Does dietary source of trans fatty acids affect risk for cardiovascular disease in humans?

David J. Baer, Ph.D.

USDA, ARS Beltsville Human Nutrition Research Center, Beltsville, MD 20705

Dietary trans fatty acids (TFA) come from partially hydrogenated oils (sometimes referred to as "industrial TFA" or iTFA) or from the fat of ruminants (for example, cows, sheep, goats and buffalo) (sometimes referred to as "natural TFA" or rTFA). Industrial TFA are formed during the hydrogenation of vegetable oils using catalysts. Depending on the hydrogenation conditions, a mixture of isomers is formed, however, elaidic (*trans*-9 18:1) acid is the primary isomer formed (Lock and Bauman). Under some industrial conditions, vaccenic (*trans*-11 18:1) acid (VA) can be formed as well. In contrast, ruminant animals produce primarily vaccenic acid through the biohydrogenation of linoleic and alpha-linolenic acids in the rumen. Through further hydrogenation, ruminants also produce stearic acid (a fully saturated fatty acid). On the other hand, vaccenic acid can further be desaturated in the rumen or extraruminal tissue (for example, mammary gland) to produce rumenic acid (cis-9, trans-11, 18:2), a conjugated linoleic acid (CLA) (Lock and Bauman). CLA from ruminants has been shown in some animal (preclinical studies) to reduce risk for cancer (Gebauer et. al.[1]). Thus, dietary sources of CLA could be used to decrease risk for cancer. However, animal feeding practices that increase CLA also increase vaccenic acid (Lock and Bauman).

In addition to differences in the isomers between iTFA and rTFA, there are differences in the amount of TFA formed. In the production of industrial TFA, the amount of TFA formed depends on several factors including the extent of the hydrogenation process. In partial hydrogenation of vegetable oils, the TFA content can range from 1 to 60% of total fatty acids. However, in ruminants, biohydrogenation in the rumen is tightly regulated and the concentration of *trans*-18:1 acids range naturally from 2 to 5% of total fatty acids but this can be manipulated by the type of diet fed to the animal (Lock and Bauman).

The physiological effects of iTFA on chronic disease risk factors, specifically risk factors for coronary heart disease, are well established. However, the health effects of rTFA are less studied and less known. Animal studies (preclinical studies) and studies of cells (in vitro studies) suggest that the effects of rTFA may differ from those of iTFA (Gebauer et. al.[1]). Further, results from these studies suggest that VA and c9,t11-CLA may lower cholesterol and reduce risk for coronary heart disease (Gebauer et. al.[1]). Further, results of some epidemiologic studies are consistent with the results from preclinical and in vitro studies (Ascherio et. al.) (Liu et. al.) (Pietinen et al.) (Willet et. al.). Other studies suggest that risk for coronary heart disease is similar for all isomers of TFA, regardless of dietary source (Oomen et. al.).

There are few human clinical studies (Chardigny et. al.) (Lacroix et. al.) (Motard-Belanger et. al.) (Tholstrup et.al.), and these studies are heterogenous with respect to study design.

Some studies lack a proper control group making comparisons difficult (Chardigny et. al.). Other studies appear to be underpowered (Motard-Belanger et. al.). Several studies have been conducted with a free-living cohort without sufficient control of the diet (Tholstrup et. al.) (Chardigny et. al.) whereas other studies had controlled diets but the saturated and unsaturated fatty acid composition of the diets was not adequately matched to eliminate their effect on cholesterol concentration (Motard-Belanger et. al.). For some studies, dairy cattle diets were manipulated to produce dairy products enriched with rTFA (Bauman et. al.); however, this approach changes the concentration of other fatty acids (for example, decreasing saturated fatty acids that are hypercholesterolemic (heightens blood cholesterol levels) and increasing fatty acids that are neutral and hypocholesterolemic (lowers blood cholesterol levels) (for example, stearic acid).

The current mean estimate of TFA intake in the US is 1.3 g/person/day (Doell et.al.) and has decreased from 4.6 g/person/day. However, current estimates of rTFA intake have remained stable over the past decade at approximately 1.2 g/person/day in the United States. Based on current estimates, rTFA is now 48% of total TFA intake and has increased from 21% due to the decrease in iTFA intake in the United States. Given the shift in dietary source of TFA, it has become increasing important to understand differences in how different TFA isomers effect risk factors for coronary heart disease, especially as it may impact food labeling, other regulatory processes, and trade.

A human feeding study was conducted to compare the effects of iTFA and rTFA (VA) on risk factors of coronary heart disease. Vaccenic acid was used as it is the predominant isomer of rTFA and this approach eliminates the effect of changes from other fatty acids when rTFA enriched dairy fat is used.

The study is registered with ClinicalTrials.gov (NCT00942656) and details of study design and results have been previously described (Gebauer et. al. [2]; Gebauer et. al. [3]). This was a double-blind study with investigators, subjects, phlebotomists, analysts, and statisticians blinded to the treatments until after statistical analyses were completed. For the feeding, there were four treatment periods, representing 4 treatments. Each treatment period lasted 24 days. During each treatment period, volunteers received a controlled diet for which stearic acid was replaced with 1) 3.3% energy from VA, 2) 3.3% of energy from mixed isomers of TFA from partially hydrogenated vegetable oil (PHVO; iTFA), and 3) 0.9% energy from c9,t11-CLA (rumenic acid, RA). Stearic acid was used as the fatty acid to be replaced among the diets since changes in intake of stearic acid do not affect circulating cholesterol concentration.

Characteristics of the volunteers who completed the intervention are presented in Table 1. Of the 119 volunteers who were randomized, partial or complete data were obtained from 106. Composition of the four diets is presented in Table 2. Effect of the different diets on LDL cholesterol, HDL cholesterol and triglycerides is presented in Table 3. Effect of dietary intake on LDL cholesterol, HDL cholesterol and triglycerides from different TFA

Table 1. Baseline characteristics of study participants[1]

	Mean ± SD
Sex, n M F	 47 59
Age, y	47 ± 10.8
Body weight, kg	80.5 ± 13.9
BMI, kg/m^2	28.5 ± 4.0
TC, mmol/L	5.00 ± 0.74
LDL-C, mmol/L	3.25 ± 0.63
HDL-C, mmol/L	1.24 ± 0.30
TG, mmol/L	1.12 ± 0.50
SBP, mm Hg	127.6 ± 13.6
DBP, mm Hg	76.1 ± 9.2
Glucose, mmol/L	5.17 ± 0.5

[1]n = 106. Values are expressed as mean ± SD, *n* = 106. DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol.

Adapted from (Gebauer et. al. [2]).

isomers is presented in Table 3. The increase in LDL cholesterol concentration after consumption of the PHVO (iTFA) diet compared to the control diet is consistent with many published studies – iTFA increases LDL cholesterol concentration. Similarly, HDL cholesterol concentration after consumption of the iTFA diet compared to the control diet is consistent with published literature – iTFA do not alter HDL cholesterol, at least at modest intakes of iTFA. rTFA (VA) increased LDL cholesterol concentration compared to the control, and LDL cholesterol concentration was higher after consumption of the rTFA diet compared to the iTFA diet. On the other hand, whereas iTFA did not change HDL cholesterol concentration compared to the control diet, rTFA increased HDL cholesterol concentration compared to the control and compared to the iTFA diet. LDL cholesterol and HDL cholesterol concentrations were not different after consumption of the RA diet compared to the control but triglyceride concentration was lower.

There has been uncertainty about differentiating rTFA from iTFA for food labeling, regulations, and dietary guidance. Results from this published study suggest that rTFA (VA) and iTFA have similar LDL cholesterol raising effects, at least at higher than usual intakes. The results from the present study demonstrate that at higher than usual intakes, isolated VA and iTFA both adversely affect LDL cholesterol concentrations when replaced with energy from stearic acid. However, rTFA raise HDL cholesterol whereas iTFA do not. The results of this large, well-controlled dietary intervention support the current labeling regu-

lation, with the requirement of VA, but not c9,t11-CLA, to be listed under TFA on the Nutrition Facts Panel.

Table 2. Chemical analysis of treatment diets (% of energy)[1]

	Control[2]	iTFA	VA	c9,t11-CLA
Protein	17.0±0.13	17.2±0.07	17.0±0.12	17.1±0.12
Fat	33.3±0.31	33.0±0.19	33.4±0.23	33.3±0.17
Carbohydrate	49.7±0.25	49.8±0.15	49.5±0.20	49.6±0.15
Saturated fatty acids	15.9±0.04	12.5±0.05	12.4±0.02	14.9±0.04
Lauric	0.43±0.006	0.42±0.007	0.40±0.003	0.43±0.005
Myristic	0.05±0.005	0.05±0.003	0.05±0.002	0.05±0.004
Palmitic	5.74±0.055	5.90±0.104	5.93±0.025	5.84±0.063
Stearic	9.24±0.105	5.68±0.115	5.61±0.058	8.18±0.126
Other	0.44±0.002	0.44±0.002	0.38±0.002	0.44±0.003
Monounsaturated fatty acids	9.85±0.070	9.77±0.089	9.74±0.047	9.87±0.048
Oleic	9.17±0.138	8.92±0.176	8.94±0.090	9.18±0.092
Other	0.68±0.002	0.86±0.002	0.81±0.003	0.69±0.004
Polyunsaturated fatty acids	5.67±0.015	5.89±0.026	5.77±0.031	5.81±0.017
Linoleic	5.10±0.037	5.30±0.062	5.20±0.080	5.23±0.041
Alpha linolenic	0.45±0.005	0.46±0.012	0.45±0.009	0.46±0.005
Other	0.13±0.002	0.13±0.004	0.13±0.003	0.12±0.004
Trans fatty acids	0.28±0.002	3.26±0.014	3.93±0.012	0.32±0.009
Palmitelaidic	0.02±0.000	0.02±0.001	0.02±0.001	0.02±0.001
Elaidic	0.21±0.007	2.87±0.050	0.00±0.000	0.24±0.032
trans-Vaccenic	0.02±0.001	0.30±0.005	3.86±0.044	0.02±0.003
Other	0.03±0.000	0.06±0.000	0.05±0.001	0.04±0.001
Conjugated linoleic acids	0.04±0.002	0.06±0.002	0.04±0.002	0.84±0.018
Linoelaidic	0.02±0.001	0.04±0.001	0.02±0.001	0.02±0.001
Conjugated linoleic	0.02±0.002	0.02±0.003	0.03±0.003	0.82±0.034

[1]Values are presented as mean ± SEM, *n*=8 samples for each treatment diet. Chemical composition of diets from chemical analyses of weekly composites of food collected throughout the study intervention period. c9,t11-CLA indicates *cis*-9, *trans*-11 conjugated linoleic acid; iTFA, industrially produced *trans* fatty acids; VA, vaccenic acid.

[2]Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,t11-CLA.

Adapted from (Gebauer et. al. [2]).

Table 3. Effect of treatment diets on lipids[1]

	Control[2]	iTFA	VA	c9,t11-CLA	*P*-values			
					VA vs iTFA	VA vs Control	iTFA vs Control	CLA vs Control
LDL-C, mmol/L	2.94 ± 0.04	3.04 ± 0.04*	3.12 ± 0.04*†	2.93 ± 0.04	0.0114	<0.0001	0.0028	0.6054

HDL-C, mmol/L	1.40 ± 0.02	1.40 ± 0.02	1.43 ± 0.02*†	1.39 ± 0.02	0.0026	0.0110	0.6315	0.2927
TG, mmol/L	1.13 ± 0.03	1.11 ± 0.03	1.16 ± 0.03†	1.06 ± 0.03*	0.0290	0.1488	0.4518	0.0026

[1] All values are means ± SEM, *n*=106. All statistical analyses were performed with SAS (ver. 9.2, Statistical Analyses System, Cary, NC) using a mixed model analysis (PROC MIXED) to determine whether effects were significant ($P \leq 0.05$). Contrast statements were used to make the following comparisons: iTFA vs control, VA vs control, iTFA vs VA, and c9,t11-CLA vs control.
*Significant difference vs control as defined by $P \leq 0.05$;
†significant difference between iTFA and VA as defined by $P \leq 0.05$.
c9,t11-CLA, *cis*-9, *trans*-11 conjugated linoleic acid; HDL-C, high-density lipoprotein-cholesterol; iTFA, industrially produced *trans* fatty acids; LDL-C, low-density lipoprotein-cholesterol; *P*-value, *P*-value of overall treatment effect; SEM, standard error of the mean; TG, triacylglycerol; VA, vaccenic acid.
[2]Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,t11-CLA.

Adapted from (Gebauer et. al. [2]).

References

Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. Trans-fatty acids intake and risk of myocardial infarction. Circulation 1994;89(1):94-101.

Bauman DE, Barbano DM, Dwyer DA, Griinari JM. Technical note: production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J Dairy Sci 2000;83(11):2422-5.

Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues JB, Chaumont P, et al. Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. Am J Clin Nutr 2008;87(3):558-66.

Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. Food Addit. Contam. Part A. Chem Anal Control Expo Risk Assess 2012;29(6):861-74.

Gebauer SK [1], Chardigny JM, Jakobsen MU, Lamarche B, Lock AL, Proctor SD, Baer DJ. Effects of Ruminant trans Fatty Acids on Cardiovascular Disease and Cancer: A Comprehensive Review of Epidemiological, Clinical, and Mechanistic Studies. Adv Nutr 2011;2(4):332-54.

Gebauer SK [2], Destaillats F, Dionisi F, Krauss RM, Baer DJ. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. Am J Clin Nutr 2015;102(6):1339-46.

Gebauer SK [3], Destaillats F, Mouloungui Z, Candy L, Bezelgues JB, Dionisi F, Baer DJ. Effect of trans fatty acid isomers from ruminant sources on risk factors of cardiovascular disease: study design and rationale. Contemp Clin Trials 2011;32(4):569-76.

Lacroix E, Charest A, Cyr A, Baril-Gravel L, Lebeuf Y, Paquin P, Chouinard PY, Couture P, Lamarche B. Randomized controlled study of the effect of a butter naturally enriched in trans fatty acids on blood lipids in healthy women. Am J Clin Nutr 2012; 95(2):318-25.

Liu XR, Deng ZY, Hu JN, Fan YW, Liu R, Li J, Peng JT, Su H, Peng Q, Li WF. Erythrocyte membrane trans-fatty acid index is positively associated with a 10-year CHD risk probability. Br J Nutr 2013;109(9):1695-703.

Lock AL, Bauman DE. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 2004;39(12):1197-206.

Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 2008;87(3):593-9.

Oomen CM, Ocke MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. Lancet 2001;357 (9258):746-51.

Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Am J Epidemiol 1997; 145(10):876-87.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. Am J Clin Nutr 2006;83(2):237-43.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 1993;341(8845):581-5.