Declaration of Erich O. Grosz

Exhibit 6

Case No. 1:07-CV-01092 (RJL)

Letter Report on Dietary Reference Intakes for *Trans* Fatty Acids

Drawn from the Report on

Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids

A Report of the
Panel on Macronutrients,
Subcommittees on Upper Reference Levels of Nutrients
and on Interpretation and Uses of Dietary Reference Intakes, and the
Standing Committee on the Scientific Evaluation
of Dietary Reference Intakes

Food and Nutrition Board

INSTITUTE OF MEDICINE

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

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This letter report has been posted as a pdf file on the National Academies websit at **www.iom.edu/fnb**. It is excerpted from the full report, *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, which will be available for sale in late summer from the National Academy Press, 2101 Constitution Avenue, N.W., Box 285, Washington, DC 20055; call (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area), or visit the NAP's on-line bookstore at **http://www.nap.edu**.

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Willing is not enough; we must do.

—Goethe

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DIETARY REFERENCE INTAKES FOR *TRANS* FATTY ACIDS

ORIGIN OF THIS REPORT

At the request of the U.S. Food and Drug Administration (FDA), the Food and Nutrition Board of the Institute of Medicine (IOM) has prepared this letter report on *trans* fatty acids. It is based on part of the chapter, Dietary Fats: Total Fat and Fatty Acids, contained in the forthcoming IOM report, *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.*

We hope this letter report will be helpful to FDA as it considers a petition to label foods with their *trans* fatty acid content. While this report might not be identical to the corresponding section of the full report once it is released, any changes will be editorial in nature. The findings in this letter report have full standing as an IOM report.

BACKGROUND ON *TRANS* FATTY ACIDS

Trans fatty acids are unsaturated fatty acids that contain at least one double bond in the *trans* configuration. The *trans* double bond configuration results in a greater bond angle than the *cis* configuration. This results in a more extended fatty acid carbon chain more similar to that of saturated fatty acids rather than that of *cis* unsaturated double bond containing fatty acids. The conformation of the double bond(s) impacts on the physical properties of the fatty acid. Those fatty acids containing a *trans* double bond have the potential for closer packing or aligning of acyl chains, resulting in decreased mobility; hence fluidity is reduced when compared to fatty acids containing a *cis* double bond. Partial hydrogenation of polyunsaturated oils causes isomerization of some of the remaining double bonds and migration of others, resulting in an increase in the *trans* fatty acid content and the hardening of fat. Hydrogenation of oils, such as corn oil, can result in both *cis* and *trans* double bonds, anywhere between carbon 4 and carbon 16. A major *trans* fatty acid is elaidic acid (9*trans* 18:1), but during hydrogenation of polyunsaturated fatty acids, small amounts of several other *trans* fatty acids (9*trans*,12*cis* 18:2; 9*cis* and 12*trans* 18:2) are produced. In addition to these isomers, dairy fat and meats contain 9*trans* 16:1 and conjugated dienes (9*cis*,11*trans* 18:2). Foods containing hydrogenated oils tend to have a higher *trans* fatty acid content than those that do not contain hydrogenated oils (Emken, 1995).

Conjugated linoleic acid (CLA) is a collective term for a group of geometric and positional isomers of linoleic acid in which the *trans/cis* double bonds are conjugated; that is, the double

bonds occur without an intervening carbon atom not part of a double bond. At least nine different isomers of CLA have been reported as minor constituents of food (Ha et al., 1989), but only two of the isomers, *cis*-9,*trans*-11 and *trans*-10,*cis*-12, possess biological activity (Pariza et al., 2001). There is limited evidence to suggest that the *trans*-10,*cis*-12 isomer reduces the uptake of lipids by the adipocyte and that the *cis*-9, *trans*-11 isomer is active in inhibiting carcinogenesis. Similarly, there are limited to data to show that *cis*9,*trans*11 and *trans*10,*cis*12 isomers inhibit atherogenesis (Kritchevsky et al., 2000).

CLA is naturally present in dairy products and ruminant meats as a consequence of biohydrogenation in the rumen. *Butyrivibrio fibrisolvens*, a ruminant microorganism, is responsible for the production of the *cis*-9,*trans*-11 CLA isomer that is synthesized as a result of the biohydrogenation of linoleic acid (Noble et al., 1974). The *cis*-9,*trans*-11 CLA isomer may be directly absorbed or further metabolized to *trans*-11 octadecenoic acid (vaccenic acid) (Pariza et al., 2001). After absorption, vaccenic acid can then be converted back to *cis*-9,*trans*-11 CLA within mammalian cells by Δ9 desaturase (Adlof et al., 2000; Chin et al., 1994; Griinari et al., 2000; Santora et al., 2000). Additionally, the biohydrogenation of several other polyunsaturated fatty acids has been shown to produce vaccenic acid as an intermediate (Griinari and Bauman, 1999), thus providing additional substrate for the endogenous production of *cis*-9,*trans*-11 CLA. Griinari and coworkers (2000) estimate that approximately 64 percent of the CLA in cow milk is of endogenous origin.

Verhulst and coworkers (1987) isolated a microorganism, *Propionibacterium acnes*, that appears to have the ability to convert linoleic acid to *trans*-10,*cis*-12 CLA, an isomer of CLA that is found in rumen digesta (Fellner et al., 1999). *Trans*-10 octadecenoic acid is formed in the rumen via biohydrogenation of *trans*-10,*cis*-12 CLA, and both have been reported to be found in cow milk (Griinari and Bauman, 1999). However, endogenous production of *trans*-10,*cis*-12 CLA from *trans*-10 octadecenoic acid does not occur because mammalian cells do not posses the Δ12 desaturase enzyme (Adlof et al., 2000; Pariza et al., 2001). Therefore, any *trans*-10,*cis*-12 CLA isomer that is reported in mammalian tissue or sera would likely originate from gastrointestinal absorption.

Small amounts of *trans* fatty acids and CLA are present in all diets. They can serve as a source of fuel energy for the body. However, there are no known requirements for *trans* fatty acids and CLA for specific body functions.

Absorption

Similar to other fatty acids, the coefficient of absorption of elaidic acid (18:1*t*) is about 95 percent (Emken, 1979). Studies in humans using pure triacylglycerols containing deuterated *cis* and *trans* octadecenoic acid isomers varying in melting point and double bond position suggest that the presence of *trans* double bond(s) in the fatty acyl chain has no measurable effect on efficiency of absorption (Emken, 1979, 1984).

Transport

Trans fatty acids are transported similarly to other dietary fatty acids and are distributed within the cholesteryl ester, triacylglycerol, and phospholipid fractions of lipoproteins (Vidgren et al., 1998). Platelet lipids also contain *trans* fatty acids, and their composition reflects *trans* fatty acid intake as do other tissues, with the exception of the brain (Mensink and Hornstra, 1995).

Metabolism

The *trans* isomers of oleic acid and linoleic acid that are formed during partial hydrogenation of unsaturated vegetable oils have been suggested to have potential adverse effects in fetal and infant growth and development through inhibition of the desaturation of linoleic acid and α-linolenic acid to arachidonic acid and docosahexaenoic acid (DHA), respectively (Koletzko, 1992; van Houwelingen and Hornstra, 1994). Many animal and in vitro studies, however, have involved much higher amounts of *trans* than all *cis* polyunsaturated fatty acids (Hwang et al., 1982; Shimp et al., 1982). Other animal studies have suggested that the deleterious effects seen with high intakes of *trans* fatty acid intake do not occur with amounts comparable to those consumed in a normal human diet containing sufficient amounts of linoleic acid (Bruckner et al., 1982; Zevenbergen et al., 1988).

Available animal and human data indicate that adipose tissue *trans* fatty acid content reflects the content of the diet, and that selective accumulation does not occur (Emken, 1984). More recent attention has been focused on validating the use of adipose *trans* fatty acid content as a measure of long-term dietary intake. In a study of Canadian subjects, Chen and colleagues (1995b) reported that adipose tissue *trans* fatty acid patterns, particularly those isomers found in partially hydrogenated vegetable fat, reflected dietary sources. Garland and coworkers (1998) also reported that adipose tissue *trans* fatty acid patterns correlated with intake and noted a stronger relationship with the isomers found in vegetable rather than animal fat. The authors cautioned that the later conclusion may have been due to the smaller between-person variability with animal versus vegetable *trans* fatty acid intake. In a letter to the editor regarding this study, Aro and Salminen (1998) suggested that the stronger correlation between adipose tissue *trans* fatty acid isomers found in hydrogenated vegetable rather than animal fat may be attributable to different rates of metabolism of the *trans* isomers. Two groups have used adipose tissue *trans* fatty acid to corroborate dietary *trans* fatty acid intake derived from food frequency questionnaires and found a strong relationship (Lemaitre et al., 1998; London et al., 1991). Despite these observations, it should be noted that adipose tissue *trans* fatty acid profiles can be confounded by the retention of intermediate products of β-oxidation (Emken, 1995).

Excretion

Trans fatty acids are completely catabolized to carbon dioxide and water.

Impact of *Trans* Fatty Acids on *n*-6 and *n*-3 Metabolism

The *trans* isomers of oleic acid and linoleic acid, which are present in hydrogenated vegetable oils and meats, have been suggested to have adverse effects on growth and development through inhibition of the desaturation of linoleic acid and α-linolenic acid to arachidonic acid and DHA, respectively (Sugano and Ikeda, 1996). Desaturation and elongation of *trans* linoleic and α-linolenic acid isomers containing a double bond at the *cis*-12 and *cis*-15 position, respectively, with formation of 20 and 22 carbon chain metabolites that could be incorporated into membrane lipids, has also been suggested. In vitro studies and studies with animals fed diets high in *trans* fatty acids have found evidence of reduced essential *n*-6 and *n*-3 fatty acid desaturation (Cook, 1981; Rosenthal and Doloresco, 1984). An inverse association between total *trans* fatty acids and arachidonic acid and DHA concentrations in plasma cholesteryl esters, and between plasma cholesteryl esters, elaidic acid (18:1*trans*), and birth

weight of premature infants has been reported (Koletzko, 1992). Studies in term infants found no relation between *trans* fatty acids and length of gestation, birth weight, or birth length (Elias and Innis, 2001). Similarly, an inverse association between plasma phospholipid *trans* fatty acids and arachidonic acid has been found for children aged 1 to 15 years (Decsi and Koletzko, 1995). The industrial hydrogenation of vegetable oils results in destruction of *cis* essential *n*-6 and *n*-3 fatty acids and the formation of *trans* fatty acids (Valenzuela and Morgado, 1999). It is not clear if differences in dietary intakes of *n*-6 and *n*-3 fatty acids, rather than inhibition of linoleic acid and α-linolenic acid desaturation by *trans* fatty acids, explains the statistical inverse associations between *trans* and *n*-6 and *n*-3 fatty acids reported in some studies (Craig-Schmidt, 2001). Based on the much greater affinity of the Δ6 desaturase for *cis* *n*-3 and *n*-6 fatty acids than monounsaturated fatty acids (Brenner, 1974; Castuma et al., 1977) and experimental work to show inhibition of the Δ6 desaturation of linoleic acid is not of concern with linoleic acid intakes above about 2 percent of energy (Zevenbergen et al., 1988), it seems unlikely that inhibition of essential fatty acid metabolism by *trans* fatty acids is of concern for practical human diets.

FINDINGS BY LIFE STAGE AND GENDER GROUP

There are no data available to indicate a health benefit from consuming *trans* fatty acids. Therefore, an Adequate Intake, Estimated Average Requirement, and Recommended Dietary Allowance are not established for *trans* fatty acids.

FOOD SOURCES OF *TRANS* FATTY ACIDS

Reports listing the *trans* fatty acid level in selected food items are available from the United States (Enig et al., 1990; Litin and Sacks, 1993; Michels and Sacks, 1995), Canada (Ratnayake et al., 1993), and Europe (Aro et al., 1998a, 1998b, 1998c; Michels and Sacks, 1995; van Erp-baart et al., 1998; van Poppel et al., 1998). More recently, a comprehensive U.S. database was compiled by the U.S. Department of Agriculture's Agricultural Research Service (ARS, 2001), which included a description of the methodology used to formulate the nutrient values (Schakel et al., 1997). *Trans* fatty acids are present in foods containing traditional stick margarine (3.04 g *trans* fatty acids/serving) and vegetable shortenings (2.54 g/serving) that have been subjected to hydrogenation, as well as in milk (0.22 g/serving), butter (0.40 g/serving), and meats (0.01 to 0.21 g/serving) (Emken, 1995). Therefore, foods that are contributors of *trans* fatty acids include pastries, fried foods (e.g., doughnuts and french fries), dairy products, and meats. Human milk contains approximately 1 to 5 percent of total energy as *trans* fatty acids (Table 1).

DIETARY INTAKE OF *TRANS* FATTY ACIDS

Estimating the amount of *trans* fatty acids in the food supply has been hampered by the lack of an accurate and comprehensive database on which to derive the data and the trend towards the reformulation of products over the past decade to reduce levels. This later issue complicates analysis of historical food intake data. Additionally, the variability in the *trans* fatty acid content of foods within a food category is extensive and can introduce substantial error when the calculations are based on food frequency questionnaires that heavily rely on the grouping of similar foods (Innis et al., 1999). *Trans* fatty acid intake is not currently collected in U.S. national surveys.

TABLE 1 *Trans* Fatty Acid Content in Term Human Milk of Women in the United States and Canada

Reference	Study Population/Stage of Lactation[a]	*Trans* Fatty Acid	Content in Human Milk	
			% of Total Fatty Acids	% of Total Energy[b]
Gibson and Kneebone, 1981	120 women, 40–45 d pp	16:1	Trace	Trace
		18:1	~10	~5.46
Chappell et al., 1985	7 women, 1–37 d pp	18:1(9)	2.6 ± 0.4	1.42
		18:1(7)	0.1 ± 0.03	0.05
		18:1(5)	0.1 ± 0.04	0.05
		18:2(6) c,t + t,c[c]	0.1 ± 0.4	0.05
		Total	2.9	1.57
Chen et al., 1995a	198 samples, 3–4 weeks pp	Total *trans*	7.19 ± 3.03	3.92
Innis and King, 1999	103 women, 2 mo pp	Total *trans*	7.1 ± 0.32	3.88

[a] pp = postpartum.
[b] Calculated using the following values: 40g fat/L milk, 8.87 kcal/g fat, 650 kcal/L milk.
[c] c,t + t,c = *cis, trans* and *trans, cis*.

Early reports suggested a wide range of *trans* fatty acid intakes, 2.6 to 12.8 g/day (Emken, 1995). The lower estimated intakes tended to be derived from food frequency data, whereas the higher estimated intakes tended to be derived from food availability data. More recent data from food frequency questionnaires collected in the United States suggest average *trans* fatty acid intakes of 1.5 to 2.2 percent of energy (Ascherio et al., 1994; Hu et al., 1997) or 5.2 percent of total dietary fat (Lemaitre et al., 1998). Intakes of about 1 to 2 percent of energy have been reported for women in Canada, although the range of intake was wide (Elias and Innis, 2001, 2002). Most recently, *trans* fatty acid intake was estimated using data from the Continuing Survey of Food Intakes by Individuals (Allison et al., 1999). The mean *trans* fatty acid intake for the U.S. population aged 3 years and older was 2.6 percent of total energy intake.

ADVERSE EFFECTS OF *TRANS* FATTY ACIDS

Hazard Identification

Total and Low-Density Lipoprotein Cholesterol Concentrations

Prior to 1980 there was generally little concern about the trend toward increased consumption of hydrogenated fat in the U.S. diet, especially when the hydrogenated fats displaced fats relatively high in saturated fatty acids (Denke, 1995). During the early 1980s studies showed a hypercholesterolemic effect of *trans* fatty acids in rabbits (Kritchevsky, 1982; Ruttenberg et al., 1983). Renewed interest in the topic of hydrogenated fat in human diets, or more precisely *trans* fatty acid intake, started in the early 1990s. The availability of a methodology able to distinguish the responses of individual lipoprotein classes to dietary

modification expanded the depth to which the topic could be readdressed. A report from the Netherlands suggested that a diet enriched in elaidic acid (a subfraction of 18:1 *trans*), compared to one enriched in oleic acid (18:1 *cis*), increased total and low-density lipoprotein (LDL) cholesterol concentrations and decreased high-density lipoprotein (HDL) cholesterol concentration, hence resulting in a less favorable total cholesterol/HDL cholesterol ratio (Mensink and Katan, 1990). Consumption of a diet enriched with saturated fatty acids resulted in LDL cholesterol concentrations similar to those observed after subjects consumed a diet high in elaidic acid, but HDL cholesterol concentrations were similar to those observed after subjects consumed the diet high in oleic acid. A number of studies on the topic have been published since then and have reported that hydrogenated fat/*trans* fatty acid consumption increases LDL cholesterol concentrations (Aro et al., 1997; Judd et al., 1994, 1998; Louheranta et al., 1999; Müller et al., 1998; Sundram et al., 1997) (Tables 2, 3, and 4). Recent data have demonstrated a dose-dependent relationship between *trans* fatty acid intake and the LDL:HDL ratio and when combining a number of studies, the magnitude of this effect is greater for *trans* fatty acids compared to saturated fatty acids (Figure 1) (Ascherio et al., 1999).

Similar to the metabolic/clinical trial data, studies in free-living subjects asked to substitute hydrogenated fat for other fat in their habitual diet resulted in higher concentrations of total and LDL cholesterol (Table 4) (Nestel et al., 1992b; Noakes and Clifton, 1998; Seppänen-Laakso et al., 1993).

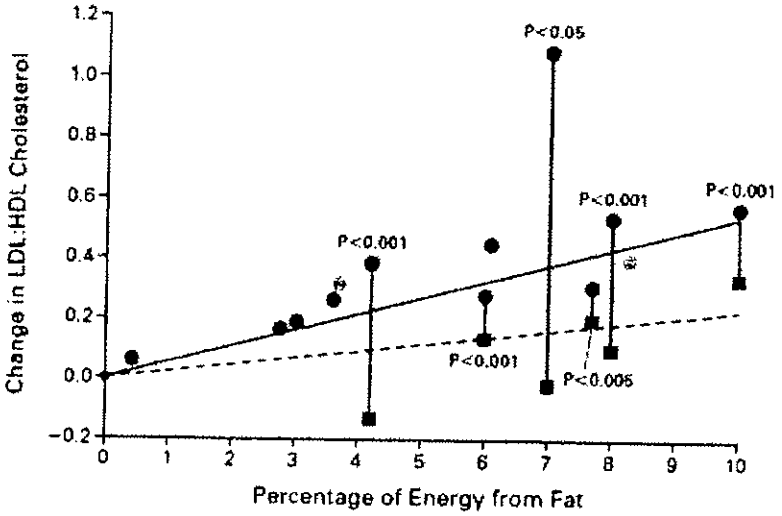

FIGURE 1 Change in the low-density lipoprotein (LDL):high-density lipoprotein (HDL) cholesterol concentration with increasing energy intake from saturated and *trans* fatty acids. Solid line represents the best-fit regression for *trans* fatty acids. Dotted line represents the best-fit regression for saturated fatty acids. Reprinted, with permission, from Ascherio et al. (1999). Copyright 1999 by the Massachusetts Medical Society.

TABLE 2 Dietary *Trans* Fatty Acids (TFA) and Blood Lipid Concentration: Controlled Feeding Trials

Reference	Study Population	Diet[a]	TFA (% of energy)	Blood Lipid Concentrations[b] LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (mg/L)
Mensink and Katan, 1990; Mensink et al., 1992	79 men and women, avg 25–26 y	3-wk crossover, 40% fat				
		10% 18:1	0	2.67[c]	1.42[c]	32[c]
		10% SFA	1.8	3.14[d]	1.42[c]	26[d]
		10% TFA	10.9	3.04[e]	1.25[d]	45[e]
Zock and Katan, 1992	56 healthy men and women	3 wk crossover, 41% fat				
		18:2	0.1	2.83[c]	1.47[c]	
		18:0	0.3	3.00[d]	1.41[d]	
		TFA	7.7	3.07[d]	1.37[d]	
Judd et al., 1994	58 men and women	6-wk crossover, 40% fat				
		18:1	0.7	3.34[c]	1.42[c]	
		SFA	0.7	3.64[d]	1.40[c,d]	
		moderate TFA	3.8	3.54[e]	1.47[c]	
		high TFA	6.6	3.60[d,e]	1.38[d]	
Aro et al., 1997	80 healthy men and women, 20–52 y	5-wk intervention, 33% fat				
		18:0	0.4	2.89[c]	1.42[c]	270[c]
		TFA	8.7	3.13[d]	1.22[d]	308[d]
Sundram et al., 1997	27 men and women, 19–39 y	4-wk crossover, 31% fat				
		18:1	0	3.17	1.25	128.3
		16:0	0	3.15	1.26	122.0
		12:0 + 14:0	0	3.57	1.18	134.3
		TFA	6.9	3.81	1.05	153.3
Louheranta et al., 1999	14 healthy women, avg 23 y	4-wk crossover, 37% fat				
		18:1	0	2.53	1.37	225 (units/L)
		TFA	5.1	2.64	1.31	220 (units/L)

[a] SFA = saturated fatty acid.
[b] LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, Lp(a) = lipoprotein(a).
[c,d,e] Different lettered superscripts within each study indicates values were significantly different.

TABLE 3 Hydrogenated Fat Intake and Blood Lipid Concentrations: Controlled Feeding Trials

Reference	Study Population	Diet[a]	TFA[b] (% of energy)	Blood Lipid Concetrations[c] LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (mg/L)
Lichtenstein et al., 1993	14 men and women, 44–78 y	32-d crossover, 30% fat				
		Baseline	0.77	3.96[d]	1.24[d]	140[d]
		Corn oil	0.44	3.23[e]	1.14[e]	160[d]
		Corn oil margarine	4.16	3.49[e]	1.11[e]	130[d]
Almendingen et al., 1995	31 men, 21–46 y	3-wk crossover, 33–36% fat				
		Butter	0.9	3.81[d]	1.05[d]	194[d]
		PHFO	8.0	3.94[d,f]	0.98[e]	234[e]
		PHSO	8.5	3.58[e]	1.05[d]	238[e]
Judd et al., 1998	46 men and women, 28–65 y	5-wk crossover, 34% fat				
		PUFA-M	2.4	3.21[d]	1.24[d]	197[d]
		Butter	2.7	3.44[e]	1.27[d]	186[e]
		TFA-M	3.9	3.27[f]	1.24[d]	202[d]
Müller et al., 1998	16 healthy females, 19–30 y	14-d crossover, 31–32% fat				
		Vegetable oil	1.1	2.63[d]	1.32[d]	212[d]
		PHFO	7.7	2.87[e]	1.28[d]	225[d]
Lichtenstein et al., 1999	36 men and women, > 50 y	35-d crossover, 30% fat				
		Soybean oil	0.55	3.98[d]	1.11[d,e]	230
		Semiliquid margarine	0.91	4.01[d,e]	1.11[d,e]	230
		Butter	1.25	4.58[f]	1.16[e]	220
		Soft margarine	3.30	4.11[d,e]	1.11[d,e]	240
		Shortening	4.15	4.24[e]	1.11[d,e]	240
		Stick margarine	6.72	4.34[e]	1.01[d]	240

[a] PHFO = partially hydrogenated fish oil, PHSO = partially hydrogenated soybean oil, PUFA-M = margarine containing polyunsaturated fatty acids, TFA-M = margarine containing *trans* fatty acids.
[b] TFA = *trans* fatty acids.
[c] LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, Lp(a) = lipoprotein(a).
[d,e,f] Different lettered superscripts within each study indicates values were significantly different.

High-Density Lipoprotein Cholesterol Concentrations

The data related to the impact of hydrogenated fat/*trans* fatty acids compared to unhydrogenated oil /*cis* fatty acids on HDL cholesterol concentrations are less consistent than for LDL cholesterol concentrations (Tables 2, 3 and 4). As reported for LDL cholesterol concentrations, the effect of hydrogenated fat/*trans* fatty acids on HDL cholesterol concentrations, if present, is likely to be dose dependent (Judd et al., 1994). The preponderance of the data suggest that hydrogenated fat/*trans* fatty acids, relative to saturated fatty acids, result in lower HDL cholesterol concentrations (Ascherio et al., 1999; Zock and Mensink, 1996; Zock et al., 1995). Because of the potentially differential effects of hydrogenated fat/*trans* fatty acids on LDL and HDL cholesterol concentrations, concern has been raised regarding their effect on the total cholesterol or LDL cholesterol:HDL cholesterol ratio (Ascherio et al., 1999). However, with respect to dietary fat recommendations, the strategy to improve the total cholesterol or LDL cholesterol:HDL cholesterol ratio would not be different from that to decrease LDL cholesterol concentrations.

Lipoprotein(a) Concentrations

Lipoprotein(a) (Lp(a)) concentrations in plasma have been associated with increased risk for developing cardiovascular and cerebrovascular disease, possibly via inhibition of plasminogen activity (Lippi and Guidi, 1999; Nielsen, 1999; Wild et al., 1997). Lp(a) is a lipoprotein particle similar to LDL with respect to its cholesterol and apolipoprotein B100 content, but it also contains an additional apolipoprotein termed apo(a) (Lippi and Guidi, 1999; Nielsen, 1999). Lp(a) concentrations have been reported by some investigators to be increased after the consumption of diets enriched in hydrogenated fat/*trans* fatty acids (Tables 2, 3, and 4) (Almendingen et al., 1995; Aro et al., 1997; Lichtenstein et al., 1999; Mensink et al., 1992; Nestel et al., 1992b; Sundram et al., 1997), but not all (Chisholm et al., 1996; Judd et al., 1998; Lichtenstein et al., 1993; Louheranta et al., 1999; Müller et al., 1998). The magnitude of the mean increases in Lp(a) concentrations associated with *trans* fatty acid intake would not have a physiologically significant effect on cardiovascular disease risk. However, an unresolved issue at this time is the potential effect of relatively high levels of *trans* fatty acids in individuals with initially high concentrations of Lp(a).

Hemostatic Factors

The effect of *trans* fatty acids on hemostatic factors has been assessed by a number of investigators (Almendingen et al., 1996; Mutanen and Aro, 1997; Sanders et al., 2000; Turpeinen et al., 1998; Wood et al., 1993b) (see Table 5). In general, these researchers have concluded that hydrogenated fat/*trans* fatty acids had little effect on a variety of hemostatic variables. Similarly, Müller and colleagues (1998) reported that hemostatic variables were unaffected by the substitution of a vegetable oil-based margarine relatively high in saturated fatty acids when compared to a hydrogenated fish oil-based margarine.

Susceptibility of Low-Density Lipoprotein to Oxidation

Hydrogenated fat/trans fatty acids have consistently been reported to have little effect on the susceptibility of LDL to oxidation (Cuchel et al., 1996; Halvorsen et al., 1996; Nestel et al., 1992b; Sørensen et al., 1998) (Table 5).

TABLE 4 Dietary *Trans* Fatty Acids (TFA), Hydrogenated Fat, and Blood Lipid Concentrations: Free Living Trials

Reference	Study Population	Diet[a]	TFA (% of energy)	Blood Lipid Concentrations[c] LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (units/L)
Nestel et al., 1992a	26 mildly hypercholesterolemic men, 27–57 y	4-wk crossover, 42% fat				
		Control 1	3.8	4.13[c]	1.11[c]	
		Control 2	3.7	4.03[c,d]	1.15[c]	
		Blend 1	6.7	3.92[d,e]	1.10[c]	
		Blend 2	6.6	3.83[e]	1.11[c]	
Nestel et al., 1992b	27 mildly hypercholesterolemic men, 30–63 y	3-wk crossover, 36–37% fat				
		Control	< 1	4.22[c]	0.98[c]	235[c]
		18:1	1.4	3.90[d]	0.98[c]	236[c]
		TFA	5.7	4.27[c]	0.98[c]	296[d]
		16:0	< 1	4.16[c]	1.09[d]	249[e]
Seppänen-Laakso et al., 1993	57 men and women, middle-aged	12-wk crossover to 1 of 2 diets, 39–43% fat		Change from baseline	Change from baseline	
		Margarine	2.9	–0.20	+0.05	
		Rapeseed	0	–0.30	–0.01	
		Olive oil	0	–0.32	0.00	
Wood et al., 1993a	38 healthy men, 30–60 y	6-wk crossover, 38% fat				
		Butter	2.1	3.78[c]	1.22[c]	
		Butter-sunflower	1.0	3.49[d]	1.19[c]	
		Butter-olive	1.0	3.59[d]	1.22[c]	
		Hard margarine	11.1	3.47[d]	1.16[c]	
		Soft margarine	0	3.26[e]	1.16[c]	
Wood et al., 1993b	29 healthy men, 30–60 y	6-wk crossover, 37% fat				
		Butter	0.2	3.52[c]	1.03[c]	
		Crude palm	0	3.36[c]	1.03[c]	
		Margarine	3.0	3.36[c]	1.00[c]	
		Refined palm	0	3.41[c]	1.06[d]	
		Refined palm+sunflower	0	3.41[c]	1.03[c]	
		Sunflower oil	0	3.23[d]	1.00[c]	

Chisholm et al., 1996	49 hypercholesterolemic men and women, avg 47 y	6-wk crossover, 26–27% fat				
		Butter	1.4	4.21[c]	1.26[c]	223[c]
		Margarine	3.6	3.82[d]	1.24[c]	249[c]
Noakes and Clifton, 1998	38 mildly hyperlipidemic men and women	3-wk crossover, 2 groups, 31–35% fat				
		Canola + TFA	3.3	3.64[c]	1.19[c]	
		TFA-free canola	0	3.61[c]	1.28[c]	
		Butter	1.1	4.14[d]	1.20[c]	
		PUFA + TFA	3.6	4.23[c]	1.17[c]	
		TFA-free PUFA	0	3.98[d]	1.23[c]	
		Butter	1.2	4.70[e]	1.27[c]	

[a] PUFA = polyunsaturated fatty acids
[b] LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, Lp(a) = lipoprotein(a).
[c,d,e] Different lettered superscripts within each study indicates values were significantly different.

TABLE 5 *Trans* Fatty Acid (TFA) Intake and Blood Clotting, Blood Pressure, and Low-Density Lipoprotein (LDL) Oxidation

Reference	Study Population	Diet[a]	TFA (% of energy)	Results[b]		Comments
Clotting						
Wood et al., 1993b	29 men, 30–60 y	6-wk crossover, 37% fat		TXB_2 (pg/mL)	6-keto-$PGF_{1\alpha}$ (pg/mL)	
		Butter	0.2	35[c]	89[d]	
		Crude palm oil	0	41[c]	94[c]	
		Margarine	3.0	40[c]	86[d]	
		Refined palm oil	0	40[c]	87[d]	
		Refined palm+sunflower	0	36[c]	100[c]	
		Sunflower oil	0	62[c]	95[c]	
Almendingen et al., 1996	31 men, avg 27 y	3-wk crossover, 33–36% fat		Fibrinogen (g/L)	PAI-1 activity (units/mL)	For PHSO, greater PAI-1 activity than PHFO or butter
		PHSO	8.5	3.0	13.5	Increased fibrinogen with butter diet
		PHFO	8.0	2.9	10.7	No significant difference in factor VII, fibrinogen peptide A, β-thromboglobulin, or tissue plasminogen activator
		Butter	0.9	3.1	8.8	
Mutanen and Aro, 1997	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 33–34% fat		Fibrinogen (g/L)		No marked difference in factor VII coagulation activity, tissue type plasminogen activity, or PAI-1 activity
		High 18:0	0.4	3.62		
		High TFA	8.7	3.61		
Turpeinen et al., 1998	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 32–34% fat				No difference in TXB_2 production or adenosine diphosphate-induced platelet aggregation in vitro
		18:0	0.4			Significant increase in collagen induced aggregation with 18:0 diet
		TFA	8.7			
Sanders et al., 2000	16 men and women, 18–32 y	1 test-meal crossover, 7% or 65% fat		$FVII_c$ (% standard)	$FVII_a$ (ng/mL)	No significant differences in factor VII coagulation activity; factor VII-activated concentrations were significantly higher with 18:1, 18:1 *trans*, 18:0, and 16:0 diets
		18:1	0.1	124	2.7	
		18:1 *trans*	24.7	122	1.9	
		18:0	0	114	1.9	

		16:0	0.2	112	2.1	
		MCT	0	112	1.5	
		Low fat	0	99	1.4	
Oxidation						
Cuchel et al., 1996	14 men and women, 44–78 y	32 d crossover, 30% fat				No difference in susceptibility to LDL oxidation
		Corn oil	0.44			
		Corn oil+margarine	4.16			
Halvorsen et al., 1996	29 men, 21–46 y	19-d crossover, 33–36% fat		Dienes (nmol/mg LDL)	Formation rate (nmol/mg LDL × min)	No significant differences in conjugated dienes, lipid peroxides, uptake by marcophages, or electrophoretic mobility of LDL
		Butter	0.9	1,020	10	TFA does not alter susceptibility to LDL oxidation
		PHSO	8.5	1,034	10	
		PHFO	8.0	1,107	10	
Sørensen et al., 1998	47 men, 29–60 y	4 wk, consumed 30 g/d of 1 of 2 margarines	(mol % of fat)	Dienes (nmol/g)	Oxidation rate (nmol/mg × min)	Fish oil consumption compared with sunflower oil margarine had no effect on LDL size and led to minor changes in LDL oxidation resistance
		Sunflower oil	0.79	445	10.4	
		Fish-oil, enriched	0.98	468	10.2	
Blood Pressure						
Mensink et al., 1991	59 men and women, 19–57 y, normo-tensive	3-wk crossover, 39–40% fat		SBP (mmHg)	DBP (mmHg)	No effect of TFA intake on blood pressure
		18:1	0	113	66	
		TFA	10.9	112	67	
		SFA	1.8	112	67	
Zock et al., 1993	55 men and women, 19–49 y	3-wk crossover, 40–43% fat		SBP (mmHg)	DBP (mmHg)	No effect of TFA intake on blood pressure
		18:2	0.1	114	68	
		18:0	0.3	113	70	
		TFA	7.7	113	69	

[a] PHSO = partially hydrogenated soy bean oil, PHFO = partially hydrogenated fish oil, MCT = medium-chain triacylglycerol, SFA = saturated fatty acid.
[b] TXB_2 = thromboxane B_2, 6-keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$, PAI-1 = plasminogen activator inhibitor type 1, $FVII_c$ = factor VII coagulant activity, $FVII_a$ = factor VII activated, SBP = systolic blood pressure, DBP = diastolic blood pressure.
[c,d] Different lettered superscripts within each study indicates values were significantly different.

Blood Pressure

A few reports addressed the issue of trans fatty acid intake and blood pressure (Mensink et al., 1991; Zock et al., 1993) (Table 5). The authors concluded that consumption of diets high in saturated, monounsaturated, or trans fatty acids resulted in similar diastolic and systolic blood pressures.

Coronary Heart Disease

Similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentrations (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). Some evidence also suggests that *trans* fatty acids result in lower HDL cholesterol concentrations (Table 6). Hence, the net result is a higher total cholesterol (or LDL cholesterol):HDL cholesterol ratio (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). This finding, combined with data from prospective cohort studies (Ascherio et al., 1996; Gillman et al., 1997; Hu et al., 1997; Pietinen et al., 1997; Willett et al., 1993) (Table 6), has lead to the concern that dietary *trans* fatty acids are more deleterious with respect to coronary heart disease than saturated fatty acids (Ascherio et al., 1999).

Summary

There is a positive linear trend between *trans* fatty acid intake and total and LDL cholesterol concentration, and therefore increased risk of CHD, thus suggesting a Tolerable Upper Intake Level (UL) of zero. Because *trans* fatty acids are unavoidable in ordinary diets, achieving such a UL would require extraordinary changes in patterns of dietary intake. Such extraordinary adjustments may introduce other undesirable effects (e.g., elimination of foods, such as dairy products and meats, that contain *trans* fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks may be introduced by any extreme adjustments in dietary pattern. For these reasons, no UL is proposed. Nevertheless, it is recommended that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet.

TABLE 6 Dietary *Trans* Fatty Acids (TFA): Epidemiological Studies

Reference	Study Design[a]	Dietary and Other Information	Results[b]			Comments
Lipoprotein concentration						
Siguel and Lerman, 1993	47 CAD cases 56 controls Case-control	No dietary intake information	Plasma TFA (%) HDL (mmol/L) LDL (mmol/L) TAG (mmol/L)	Case 1.38 0.88 3.78 1.78	Control 1.11 1.34 2.97 0.97	TFA negatively associated with HDL TFA positively associated with LDL and TAG
Coronary heart disease (CHD)						
Hudgins et al., 1991	76 men, 23–78 y Cross sectional	No dietary intake information	Total TFA in adipose tissue was 4.4% of total fatty acids			Total TFA content in adipose tissue was not significanlty related to risk factors of CHD (e.g, age, body mass index, LDL, cholesterol, blood pressure)
Troisi et al., 1992	748 men, 43–85 y Cross sectional	Food frequency questionnaire, multivariate analysis	TFA intake was directly related to total (r = 0.07, P = 0.04) and LDL (r = 0.09, P = 0.01) cholesterol			An increased TFA intake from 2.1 to 4.9 g/d increased the risk of MI by 27%
Willett et al., 1993	Women, 431 CHD cases Cohort, 8-y follow-up,	Food frequency questionnaire, multivariate analysis	TFA intake (% energy) 1.3 1.8 2.2 2.6 3.2		RR of CHD 1.0 1.4 1.25 1.55 1.8	Positive association with TFA intake and risk of CHD
Ascherio et al., 1994	239 MI cases 282 controls Case-control	Food frequency questionnaire, multivariate analysis	TFA intake (g/d) 1.69 2.48 3.35 4.52 6.51		RR of MI 1.0 0.73 1.24 1.63 2.28	Positive association of TFA intake and risk of myocradial infarction

continues

TABLE 6 Continued

Reference	Study Design[a]	Dietary and Other Information	Results[b]		Comments
Kromhout et al., 1995	12,763 men, 40–59 y Cohort, 25-y follow-up	Weighed food record	Correlation between 18:1*trans* intake and CHD mortality is 0.78 ($p < 0.001$)		
Ascherio et al., 1996	43,757 men, 40–75 y Cohort, 6-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (g/d) 1.5 2.2 2.7 3.3 4.3	RR of MI 1.0 1.20 1.24 1.27 1.40	TFA intake directly associated with risk of MI
Hu et al., 1997	Women, 34–59 y 939 MI cases Cohort, 14-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (% energy) 1.3 1.7 2.0 2.4 2.9	RR of MI 1.0 1.07 1.10 1.13 1.27	RR for 2% increment in energy from TFA intake was 1.93
Gillman et al., 1997	Men, 45–64 y 267 CHD cases Cohort, 21-y follow-up	24-h recall, multivariate analysis	Margarine intake (tsp/d) 0 1–4 ≥ 5	No. of events (/1,000) Period 1 / Period 2 77 / 65 42 / 35 18 / 30	RR for CHD for each increment of 1 tsp/d was 0.99 for follow-up period 1 and 1.12 for period 2 Modest risk of CHD with increasing margarine intake
Pietinen et al., 1997	Smoking men, 50–69 y 1,399 coronary events 635 coronary deaths Cohort, 6.1-y follow-up	Food frequency questionnaire, multivariate analysis	TFA intake (g) 1.0 1.7 2.0 2.7 6.2	RR of major coronary event 1.00 1.10 0.97 1.07 1.14	Positive association between TFA intake and risk of coronary death

			TFA intake (g)	RR of coronary death	
			1.0	1.00	
			1.7	1.05	
			2.0	1.12	
			2.7	0.90	
			6.2	1.39	
Tavani et al., 1997	Women, 18–74 y 429 MI cases 866 controls Case-control	Questionnaire on selected indicator foods, multivariate analysis	Margarine intakes	RR of MI	The association with margaine could explain about 6% of MI in this population
			No or low	1.0	
			Medium or high	1.5	
Cancer					
Kohlmeier et al., 1997	Women, 50–74 y 291 breast cancer cases 407 controls Case-control	No diet information	Adipose TFA concentration	OR of breast cancer	Risk for breast cancer is based on the relative concentration of TFA and PUFA
			TFA	1.46	
			TFA within lowest PUFA tertile	3.65	
			TFA within highest PUFA tertile	0.97	
Tuyns et al., 1988	35–75 y 453 colon cancer cases 365 rectal cancer cases 2,851 controls Case-control	Dietary history			There was no increased risk of either cancers from with increased consumption of margarine

[a] CAD = coronary artery disease, MI = myocardial infarction.
[b] HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, TAG = triacylglycerol, RR = relative risk, OR = odds ratio, PUFA = polyunsaturated fatty acid.

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