structure, which is similar to that of saturated fatty acids. Membranes built of TFAs can therefore form a more rigid package of the phospholipids than membranes with *cis* fatty acids. The measurement of phase transition temperatures of membranes built from identical phospholipids with *trans* rather than of *cis* unsaturated fatty acids results in significantly higher transition temperatures (Wever *et al.*, 1994). The replacement of one *cis* acyl chain by a *trans* fatty acid in phosphatidylethanol amine increases the transition temperature by 18-31°C, depending on the structure of the other acyl chain of the lipid molecule. Therefore, the conversion of *cis* unsaturated fatty acids into their *trans* configuration results in a significant reduction of the membrane fluidity which is, however, smaller than the replacement of *cis* by saturated fatty acids.

9-B Effects of TFAs on cellular and tissue function

TFAs have been implicated in the etiology of various metabolic and functional disorders. They increase erythrocyte fragility (Decker & Mertz, 1967), produce mitochondrial swelling, thereby reducing its oxygen consumption and ATP synthesis (Zevenbergen *et al.*, 1988), and enhance the arrhytmogenicity of cardiac miocytes in experimental animals, probably by modulating or by diminishing membrane fluidity (Wenzel & Kloepell, 1980). Because of the effects of TFAs on the metabolism of gamma-linolenic and arachidonic acid (Kinsella *et al.*, 1981), ingestion of *trans* isomers can affect the metabolism of prostaglandins and other eicosanoids and may alter platelet aggregation and vascular function as well (Asherio *et al.*, 1994). TFAs also show competitive interactions with essential fatty acid metabolism (EFA) by inhibiting its incorporation into membrane phospholipids and by reducing the conversion of EFAs to eicosanoids in different animal cells (Peacock & Wahle, 1989). *Trans* isomer ingestion results in essential fatty acid deficiency which may be prevented by increasing EFA availability (Kinsella *et al.*, 1981; Zevenbergen & Haddeman, 1989). In addition, the incorporation of TFAs into membrane phospholipids may influence the physical properties of the membrane as well as the activities of the membrane-associated enzymes (Holman *et al.*, 1991). *Trans* isomers have a weaker inhibitory effect on collagen-induced platelet aggregation than do *cis* isomers (Peacock & Wahle, 1988). Further support for a specific effect of isomeric fatty acids derives from evidence that dietary TFAs

inhibit the activities of membrane-bound enzymes such as Na+/K+ ATPase and adenylate cyclase, and reduce the density of beta adrenergic receptors in the plasma membrane of heart tissue taken from animals that were not deficient in EFAs (Alam *et al.*, 1989). However, TFAs do not appear to have significant effect on reproduction, longevity, or the incidence of cancer (Senti, 1985).

Perhaps the primary concerns about the health effects of TFAs have arisen because these isomers are structurally similar to saturated fats, lack the essential metabolic functions of their parent polyunsaturated fats, and compete with EFAs in many complex metabolic pathways (Mann, 1994). Recently important new data on the health effects of TFAs have become available. It has been demonstrated that TFA ingestion increases low density lipoprotein (LDL) cholesterol to a degree similar to that of saturated fats (Mensink & Katan, 1990). The increase in LDL concentration has been attributed in part to the down-regulation of the LDL receptor (Hayashi *et al.*, 1993). In contrast to other forms of fats, *trans* isomers decrease high-density lipoprotein (HDL) cholesterol. The mechanism of this decrease has been postulated to be due to the stimulation of cholesteryl ester transfer lipoprotein activity, which transfers cholesteryl esters from HDL to VLDL and LDL (Abbey & Nestel, 1994). However, TFA feeding is also known to decrease HDL in animal species such as pigs (Jackson *et al.*, 1977) and rats (Sano & Privett, 1980; Morgado *et al*, 1999), which do not have cholesteryl ester transfer protein activity, suggesting that additional mechanisms may be operative. Recently Subbaiah *et al.*, (1998) suggested that TFAs may decrease HDL through the inhibition of lecithin:cholesterol acyltransferase. This enzyme esterifies free cholesterol in plasma by transferring an acyl group from phosphatidylcholine (Glomset & Norum, 1973). Both human and rat lecithin: cholesterol acyltransferases, which are normally specific for the sn-2 acyl group of phosphatidylcholine, exhibit an alteration in their positional specificity when TFAs containing-phosphatidylcholine is used as a substrate. This may induce an inhibition of the enzyme and may allow the formation of more saturated cholesteryl esters, which are more atherogenic due to their increased deposition and reduced clearance from arterial tissue (Phillips *et al.*, 1987). Thus the increase in the ratio of LDL cholesterol to HDL cholesterol for TFAs is approximately double that of saturated fatty acids (Mensink & Katan, 1990). And therefore the effect of TFAs on the serum lipoprotein profile is at least as unfavorable as that of the cholesterol-raising saturated fatty acids, because they not only raise LDL-cholesterol levels, but also lower HDL-cholesterol levels. Similar adverse effects were confirmed in other studies. Unlike other fats, TFAs were found to increase lipoprotein(a), another risk factor for coronary heart disease (Mensink *et al.*, 1992).

Partially hydrogenated marine oils, which are used extensively in the manufacture of cheaper margarines, may contain up to 50% *trans* bonds and considerably high amounts of 20 and 22 monoenes isomers. These isomers, whose tissue distribution is quite different from those found in natural fats, are not incorporated into the membrane phospholipids, but induce peroxisome proliferation and are beta-oxidized to chain lengths of 18 and 16 carbons in the peroxisomes (Hoy, 1991). The marine oils hydrogenated fatty acids *trans* isomers interfere with delta-5 desaturases in the liver (Rosenthal and Doloresco, 1984), resulting in an accumulation of 18:2 n-6 and 20:3 n-6 fatty acids (Hoy, 1991).

9-C TFAs and lipoprotein(a).

Lipoprotein(a) (Lp(a)) is a macromolecular complex, made up of apoprotein B, cholesterol and other lipids, and a protein called apo(a). Apo(a) shows sequence homology to plasminogen (Mc Lean *et al.*,1987). Lp(a) concentration in the blood is largely under genetic control and does not change significantly with age (Dahlén, 1990). Most subjects have Lp(a) levels below 150 mg/L, but levels in some individuals may exceed 400 mg/L. Such

subjects have a markedly increased risk for coronary heart disease, a relationship that is different and must not be confused with serum low-density or high-density lipoprotein (LDL or HDL) cholesterol levels (Sandkamp *et al.*, 1990). In spite of the structural resemblance between Lp(a) and LDL, determinants of serum Lp(a) levels are distinctly different from those of LDL. Although niacin used with neomycin has been reported to decrease Lp(a) levels (Guraker *et al.*, 1985), attempts to modify Lp(a) by drugs (Vessby *et al.*, 1982) or diet (Katan & Beynen, 1987) have not been successful. Hornstra *et al.* (1991), however, have shown that dietary fatty acid composition may affect Lp(a) levels. Although the Lp(a) levels are under genetic regulation and, unlike LDL, Lp(a) levels are insensitive to diet, this suggestion appears to be correct as far as dietary cholesterol is concerned. Dietary fat composition does affect Lp(a) concentrations in a way that correlates with the saturated fatty acid and the *trans* fatty acid composition of the diet. Mensink *et al.* (1992) demonstrated that *trans* monounsaturated fatty acids (*trans*-C18:1) increased Lp(a) levels relative to three other fatty acids with 18 carbon atoms: stearic acid (C18:0), oleic acid (*cis*-C18:1), and linoleic acid (*cis*, *cis*-C18:2). Results of Mensink *et al.*, are also in good agreement with those of Nestel *et al.* (1992), who reported that a diet rich in the TFA elaidic acid (*trans*-C18:1) elevated the levels of Lp(a) in middle hypercholesterolemic men. The general conclusion of these studies is that short-term dietary experiments suggest that diets high in *trans* monounsaturated fatty acids may increase human serum levels of Lp(a).

9-D Conjugated linoleic acid, a special class of trans *fatty acid isomer.*

Conjugated linoleic acid (CLA) is formed, as are other *trans* isomers, in the first stomach of ruminants as an intermediate of the hydrogenation of dietary unsaturated fatty acids during bacterial fermentation. The first step in this biohydrogenation is the isomerization of linoleic acid to mainly *cis*, *trans* C18:2 n-9, n-11, a conjugated form of linoleic acid, catalyzed by the anaerobic bacterium *Butyrivibrio fibrisolvens* (Kepler *et al.*, 1966). These intermediates are then hydrogenated to form a mixture consisting mainly of *trans* vaccenic and elaidic acid. However, a small fraction remains as CLA. This fatty acid can also be formed through the autoxidation of linoleic acid by free radicals, followed by reprotonation of the pentadienyl radical by proteins, for instance whey protein (Fritsche & Steinhart, 1998). Chin *et al.* (1994) investigated the ability of nonruminants (rats) to produce CLA. They supplemented the diet with 5% free linoleic acid or 8.6% corn oil (equivalent to 5% free linoleic acid as triglyceride) and observed higher tissue CLA concentrations in rats fed free linoleic acid than in control animals. These investigators concluded that the intestinal bacterial flora of rats is capable of converting free linoleic acid but not linoleic acid esterified in triglycerides, to *cis*, *trans*, C18:2 n-9, n-11 and *trans*, *cis* C18:2 n-9, n-11.

Strong hydrogenation of soybean oil allows the formation of variable small amounts of CLA (Mossoba *et al.*,1991). Chin *et al.* (1992) found CLA in coconut and olive oil in an order of approximately 0.02 g/100g fat. Another source of CLA was reported by Spitzer *et al.* (1991) who identified this *trans* isomer in exotic seed oils, *e.g.*: in *Acioa edulis* oil, which is used for cooking in areas of Brazil. Dairy products are also a major source of CLA. The content in milk fat ranged from 0.24% to 1.77% (Fritsche & Steinhart, 1998). This wide variability is caused by the range of CLA content in the raw material, which is probably influenced by different dairy cow breeds and feeding systems as well as processing parameters. The CLA content in meat from ruminants is higher than in the meat of non-ruminants (*e.g.*: 1.20% in lamb and 0.12% in pork). In the case of non-ruminants, CLA may occur from dietary sources such as feeding powdered meat and tallow (Fritsche & Steihart, 1998).

CLA shows unique chemoprotective actions. It has been shown to protect against

some types of cancer in both animals and in humans (Ha *et al.*,1990; Ip *et al.*, 1991; Visonneau *et al.*, 1997). In addition to the chemoprotective properties of CLA, this *trans* isomer has also been linked to an influence on the growth and development of rats (Belury & Kempa-Steczko, 1997). A beneficial influence on the development of atherosclerosis has also been associated with CLA (Lee *et al.*, 1994). Another remarkable property of this *trans* isomer is its antioxidant action. In some experimental models, CLA has been shown to be a more potent antioxidant than a-tocopherol and almost as effective as BHT (Ha *et al.*, 1990). To date, all physiological studies on CLA have been carried out with mixtures of CLA isomers (mainly *cis*, *trans* C18:2 n-9, n-11). Therefore, the biologically active isomer(s) remain(s) unknown. Although it is controversial to directly extrapolate from animal studies or cell cultures studies to human beings, CLA may be able to render lasting protection against subsequent cancer risk. It might be desirable to enhance the CLA concentrations in foods to obtain a beneficial level. A supplementation of CLA should therefore be considered (Fritsche & Steinhart, 1998).

9-*E* Trans *fatty acids and cancer.*

Numerous epidemiological studies have reported a positive correlation between increased dietary fat intake and cancers of the breast, colon and prostate (BNF report, 1987). A more specific association between these forms of cancer in humans and the intake of hydrogenated vegetable fats has also been reported (Enig *et al.*, 1978). However, a restricted number of research papers about TFAs and cancer have been published. These papers represent the literature available on *trans* fat and cancer in animal models. The general conclusion is that increasing the intake of TFAs (at expense of *cis* fat) does not produce an adverse outcome with respect to cancer risk. Furthermore, it has even been reported that high *trans* fat ingestion is less tumorigenic than a blend of fat with a lower *trans* content which simulated the composition of dietary fat commonly found in the western diet (Hunter & Applewhite, 1991). Inhibition of prostaglandin E2 synthesis reduces tumor proliferation and metastasis (Karmali, 1980). Therefore, considering the reported inhibitory effects of TFAs on essential fatty acid metabolism and eicosanoid synthesis (Koletzko, 1991), it is conceivable that isomeric *trans* fatty acids could actually reduce tumor growth and metastasis. Although the most recent paper on the subject was published in 1996 (Ip & Marshall), there has been no evidence to date to indicate that TFA intake presents a risk factor for cancer under properly-controlled conditions. The evidence that breast cancer is related to diet stems less from epidemiological evidence than from animal experimentation and from the analysis of ecological data (Selenskas *et al.*, 1984). The epidemiological evidence is that the impact of fat intake in general upon breast cancer risk is slight to negligible (Erickson *et al.*, 1984). There is at present no strong evidence that the intake of TFAs is related to an increased risk of breast cancer (van den Brandt *et al.*, 1993).

There is however, good and increasing evidence that dietary fat is related to the risk of cancer of the colon and rectum (Freudenhein & Graham, 1989). This evidence centers on the intake of saturated fats or animal fat as increasing risk and the intake of fiber and vegetable products as decreasing risk. The clinical trials that are presently underway are evaluating dietary restriction of fat intake and supplementation of fiber and fruit and vegetable intake (Greenberg *et al.*, 1994). No evidence indicates that the intake of TFAs is related to increased risk of cancer of the colon and rectum (Giovannuci *et al.*, 1994). There is good evidence that fat intake might be related to the risk of prostate cancer (Freudenhein & Graham, 1989). Most of this evidence has focused on the consumption of saturated fat and fats of animal origin. The intake of TFAs has not been correlated to the risk of prostate cancer. The one study that directly examined the relevance of TFAs reported that they are not related to risk (Giovannuci *et al.*, 1993). In summary, there is little

evidence that TFAs are related to the risk of cancer at any of the major cancer sites (Ip & Marshall, 1996).

10- EFFORTS TO REDUCE THE CONSUMPTION OF *TRANS* FATTY ACIDS

In response to the controversy provoked by studies of TFA consumption and human health, some companies are now taking steps to develop TFA-reduced or TFA-free products. As margarines are the primary target of criticism, the food industry has focused its efforts on reducing or eliminating the *trans* content of these products. One strategy has been to blend fully-hydrogenated vegetable oils without *trans* isomers with unhydrogenated liquid oils, which naturally have no *trans* isomers. The hardness and the spreadability of the final product must be adjusted by varying the proportion of the solid and the liquid portions of the blend. These procedures have made it possible to reduce the TFA content of stick and tub margarine from 50% to 10% or less. "Low fat," "diet," or "light" margarines, with less than 40% fat, have a *trans* content ranging from 5% to 10%.

Another strategy that has not yet been fully developed is the interesterification of highly-hydrogenated fats with liquid oils by means of chemical or enzymatic-drive procedures. Unlike hydrogenation, interesterification neither affects the degree of saturation nor causes isomerization of the double bonds. Thus it does not change the fatty acid profile of the starting material. Instead, it rearranges the fatty acids in the glycerol molecule. Random interesterification changes the crystal form of an oil or blend to produce the desired solid fat content curves or to produce blends with high levels of polyunsaturated fatty acids. By this mixed procedure, hydrogenation-interesterification, it will be possible to obtain margarine with virtually zero *trans* content. However, to achieve improved results further developments are necessary (Fitch-Haumann, 1994).

The latest development in Europe has been the introduction of a fish oil spread manufactured by FDB Viby Fabrikker of Denmark. The spread consists of 20-25% highly deodorized liquid fish oil and 70-80% of highly hydrogenated vegetable fat. The product called Tobis has *trans* isomer contents lower than 15%. It contains no water, has a slightly yellow color, and is stable to oxidation (Inform, 1990). A product with different characteristics was developed by Unilever NV of the Netherlands. This is a blend of unhydrogenated marine oil and vegetable oil in a ratio of marine to vegetable below 1:3. This product, which has non-*trans* isomers, can be used for frying and baking as well as for making margarine and soft spreads (Inform, 1990). As another approach, non-caloric or hypocaloric fat substitutes are now used to develop non-*trans* and non-cholesterol butter and margarine (Singhal *et al.*, 1991).

Meanwhile, Lipton Canada has obtained two temporary marketing authorizations for including a TFA declaration on food product labels. The company began using the first temporary marketing authorization for TFAs on its Becel line of margarine products in late 1997. Declarations on two of the Becel products – Becel RSF, which contains 26% fat, and its Light version containing 40% fat – list *trans* fat content as zero because of the low fat level per serving. The other two Becel products – its full-fat version and a salt-free margarine – list the *trans* fatty acid content as 0.1 gram per 10 gram (two teaspoon) serving. Also from Lipton is the Fleischmann margarine line, whose products also list their TFA content as 0.1 gram per 10-gram serving. Products containing 0.1 gram of *trans* fat per serving include the statement "Virtually no *trans* fat." The declarations are part of the nutrition listing label.

The replacement of partially hydrogenated soybean oil (or partially hydrogenated fish oil) by palm oil in margarine is another technological alternative used to considerably reduce the *trans* content of the final product. Palm oil, unlike soybean or fish oil, can be used without hydrogenation to achieve a certain hardness of margarine products because of its semisolid texture at room temperature.

Palm oil consists of 50% saturated and 50% unsaturated fatty acids and has a content of 44% palmitic acid and 10.6% linoleic acid. Muller *et al.* (1998) recently demonstrated that a margarine based on palm oil was less atherogenic (based on examination of LDL-cholesterol and HDL-cholesterol profiles) than a soybean-oil, partially-hydrogenated *trans*-containing margarine. The product, however, is less favorable than one based on a more polyunsaturated vegetable oil.

11- FUTURE PROSPECTS FOR *TRANS* FATTY ACIDS

The food industry could voluntarily phase out production of TFAs, but at present producers resist even acknowledging that their products have adverse effects. A voluntary phase-out is therefore unlikely, although some of the largest producers have publicly committed themselves to reducing the *trans* isomer content of their products. An alternative is to develop policies to regulate TFAs in foods by labeling their contents. Some have suggested that the *trans* fatty acids be listed along with the saturated fat on the label, but this must be accompanied by efforts to educate the public about TFAs and the potential risks associated with consuming hydrogenated fats. Warning labels are already applied to alcohol and tobacco products; such action is even further justifiable for products containing partially-hydrogenated fats, as their presence is not readily visible to the consumer.

As a matter of fact, some conclusions may be drawn from the current information and summarized as follows:

- Intake of TFAs cannot be avoided when the diet includes milk fat, ruminant fat, and/or hydrogenated fats in margarine and shortenings.
- The average dietary intake of TFAs will tend to decline due to a general reduction in fat content in foods.
- Nutritionally, TFAs should be of secondary priority after saturated fats. However, high intakes may have adverse effects on the plasma LDL/HDL cholesterol ratio.
- There appear to be many ways to avoid TFAs, but the healthiest requires changes in individual eating habits.
- The U.S. Food and Drug Administration (FDA) is still working on provisions for possible TFA labeling on foods, but does not anticipate releasing a proposal during 1998 (Inform, 1998).

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