Original Article

Cholesterolaemic effect of palmitic acid in relation to other dietary fatty acids

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The effect of dietary intake of high palmitic acid levels in combination with other fatty acids in normal subjects was assessed. Palmitic acid (10% of energy) was fed in conjunction with decreasing levels of linoleic acid to determine if a threshold level of linoleic acid prevented palmitic acid from being hypercholesterolaemic. Healthy subjects received each of the diet treatments for 21 days, followed by washout periods of 7 days. In a second experiment, the effect of exchanging palmitic acid for *trans* fatty acids on plasma lipoprotein cholesterol levels and on rates for endogenous synthesis of cholesterol in normal subjects was investigated. Diet treatment lasted for 30 days. On day 30 of each diet treatment, a priming dose of deuterium was consumed, followed by a subsequent blood sample at 24 h. Blood cholesterol fractions were isolated and analysed by isotope ratio mass spectrometry to measure cholesterol fractional synthetic rates. In the first experiment, total plasma cholesterol levels increased as the percentage of linoleic acid decreased. The data indicated that high levels of palmitic acid were not hypercholesterolaemic if intake of linoleic acid was greater than 4.5% of energy. When the diet contained *trans* fatty acids plasma total and low-density lipoprotein-cholesterol increased and cholesterol synthesis increased with a decrease in high-density lipoprotein-cholesterol.

Key words: cholesterol, deuterium, human, palmitic acid, plasma, *trans* fatty acids.

Introduction

Saturated fatty acids and hydrogenated oils are common components of margarines and prepared foods. Specific dietary fatty acids may raise total serum cholesterol levels and low-density lipoprotein (LDL)-cholesterol levels. Understanding the effects of individual saturated fatty acids is complicated by evidence showing that all saturated fats do not affect lipoprotein profiles equally.[1–3] Stearic acid (18:0) has little effect on serum lipid levels, similar to some monounsaturates, while lauric (12:0) and myristic (14:0) acids have potent cholesterol-raising effects.[4–6] According to the original Keys hypothesis,[1,7] palmitic acid (16:0) should increase blood cholesterol levels, but these commonly cited equations fail to separate the effect of three saturated fatty acids (12:0, 14:0 and 16:0), defining each as equally hypercholesterolaemic. When the Keys equation is modified to treat palmitic acid as neutral (similar to stearic acid), the equation is a better predictor of changes observed in serum cholesterol levels.[8] In a study that exchanged 5% of energy from 12:0 and 14:0 for 16:0 in healthy young men consuming a low-cholesterol diet, the dietary combination of 12:0 plus 14:0 produced significantly higher serum cholesterol levels than 16:0.[9] Other studies have failed to demonstrate elevated plasma cholesterol following palmitic acid consumption.[10] Ng *et al.* compared the effects of palmitic acid and oleic acid in normolipaemic subjects.[10] Exchanging 7% of energy for either palm oil or olive oil produced identical lipoprotein profiles suggesting that in healthy humans, exchanging dietary palmitic acid for oleic acid within the normally present range of these fatty acids would not affect serum cholesterol concentration.

Cook *et al.* have suggested that other factors may be present in the diet that influence the cholesterolaemic effect of palmitic acid.[11] These researchers fed four diets to normocholesterolaemic subjects with all four combinations of low (approximately 3% of energy) or high (approximately 10% of energy) of palmitic acid and linoleic acid (18:2). When the dietary level of linoleic acid was low, increasing the level of palmitic acid produced a cholesterol-raising effect in plasma. However, when the linoleic acid level was increased to current dietary recommended levels (10% of energy), then the effect of increasing palmitic acid had no significant effect on serum total or LDL-cholesterol levels. Differing levels of linoleic acid in dietary treatments that were used in previous studies to investigate palmitic acid could explain the contradictory results obtained.

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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[12] and epidemiological[13] studies have suggested that hydrogenated fats containing *trans* fatty acids increase plasma total cholesterol, LDL-cholesterol and lipoprotein a while depressing high-density lipoprotein (HDL)-cholesterol levels. The metabolic basis for these observations is unknown but these metabolic effects will tend to increase risk of cardiovascular disease,[14] particularly if dietary *trans* fatty acids increase endogenous rates of cholesterol synthesis.

The first experiment conducted was designed to assess the effect of decreasing dietary levels of linoleic acid while maintaining high levels of palmitic acid to determine if a threshold level of dietary linoleic acid existed that prevents an increase in plasma lipoprotein cholesterol levels. A second study investigated if substitution of palmitic acid for hydrogenated fat containing *trans* fatty acids at a level of linoleic acid intake of 6% of energy would mitigate the hypercholesterolaemic effects of dietary *trans* fatty acids.

Materials and methods

The Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee (experiment 1) and the Malaysian Palm Oil Board Ethical Committee (experiment 2) approved all procedures. Subjects gave informed written consent. An in-depth questionnaire was completed by all subjects to screen for medical problems known to affect lipoprotein levels, and to characterise the subjects' activity levels and sleep patterns. Subjects were non-smokers and were not taking any medication or vitamin supplements during the study.

Subjects and diets

Three female and three male subjects were recruited for experiment 1 (Table 1). Caloric requirements were determined and maintained as described earlier.[15] All meals were prepared in the metabolic research kitchen and consumed at fixed times. Adjustments to energy intake were made if sustained weight changes were observed. Because the duration of the experiment was eight months, some minor adjustments were needed to account for seasonal activity. The study consisted of eight different diet treatments with a break of at least seven days between treatments (Table 2). Each diet treatment lasted for 21 days and consisted of a seven-day rotating menu. The menus remained the same throughout the experiment with only the fat composition changing in each diet treatment. Diets were formulated from food composition tables to contain an average of 30% of energy from fat, 55% from carbohydrate, 15% from protein and a low cholesterol content (110 mg cholesterol/day).[16] The diets provided a constant level of palmitic acid (10% of energy) throughout the experiment. The level of linoleic acid was gradually decreased in approximately equal steps from 10 to 2.5% of energy over the eight diet treatments, the remaining fat being provided by monounsaturated fat. To achieve the dietary design palm stearin, safflower, high oleic safflower and olive oils were used. Diets were balanced for n-3 fatty acids, cholesterol and fibre content.

Table 1. Descriptive characteristics of subjects consuming the experimental diets

Demographic parameters	Normal subjects
Experiment 1	
Age (years)	25.0 ± 1.7
Bodyweight (kg)	65.5 ± 3.8
Height (cm)	173.7 ± 4.4
BMI	21.3 ± 0.6
Experiment 2	
Age (years)	NA
Bodyweight (kg)	56.86 ± 2.41
Height (cm)	154.2 ± 1.9
BMI	23.89 ± 0.8
Initial TC (mmol/L)	5.37 ± 0.35

BMI, body mass index; NA, not assessed; TC, total cholesterol.

Table 2. Dietary formulation for experiment 1

Energy source	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Calories (kcal)	3104	2984	2946	2906	2954	2956	2888	2964
Total fat	30.9	30.3	30.1	29.8	29.6	30.1	29.5	29.2
16:0	10.2	10.0	10.1	10.1	10.0	9.9	10.0	10.4
18:0	1.1	1.2	1.2	1.2	1.2	1.3	1.3	1.5
MUFA	7.3	8.0	9.2	9.5	10.1	12.7	12.2	11.7
18:2 (n-6)	10.0	8.5	7.5	6.6	5.5	4.5	3.5	2.5
18:3 (n-3)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
20:5 (n-3)	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01
22:6 (n-3)	0.03	0.04	0.04	0.04	0.04	0.05	0.04	0.03
Cholesterol (mg)	98	108	101	101	105	105	113	125

Diets were formulated using Food Processor II nutrient analysis software (Esha Research, Salem, OR, USA). Meals were analysed in duplicate and values pooled to obtain per day values (mean ± SD). Fatty acids were analysed in duplicate portions of food as methyl esters by gas-liquid chromatography. Analysed values were calculated as mean ± SD of 3 day menu in duplicate (n = 6) and are expressed as percentage energy. MUFA, monounsaturated fatty acid.

Experiment 2 was carried out with 10 normolipaemic female Malaysian subjects (Table 1). Subjects began the study after a three-week control period that represented the habitual Malaysian diet normally consumed by these volunteers (Table 3). The habitual (or baseline) diet contained 29% of energy as fat and incorporated typical local Malaysian recipes and nutrient content.[17] A mixture of palm olein and coconut oil in the approximate ratio of 90:10 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. Energy requirements were determined to maintain the subjects' current weight.[14] Subjects were weighed weekly to verify maintenance of bodyweight. Subjects consumed each of the two diets containing 30% of energy as fat for a 30-day period. The high saturated fat diet was high in palmitic acid (10.6% of energy) and the high *trans* fat diet 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. The dietary design reflects a real-life situation wherein consumers purchase high linoleic acid products containing hydrogenated fatty acids. Using a 6-day rotating menu, subjects were provided with three meals (breakfast, lunch and dinner), which were prepared fresh each day in a central laboratory kitchen by trained personnel. 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.

Experimental design

Subjects in the first experiment were fed the diets for 21 days with a washout period of 7 days between diet treatments. On day 21 (background day) of each treatment, a fasting blood sample (30 mL) was taken. Subjects then consumed a priming dose of deuterium oxide (CDN Isotopes, Pointe-Claire, Quebec, Canada; 0.5 g/kg estimated body water) and a maintenance dose (1.0 g/kg estimated body water in 2 L unlabelled water) over the next 24 h.[18] Exactly 24 h later (day 22, test day), a second fasting blood sample (30 mL) was collected.

Subjects in the second experiment were fed either the high saturated fat or the high *trans* fat diet for 30 days, separated by a washout period of four weeks. The deuterium administration protocol was exactly the same as in experiment 1.

Serum was analysed for lipoprotein cholesterol levels. The remaining blood from the background day and test day were centrifuged at 1850 g for 15 min at 4°C (Jouan refrigerated centrifuge, CR 4.11, Sainte Nazaire, France) to obtain plasma. Plasma from the background day was used to determine background deuterium enrichment and plasma from the test day was used to measure deuterium enrichment at 24 h in cholesterol, cholesteryl ester and plasma water.

Table 3. Nutrient intake per day in study 2

	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
SFA	13.2 ± 2.3	13.0 ± 1.7	8.1 ± 1.1
10:0	0.2 ± 0.2	ND	0.1 ± 0.1
12:0	1.2 ± 1.1	0.5 ± 0.4	0.9 ± 0.4
14:0	0.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.2
16:0	9.8 ± 1.7	10.6 ± 1.5	4.5 ± 0.6
18:0	1.4 ± 0.3	1.3 ± 0.2	2.1 ± 0.3
MUFA	11.9 ± 2.0	12.9 ± 1.5	8.0 ± 0.9
16:1(n-9)	0.4 ± 0.4	0.2 ± 0.1	0.3 ± 0.2
18:1(n-9)	11.4 ± 1.8	12.7 ± 1.5	7.8 ± 0.9
PUFA	3.4 ± 0.6	3.8 ± 0.5	6.5 ± 1.1
18 : 2 (n-6)	3.2 ± 0.6	3.5 ± 0.5	5.8 ± 1.1
18 : 3 (n-3)	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.3
22 : 6 (n-3)	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2
Trans FA	ND	ND	5.6 ± 1.1
18 : 1 te	ND	ND	3.1 ± 0.7
18:1 (n-11t)	ND	ND	0.7 ± 0.2
18:1 (n-13t)	ND	ND	1.1 ± 0.4
18:2 (n-6tt)	ND	ND	1.6 ± 0.1
18:2 (n-6tc)	ND	ND	0.3 ± 0.1
P/S ratio	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.2

Values are expressed as percentage energy. Nutrients were analysed from double portions of food consumed by volunteers (n = 10). 18 : 1 te, *trans* elaidic acid; FA, fatty acid; MUFA, monounsaturated fatty acids; ND, not detectable; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; t, *trans*; tt, *trans trans*; tc, *trans cis*.

Statistical analysis

Repeated measures analysis of variance and regression procedures determined the significance of the dietary treatment effects.

Results

Experiment 1

Subject compliance in consuming all food provided by the metabolic research kitchen was high. Weight changes were minimal throughout the study period. There were no significant differences between diets in regard to total fat intake. The analysed fat content of the meals in each diet treatment were close to the values formulated and consistent within each diet treatment on a daily basis.

Total cholesterol. The normal levels of cholesterol for individuals within the age range of the subjects studied are 3.20–5.20 mmol/L. One subject's total cholesterol level was in the borderline high range (<5.70 mmol/L) after the initial screening measurement, but dropped to within the normal range after consuming the test diets. As the dietary linoleic acid content decreased from 10 to 2.5% the mean total cholesterol level increased by 12 ± 3.7% (Fig. 1). Four out of six subjects exhibited a maximum total cholesterol level when consuming the diets that contained less than 4.5% linoleic acid. A linear regression of mean percentage change of total plasma cholesterol levels and intake of 18:2 showed a transition at 4.5% 18:2, with an R^2-value of 0.91 between 4.5 and 10% 18:2. Below 4.5% the R^2-value was 0.63 (Fig. 1). Therefore, total plasma cholesterol tends to increase linearly as the dietary linoleic acid content is decreased from 10 to 4.5%.

LDL-cholesterol. The normal LDL-cholesterol range is 1.7–3.4 mmol/L. With decreasing levels of linoleic acid in the diet from 10 to 2.5%, LDL-cholesterol levels increased by 23.4 ± 7.2%. Several subjects exhibited a change in LDL-cholesterol levels at a dietary 18:2 intake of approximately 4.5% (Fig. 1). At intakes of 18:2 above 4.5% the R^2-value was 0.95, but when intakes of 18:2 were between 2.5 and 4.5% the R^2-value dropped to 0.37 reflecting the behaviour of the total cholesterol response. The rate of change of LDL-cholesterol with 18:2 content was higher than for total cholesterol.

HDL-cholesterol. The normal range for HDL-cholesterol is 0.9–2.2 mmol/L. The mean HDL-cholesterol increased gradually from 1.30 ± 0.10 mmol/L after consuming the high linoleic acid diet to 1.50 ± 0.16 mmol/L after consuming the lowest linoleic acid diet.

Experiment 2

Total cholesterol. The 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 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 consumed, compared to the high saturated fat diet (Fig. 2). Total cholesterol values for eight out of 10 subjects increased and one remained unchanged after the high *trans* fat diet was consumed, relative to the high saturated fat diet.

LDL-cholesterol. The mean LDL-cholesterol level after consumption of 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 consumed. When the *trans* fatty acid diet was ingested, mean LDL-cholesterol levels increased by 11.5% over the high saturated fat diet ($P < 0.05$) (Fig. 2). A similar pattern to total cholesterol levels was observed for LDL-cholesterol levels in individual subjects.

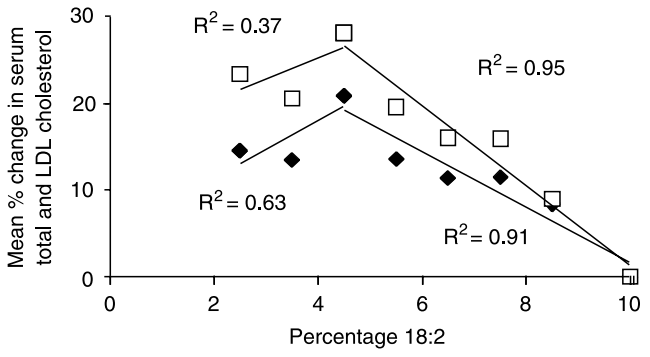

Figure 1. Mean percentage change in total cholesterol (TC) and low-density lipoprotein (LDL)-cholesterol in a diet of 10% 18:2n-6 fatty acids. With decreasing intake of 18:2n-6, total and LDL-cholesterol increase linearly ($R^2 = 0.91$ and 0.95, respectively). A change occurs when approximately 4.5% of energy comes from 18:2n-6 fatty acids. Values represent mean ± SEM for six normolipaemic subjects (three women and three men). □, TC; ◆, LDL.

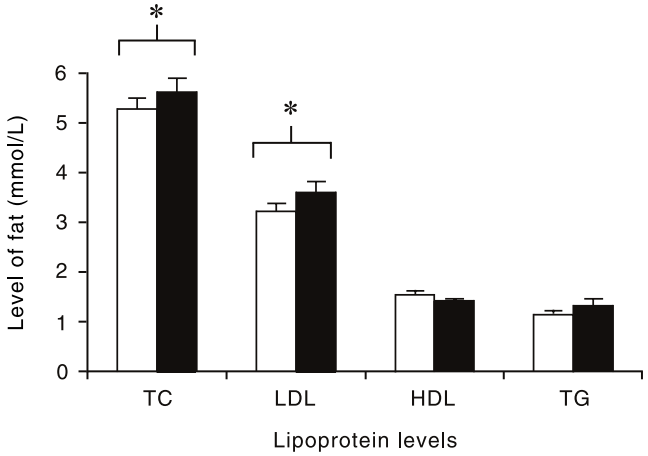

Figure 2. The effect of consuming (□) high saturated fat and (■) high *trans* fat diets on plasma lipoprotein cholesterol levels ($n = 10$; experiment 2). *$P < 0.05$. Values represent mean ± SEM. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

HDL-cholesterol. The HDL-cholesterol levels of the subjects averaged 1.44 ± 0.09 mmol/L after consuming the baseline diet, and increased to 1.55 ± 0.08 mmol/L after the high saturated fat diet was consumed. Mean HDL levels dropped close to those of the baseline diet when the high *trans* fat diet was consumed (Fig. 2). The mean HDL-cholesterol of the subjects decreased by 7.7% when the high *trans* fat diet was consumed compared with the high saturated fat diet. The HDL-cholesterol levels of all subjects decreased when a high *trans* fat diet was consumed. When the high saturated fat diet was consumed, the LDL/HDL ratio averaged 2.12 ± 0.11 but increased to 2.58 ± 0.20 when the fat source was the high *trans* fat diet.

Cholesterol fractional synthetic rate. 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 synthesised cholesterol molecules relative to the initial cholesterol enrichment. In the second experiment, the total cholesterol synthetic rate (FSR_{tot}) was measured to be 0.020 ± 0.005 when the high saturated fat diet was consumed and 0.026 ± 0.004 when the high *trans* fat diet was consumed (Fig. 3). However, when free cholesterol and cholesterol ester fractions were considered separately, the fractional synthetic rate (FSR) of the free cholesterol fraction was significantly greater when the high *trans* fat diet was consumed ($FSR = 0.046 \pm 0.006$) compared to the high saturated fat diet ($FSR = 0.032 \pm 0.006$). Eight out of 10 subjects increased their FSR_{tot} when a high *trans* fat diet was consumed, but this did not attain significance when compared with the high saturated fat diet.

Discussion

In North America, palmitic acid is the predominant saturated fatty acid in the diet, contributing approximately 7–8% of the total energy intake. Previous studies from this laboratory supported the hypothesis that increasing levels of 18:2n-6 fatty acids in the diet result in a decrease in both total and LDL-cholesterol levels.[11] At low levels of dietary 18:2n-6 (3% energy), increasing the content of palmitic acid resulted in significant increases in both total and LDL-cholesterol levels. These data lend support to previous research suggesting that palmitic acid is 'conditionally' hypercholesterolaemic.[19,20]

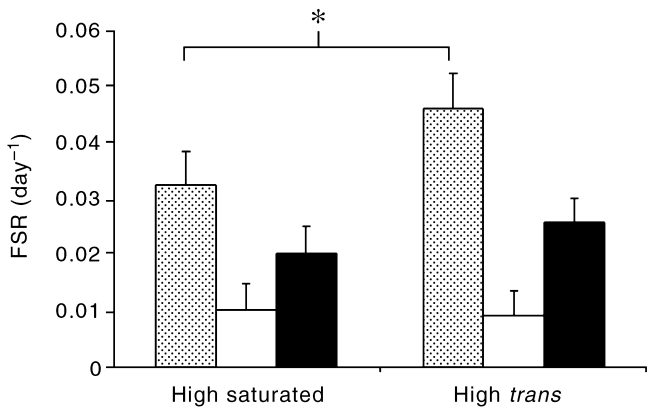

Figure 3. Effect of diet treatment on mean fractional synthetic rate (FSR) for free cholesterol (FSR_C), cholesteryl ester (FSR_{CE}) and total cholesterol (FSR_{tot}). The FSR of free cholesterol increases significantly when a high *trans* fat diet is consumed ($P < 0.05$). (⚃), FSR_C; (□), FSR_{CE}; (■), FSR_{tot}.

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. This notion does not consider recent studies suggesting that saturated fatty acids are not uniformly cholesterolaemic, with stearic acid being described as neutral[20] while myristic acid could be assigned the highest cholesterol-raising potency.[21] During the first experiment total fat and palmitic acid intakes were kept constant and small decrements in the linoleic acid content were made. As a result, small changes in plasma lipoprotein levels could be followed. Lower linoleic acid intakes resulted in increased levels of total cholesterol and LDL-cholesterol when the intake of palmitic acid is maintained at a high level. The serum cholesterol levels observed at the extremes of the linoleic acid intakes support those found in the high 16:0, low 18:2n-6 and high 16:0, high 18:2n-6 diets in a previous experiment.[11]

Hayes and Khosla calculated that the ratio of percentage energy from 18:2n-6/14:0 intake is the best predictor of the cholesterolaemic effect of a diet.[21] As the ratio of 18:2n-6 to 14:0 increases, the observed plasma cholesterol concentration decreases exponentially until a plateau is reached. The resultant curve predicted that for moderate intakes of 14:0 (<2% of energy), consumption of more than 5–6% energy of 18:2 (n-6) would not produce a further decrease in plasma cholesterol. The present study did not show an exponential relationship between 18:2n-6/16:0, but it did show a linear decrease (data not shown). In experiment 1, the total fat level was constant and the dietary palmitic acid level was maintained at 10% of the energy consumed throughout the study, but HDL levels tended to increase as percentage 18:2n-6 levels decreased. This observation may be contrary to current dogma.

Serum values for five subjects indicate a clear continuous reduction in total cholesterol and LDL-cholesterol when the 18:2 intake is 4.5% or greater,which suggests that the minimum level of linoleic acid required in the diet to prevent a significant increase in total and LDL cholesterol at high 16:0 levels is approximately greater than or equal to 4.5% of energy.

In the second experiment, the current results extend previous findings that a moderate intake of *trans* fatty acids increase the LDL/HDL-cholesterol ratio. Subjects consuming high saturated or high *trans* fat diets had similar energy intakes, which consequently cannot explain the changes in LDL-cholesterol or rates of endogenous cholesterol synthesis observed.[22] 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 hypocholesterolaemic effect when consumed in conjunction with *trans* fatty acids as it does with palmitic acid, as was observed in the first study. This observation is also supported by data from Matthan *et al.*[23]

Judd *et al.*[24] reported the relative effects 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 dietary treatments. In the present study, the order of the cholesterolaemic effects of high *trans* compared with 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 mean total cholesterol was 6.6% lower than that observed with a high *trans* diet containing 5.8% of energy from linoleic acid. There are instances where saturated fats resulted in lower cholesterol levels than *trans* fatty acids. Sundram *et al.* compared the effects of exchanging *cis* 18:1, 16:0 or 12:0 + 14:0 for *trans* elaidic acid in humans.[17] The *trans*-enriched diet significantly elevated total and LDL-cholesterol levels relative to the 16:0- and 18:1-enriched 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 lipoprotein at levels relative to all other dietary treatments. These effects were apparent despite the fact that the linoleic acid content of the *trans*-rich diet was significantly higher than *cis* 18:1- and saturated 16:0-rich diets. Therefore, in the present study, the linoleic acid content is not likely to be responsible for the higher cholesterolaemic 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.[25] Saturates, in addition to raising total and LDL-cholesterol, do not appear to affect HDL-cholesterol levels to the same extent,[26] nor are they capable of raising the level of beneficial HDL-cholesterol.[27] 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 the risk of cardiovascular disease. The resulting LDL/HDL-cholesterol ratio is therefore significantly lowered by *trans* fatty acid-enriched diets.[25,26]

Cholesterol ester transfer protein (CETP) activity has been postulated as a possible mechanism to explain the observed shifts in the LDL/HDL ratio (i.e., by enhanced transfer of cholesteryl ester (CE) from HDL to LDL).[28] In individuals whose LDL-receptors are downregulated, 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 were 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.[29]

The effect of the 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.*[23] found that as the degree of *trans* fatty acids in the diet increased, the FSR for free cholesterol (FSR_C) decreased. This is opposite to the effect 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[30] so it is difficult to attribute the increase observed in cholesterol synthesis to either increased linoleic or increased *trans* fatty acids. Diets that contain high levels of linoleic acid and *trans* fatty acids appear not to have the hypocholesterolaemic effect observed when linoleic acid is consumed in conjunction with high palmitic acid levels. Increasing *trans* fatty acids in the diet seems to promote 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 labelling would enable the consumer to make a choice between these two fat sources. This present study has shown that substitution of palmitic acid for hydrogenated fat containing *trans* fatty acids at a usual level of linoleic acid intake (6% of energy) mitigates the hypercholesterolaemic effects of dietary *trans* fatty acids.

It is concluded that for normolipaemic subjects who typically consume a relatively low-fat diet (30% energy from fat) containing the recommended intake of n-6 polyunsaturated fat, it is unlikely that consumption of palmitic acid will have an appreciable effect on lipoprotein profiles. Evidence for the benefits of lowering the level of saturated fat in the diet below 10% of energy is lacking, leading one to question the benefit of reductions beyond this point. This finding may be of particular importance as low and even negligible levels of dietary saturated fat are frequently recommended by health professionals to promote lowered lipid levels. Because palmitic acid is an abundant fatty acid in palm oil, meat and dairy products, and food items that are high in other nutritients, re-evaluation of the recommendations to limit consumption of these food items is warranted.

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