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REVIEW

Improving hydrogenated fat for the world population

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Summary Hydrogenated fat made from vegetable or fish oils has served as an increasing source of calories in both developed and developing countries since its introduction into the diet in 1919. The *trans* isomers formed during hydrogenation serve as an energy source and have been assumed to act in the same way as the *trans* fatty acid in butterfat or the tallow rendered from the fat of ruminant animals. More recent studies with porcine fed butter fat vs. hydrogenated fat using corn oil as a control indicated, however, that *trans* fatty acids in hydrogenated fat inhibit acyl-CoA desaturase enzyme activities. Such activities are involved in the metabolic conversion of essential fatty acids (EFA) to polyunsaturated fatty acids (PUFA), which remodel the vascular cell membrane fatty acids composition resulting in calcification of vascular tissue. The *trans* fatty acids in butter and ruminant fat do not inhibit acyl-CoA desaturase or remodel vascular cell membrane fatty acid composition. *Trans* fatty acids in hydrogenated fats increase the production of inflammatory cytokines associated with the pathophysiology of atherosclerosis. Cytokines have a stimulating effect on cyclooxygenase (COX-2) and may inhibit prostacyclin synthase. The elimination of *trans* fatty acids from hydrogenated fat is desirable for three reasons: they remodel vascular cell membrane fatty acid composition so that the influx of calcium increases; they inhibit prostacyclin synthesis; and they increase the production of proinflammatory cytokines. Vegetable oils can be hydrogenated and rearranged with unhydrogenated vegetable oils to contain no *trans* or isomeric fatty acids.

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The history of *trans* fatty acids

Trans fatty acids are formed during the partial hydrogenation of vegetable or fish oils. In 1919, a French chemist found that a liquid oil could be converted to a solid fat by adding hydrogen atoms under pressure to the heated oil [1]. The oils are liquid at room temperature similar to olive oil that has been used for centuries in southern Europe as an important source of fat in the diet. Butter, lard and beef tallow are saturated fats that have been used for centuries as a fat source in the diet in northern Europe. Both oils and fats are triglycerides made from three fatty acids attached to glycerine. If a triglyceride contains more unsaturated fatty acids such as linoleic or linolenic acid than a saturated fatty acid such as stearic or palmitic acid, it is a liquid at room temperature. If it contains more saturated fatty acids, it is a solid at room temperature. The term unsaturated means that the 18 carbon chain of linoleic or linolenic acid does not contain as many hydrogen atoms in the chain as stearic or palmitic acid such as butter fat, lard, or tallow.

Prior to 1952, the fat industry had little control over the hydrogenation of vegetable or fish oils which was carried out in stainless steel tanks in 20,000 lb batches. No reliable instrument to measure the fatty acid composition of hydrogenated fat was available before studies by Martin and Synge [2] on partition chromatography. They received the Nobel Prize in Chemistry in 1952 for their discovery. James and Martin [3] applied partition gas chromatography to the analysis of margarine, which revealed a shifting of double bonds in linoleic, linolenic, or fish oil fatty acids during hydrogenation. Also, a change in the configuration and the position of the double bonds results in the formation of geometrical isomers named ''*trans*'' fatty acids. The change in position of the unsaturation on the chain of carbon atoms during hydrogenation produces a new series of unsaturated fatty acids that add to the semi plastic condition to copy the characteristics of butter fat.

The process by which hydrogenated fat became available in 1919 provided less than 12 g/capita/day in the United States (US) [4] compared to 62.7 g/capita/day by 1990. A similar increase in consumption occurred in other developed countries with somewhat more modest increases in developing countries. Daily intake of *trans* fatty acids in hydrogenated fat in the US was estimated by the Food and Drug Administration (FDA) to be 6.862 g/capita/day in men and 4.776 g/capita/day for women [5] in 2003. Ratnayake and Chen [6] estimated the *trans* fatty acid intake in the general Canadian population to range from 0.5 to 26.1 with a mean of 8.4 g/capita/day in 1995. In northern Europe intake is estimated at 4.5—17 g/capita/day, in southern Europe at 1.34— 4.9 and in Israel 6.5 [7]. The consumption of less *trans* fatty acids in southern Europe, such as in Greece, Italy, Portugal and Spain, is likely due to the availability of olive oil in these countries. The climate, rainfall and soil in northern Europe have provided conditions that favor the production of butter by the dairy industry.

In India, 2.7—4.8 g/capita/day of *trans* fatty acids are consumed with higher intake in the urban than rural areas [8,9]. As New Zealand and Australia have a large dairy industry, only 2.7—4.8 g/capita/day of *trans* fatty acids from hydrogenated oils are consumed in these countries [11]. The least amount of *trans* fatty acids is consumed in Hong Kong, Japan, Korea, and China at 1.5—3 g/capita/day [10]. A large hydrogenation plant is located in a suburb of Tokyo that uses both fish and vegetable oils. The hydrogenation plant in Beijing hydrogenates soybean oil almost exclusively for the production of margarine. In Argentina, Chile, Peru, Ecuador and Brazil from 3 to 19 g/capita/day of *trans* fat are consumed [11]. In Chile, Peru and Ecuador, up to 53% of the fat consumed includes hydrogenated fish oil.

An interesting comparison can be made with the US and Cuba since the latter does not have hydrogenated fat to purchase because of the US embargo, nor does it have any hydrogenation plants. In 1976,

the mortality rate from heart disease, for those over 65 years of age, was higher in the US than in Cuba, or 2183/100,000 and 976/100,000, respectively [12]. The mortality rate from heart disease for those over 65 years of age decreased to 1712 per 100,000 in the US in 2000, possibly due to a decrease of the *trans* fatty acid concentration in hydrogenated fat in the US; it has remained essentially the same in Cuba since 1976.

MacKay and Mensah [13] reported a 39–42% decrease in the death rate from coronary heart disease in northern European countries and a 21–61% increase in the former Soviet Union block countries between 1988 and 1992. The increase in death rate in the former Soviet Union could result from a number of factors such as stress, cigarette smoking, poor diet and excessive alcohol consumption. The higher death rate may also be influenced by the high concentration of *trans* fatty acids in the hydrogenated fat available in the former Soviet Union countries such as Poland. An analysis of Polish margarine indicated that stick margarine contained 41% and baker margarine 55% *trans* fatty acids [14], a higher concentration than in any other northern European country.

Oomen et al. [15] found in a study in the Netherlands that the average *trans* fatty acid intake decreased from 4–3% to 1–9% of energy between 1985 and 1995. After adjustment for age, body mass index, smoking and dietary covariates, the *trans* fatty acid intake at baseline was positively associated with the 10-year risk of coronary heart disease. Oomen et al. concluded that a high intake of *trans* fatty acids (all types of isomers) contribute to the risk of coronary heart disease. A substantial decrease in *trans* fatty acid intake, due to industrial lowering of *trans* content in Dutch edible fats could, therefore, have had a large public-health impact.

In 1968, Dr. Campbell Moses, medical director of the American Heart Association (AHA), appointed a five-member subcommittee on fats from the AHA nutrition committee to revise the 1961 version of Diet and Heart Disease [16]. As a member of this subcommittee, I urged Dr. Moses to ask the Institute of Shortening and Edible Oils Inc to urge its members to decrease the amount of *trans* fatty acids and increase the amount of essential fatty acids (EFA) in their shortenings and margarines. At the time, it was known that an increase in the EFA content of a dietary fat would lower plasma cholesterol levels, and there was strong evidence that *trans* fatty acids increase plasma cholesterol levels [4]. Industry agreed to lower the *trans* fatty acids and increase the level of EFA in shortenings and margarine. The average *trans* fatty acid content of shortenings was decreased from 30% to 20% and margarine from 40% to 27%, and the linoleic acid content of shortening was increased from 8% to 24% and margarine from 11% to 25% after 1968. Dr. Levy, director of the National Heart, Lung, and Blood Institute at the time, believed 1968 to be a watershed as the incidence of coronary heart disease (CHD) has steadily decreased in the US since that year [17].

The *trans* fatty acids formed during commercial hydrogenation differ from those in ruminant fat

Commercially, hydrogenated fat contains a mixture of isomers not present in beef tallow or butter fat. They are *trans* 18:1 isomers ranging from *trans* Δ^3 to Δ^{16} 18:1 [18]. Feeding hydrogenated fat which contained a mixture of *trans* 18:1 isomers to animals decreased the conversion rate of linoleic acid to arachidonic acid due to their inhibitory effect on Δ^6 and Δ^5 desaturases compared to ruminant and butterfat which contain only *trans* Δ^{11} 18:1 acid. The position of the double bond in the *trans* 18:1 acid is important in determining the degree of inhibition. An in vitro study [19] showed that *trans* Δ^3, Δ^4, Δ^7, and Δ^{15} 18:1 isomers are strong inhibitors for Δ^6 desaturase while *trans* Δ^3, Δ^9, Δ^{13}, Δ^{15} 18:1 isomers are strong inhibitors for Δ^5 desaturase. The *trans* Δ^{11} or stearic acid in ruminant or butter fat has no inhibitory effect on Δ^5 or Δ^6 desaturase. Consequently, the presence of mixed *trans* 18:1 isomers could have more collective inhibitory effect on Δ^6 and Δ^5 desaturases than *trans* Δ^{11} 18:1 isomer alone and could explain why *trans* acids in hydrogenated fat can inhibit EFA metabolism while that in ruminant or butterfat does not.

A diet containing 18.0% *trans* fatty acid and 32.8% linoleic acid fed to rats had no inhibitory effect on prostaglandin synthesis while rats fed a diet containing 33.0% *trans* fatty acid and 0.3% linoleic acid released significantly less prostacyclin (PGI_2) and thromboxane (TXB_2) by aorta and platelets as a result of a reduced level of arachidonic acid in their membrane phospholipid [20]. Prostaglandin synthesis in the aorta and platelets was, therefore, influenced by the amount of *trans* fatty acid and linoleic acid in the diet.

Trans free margarines are now available, but they may contain positional isomers which can be converted to polyunsaturated fatty acid (PUFA) that may not have EFA activity. Holman et al. [21] found feeding an EFA-deficient diet or partially

hydrogenated soybean oil induced measurable amounts of unusual PUFA. These PUFA may compete in metabolism of normal PUFA and are substrates for formation of prostaglandin of unknown structure and function.

The *trans* fatty acids in hydrogenated fat affect the integrity of the vascular cell membrane. Inhibition of desaturase activity in the endoplasmic reticulum causes alterations in the physical structure and function of the vascular cell membrane. This observation is supported by the fact that *trans* fatty acids have an inhibiting effect on Δ^6 desaturase in vitro [19] and that dietary hydrogenated fat inhibits desaturase activities in rat liver microsomes in vivo [18]. This explains why the *trans* isomers in hydrogenated fat, but not the saturated fatty acids or *trans* fatty acids in butterfat, act as a competitive inhibitor for Δ^6 desaturase enzymes [20].

Dietary fat is digested within hours and *trans* fatty acids are available in the plasma for inhibition of prostaglandin synthesis essential to blood fluidity [20]. Someone eating a bag of potato chips at dinner and a bag of popcorn in the evening may consume 20 g *trans* fatty acids devoid of EFA activity [7]. The stage may be set for the inhibition of acyl-CoA desaturase enzyme activity and less prostacyclin in the plasma to keep the blood fluid. In addition, the high salt content of potato chips and popcorn will increase blood pressure [22], ''the silent killer'' that contributes to sudden heart attacks.

Trans fatty acids incorporate into human tissue as shown by the analysis of 24 human specimens in 1957 [23]. The aorta contained up to 8.8%, the heart up to 9.3%, adipose tissue up to 12.2% and the liver up to 14.4% *trans* fatty acids. The fact that *trans* fatty acids in hydrogenated fat incorporate into vascular cell membranes and inhibit EFA metabolism was verified in piglets from porcine fed hydrogenated fat [24].

Hydrogenated fat had a distinctly different effect on the percentage of $n-3$ and $n-6$ PUFA metabolites incorporated into the arterial cell membrane of the piglets from mothers fed hydrogenated fat compared with those from mothers fed butterfat. A change in the percentages of the fatty acids in the aorta 3 days after birth and 48 days after birth in piglets born to porcine fed hydrogenated fat indicated that the *trans* fatty acids incorporated into their cellular membrane inhibited the metabolic conversions of linoleic and linolenic acid to longer chain $n-6$ and $n-3$ PUFA metabolites. The significant decrease in the percentage of $n-6$ and $n-3$ PUFA metabolites in aortic phospholipids of piglets born to porcine fed hydrogenated fat indicates an impairment of these metabolic conversions at a time most crucial for these PUFA for vascular cell membrane modeling [25], growth [26], visual acuity [27] and brain development [28].

Although the dietary level of 17.5% *trans* fatty acid was substantially greater for porcine fed hydrogenated fat than what pregnant women would be expected to consume in developed countries [29–31], 2.2–18.7% *trans* fatty acids were found in human milk fat and 1.9–15.6% *trans* fatty acids were found in plasma triacylglycerol in 62 exclusively breast fed infants at 2 months of age in Canada [32]. A lactating mother who consumes snacks that contain *trans* fatty acid (or consumes *trans* fatty acids in other foods) would have a substantial amount of *trans* fatty acids in her milk supply [33] and could pass those to her infant. A local collection of 30 human milk samples contained a mean of 4.72% *trans* fatty acids [34], a concentration which inhibited the conversion of EFA to long chain PUFA in porcine piglets [24]. To date, the FDA has not considered the daily intake of *trans* fatty acids relevant to the health of small children since they do not exhibit overt heart disease. This is shortsighted thinking. Stary [35,36] found fatty streaks and minute amounts of calcium visible under an electron microscope (type II lesions) in the aorta of 99% of human children examined, although *trans* fatty acids were not specifically implicated in those observations. The influence of dietary *trans* fatty acids on the fatty acid composition of the phospholipid fraction from the coronary arteries of human infants is not readily determined. Scott et al. [37] found only 2.4% linoleic acid in the coronary arteries of infants <24 h of age. Only 1.2% linoleic acid was found in the artery from human placenta [38].

Health effects of *trans* fatty acids in hydrogenated fats

In patients that died from primary cardiac arrest, an increase of *trans* fatty acids in red cell membrane was also accompanied by a decrease of total $n-3$ fatty acids [39]. A low blood level of $n-3$ PUFA fatty acids in the plasma is considered a risk for sudden death [40]. The decrease of total $n-3$ PUFA ($20{:}5n-3 + 22{:}6n-3$) in aortic phospholipid can also decrease the systemic arterial compliance which increases the pulse pressure and total vascular resistance and can increase the risk of adverse cardiovascular events [41].

Dietary studies on coronary heart disease during the 1950s and 1960s had typically not considered

the percentage of *trans* fatty acids or the pathophysiology of heart disease [42—48]. An exception was Pilegram who discussed in two reviews the biochemical mechanism in atherogenesis [49,50]. It was shown that the mitochondria in heart smooth muscle cells use fatty acids as a source of energy through oxidative phosphorylation, a process that involves many steps before the mitochondria can use that energy [51]. The *trans* fatty acid (elaidic acid) in hydrogenated fat metabolizes more slowly than the oleic acid in unhydrogenated vegetable oil [52,53]. Whether this is significant to a heart under the stress of a heart attack remains unknown.

One of the most comprehensive studies on the possible role of dietary fat (The National Heart Study) [54] in heart disease was carried out in the 1960s. In this study, persons consuming margarine C (with 12% *trans* fatty acids and 62% linoleic acid) had serum cholesterol levels 20 mg% lower than those consuming margarine D (with 38% *trans* fatty acids and only 12% linoleic acid). Margarine D had no essential fatty acid activity. (The director of research of the company furnishing these fats, supplied these figures in a personal communication.) These diet studies used gas chromatography as the standard method recommended by the FDA to measure the linoleic acid content of a hydrogenated fat, but the gas chromatography process does not indicate essential fatty acid activity. Unless hydrogenated fat is blended with unhydrogenated fat, there is no assurance that it has essential fatty acid activity. However, the human diet contains both saturated and unsaturated fatty acids. When both were fed, there was no change in plasma cholesterol level [55,56]. Essential fatty acid activity can be established by feeding the fat to an animal and noting weight gain.

FDA response to *trans* fatty acids in the diet

The significance of *trans* fatty acids in human nutrition was not apparent until 1979 in a symposium of 13 participants with only one member from a medical school. The preface to the symposium [57] stated:

As a result of advances in processing technology, particularly catalytic hydrogenation, nutritionists, biochemists, and medical researchers were faced with a new and highly complex problem. New analytical methodology showed that hydrogenated oil contained a large number of both geometrical and positional fatty acid isomers whose nutritional value and biological effects were unknown. Concern was voiced among the scientific community that isomeric fats might not be safe since biological organisms might not be able to properly utilize these ''new'' isomeric fats that were produced during hydrogenation.

On July 23, 2003, the FDA in the US issued a directive that required labeling by January 1, 2006 of foods that contain *trans* fatty acids [58]. The FDA based this directive on 160 peer-reviewed articles and not on the symposium published in 1979. The FDA's major concern was the role of *trans* fatty acids in increasing the plasma cholesterol concentration of low-density lipoprotein (LDL-C) considered an established risk factor for coronary heart disease. A series of clinical studies in the Netherlands at the Department of Human Nutrition in the Agricultural University at Wageningen focused on the negative effect of the *trans* fatty acids in hydrogenated fat on the level of LDL-C and high density lipoprotein (HDL-C) plasma levels in healthy subjects [59—63]. They found that the replacement of 10% of energy from saturated fatty acids by *trans* fatty acids decreased serum HDL-C cholesterol by 21% and impaired flow mediated vasodilation (FMD) as an endpoint in dietary intervention [64,65].

That both hydrogenated fat and animal fats contain *trans* fatty acids [66,67] provided a reason for the FDA to recommend a total daily intake of 2.6% of *trans* fatty acid/day in the diet [68]. The *trans* Δ^{11} 18:1 in beef contributes to the total *trans* fatty acid intake but it has no influence on cell modeling or prostaglandin synthesis. Beef, an excellent source of protein, needs to remain in the diet. The FDA assumed that approximately 50% of the *trans* fatty acids in the diet are furnished by beef. Animal fat (*trans* Δ^{11}-18:1) does not interfere with EFA metabolism as our study on porcine piglets indicated [24].

The hazards of *trans* and isomeric fatty acids in hydrogenated fat were not fully appreciated until the study of Han et al. [69] in 2002. They found that the consumption of a diet that contained 6.7% of the energy from *trans* fatty acids increased production of inflammatory cytokines that have been associated with the pathophysiology of atherosclerosis. Mozaffarian et al. recently found [70] that the *trans* fatty acids in hydrogenated fat are strongly associated with systemic inflammation in patients with heart disease. The most recent study from this group [71] suggested that a higher intake of *trans* fatty acids could adversely affect endothelial function. Fitzgerald and Patrono [72] found cytokine to upregulate the cyclooxygenase-2 (COX-2) expression from which they concluded

that COX-2 could affect the rate of prostacyclin (PGI_2) synthesis. Vila [73] in a review on cyclooygenase and atherosclerosis stated that cytokines over-expressed COX-2, and that prostacyclin synthase (PGIS) was inactivated by the peroxynitrite radical produced from the interaction between hydroxyl radicals and NO leading to a reduction of PGI_2 synthesis [74]. Linking the studies of Fitzgerald, Patrono and Vila reveals that *trans* fatty acids could have an effect on COX-2 and PGI_2 synthesis. Prostacyclin is part of a homeostatic defense mechanism that limits the consequences of platelet activation. Suppression of COX-2 dependent formation of prostaglandin by coxibs such as Vioxx may predispose patients to myocardial infarction or thrombotic stroke [75]. The direct effect of hydrogenated oils on COX-2 and prostacyclin synthesis requires further study.

For the FDA to allow the *trans* fatty acids in hydrogenated vegetable oils to remain in food products, on the assumption of limiting consumption to 2.6% *trans* fatty acids out of the total daily fat intake of which 50% may be from Δ^{11} 18:1 from animal protein sources in the diet, may not, however, prevent an increase in the production of inflammatory cytokines or the remodeling of vascular cell membranes by *trans* fatty acids. The only safe way to continue to use hydrogenated vegetable and fish oil as an essential food product is to eliminate *trans* fatty acids in hydrogenated fat from the food supply. Over 50 years ago, the director of research of an international company had indicated that his company could produce *trans* free hydrogenated fat for an additional two cents per lb in processing costs, but he could not do it unless other companies did the same. Obviously an FDA mandate is required for companies to stay competitive in the manufacturing field and remain financially solvent. On November 18, 2004, Health Canada announced a multi-stakeholder task force to find ways to reduce *trans* fat in Canadian food.

Conclusion

The assumptions of the FDA to allow *trans* fatty acids to continue at an average of 2.6%/capita of total fat in the daily diet based on the fact that *trans* fatty acid also naturally exist in animal fat is very tenuous. The *trans* fatty acid in ruminant fat does not affect normal vascular cell modeling. The industry is technologically capable of producing a *trans* free hydrogenated fat to provide an essential source of calories for both the developed and developing countries.

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