Dietary Hydrogenated Soybean Oil Affects Lipid and Vitamin E Metabolism in Rats

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Summary Fatty acids containing stearic acid, which are found in hydrogenated fat, may have a detrimental effect on the cholesterol and triacylglycerol (TAG) content of plasma lipoproteins, and on the absorption of fatty acids and fat-soluble vitamins. The aim of our study was to examine the tissue concentration of lipids and vitamins A and E after feeding a hydrogenated soybean oil (HSO) diet to rats. Twenty male Sprague-Dawley rats were randomly divided into two groups, fed on coconut oil (control) and HSO, respectively in amounts corresponding to 15% of the total feed. Plasma total cholesterol, VLDL- and LDL-cholesterol, lipid peroxidation and daily excretion of the TAG and cholesterol in feces were higher in the HSO than in the control group. TAG values in plasma and liver, and HDL-cholesterol levels in plasma were lower in the HSO than in the control group. The same was true for phospholipids in plasma and for saturated fatty acids, mono- and polyunsaturated fatty acids levels in the liver and vitamin E in plasma. LDL and adipose tissue. The results of this study provide new evidence concerning the effect of dietary hydrogenated fat on lipid, TAG and vitamin E status, which are important for maintenance of good health. Consumption of dietary HSO may be associated with cardiovascular disease.

Key Words vitamin E, vitamin A, absorption, lipoproteins, cardiovascular disease

During the past decade, reduction in fat intake has been the main focus of national dietary recommendations to decrease the risk of cardiovascular disease (CVD) (1–4). Several lines of evidence, however, have indicated that certain type of fats have a more important role in determining risk of CVD than the total amount of fat in the diet (2). Recent evidences suggest that dietary fatty acid composition influences numerous values including the cholesterol and triacylglycerol (TAG) content of plasma lipoproteins (2–4). The margarine-enriched diet, relative to butter, canola, and soybean oil, was associated with a less favorable antioxidant status and increased indexes of oxidative stress (4). Hydrogenated fat is used in the manufacture of margarine and vegetable shortening and is therefore present in foods prepared with these products (3). However, whether hydrogenated soybean oil (HSO) affects lipid peroxide, cholesterol and TAG in rats is currently unknown and warrants further study.

A number of studies suggest that the poor availability of conventional fats such as cocoa butter is related to their content of stearic acid (18:0) and specifically tristearin (5–8). Generally, the higher the content of stearic and tristearin in a fat, the poorer the digestion and absorption (1, 2, 6). Another potential source of tristearin is in highly hydrogenated fatty sources. The hydrogenation of natural fats and oils that contains a high content of 18-carbon unsaturated fatty acids will result in an increased content of stearic acid and tristearin, presumably making these fats less available (5, 6). HSO, a fat source that currently is consumed in North America and Western Europe, as a component of liquid shortening, would fall into this category. This oil is soybean oil that essentially has been hydrogenated to completion (1, 3).

The most frequently studied antioxidant vitamins are A and E. Vitamin A, retinol, serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (9). Vitamin E, a-tocopherol, is the most important antioxidant in the lipid phase. Vitamin E acts to protect cell against the effects of free radicals, which are potentially damaging byproducts of the body’s metabolism (10). A number of studies have reported the existence of vitamin E deficiency in hypocholesteremic patients (reviewed in Ref. 10). Vitamin E supplementation affects plasma total cholesterol and LDL- and VLDL-cholesterol levels and one can hypothesize that the antioxidant power of it could preserve LDL-cholesterol from the peroxidation phenomenon (11). There is also some evidence that a poorly digested fat such as olestra can potentially decrease the absorption of fat-soluble vitamins such as vitamins A and E (7, 8).

To our knowledge, there is no information on the
digestibility, as well as plasma and tissue concentrations of lipid profiles, of vitamins A and E after feeding diets rich in HSO as hydrogenated fats. We investigated the effects of HSO on the apparent absorption of dietary fats by monitoring levels of lipids and vitamin A and E in plasma, liver and feces in rats. Knowledge of the effects of HSO in this experimental dietary model could contribute to determination of the potential risk of HSO for some human diseases caused by nutritional factors.

MATERIALS AND METHODS

Rats and diets. In a two-system design, 20 male Sprague-Dawley rats with an average initial body weight of 54.1 ± 2.38 g were divided into 2 groups of 10 animals each. The rats were kept singly in plastic cages in a temperature-controlled environment. They were maintained on a 12 h light:dark cycle and were given free access to water. The Nutritional Institute Committee Director of Martin Luther University approved the study and ethics protocol.

Both groups received a semi-synthetic diet including 0.5% cholesterol and 15% of a fat mixture as the main component. Both diets used in our study were nutritionally-adequate purified mixtures formulated to be isoenergetic containing 15% of fat. The diet composition is shown in Table 1.

The fatty acid composition of each diet is shown in detail in Table 2. The fatty acid compositions of HSO (Henry Lamotte GmbH, Bremen, Germany), coconut oil (CNO) and safflower oil (Union Deutsche Lebensmittelwerke GmbH, Hamburg, Germany) were determined by gas chromatography as described below. Some fatty acid composition values of these fats were also provided by the manufacturers but these values were not used in our assessments. Because HSO is solid, it is extremely hard in texture and difficult to incorporate into a homogenous diet. HSO was melted before incorporation into the diet mixture but the fat formed into small pellets so that the rats could avoid ingestion of the fat if they found it unpalatable.

Experimental design. The diets of HSO and CNO were supplemented with 3,000 IU of vitamin A (retinyl acetate) and 25 mg of vitamin E (dl-α-tocopherol equivalent) per kg of feed. One group received HSO as a fat mixture and the other (control) group received CNO. Both fat mixtures included 5% safflower oil for adequate supply of essential fatty acids. A restrictive feeding system was used in which all animals were administered identical quantities of diet. The quantity was increased gradually with increasing body weight of the animals from 8 to 16 g per day.

Body weight gains were monitored weekly at the same time during the light cycle throughout the experiment. Feces of five animals per group were collected at day 13 and 28, for 5 d each to determine the digestibility coefficient for each fat source. The digestibility coefficient is a measure of bioavailability expressed as the percentage of ingested fat that was not excreted in the feces (6). After 32 d, the rats were killed by decapitation after a light anesthesia with diethyl ether. Last feed was given 12 h before slaughtering time. The blood was collected in heparinized polyethylene tubes. Plasma was separated by centrifugation at 1,400×g for 20 min at 4°C. Plasma samples were stored at −20°C for analysis. Liver and adipose tissues were excised, immediately frozen in liquid nitrogen and stored at −20°C until analysis. Plasma and tissue collection was performed in the dark cycle because certain enzymes in lipogenesis and oxidative stress may be subject to circadian rhythm.

Plasma lipoproteins analyses. Plasma lipoproteins

| Components | Amount (g/kg) |
|------------|--------------|
| Casein | 200 |
| Corn starch | 280 |
| Saccharose | 273 |
| Fat mixture¹ | 150 |
| Fiber | 30 |
| Mineral mixture² | 40 |
| Vitamin mixture³ | 20 |
| dl-Methionine | 2 |
| Cholesterol | 5 |

¹ Fat mixture: HSO group: hydrogenated soybean oil and safflower oil (19+1) and 3,000 IU vitamin A (retinyl acetate) plus 25 mg dl-α-tocopherol equivalent. Control group: coconut oil and safflower oil (19+1) and 3,000 IU vitamin A (retinyl acetate) plus 25 mg dl-α-tocopherol equivalent.

² Minerals supplemented per kg diet: 6.88 g monocalcium phosphate, 10.83 g CaCO₃, 9.0 g K₂SO₄, 2.59 g NaCl, 1.01 g MgO, 116.7 g FeSO₄·7H₂O, 38 mg ZnO, 24 mg CuSO₄·5H₂O, 16 mg MnO, 0.32 mg Ca₃(PO₄)₂, 0.33 mg Na₂SeO₃, 0.13 mg CaCl₂·2H₂O, 0.02 mg iodine.

³ Vitamins supplemented per kg diet: 1,000 IU cholecalciferol, 1 mg menadione sodium bisulphite, 5 mg thiamine-HCl, 6 mg riboflavin, 6 mg pyridoxine-HCl, 30 mg nicotinic acid, 15 mg Ca pantothenate, 2 mg folic acid, 0.2 mg biotin, 0.025 mg cyanocobalamine, 1.000 mg choline chloride.

Table 2. Fatty acid composition of dietary fats (% of total fatty acids).

| Fatty acid | HSO soybean oil | CNO coconut oil | SAFF safflower oil |
|-----------|----------------|----------------|-------------------|
were separated by step-wise ultracentrifugation (Mikro-
ultracentrifuge, Sorvall Products, Bad Homburg, Ger-
many) at 90,000×g at 4°C for 1.5 h (12). The lipopro-
tein fractions (VLDL, δ<1.006 g/mL; LDL, 1.006 g/mL;
<δ<1.063 g/mL; HDL, δ>1.063 g/mL) were removed by
suction. Concentrations of cholesterol and TAG were
determined in plasma using enzyme reagent kits
(Merck, Darmstadt, Germany and BioMerieux, Marcy-
l’Etoile, France).

Determination of tissue and feces fatty acids. Lipids of
liver and feces were extracted using a low-toxicity sol-
vent mixture of n-hexane and isopropanol (3:2, v/v)
(13). Total lipids were methylated with trimethylsulfu-
nium hydroxide. Fatty acid methyl esters were sepa-
rated by a gas chromatograph (HP 5890, Hewlett Pack-
ard GmbH, Waldbronn, Germany), equipped with a
polar capillary column (30 m FFAP, 0.53 mm ID, Mach-
erey and Nagel, Düren, Germany), a flame ionization
detector and an automatic on-column injector. Helium
was used as carrier-gas with a flow of 5.4 mL/min.
Fatty acid methyl esters were identified by comparing
their retention times with those of individually purified
standards and quantified using an internal standard (C
15:0). For determination of liver and feces total choles-
terol and TAG, aliquots of the extracts were dissolved
in Triton X-100. Concentrations of total cholesterol
and TAG were determined using enzyme reagent kits
obtained from Merck. The apparent digestibility of cho-
lesterol and TAG was calculated from concentrations in
diets.

Lipid peroxidation (LP) assay in plasma. LP levels in
plasma were measured with the thiobarbituric acid
reaction by the method of Placer et al. (14) as described
in previous studies (11, 15). The quantification of
thiobarbituric acid reactive substances was determined
by reference to a standard curve of malondialdehyde
equivalents generated by acid-catalyzed hydrolysis of
1,1,3,3-tetramethoxypropane. The values of LP were
expressed as nmol/mL for plasma. The assay coefficients
of variation for LP were less than 3%.

Determinations of vitamins A and E in blood, liver and
adipose tissue. Concentrations of tocopherols in plasma,
LDL, liver, adipose tissue, oil and diet were determined
by high-performance liquid chromatography (HPLC,
HP 1100, Hewlett Packard GmbH) according to stan-
dard analysis (16) as described in previous studies (11,
15). Samples were saponified with potassium hydroxide in
the presence of pyrogallol (saturated form in water) as
an antioxidant for 30 min at 70°C (11). The tocophe-
rols were extracted with n-hexane. The tocopherol iso-
mers were separated on a Si-60 column (Merck) using a
mixture of n-hexane and 1,4-dioxane (96:4, v/v) as eluent and detected by fluorescence (excitation: 295 nm,
emission: 330 nm).

Retinol was determined in plasma and liver by HPLC
as described in our previous studies (11, 15). Samples
were saponified with potassium hydroxide in the pres-
ence of ascorbic acid as an antioxidant for 30 min at
70°C. Retinol was extracted with n-hexane. The solvent
was evaporated under nitrogen. Retinol was resolved in
a small volume of methanol, applied on a Nucleosil 100
column (Knauer, Germany), using acetonitrile as elu-
ent, and detected by fluorescence (excitation: 330 nm,
emission: 470 nm).

Statistical analysis. Data are represented as means±
standard deviation (SD). To determine the effect of
treatment, data were analyzed using one-way ANOVA
repeated measures; p-values of less than 0.05 were
regarded as significant. Significant values were assessed
with Tukey’s multiple range test. Data were analysed
using the SPSS statistical program (version 10.0 soft-
ware, SPSS Inc., Chicago, Illinois, USA).

RESULTS

Animal performance

Table 3 shows data on performance of animals receiving
HSO and control diets. Body weights at the start of the
study were similar for the two groups, and the

The Effect of Hydrogenated Soybean Oil on the Absorption of Fat

|                | Control | HSO       |
|----------------|---------|-----------|
| Body weight gain (g) | 167±7.94 | 158±9.25  |
| Feed conversion ratio | 0.44±0.03 | 0.43±0.02  |
| (g body weight gain/g feed) |         |           |

Values are means±SD with n=10 per group.
the control although plasma LP values were decreased \((p<0.05)\). There was no statistical significance in the liver vitamins A and E and plasma vitamin A as expressed as nmol/g.

**Cholesterol, TAG and phospholipids in plasma and lipoproteins in plasma and liver**

Adjusted means, calculated from an analysis of ANOVA after 32 d, are presented in Table 6 for cholesterol, TAG and phospholipids in plasma and lipoproteins. Compared with the HSO group, the plasma phospholipid \((p<0.01)\) and LDL-phospholipid \((p<0.01)\) value was higher in the control. Plasma-TAG \((p<0.01)\), VLDL-TAG \((p<0.01)\), HDL-TAG \((p<0.05)\), and HDL-cholesterol \((p<0.01)\) values were significantly lower in the HSO group than in the control group whereas plasma \((p<0.01)\), VLDL- \((p<0.001)\), LDL-cholesterol \((p<0.05)\) and LDL-TAG \((p<0.05)\) values were increased in the HSO groups.

**Liver lipids**

Table 7 shows the concentration of liver lipids. Concentrations of liver TAG and total fatty acids were significantly \((p<0.001)\) higher in rats fed the control diet than in the HSO diet. Dietary HSO exerted no effects on the concentration of cholesterol in the liver. SFA and MUFA concentrations were generally lower in the HSO than in the control group.

**DISCUSSION**

The incomplete bioavailability of HSO demonstrated in our study is supported by the results of two studies.
that suggested that high HSO feed is poorly absorbed (5, 6). Thus it appears that this fat is poorly absorbed at both high and low fat levels and when it is blended with other oils. The explanation for incomplete bioavailability of HSO may relate to its high content of long-chain saturated fatty acids. Evidence suggests that stearic acid and tristearin are poorly digested and absorbed in rats (1, 19). Because HSO is composed of stearic acid, a large component of this fat must be made up of tristearin. Furthermore, in the present study, the higher the content of stearic acid in a fat source, the poorer was the availability of fatty acids, the total cholesterol and TAG. Due to excretion of TAG in feces, TAG levels in plasma and lipoprotein fractions were significantly lower in the HSO groups than in the control groups.

Numerous well-controlled human trials have shown that dietary trans fatty acids, compared with oleic acid, negatively affect the plasma lipoprotein profile by increasing LDL-cholesterol concentrations (1, 19) and may be associated with cancer (6). However, this is not a concern with completely hydrogenated fats because they are virtually devoid of trans fatty acids. Another concern with HSO is its high content of saturated fatty acids, which is often linked to high cholesterol levels. However, most HSO contains stearic acid, which has been shown in numerous studies to be less atherogenic (2, 3, 5). Nevertheless, it is possible that the absorbed portion of HSO may contain more cholesterolemic fatty acids, such as palmitate. However, it has been reported that highly hydrogenated soybean oil prevented an expected dietary cholesterol-induced rise in plasma total cholesterol and reduction (5). In the study, vitamin E concentrations in plasma, LDL and adipose tissue were decreased in the HSO group although plasma, VLDL- and LDL-cholesterol values increased. Finally, a poorly digestible fat such as HSO could decrease the absorption of vitamin E. Therefore, food containing a high concentration of HSO should be supplemented with vitamin E.

To our knowledge, there is scarce information in the literature directly assessing values in the blood and tissues of hydrogenated fatty acid in animals and subjects. A decrease in liver and plasma concentrations of TAG in response to HSO supplementation has been seen in a rat study (5). This behavior is consistent with the fact that liver stores of TAG are mobilized rapidly when TAG absorption is reduced; they become depleted in parallel with the circulating pool (19–22). In our study, liver and plasma TAG concentrations and vitamin E concentrations of plasma, LDL and adipose tissue responded in a parallel manner to an addition of HSO to the basal diet. TAG concentrations in the plasma, VLDL and HDL and vitamin E levels in the plasma, LDL and adipose tissue were lower in the HSO group than in the control group. The cause of the decreased TAG and vitamin E concentrations in the HSO groups may reflect the effect of HSO on reducing the absorption of fatty acids observed in our study. Thus, we observed the TAG and vitamin E-lowering effect of HSO.

In our study, LP concentration was used as an index of oxidative stress status in the rats. Our findings show that LP levels in the plasma of the HSO group were higher than in the control group. A possible explanation for the enhancement of LP concentration may be decreased formation of vitamin E in plasma, LDL and adipose tissue of the HSO group, which in view of augmented activity of reactive oxygen substances allows a consequent increase in LP production because vitamin E is, under physiological conditions, an important antioxidant with lipophilic properties acting in cellular membranes (9–11).

We concluded that the effects of hydrogenated fatty acids on the plasma lipoprotein and vitamin E profile is at least as unfavorable as that of the LP-inducing and cholesterol-raising saturated fatty acids. The hydrogenated fatty acids not only raise LDL- and VLDL-cholesterol and plasma LP levels but also lower HDL- cholesterol and LDL vitamin E levels. The HSO also decreases plasma, lipoprotein and liver TAG concentrations because HSO containing a high content of 18-carbon saturated fatty acid will result in an increased content of stearic acid and tristearin, presumably making these fats less available.

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REFERENCES

1) Mensink RP, Katan M. 1990. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* **323**: 439–445.

2) Lichtenstein AH. 1998. Trans fatty acids and blood lipid levels. *Lipids* (a), parameters of cholesterol metabolism, and hemostatic factors. Review. *Nutr Biochem* **9**: 244–248.

3) Lichtenstein AH, Ausman LM, Carrasco W, Jenner JL, Or dovás JM, Schuefer EJ. 1993. Hydrogenation impairs the hypolipidemic effect of corn oil in humans. Hydrogenation, trans fatty acids, and plasma lipids. *Arterioscler Thromb* **13**: 154–161.

4) Sanchez-Moreno C, Dorfman SE, Lichtenstein AH, Martin A. 2004. Dietary fat type affects vitamins C and E and biomarkers of oxidative status in peripheral and brain tissues of golden Syrian hamsters. *J Nutr* **134**: 655–660.

5) Kamei M, Ohgaki S, Kanbe T, Niiya I, Mizutani H, Matsui-Yuasa I, Otani S, Morita S. 1995. Effects of highly hydrogenated soybean oil and cholesterol on plasma, liver cholesterol, and fecal steroids in rats. *Lipids* **30**: 533–539.

6) Kaplan RJ, Greenwood CE. 1998. Poor digestibility of fully hydrogenated soybean oil in rats: a potential benefit of hydrogenated fats and oils. *J Nutr* **128**: 875–880.

7) Koonsvitsky BP, Berry DA, Jones MB, Lin P, Cooper DA, Jones DY, Jackson JE. 1997. Olestra affects serum concentrations of α-tocopherol and carotenoids but not vitamin D or vitamin K status in free living subjects. *J Nutr* **127**: 1636S–1645S.

8) Cooper DA, Berry DA, Jones MB, Kiorpes AL, Peters JC. 1997. Olestra’s effects on the status of vitamins A, D and E in pig can be offset by increasing dietary levels of these vitamins. *J Nutr* **127**: 1589S–1608S.

9) Czernichow S, Hersberg S. 2001. Interventional studies concerning the role of antioxidant vitamins in cardiovascular disease: A review. *J Nutr Health Aging* **5**: 188–195.

10) Halliwell B, Gutteridge JMC. 1999. Free radicals, other reactive species and disease. *In: Free Radicals in Biology and Medicine* (Halliwell B, Gutteridge JMC, eds), 3rd ed. p 639–645. Oxford University Press, Oxford, UK.

11) Naziroğlu M, Simşek M, Simşek H, Aydilek N, Özcan Z, Atılgan R. 2004. The effects of hormone replacement therapy combined with vitamins C and E on antioxidants levels and lipid profiles in postmenopausal women with Type 2 diabetes. *Clin Chim Acta* **344**: 63–71.

12) Tiedink HGM, Katan MB. 1989. Variability in lipoprotein concentrations in serum after prolonged storage at −20°C. *Clin Chim Acta* **180**: 147–156.

13) Hara A, Radin NS. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem* **90**: 420–426.

14) Placer ZA, Cushman L, Johnson BC. 1966. Estimation of product of lipid peroxidation (as malonyl dialdehyde) in biological fluids. *Anal Biochem* **16**: 359–364.

15) Naziroğlu M, Karaoğlu A, Aksoy A. 2004. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology* **195**: 221–230.

16) Balz MK, Schulte E, Thier HP. 1993. Simultaneous determination of α-tocopherol acetate, tocopherols and tocotrienols by HPLC with fluorescence detection in foods. *Fat Sci Technol* **95**: 215–220.

17) Colandre ME, Diez RS, Bernal CA. 2003. Metabolic effect of trans fatty acids on an experimental dietary model. *Br J Nutr* **89**: 631–639.

18) Huang X, Fang C. 2000. Dietary trans fatty acids increase hepatic acyl-CoA: Cholesterol acyltransferase activity in hamsters. *Nutr Res* **20**: 547–558.

19) Wilson TA, McIntyre M, Nicolosi RJ. 2001. Trans fatty acids and cardiovascular risk. *J Nutr Health Aging* **5**: 184–187.

20) Bracco U. 1994. Effects of triglycerides structure on fat absorption. *Am J Clin Nutr* **60**: 10028–10098.

21) Sakono M, Takagi H, Sonoki H, Yoshida H, Iwamoto M, Ikade I, Imaizumi K. 1997. Absorption and lymphatic transport of interesterified or mixed fats rich in saturated fatty acids and their effect on tissue lipids in rats. *Nutr Res* **17**: 1131–1141.

22) Thurnham DI, Davies JA, Crump BJ, Situnayake RD, Davis M. 1986. The use of different lipids to express serum tocopherol: lipid ratios for the measurement of vitamin E status. *Ann Clin Biochem* **23**: 514–520.