Eur J Nutr 42 : 188 – 194 (2003)
DOI 10.1007/s00394-003-0411-9

ORIGINAL CONTRIBUTION

Kalyana Sundram
Margaret A. French
M. Thomas Clandinin

Exchanging partially hydrogenated fat for palmitic acid in the diet increases LDL-cholesterol and endogenous cholesterol synthesis in normocholesterolemic women

Summary Partial hydrogenation of oil results in fats containing unusual isomeric fatty acids characterized by *cis* and *trans* configurations. Hydrogenated fats containing *trans* fatty acids increase plasma total cholesterol (TC) and LDL-cholesterol while depressing HDL-cholesterol levels. Identifying the content of *trans* fatty acids by food labeling is overshadowed by a reluctance of health authorities to label saturates and *trans* fatty acids separately. Thus, it is pertinent to compare the effects of *trans* to saturated fatty acids using stable isotope methodology to establish if the mechanism of increase in TC and LDL-cholesterol is due to the increase in the rate of endogenous synthesis of cholesterol. Ten healthy normocholesterolemic female subjects consumed each of two diets containing approximately 30 % of energy as fat for a four-week period. One diet was high in palmitic acid (10.6 % of energy) from palm olein and the other diet exchanged 5.6 % of energy as partially hydrogenated fat for palmitic acid. This fat blend resulted in monounsaturated fatty acids decreasing by 4.9 % and polyunsaturated fats increasing by 2.7 %. The hydrogenated fat diet treatment provided 3.1 % of energy as elaidic acid. For each dietary treatment, the fractional synthesis rates for cholesterol were measured using deuterium-labeling procedures and blood samples were obtained for blood lipid and lipoprotein measurements. Subjects exhibited a higher total cholesterol and LDL-cholesterol level when consuming the diet containing *trans* fatty acids while also depressing the HDL-cholesterol level. Consuming the partially hydrogenated fat diet treatment increased the fractional synthesis rate of free cholesterol. Consumption of hydrogenated fats containing *trans* fatty acids in comparison to a mixture of palmitic and oleic acids increase plasma cholesterol levels apparently by increasing endogenous synthesis of cholesterol.

Key words *trans* fatty acids – palmitic acid – deuterium – cholesterol

Received: 27 May 2002
Accepted: 23 January 2003

K. Sundram
Malaysian Palm Oil Board
Kuala Lumpur, Malaysia

M. A. French · Dr. M. T. Clandinin (✉)
Nutrition and Metabolism Group
Dept. of Agricultural, Food and Nutritional Science
4–10 Agriculture/Forestry Centre
University of Alberta
Edmonton, Alberta, T6G 2P5, Canada
Tel.: +1-780/492-5188
Fax: +1-780/492-8855
E-Mail: tclandin@ualberta.ca

M. T. Clandinin
Dept. of Medicine
University of Alberta
Edmonton, AB, Canada

Introduction

Partial hydrogenation of oils is widely used to produce edible fat products having specific physical and textural properties. This process results in conversion of the *cis* bond to *cis* or *trans* configurations at a variety of positions along the fatty acid chain. Clinical [1] and epidemiological [2] studies have suggested that hydrogenated fats containing *trans* fatty acids increase plasma total cholesterol, LDL-cholesterol and lipoprotein Lp(a) while depressing HDL-cholesterol levels. The metabolic basis for these observations is unknown but these metabolic effects will tend to increase risk of cardiovascular disease [3] particularly if dietary *trans* fatty acids increase endogenous rates of cholesterol synthesis.

The effect of different dietary regimens on cholesterol synthesis has been studied using several tech-

EJN 411

niques: assessment of cholesterol precursor levels [4, 5], sterol balance [6, 7], deuterium incorporation [8, 9] and mass isotopomer distribution analysis (MIDA) [10, 11]. Studies of diet fat type [12], caloric restriction [13] and feeding frequency [14] have shown that the deuterium incorporation method is a sensitive technique, which correlates closely with results obtained from the sterol balance method [15] and MIDA [16]. Unesterified cholesterol synthesis increases as increased polyunsaturated fat is consumed, although serum cholesterol levels decrease [12]. The mechanism for these observations is thought to be due to increased LDL catabolic rates when high polyunsaturated fat diets are fed.

Recent studies [17, 18] suggest that palmitic acid is hypercholesterolemic when the diet is low in linoleic acid (~2 % of energy) but not when higher (~10 % of energy) levels of linoleic acid are fed. Replacing partially hydrogenated fats high in *trans* fatty acids with palm olein-based fat blends in food formulations requiring more solid fats may avoid the cardiovascular risk associated with consumption of *trans* fatty acids if the diet contains sufficient linoleic acid to eliminate potential hypercholesterolemic effects of palmitic acid. Thus the present study was designed to reflect real life situations wherein consumers are confronted by high linoleic containing hydrogenated products and thus determine if substitution of palmitic acid for hydrogenated fat containing *trans* fatty acids at a usual level of linoleic acid intake (6 % of energy) would mitigate the hypercholesterolemic effects of dietary *trans* fatty acids.

Methods

Subjects and diets

The study was supported by a grant from the Malaysian Palm Oil Board and the National Sciences and Engineering Research Council of Canada.

Subjects were recruited from employees of the Malaysian Palm Oil Board (MPOB) and screened on the basis of a questionnaire and medical examination. Subjects were healthy, non-smokers, consumed no alcohol and were normolipemic [total cholesterol (TC) < 6.2 mmol/L, triglycerides (TG) < 2.0 mmol/L]. Approval for the study was obtained from the institutional Ethical Committee and informed written consent was obtained from all subjects.

Experimental design

Ten female subjects were recruited and began the study after a three-week control period that represented the habitual Malaysian diet normally consumed by these volunteers, which is very low in *trans* fatty acids. The habitual or baseline diet, containing 29 % of energy as fat, incorporated typical local Malaysian recipes and nutrient content [19]. A mixture of palm olein and coconut oil in the approximate ratio of 90:10 typically represented the fat blend during this habitual diet. At the end of the control period subjects were randomly assigned to one of two dietary test periods in which a minimum of two-thirds of the fat energy was replaced by a test fat (Table 1). Energy requirements were determined to maintain the subjects' current weight [20]. Subjects were weighed weekly to verify maintenance of body weight. Subjects consumed each of the two diets containing 30 % of energy as fat for a 30 d period. The high saturated fat diet was high in palmitic acid (10.6 % of energy) from palm olein and the other diet, high *trans* fat, exchanged 5.6 % of energy as *trans* fatty acids for palmitic acid. This partially hydrogenated diet fat treatment provided 3.1 % of energy as elaidic acid and 2.6 % as other *trans* isomers. Subjects were fed either the high saturated fat or the high *trans* fat diet for 30 d separated by a washout period of four weeks. Using a 6-day rotating menu, subjects were provided with three meals (breakfast, lunch and high tea), which were prepared fresh each day in a central laboratory kitchen by a trained caterer. These three meals accounted for more than 75–80 % of the daily caloric intake of the volunteers throughout the study. A dietitian monitored adherence to the pre-set menus and cooking oil allotments in the kitchen during all meal preparations. Meals were provided from Monday to Saturday of each week. To further enhance compliance the test oils were provided to the volunteers' families for preparation of dinner and all meals on Sunday. The use of the oils in their homes was recorded in a diary, which also served as an additional measure of compliance. Volunteers were randomly chosen to provide double portions of the meals consumed at home, which were mixed with the three cooked meals provided to them and analyzed in the laboratory. Subjects were also monitored to

Table 1 Demographic parameters of subjects

Subject	Height (cm)	Weight (base)	Weight (high Sat)	Weight (high trans)
1	153.8	49.11	49.2	49.6
2	166.2	65.91	65.6	65.8
3	159.0	59.91	59.3	60.4
4	145.0	47.41	46.7	46.3
5	159.0	65.10	65.7	66.4
6	151.2	49.50	49.9	50.3
7	151.0	64.89	64.8	64.4
8	148.0	58.51	58.9	59.8
9	151.0	47.90	47.8	48.1
10	157.5	60.73	60.2	59.6
Mean ± SEM	154.2 ± 1.9	56.86 ± 2.41	56.8 ± 2.3	57.1 ± 2.3

ensure a relatively constant food intake during the test periods, which resulted in a stable BMI as determined by weekly body weight records.

Composition of fat blends and 6-day rotating menus

The dietary design tested the response of plasma lipids to consumption of a high saturated fat diet compared to a high *trans* fat diet, which resulted in a change in three major fatty acids. The total 18:1 (*cis* and *trans*) was the same in both the high saturated and high *trans* fat diets. In addition, the sum of *trans* and saturates was the same in both diets. If linoleic acid had been equalized in both diets the *trans* content would have been much higher, over 9 % of energy, which is unrealistic in current consumption patterns.

Currently, liquid oils are hydrogenated to a specific melting point for various margarine and solid fat formulations. The hydrogenated oils are then retrospectively diluted with unhydrogenated oils to achieve the final required melting characteristics that are so vital in the formulation of spreadable (tub) margarine. The high *trans* diet was formulated to reflect this real life situation. Soybean oil was hydrogenated in the MPOB's food technology pilot plant facility using a nickel-sulfur-poisoned catalyst under controlled conditions to produce partially hydrogenated soybean oil. The partially hydrogenated soybean oil had a melting point of 32 °C, which was then mixed with native soybean oil so that the final *trans* fatty acid content was 5.6 % of energy. The composition of the high *trans* fat was determined by infrared spectroscopy and capillary gas chromatography. Of this composition, 3.1 % was elaidic acid. The source of oil for the high saturated fat diet (predominantly as palmitic acid) was solely palm olein in its natural state.

Laboratory methods

Subjects were assigned a randomly coded three-digit number that was used for labeling all blood and plasma tubes to avoid introduction of bias during sample analyses. On d 30 of each treatment, a fasting blood sample (20 ml) was taken by venipuncture at 0730 h. Subjects then consumed a priming dose of deuterium oxide (99.8 atom percent excess, C/D/N Isotopes Inc., Pointe-Claire, Quebec, Canada) at 0.5 g D_2O/kg estimated body water (60 % of body weight). A maintenance dose of 1.0 g D_2O/kg estimated body water was provided in a 2 L bottle of water to be consumed gradually over the next 24 h, to maintain plasma deuterium enrichment at a plateau and to compensate for unlabeled water obtained from the diet. Exactly 24 h later (d 31), a second fasting blood sample (20 ml) was drawn.

Plasma obtained on days 30 and 31 from each volunteer was analyzed enzymatically for total cholesterol, HDL-cholesterol and triglycerides using commercial enzymatic kits (Gilford Diagnostics, UK) and measured with a clinical autoanalyzer (Express 550, Corning, Corning, NY). LDL-cholesterol was measured after isolation of very low-density lipoproteins according to the Lipid Research Clinic procedures (NIH 1974). Serum apolipoproteins A1 and B were measured using the Sigma immuno-turbidimetric test kits (St Louis, MO, USA). All samples from a volunteer were analyzed at the same time. The remaining blood from d 30 and 31 was centrifuged at 3000 rpm for 15 min at 4/C (in a Sigma 3K12 refrigerated centrifuge, B. Braun, Germany) to obtain plasma. Day 30 plasma was used to determine background enrichment of plasma water, free cholesterol and cholesteryl ester, and d 31 plasma was used to measure deuterium enrichment at 24 h in the same fractions. For d 31 plasma water measurements, plasma was diluted twenty-fold with 5 % bovine serum albumin to reduce the deuterium enrichment to within the analytical limits of the mass spectrometer; d 30 plasma water samples were used directly. Free cholesterol and cholesteryl ester samples were prepared as described previously [21].

Fat analysis of diets

The energy density, total fat, fatty acids, protein, carbohydrate and cholesterol content of the 6-day menus and daily consumption of the test diets by individual volunteers was established by analysis as described previously [22].

Measurement of endogenous cholesterol synthesis

The cholesterol fractional synthesis rate (FSR), which represents the fraction of the rapidly turning over free cholesterol pool synthesized per day, was measured as described earlier [17, 23, 24]. Deuterium enrichment was measured in plasma water, plasma cholesterol and plasma cholesteryl ester against hydrogen prepared from a water standard using a Finnigan MAT251 Isotope Ratio Mass Spectrometer (Bremen, Germany). The mass three abundance was corrected for H^{3+} contribution [25]. Each sample was analyzed in duplicate. Cholesterol fractional synthesis rates (FSR) were determined from the initial incorporation rate of deuterium-labeled cholesterol into the rapid exchangeable cholesterol pool, relative to the initial precursor enrichment as determined using the body water deuterium level [24]. Maximum attainable enrichment was calculated as the body water pool enrichment corrected for the fraction of protons in *de novo* synthesized cholesterol that derive from water, relative to non-water sources [24].

The deuterium-uptake method determines short-

term cholesterol synthesis in humans by measuring the rate of D_2O uptake from the body water pool into newly synthesized cholesterol over the 24 h test period. From the increase in enrichment the fractional synthetic rate (FSR)

$$FSR_{FC} = \frac{\delta_{FC}}{(\delta_{PW}\, X0.478)}$$

can be determined as the proportion of the central pool of free sterol derived from synthesis, where δ_{FC} is the change in enrichment in the free cholesterol fraction and δ_{PW} is the change in enrichment in the plasma water over 24 h. The enrichment in each fraction of free cholesterol (FC) and cholesteryl ester (CE) are determined separately and the total FSR calculated [21].

Statistical analysis

The two lipid and lipoprotein values obtained for each subject on day 30 and 31 were averaged for statistical analysis. Repeated measures ANOVA coupled to Scheffe's test for significance ($p < 0.05$) was used to analyze the data using Statistica (Tulsa, OK). Carry-over effects of previous diets were evaluated by a diet-by-period interaction term in the analysis.

Results

The fat exchanges and their consumption during the two dietary periods were achieved as postulated by the design of the current study. The intake of total energy, fat, protein, carbohydrate and dietary cholesterol (Table 2) was not significantly different between the two dietary treatments. However, in keeping with our objectives, the GC lipid analysis shows the percentage energy from saturated fatty acids was significantly higher during the high saturated fat diet period. Conversely, during the high *trans* fat diet treatment, intake of *trans* fatty acids was apparent, whereas this was not detected during the high saturated fat treatment. In addition, the *trans* fatty acid diet was characterized by a higher availability of *cis* polyunsaturated linoleic acid, which resulted in a higher polyunsaturated/saturated (P/S) fatty acid ratio (high *trans* diet P/S = 0.82 ± 0.18, saturated fat diet P/S = 0.30 ± 0.04).

The mean height and mean body weight after consuming the baseline, high saturated and high trans diets were 154.2 ± 1.9 cm, 56.86 ± 2.41 kg, 56.8 ± 2.3 kg and 57.1 ± 2.3 kg respectively. Despite the changes in the type of fatty acid consumed during the experimental periods, all volunteers maintained a constant body weight during the diet treatments relative to their baseline entry levels.

Table 2 Nutrient intake per day (% energy)*

Nutrient	Baseline	High saturated fat	High *trans* fat
Total energy (kcal)	2112 ± 496.9	2140 ± 166.4	2070 ± 293.0
Total fat	29.3 ± 3.90	30.5 ± 1.59	29.9 ± 3.03
Protein	17.2 ± 2.90	14.5 ± 1.04	14.3 ± 2.91
Carbohydrate	53.4 ± 4.20	55.5 ± 2.24	55.7 ± 3.68
Cholesterol (mg)		213 ± 22	205 ± 19
SFA	**13.20 ± 2.34**	**13.0 ± 1.75**	**8.14 ± 1.11**
10:0	0.15 ± 0.18	n. d.	0.08 ± 0.07
12:0	1.16 ± 1.08	0.47 ± 0.42	0.86 ± 0.39
14:0	0.70 ± 0.41	0.46 ± 0.15	0.48 ± 0.15
16:0	9.77 ± 1.65	10.6 ± 1.51	4.54 ± 0.60
18:0	1.35 ± 0.25	1.31 ± 0.16	2.06 ± 0.33
MUFA	**11.85 ± 1.97**	**12.9 ± 1.45**	**8.00 ± 0.94**
16:1 (n-9)	0.43 ± 0.40	0.23 ± 0.09	0.25 ± 0.15
18:1 (n-9)	11.42 ± 1.79	12.7 ± 1.45	7.75 ± 0.92
PUFA	**3.41 ± 0.63**	**3.80 ± 0.47**	**6.54 ± 1.12**
18:2 (n-6)	3.20 ± 0.58	3.54 ± 0.46	5.80 ± 1.11
18:3 (n-3)	0.14 ± 0.04	0.11 ± 0.08	0.49 ± 0.27
22:6 (n-3)	0.08 ± 0.12	0.14 ± 0.16	0.25 ± 0.17
***Trans* FA**	**n. d.**	**n. d.**	**5.59 ± 1.12**
18:1 te	n. d.	n. d.	3.13 ± 0.67
18:1 (n-11t)	n. d.	n. d.	0.66 ± 0.20
18:1 (n13t)	n. d.	n. d.	1.14 ± 0.40
18:2 (n-6tt)	n. d.	n. d.	1.60 ± 0.10
18:2 (n-6tc)	n. d.	n. d.	0.25 ± 0.06

* *18:1 te* = *trans* elaidic acid, *t* = trans, *tt* = trans trans, *tc* = trans cis, *n. d.* = not detectable. Nutrients analyzed from double portions of food consumed by volunteers (n = 10)

Total cholesterol

Mean level of total cholesterol for the subjects after consuming the baseline diet was 5.37 ± 0.35 mmol/L. After consuming the high saturated fat diet, the mean total cholesterol level dropped to 5.28 ± 0.24 mmol/L compared to the baseline diet. Mean total cholesterol increased significantly by 6.6 % ($p < 0.05$) when the *trans* fatty acid diet was fed compared to the high saturated fat diet (Fig. 1). Total cholesterol values for eight out of ten subjects increased and one remained unchanged after the high *trans* fat diet was consumed relative to the high saturated fat.

LDL-cholesterol

The mean LDL-cholesterol in these volunteers after the baseline diet was 3.35 ± 0.27 mmol/L. LDL-cholesterol decreased marginally to 3.23 ± 0.17 mmol/L when the high saturated fat diet was fed. When the *trans* fatty acid diet was ingested, mean LDL-cholesterol levels signifi-

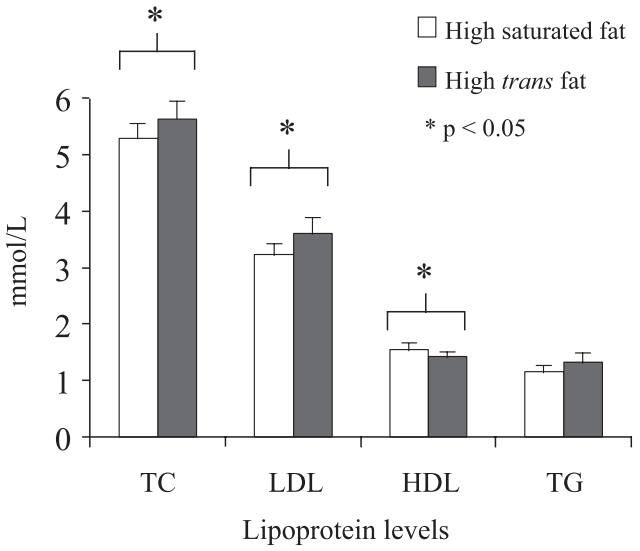

Fig. 1 The effect of consuming high saturated fat and high *trans* fat diets on plasma lipid and lipoprotein cholesterol levels (n = 10). Values represent means ± SEM

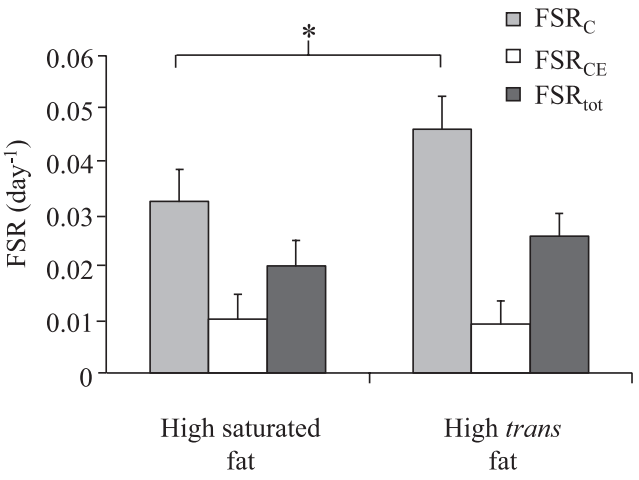

Fig. 2 Effect of diet treatment on mean fractional synthetic rate (FSR) for free cholesterol and cholesteryl ester fractions and total cholesterol. The fractional synthetic rate of free cholesterol increases significantly when a high *trans* fat diet is consumed ($p < 0.05$)

cantly increased by 11.5% over the high saturated fat diet ($p < 0.05$).

HDL-cholesterol

HDL-cholesterol of these subjects averaged 1.44 ± 0.09 mmol/L after consuming the baseline diet, which increased to 1.55 ± 0.08 mmol/L after the high saturated fat diet was fed. Mean HDL levels fell back close to the baseline diet when the high *trans* fat diet was consumed. The mean HDL-cholesterol of the subjects decreased significantly by 7.7% when the high *trans* fat diet was fed compared to a high saturated fat diet ($p < 0.05$). The HDL-cholesterol levels of all subjects decreased when a high *trans* fat diet was fed. When the high saturated fat diet was fed the LDL/HDL ratio averaged 2.12 ± 0.11 but increased significantly to 2.58 ± 0.20 when the fat source was the high *trans* fat diet ($p < 0.01$).

Cholesterol fractional synthetic rate

The total cholesterol synthetic rate (FSR_{tot}) was measured to be 0.020 ± 0.005 when the high saturated fat diet was fed and 0.026 ± 0.004 when the high *trans* fat diet was consumed (Fig. 2). However, if free cholesterol and cholesterol ester fractions were considered separately, the FSR of the free cholesterol fraction was significantly greater (FSR of 0.046 ± 0.006) when the high *trans* fat diet was consumed compared to the high saturated fat diet (FSR of 0.032 ± 0.006). Eight out of ten subjects increased their FSR_{tot} when a high *trans* fat diet was fed but this did not attain significance compared to the high saturated fat diet (Fig. 3).

Discussion

The current results extend previous findings that moderate intake of *trans* fatty acids increase the LDL/HDL-cholesterol ratio. Subjects consuming high saturated or

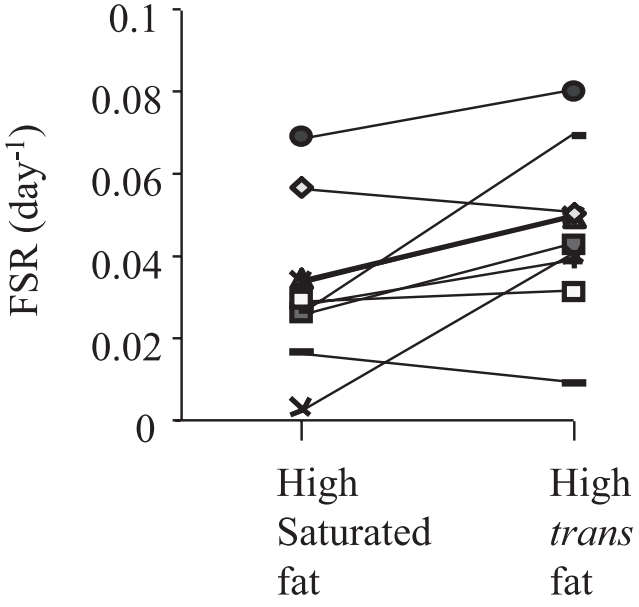

Fig. 3 Individual subjects' fractional synthetic rates of total cholesterol after consuming a diet high in saturated fat and high in *trans* fat. The FSR of eight out of ten subjects increased when consuming a diet high in *trans* fatty acids

high *trans* fat diets had similar energy intakes, which consequently cannot explain the changes in LDL-cholesterol or rates of endogenous cholesterol synthesis observed [27].

Elevated total cholesterol and LDL-cholesterol levels and low HDL-cholesterol levels are associated with increased risk of cardiovascular disease. It is commonly accepted that diets high in saturated fat raise plasma total cholesterol and LDL-cholesterol. However, recent studies suggest that the saturated fatty acids are not uniformly cholesterolemic, with stearic acid being described as neutral [28], while myristic acid has been assigned the highest cholesterol-raising potency [29]. It has been found that diets high in palmitic acid are cholesterol-raising unless they are accompanied by sufficient levels of linoleic acid [17]. When the level of linoleic acid was low, high levels of palmitic acid in the diet were found to be hypercholesterolemic, but when the level of linoleic acid was increased to 10% the hypercholesterolemic effect of high amounts of palmitic acid disappeared.

It should be noted that in the present study the level of linoleic acid in the high *trans* fat diet was higher than in the high saturated fat diet. Linoleic acid appears not to have the same hypocholesterolemic effect when consumed in conjunction with *trans* fatty acids as with palmitic acid. This observation is also supported by data from Mattan et al. [30].

Judd et al. [31] reported the relative effect of different fatty acids on total cholesterol and LDL-cholesterol levels to be oleic acid < moderate *trans* < high *trans* < saturated fats. The linoleic acid content was kept constant at 6% of energy through all of the diet treatments. In the present study, the order of the cholesterolemic effect of high *trans* vs. high saturated fat was opposite to that observed by Judd et al.; the amount of linoleic acid was 3.5% of total energy in the high saturated fat diet and the serum mean total cholesterol was 6.6% lower than that observed with a high *trans* diet containing 5.8% of energy of linoleic acid. Interpretation of the results is also confounded by the higher percentage of oleic acid in the high saturated fat diet. There are instances where saturated fats resulted in lower cholesterol levels than trans fatty acids. Sundram et al. [19] compared the effects of exchanging *cis* 18:1, 16:0 or 12:0 + 14:0 for *trans* elaidic acid in humans. The *trans*-rich diet significantly elevated total and LDL-cholesterol levels relative to the 16:0 and 18:1-rich fat but attained no significance compared to the saturated 12:0 + 14:0-rich dietary fat. However, the *trans* diet uniquely lowered HDL-cholesterol and elevated Lp(a) relative to all other dietary treatments. These effects were apparent despite the fact that linoleic acid content in the *trans*-rich diet was significantly higher than *cis* 18:1 and saturated 16:0-rich diets. Therefore in the present study linoleic acid content is not responsible for the higher cholesterolemic effect of *trans* fat compared to saturated fat.

Trans fatty acids decrease HDL-cholesterol, and this is currently viewed as a major public health concern [32]. Some saturated fatty acids, in addition to raising total and LDL-cholesterol, do not appear to affect HDL-cholesterol levels to the same extent [33] or are even capable of raising the beneficial HDL-cholesterol level [34]. The effect of *trans* fatty acids in elevating LDL-cholesterol levels and depressing HDL-cholesterol can have a large effect on the LDL/HDL ratio which is used to assess risk of cardiovascular disease. Thus the resulting LDL/HDL-cholesterol ratio is significantly lowered by *trans*-rich diets [33, 34].

Cholesterol ester transfer protein (CETP) activity has been postulated as a possible mechanism, i. e., enhanced transfer of cholesteryl ester (CE) from HDL to LDL, to explain the observed shifts in the LDL/HDL ratio [35]. In individuals whose LDL-receptors are down regulated, increased CE transfer from HDL could be expected to diminish the HDL-CE pool and overload the LDL-CE pool when LDL clearance is impaired. This was demonstrated in *Cebus* monkeys whose LDL-receptor activity and clearance of LDL are highly efficient. When fed an elaidic-rich *trans* diet, these monkeys were found to have elevated CETP activity and depressed HDL levels without affecting the LDL-pool size or LDL-clearance rate [36].

The effect of linoleic acid level in different diet treatments may also affect endogenous cholesterol synthesis. In a study using various levels of hydrogenated fat in different margarines Matthan et al. [30] found that as the degree of *trans* fatty acids in the diet increased, the FSR_{FC} decreased. This is opposite to the effect that was found in the present study. In Matthan's study the linoleic acid decreased gradually as the *trans* fatty acid content increased, whereas in the present study *trans* fatty acids were accompanied by a higher level of linoleic acid than the high saturated fat diet. High levels of polyunsaturated fats have been shown to increase cholesterol synthesis [12] so it is difficult to attribute the observed increase in cholesterol synthesis to either increased linoleic or increased *trans* fatty acids. Linoleic acid appears not to have the "protective" effect observed with high palmitic acid levels [17]. Therefore, increasing *trans* fatty acids in the diet promotes increased LDL-cholesterol by increasing endogenous cholesterol synthesis.

It is well accepted that *trans* fatty acids raise total and LDL-cholesterol levels. Food manufacturers need to find a healthy alternative in the production of processed food requiring a solid fat. Saturated fat containing high amounts of palmitic acid but not lauric or myristic acid could fill this void. Separate identification of saturated and *trans* fat content in food labeling currently appears pertinent so that it would enable the consumer to make

a choice between these two fat sources. This study has shown that substitution of palmitic acid and oleic acid for hydrogenated fat containing *trans* fatty acids at a usual level of linoleic acid intake (6% of energy) mitigates the hypercholesterolemic effects of dietary *trans* fatty acids.

References

1. Katan MB, Zock PL, Mensink RP (1995) *Trans* fatty acids and their effects on lipoproteins in humans. Ann Rev Nutr 15:473–493
2. Ascherio A, Willett WC (1995) Metabolic and atherogenic effects of *trans* fatty acids. J Internal Med 238:93–96
3. Willett WC, Stampfer MJ, Manson JE, et al. (1993) Intake of *trans* fatty acids and risk of coronary heart disease among women. Lancet 341:581–585
4. Glatz JF, Katan MB (1993) Dietary saturated fatty acids increase cholesterol synthesis and fecal steroid excretion in healthy men and women. Eur J Clin Invest 23:648–655
5. Parker TS, McNamara DJ, Brown CD, Rolb R, Ahrens Jr EH, Alberts AW (1984) Plasma mevalonate as a measure of cholesterol synthesis in man. J Clin Invest 74:795–804
6. Avigan J, Steinberg D (1965) Sterol and bile acid excretion in man and the effects of dietary fat. J Clin Invest 44: 1845–1856
7. Grundy SM (1975) Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. J Clin Invest 55:269–282
8. Dietschy JM, Spady DK (1984) Measurement of rates of cholesterol synthesis using tritiated water. J Lipid Res 25: 1469–1476
9. Jones PJ, Pappu AS, Illingworth DR, Leitch CA (1992) Correspondence between plasma mevalonic acid levels and deuterium uptake in measuring human cholesterol synthesis. Eur J Clin Invest 22:609–613
10. Hellerstein MK (1995) Methods for measurement of fatty acid and cholesterol metabolism. Curr Opin Lipidol 6: 172–181
11. Lee WN, Bassilian S, Guo Z, Schoeller D, Edmond J, Bergner EA (1994) Measurement of fractional lower case lipid synthesis using deuterated water (^2H_2O) and mass isotopomer analysis. Am J Physiol 266:E372–383
12. Mazier MJ, Jones PJH (1997) Dietary fat saturation and feeding state modulate rates of cholesterol synthesis in normolipidemic men. J Nutr 127:332–340
13. Di Buono M, Hannah JS, Katzel LI, Jones PJH (1999) Weight loss due to energy restriction suppresses cholesterol biosynthesis in overweight, mildly hypercholesterolemic men. J Nutr 129: 1545–1548
14. Jones PJH, Leitch CA, Pederson RA (1993) Meal-frequency effects on plasma hormone concentrations and cholesterol synthesis in humans. Am J Clin Nutr 57:868–874
15. Jones PJH, Ausman LM, Croll DH, Feng JY, Schaefer EA, Lichtenstein AH (1998) Validation of deuterium incorporation against sterol balance for measurement of human cholesterol biosynthesis. J Lipid Res 39:1111–1117
16. Di Buono M, Jones PJH, Beaumier L, Wykes LJ (2000) Comparison of deuterium incorporation and mass isotopomer distribution analysis for measurement of human cholesterol biosynthesis. J Lipid Res 41:1516–1523
17. Clandinin MT, Cook SL, Konrad SD, Goh YK, French MA (1999) The effect of palmitic acid on lipoprotein levels and endogenous cholesterol synthesis in hyperlipidemic subjects. Lipids 34(S): 121–124
18. Choudhury N, Tan L, Truswell AS (1995) Comparison of palmolein and olive oil: effects on plasma lipids and vitamin E in young adults. Am J Clin Nutr 61: 1043–1051
19. Sundram K, Ismail A, Hayes KC, Jeyamalar R, Pathmanathan R (1997) Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. J Nutr 127:S514–520
20. Bell L, Jones PJH, Telch J, Clandinin MT (1985) Prediction of energy needs for clinical studies. Nutr Res 5:123–129
21. Jones PJH, Schoeller DA (1990) Evidence for diurnal periodicity in human cholesterol synthesis. J Lipid Res 31: 667–673
22. Sundram K, Hayes KC, Siru OH (1994) Dietary palmitic acid lowers cholesterol relative to a lauric-myristic acid combination in normolipemic humans. Am J Clin Nutr 59:841–846
23. Jones PJH (1990) Use of deuterated water for measurement of short-term cholesterol synthesis in humans. Can J Physiol Pharmacol 88:955–959
24. Jones PJH, Leitch CA, Li Z-C, Connor WE (1993) Human cholesterol synthesis measurement using deuterated water: theoretical and practical considerations. Arterioscler Thromb 13:247–253
25. Coplen TB (1988) Normalization of oxygen and hydrogen isotope data. Chem Geol 72:293–297
26. Raeini-Sarjaz M, Vanstone CA, Papamandjaris AA, Wykes LJ, Jones PJH (2001) Comparison of the effect of dietary fat restriction with that of energy restriction on human lipid metabolism. Amer J Clin Nutr 73:262–267
27. Grundy SM, Vega GL (1986) Plasma cholesterol responsiveness to saturated fatty acids. Am J Clin Nutr 47:822–824
28. Hayes KC, Khosla P (1992) Dietary fatty acid thresholds and cholesterolemia. FASEB J 6:2600–2607
29. Zock PL, de Vries JH, Katan MB (1994) Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscl Thromb 14:567–575
30. Matthan NR, Ausman LM, Lichtenstein AH, Jones PJH (2000) Hydrogenated fat consumption affects cholesterol synthesis in moderately hypercholesterolemic women. J Lipid Res 41: 834–839
31. Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME, Podczasy JJ (1994) Dietary *trans* fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. Amer J Clin Nutr 59:861–868
32. Katan MB (1995) Exit trans fatty acids. Lancet 346:1245–1246
33. Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ (1999) Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. N Engl J Med 340:1933–1940
34. Khosla P, Sundram K (1996) Effects of dietary fatty acid composition on plasma cholesterol. Prog Lipid Res 35: 93–132
35. Abbey M, Nestel PJ (1994) Plasma cholesteryl ester transfer protein is increased when trans elaidic acid is substituted for cis-oleic acid in the diet. Atherosclerosis 106:99–107
36. Khosla P, Hajri T, Pronczuk A, Hayes KC (1997) Replacing dietary palmitic acid with elaidic acid (t-C18:1 n9) depresses HDL and increases CETP activity in cebus monkeys. J Nutr 127:531S–536S