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Cholesterolaemic influence of palmitic acid in the *sn*-1, 3 *v.* the *sn*-2 position with high or low dietary linoleic acid in healthy young men

C. E. Forsythe, M. A. French, Y. K. Goh and M. T. Clandinin*

Alberta Institute for Human Nutrition, Department of Agricultural, Food and Nutritional Science and Department of Medicine, University of Alberta, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Canada T6G 2P5

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Healthy young men were fed four diets for 2 weeks each providing natural fats containing palmitic acid (16 : 0) predominantly in the *sn*-1, 3 position of dietary TAG or containing 16 : 0 predominantly in the *sn*-2 position with low or high levels of linoleic acid (18 : 2*n*-6). Two treatments supplied 16 : 0 in the *sn*-1, 3 positions from palmstearin with low (3 % energy) or high (>7 % energy) 18 : 2*n*-6 and two treatments supplied 16 : 0 in the *sn*-2 position from lard with high or low levels of 18 : 2*n*-6. Diets contained 30–35 % energy as fat, 7–11 % energy as 16 : 0 and moderate levels of cholesterol. Fasting serum cholesterol and lipoprotein concentrations were measured. Cholesterol fractional synthesis rate (FSR) was determined by ^{2}H incorporation. Diets providing 16 : 0 in the *sn*-2 position resulted in lower fasting serum total cholesterol (TC) and a lower TC:HDL ratio than diets providing 16 : 0 in the *sn*-1, 3 positions. Diets with high levels of 18 : 2*n*-6 significantly decreased the TC:HDL ratio, reaffirming the well-known cholesterol-reducing effect of 18 : 2*n*-6. A lower non-esterified cholesterol FSR was observed with low dietary levels of 18 : 2*n*-6. No differences between dietary treatments were found for serum HDL-cholesterol, LDL-cholesterol or TAG. It is concluded that dietary fats containing 16 : 0 in the *sn*-2 position may result in slightly lower fasting TC than diets providing 16 : 0 in the *sn*-1, 3 positions, while the level of *n*-6 polyunsaturated fat influences endogenous cholesterol synthesis.

Cholesterolaemia: Palmitic acid: Lipid metabolism

Limitation of consumption of dietary fats rich in the SFA palmitic acid (16 : 0) to reduce risk factors for CVD has been debated based on investigations suggesting that 16 : 0 is not as hypercholesterolaemic as once implicated. Both animal (Khosla & Hayes, 1993; Idris & Sundram, 2002; Gupta *et al.* 2003) and human (Ng *et al.* 1992; Nestel *et al.* 1998; Clandinin *et al.* 2000; French *et al.* 2002) research has demonstrated that 16 : 0 may be considered neutral or 'conditionally' hypercholesterolaemic. This neutrality is contingent on the dietary content of linoleic acid (18 : 2*n*-6) and cholesterol (Ng *et al.* 1992; Choudhury *et al.* 1997; Nestel *et al.* 1998; Clandinin *et al.* 2000; French *et al.* 2002). When 18 : 2*n*-6 intake is greater than 5 % total energy and cholesterol is less than 400 mg/d, 16 : 0 does not increase serum total or LDL-cholesterol in healthy human subjects (Hayes & Khosla, 1992; Sundram *et al.* 1995; Hayes, 1997; Clandinin *et al.* 2000; French *et al.* 2002).

Positional distribution of 16 : 0 in dietary TAG may also be a factor determining cholesterolaemic properties (Zock *et al.* 1995). During normal intestinal digestion, fatty acids are hydrolysed from the *sn*-1, 3 positions of glycerol resulting in production of NEFA and 2-monoacylglycerols. Non-esterified SFA derived from the *sn*-1, 3 positions may be absorbed less efficiently and have lesser influence on plasma cholesterol concentrations, given that these fatty acids have the potential to form insoluble soaps with Ca and Mg, and be excreted in the faeces (Decker, 1996; Lien *et al.* 1997). In support of this, studies in infants (Filer *et al.* 1969; Innis *et al.* 1994) and adults (Yli-Jokipii *et al.* 2002; Emken *et al.* 2004) indicate that 75–80 % fatty acids in the *sn*-2 position of TAG are retained, and are incorporated into chylomicrons for transport in the circulation. Further, 16 : 0 in the *sn*-2 position of dietary TAG has been shown to be more atherosclerotic than 16 : 0 in the *sn*-1, 3 positions when fed to rabbits (Kritchevsky, 1988; Kritchevsky *et al.* 2002). In human infants (Nelson & Innis, 1999), feeding 16 : 0 in the *sn*-2 position increases fasting serum cholesterol concentrations compared with feeding 16 : 0 predominantly in the *sn*-1, 3 positions. These observations imply that 16 : 0 found in the *sn*-2 position of dietary TAG may impart more hypercholesterolaemia than 16 : 0 in the *sn*-1, 3 positions.

Investigations of normocholesterolaemic and hypercholesterolaemic adult human subjects have failed to show that feeding interesterified oils (those with randomised fatty acids) containing more 16 : 0 in the *sn*-2 position, compared with oils containing less 16 : 0 in the *sn*-2 position, result in a significant increase in serum total or LDL-cholesterol

Abbreviations: FSR, fractional synthesis rate; TC, total cholesterol.

* **Corresponding author:** Dr M. T. Clandinin, fax 1–780–492–8855, email tom.clandinin@ualberta.ca

(Meijer & Westrate, 1997; Nestel *et al.* 1998). Thus, altering the natural TAG structure of dietary fats through interesterification to provide less 16 : 0 in the *sn*-2 position may not affect fasting serum cholesterol concentrations in adults. However, few studies have compared feeding unaltered biological sources of dietary fat to adult human subjects containing fatty acids in different positional distributions on serum cholesterol and lipoprotein cholesterol levels. Palm oil and lard are two natural dietary fats containing high levels of 16 : 0 with differing positional distributions. Palm oil contains 16 : 0 esterified predominantly in the *sn*-1 and *sn*-3 position of glycerol (Kritchevsky *et al.* 2002) while lard contains 16 : 0 primarily in the *sn*-2 position (Myher & Kuksis, 1979). Research has investigated the dietary influences of palm oil in human subjects and shown several conditions under which it is not hypercholesterolaemic (Ghafoorunissa, 1995; Khosla & Sundram, 1997; Truswell, 2000); these results may be due to predominant esterification of 16 : 0 in the *sn*-1, 3 position.

Based on these observations, the purpose of the present study is to compare the cholesterolaemic response of feeding 16 : 0 in the *sn*-1, 3 positions from palmstearin with feeding 16 : 0 in the *sn*-2 position from lard in young normocholesterolaemic male subjects. The study also investigates the influence of dietary 18 : 2*n*-6 content on source of 16 : 0, given the known cholesterol-reducing effects of 18 : 2*n*-6 (Hayes & Khosla, 1992; Hayes, 1997). The hypothesis is that feeding 16 : 0 in the *sn*-1, 3 positions from palmstearin will be less cholesterolaemic than feeding 16 : 0 in the *sn*-2 position from lard, and 18 : 2*n*-6 content will modulate the cholesterolaemic response to both sources of 16 : 0. To determine cholesterolaemic effects, fasting serum cholesterol levels and 24 h cholesterol fractional synthesis rate (FSR), assessed by the ^{2}H incorporation method, are measured.

Experimental methods

Subjects

Procedures were approved by the Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee (University of Alberta, Edmonton, Canada). All subjects gave written consent before the investigation. Ten male subjects with no history of medical problems and no family history of heart disease or hyperlipidaemia were recruited through advertisements posted at the University of Alberta. Eight subjects completed all aspects of the study. Each subject was interviewed to inform them of the study purpose, to explain expected responsibility and time commitment, and to outline dietary treatments. A questionnaire was completed by each participant to characterise activity level, food intake and sleeping patterns, and to provide information about food allergies and food dislikes. Subjects were willing to consume lard and pork products for a proportion of the study, and they agreed to only consume food given to them during the study while also refraining from alcohol and caffeine. Subjects were not taking any medications or supplements that would influence blood lipids or cardiovascular function. Fasting blood samples were obtained from each subject upon entry into the study to establish baseline values of serum cholesterol and TAG concentrations and confirm that they were all normocholesterolaemic.

Subject energy requirements

Energy requirements for each subject were determined using habitual 3 d food records and the Mayo Clinic Nomogram, incorporating the subjects' height, weight, age, sex and activity factor (Bell *et al.* 1985). Subjects were weighed daily before breakfast during the dietary feeding treatments to ensure body weight remained stable. Adjustments were made to energy intake if sustained weight changes were observed or if daily energy expenditure was altered.

Dietary design

The study design provided four dietary treatments of 14 d each with a 2-week washout period between each treatment. Treatments over 2 weeks were selected based on previous investigations showing that steady-state plasma lipoproteins were achieved after 2-week diet treatments (Mattson & Grundy, 1985). Each treatment consisted of a 3 d rotational menu portioned into three isoenergetic meals with a total of approximately 30 % energy from fat, 15 % energy from protein and 55 % energy from carbohydrate. Diets were based on normal foods and designed using the Food Processor II nutrient analysis computer software program (ESHA Research, Salem, OR, USA). Each dietary treatment was balanced for *n*-3 fatty acids (0·25 ± 0·1 % total energy), cholesterol (range 250–360 mg/d) and fibre content (19–26 g/d). Fatty acids were calculated from nutrient composition tables and laboratory analysis to provide high levels of 16 : 0 (about 8 % total energy) with low and high levels of 18 : 2*n*-6 (3 and 7–9 % total energy respectively). Lard and pork products such as ham, bacon, sausage and pork chops were fed to provide a dietary source of 16 : 0 in the *sn*-2 position of TAG. Positional distribution of 16 : 0 in lard was determined from prior investigations (Renaud *et al.* 1995; Hunter, 2001) and contained approximately 65 % 16 : 0 in the *sn*-2 position and 8 % in the *sn*-1, 3 positions. Palmstearin and a small amount of low-fat dairy products were fed to provide 16 : 0 in the *sn*-1, 3 positions. Palmstearin contained approximately 23 % 16 : 0 in the *sn*-2 position and 58 % in the *sn*-1, 3 positions. A high-linoleate safflower-seed oil was fed to provide a dietary source of 18 : 2*n*-6. Depending on the dietary treatment, lard, palmstearin and safflower-seed oil were incorporated into common food items such as hash browns at breakfast, pitas and sandwiches at lunch, and pre-prepared entrées at dinner. Pre-prepared entrées obtained from Bassilis Best (Fabko Foods, Ltd, Edmonton, AB, Canada) were used for most lunch and dinner meals. These entrées contained low amounts of total fat (4·7–9·1 g) and cholesterol (31–35 mg) and were analysed before use to confirm fat content and fatty acid composition.

The four dietary treatments were fed in the following order: *sn*-2 16 : 0 low-18 : 2*n*-6; *sn*-2 16 : 0 high-18 : 2*n*-6; *sn*-1, 3 16 : 0 low-18 : 2*n*-6; *sn*-1, 3 16 : 0 high-18 : 2*n*-6. Each subject consumed the same dietary treatment during the same period. Most dietary fat in the *sn*-2 16 : 0 low-18 : 2*n*-6 diet was provided by lard and pork products.

In the *sn*-1, 3 16 : 0 low-18 : 2*n*-6 diet, the majority of fat was provided by palmstearin and monounsaturated fat from olive oil, mixed nuts and non-hydrogenated peanut butter. In all diets, the remaining fat sources were from egg yolks, beef, chicken, bagels, low-fat dinner buns, English muffins, waffles and pre-prepared entrées. Additional fruits and/or vegetables devoid of fat were permitted if needed. Clear herbal tea, decaffeinated coffee and sugar-free fruit juices were allowed.

Meals for each dietary treatment were prepared daily in the Human Nutrition Research Unit kitchen for consumption in the dining room (breakfast and lunch) or packaged for take-out (supper). Meals were consumed at regular intervals: 07.30–09.00 hours for breakfast, 11.30–13.00 hours for lunch and 17.30–19.00 hours for supper, depending on the individual participants' schedule. To assess compliance, dishware and containers were inspected upon return to ensure that all visible fat was eaten.

Fat analysis of diets

Diets were analysed for total fat and fatty acid composition. Duplicate samples for each meal in the 3 d menu of each dietary treatment were prepared. Meals were homogenised in a Sybron/Brinkman polytron (model PT 10/35; Sybron/Brinkman, Rexdale, ON, Canada) with the addition of water to a smooth paste. A sample of the blended food paste containing approximately 0·3–0·5 g fat was weighed and extracted with chloroform–methanol (2 : 1, v/v) (Folch *et al.* 1957). A known amount of tripentadecanoate (Sigma Chemical T4257; Sigma Chemical, St Louis, MO, USA) was added.

Fatty acid methyl esters were prepared with BF_3 in methanol and quantified by automated GLC (Vista 3400 CX GLC and Vista 8200 data system; Varian Instruments, Georgetown, ON, Canada). Fatty acid methyl ester separation and identification was performed on a capillary column (DB20 25 m × 0·22 mm internal diameter; SGE Inc., Austin, TX, USA). Retention times of each of the fatty acid methyl esters were compared with those of a standard fatty acid methyl ester (Nu-Check Prep, Inc., Elysian, MN, USA) containing thirty-two known fatty acid methyl esters.

Blood analysis

On day 13 of each dietary treatment, a 30 ml fasting blood sample was obtained by venepuncture between 07.30 and 09.00 hours. On day 12, subjects were instructed to perform comparable amounts of physical activity and refrain from eating after 20.00 hours. Plasma was obtained by centrifugation (3000 rpm for 10 min) and frozen at −20°C. Day 13 plasma samples were outsourced for same-day analysis of total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, TAG and C-reactive protein (Quest Diagnostics, Edmonton, AB, Canada). The plasma obtained on day 13 was used to determine baseline ^{2}H concentration in plasma water and plasma cholesterol, and the enriched plasma sample obtained on day 14 was used to determine ^{2}H enrichment over the 24 h period.

^{2}H incorporation for measurement of endogenous cholesterol biosynthesis

^{2}H incorporation is a stable isotope tracer methodology that measures cholesterolgenesis based on the rate of incorporation of ^{2}H-labelled water into *de novo* synthesised non-esterified cholesterol. ^{2}H-labelled water is a non-radioactive tracer that can be safely ingested and the enrichment of the precursor pool of cholesterol easily measured (Jones, 1990; Jones *et al.* 1993). The fundamental outcome measurement is the FSR of cholesterol, which is defined as the fraction of the rapidly turning over non-esterified cholesterol pool that is newly synthesised from a precursor pool (i.e. plasma water) over a 24 h period (Jones, 1990; Jones *et al.* 1993).

After the blood draw on day 13, subjects consumed a priming dose of ^{2}H-labelled water at 0·5 g ^{2}H-labelled water/kg estimated body water before breakfast was eaten. A maintenance dose of 1·0 g ^{2}H-labelled water/kg estimated body water was then provided in a 2 litre bottle of water for consumption over the next 24 h to maintain plasma ^{2}H enrichment at plateau and to compensate for unlabelled water obtained in the diet. Body water was estimated as 60 % total body mass (Altman, 1961; Jones *et al.* 1994*a*). On day 14, 24 h after the priming ^{2}H-labelled water dose, a second fasting blood sample was collected.

Determination of ^{2}H enrichment

^{2}H enrichment was measured in plasma non-esterified cholesterol and plasma water as described previously (Kuksis & Myher, 1989, Kuksis *et al.* 1990). To obtain non-esterified cholesterol, a 0·2 ml sample of plasma and 1 ml diethyl ether were added to a solution of 2 units phospholipase C in 2 ml 17·5 mM-tri(hydroxymethyl)-aminomethane buffer (pH 7·3) and 8 mg 1 % $CaCl_2$ (Kuksis *et al.* 1993). Samples were analysed in triplicate. The analyses were performed using an Agilent Model 6890 Thermo Finnigan Delta*Plus* XL GC-pyrolysis-isotope ratio mass spectrometer (Thermo Finnigan MAT GmbH, Bremen, Germany) with an HP-5 capillary column (30 mm × 0·32 mm internal diameter and 0·25 μm film thickness). The injector temperature was 325°C to ensure that the entire sample was volatilised. The split ratio was 10 : 1. The sample was injected on-column at 150°C, held for 1 min and then the oven temperature was increased by 30°C/min to 200°C, then 20°C/min to 325°C and held for 5 min. The analytical precision of the instrument was calculated from multiple analyses (*n* 96) of the hydrogen produced from the pyrolysis of the internal standard, tridecanoylglycerol. The coefficient of variability of the instrument was 4 %.

The ^{2}H enrichment in plasma body water was measured by the use of a Finnigan MAT 251 isotope ratio mass spectrometer (Thermo Finnigan MAT GmbH) against hydrogen prepared from a water standard as described previously (French *et al.* 2002). Samples of day 14 plasma (enriched plasma), intended for measurement of plasma water enrichment, were diluted 20-fold with 5 % bovine serum albumin solution to lower the ^{2}H enrichment to within the analytical range of the isotope ratio mass spectrometer. Baseline day 13 samples were not diluted. The mass three abundance was corrected for H_3^+ contribution and determined daily. Multiple analyses of hydrogen produced from the reduction

of laboratory water standard demonstrated the analytical precision (CV) of this instrument at $<1\,\%$. All samples were analysed in duplicate.

Determination of cholesterol fractional synthesis rate

Cholesterol FSR was determined from the initial incorporation rate of ^{2}H-labelled cholesterol into the rapid exchangeable cholesterol pool relative to the initial precursor enrichment determined using body water ^{2}H level (Jones, 1990). Maximum attainable enrichment was calculated as body water pool enrichment corrected for the fraction of protons in *de novo* synthesised cholesterol that derive from water, relative to non-water sources (Jones, 1990; Jones *et al.* 1993) using the equation:

$$FSR_{NEC} = \frac{\delta_{NEC}}{(\delta_{PW} \times 0,478)}$$

where δ_{NEC} is the change in enrichment in the total non-esterified cholesterol fraction and δ_{PW} is the change in enrichment in the plasma water over 24 h.

Statistical methods

A two-way ANOVA with repeated measures was used to determine the significance of dietary treatments on plasma lipids, C-reactive protein and cholesterol FSR (SAS version 8.2; SAS Institute Inc., Cary, NC, USA). Significant differences were determined among dietary treatments by a Duncan's multiple-range test. Statistical significance was set at $P<0{\cdot}05$.

Results

Subjects

Ten subjects were enrolled in the study. One subject withdrew from the study because he developed digestive problems from eating more fat than he was accustomed. Another subject discontinued participation due to other time commitments that restricted him from consuming meals at the Human Nutrition Research Center each day. Eight subjects successfully completed each dietary treatment and compliance to the protocol was high (Table 1). All subjects maintained their baseline weight on the energy level assigned to them at the beginning of the study. Thus, body-weight fluctuations over the individual 14 d feeding treatments were negligible.

Table 1. Descriptive data of subjects (*n* 8) completing the protocol

	Age (years)	Mean weight (kg)	Height (cm)	BMI (kg/m^2)
	28	89·3	170	28·0
	26	75·3	175	24·5
	27	64·1	173	21·7
	28	86·3	180	26·5
	26	108	187	28·3
	24	88·5	195	24·5
	21	73·8	190	20·8
	27	69·3	182	20·8
Mean	25·9	81·8	182	24·4
SD	2·4	14·1	8·7	3·1

Habitual energy intakes of subjects

Habitual dietary intake was 12 639 (SD 2570) kJ. Mean percentage energy derived from saturated fat, protein and carbohydrate was 9 (SD 2), 17 (SD 4) and 49 (SD 8) %, respectively. Mean habitual daily intake of cholesterol was 338 (SD 169) mg.

Fatty acid analysis of lard and palmstearin

Laboratory analysis of the fatty acid compositions of lard and palmstearin indicated that lard contained 40 % total SFA, 27 % 16 : 0, 15 % stearic acid (18 : 0), 43 % MUFA, 8·4 % 18 : 2*n*-6 and 95 mg cholesterol/100 g. Palmstearin contained 70 % total SFA, 63 % 16 : 0, 4·9 % 18 : 0, 24 % MUFA, 5·3 % 18 : 2*n*-6 and no cholesterol/100 g.

Dietary treatments and nutrient compositions

Formulated nutrient content of diets and laboratory analysis of diets are shown (Table 2). The contribution of total energy from fat in each diet was 30–36 %. The difference in total fat and fatty acids between the laboratory analysis of diets and diets formulated by Food Processor was negligible except for the *sn*-2 16 : 0 high-18 : 2*n*-6 diet. The *sn*-2 16 : 0 high-18 : 2*n*-6 diet provided 5 % more energy from monounsaturated fat than formulated. Cholesterol intake was maintained within the range formulated. Diets that provided *sn*-1, 3 16 : 0 from palmstearin contained moderately less cholesterol than diets that provided *sn*-2 16 : 0 from pork fat because palmstearin did not contain any cholesterol. There were no differences between dietary treatment with regards to intake of *n*-3 fatty acids (0·24 (SD 0·11) % total energy), Ca (1052 (SD 62) mg) or type and amount of dietary fibre (23·2 (SD 8·3) g). The polyunsaturated:saturated fat ratio was approximately 0·32 in the low-18 : 2*n*-6 diets and 0·82 in the high-18 : 2*n*-6 diets. Energy contribution of total 16 : 0 in the *sn*-2 position was based on data from Sheppard *et al.* (1978), with dairy lipid containing 43 % 16 : 0 in the *sn*-2 position, lard containing 65 % 16 : 0 in the *sn*-2 position, and palm oil containing 23 % 16 : 0 in the *sn*-2 position. Table 2 shows the total contribution of energy as 16 : 0 from dairy lipid, lard and palmstearin.

Plasma lipid and lipoprotein cholesterol levels

Mean plasma lipid and lipoprotein cholesterol in subjects fed each dietary treatment are shown in Table 3. Diets providing 16 : 0 in the *sn*-2 position resulted in significantly lower TC concentrations that diets providing 16 : 0 in the *sn*-1, 3 position ($P=0{\cdot}02$). Level of dietary 18 : 2*n*-6 did not significantly influence fasting TC concentrations. No significant effect of diet was observed on HDL- or LDL-cholesterol or fasting TAG concentrations. C-reactive protein levels observed in subjects during all test diets were less than 1·0 mg/l (data not shown).

Total cholesterol:high-density lipoprotein-cholesterol ratio

Consuming diets that contained 16 : 0 in the *sn*-2 position with low- or high-18 : 2*n*-6 resulted in a modest, but statistically significant ($P=0{\cdot}0001$), reduction in TC:HDL ratio when

Table 2. Nutrient content of *sn*-1, 3 16 : 0 and *sn*-2 16 : 0 dietary treatments* (Percentages of total energy)

	sn-2 16 : 0 low-18 : 2*n*-6		*sn*-1, 3 16 : 0 low-18 : 2*n*-6		*sn*-2 16 : 0 high-18 : 2*n*-6		*sn*-1, 3 16 : 0 high-18 : 2*n*-6	
Energy source	Nutrient analysis software	Laboratory analysis	Nutrient analysis software	Laboratory analysis	Nutrient analysis software	Laboratory analysis	Nutrient analysis software	Laboratory analysis
Energy (kJ/d)	12 560		12 560		12 560		12 560	
Total fat	33·1	31·5	29·6	30·2	35·7	36·8	31·0	30·1
16 : 0	8·33	7·91	9·94	11·0	7·49	6·69	9·06	9·58
18 : 0	4·05	4·00	1·42	1·66	3·81	4·47	1·44	1·47
MUFA	12·8	12·1	11·3	12·0	11·5	16·4	7·73	10·1
18 : 2*n*-6	3·06	3·11	3·02	2·69	8·21	6·77	8·96	8·68
18 : 3*n*-3	0·31	0·26	0·21	0·14	0·25	0·39	0·15	0·17
Cholesterol (mg/d)	347		259		332		296	
Dietary fibre (g/d)	19·2		25·9		23·7		24·1	
Ca (mg/d)	1000		1140		1050		1020	
Dairy 16 : 0†		0·35		1·80		0·00		1·67
Lard or palmstearin 16 : 0†		7·23		7·62		6·55		6·84
Total *sn*-2 16 : 0†		5·74		1·98		6·39		1·80
P:S ratio	0·35		0·30		0·84		0·81	

18 : 0, Stearic acid; 18 : 3*n*-3, α-linoleic acid; P:S ratio, polyunsaturated:saturated fat ratio.
* All values were derived using Food Processor II nutrient analysis software version 7.71 (ESHA Research, Salem, OR, USA). Some values were obtained from laboratory analysis. Meals were analysed in duplicate.
† 16 : 0 Values were acquired from direct laboratory analysis (Sheppard *et al.* 1978).

compared with consuming diets containing 16 : 0 in the *sn*-1, 3 position with low- or high-18 : 2*n*-6 (Table 3). Consuming diets with high-18 : 2*n*-6 resulted in a statistically lower TC:HDL ratio than consuming low-18 : 2*n*-6 diets ($P=0{\cdot}003$).

Cholesterol fractional synthesis rate

The FSR for total non-esterified cholesterol was significantly lower in subjects consuming the *sn*-1, 3 16 : 0 and low-18 : 2*n*-6 diet compared with consuming all other diets ($P=0{\cdot}01$) (Table 4). The effect of consuming *sn*-1, 3 16 : 0 compared with *sn*-2 16 : 0 on non-esterified cholesterol FSR was not significant.

Discussion

The findings of the present study indicate that feeding diets with 16 : 0 in the TAG *sn*-2 position to young, normocholesterolaemic men results in modestly lower fasting serum TC concentrations and a lower fasting TC:HDL ratio than feeding 16 : 0 in the *sn*-1, 3 position (Table 3). Further, the present study supports previous observations indicating that the provision of dietary 18 : 2*n*-6 at levels of 5 % total energy or greater has a significant cholesterol-lowering effect, especially in the presence of high dietary 16 : 0 (Hayes & Khosla, 1992; Sundram *et al.* 1995; Hayes, 1997; Clandinin *et al.* 2000; French *et al.* 2002).

The finding of lower fasting TC concentrations in response to feeding 16 : 0 in the *sn*-2 position from lard compared with the *sn*-1, 3 positions from palmstearin to normocholesterolaemic males is not similar to a previous research study investigating influence of positional distribution of 16 : 0 from palm oil or lard on blood lipids in human subjects. Zhang *et al.* (1997) fed Chinese men and women diets high in refined, bleached palm oil, or diets high in lard. The diets were administered for 6 weeks using normal Chinese foods and provided 30 % energy as total fat (Zhang *et al.* 1997). The diet fatty acid composition was similar to the present study in that 16 : 0 level

Table 3. Plasma lipid and lipoprotein cholesterol concentrations (mmol/l) in subjects consuming each diet treatment*
(Mean values with their standard errors)

	Diet treatment									
	sn-2 16 : 0 low-18 : 2*n*-6		*sn*-1, 3 16 : 0 low-18 : 2*n*-6		*sn*-2 16 : 0 high-18 : 2*n*-6		*sn*-1, 3 16 : 0 high-18 : 2*n*-6		Main effects*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*sn*-2 *v.* *sn*-1, 3 16 : 0	Low- *v.* high-18 : 2*n*-6
TC	4·4	0·2	4·6	0·2	4·2	0·2	4·4	0·2	$P=0{\cdot}02$	NS
HDL-cholesterol	1·0	0·04	1·0	0·05	1·0	0·04	1·0	0·04	NS	NS
LDL-cholesterol	2·8	0·1	2·9	0·2	2·7	0·2	2·7	0·2	NS	NS
TAG	1·2	0·2	1·6	0·3	1·2	0·2	1·5	0·5	NS	NS
TC:HDL ratio	4·3	0·3	4·8	0·3	4·2	0·3	4·4	0·3	$P=0{\cdot}0001$	$P=0{\cdot}003$

TC, total cholesterol.
* No significant interactions were found.

Table 4. Total non-esterified cholesterol fractional synthesis rates in subjects consuming each diet treatment

(Mean values with their standard errors)

	Fractional synthesis rate (pools/d)	
Diet treatment	Mean	SEM
sn-2 16 : 0 low-18 : 2*n*-6	0·054[a]	0·005
sn-1, 3 16 : 0 low-18 : 2*n*-6	0·040[b]	0·004
sn-2 16 : 0 high 18 : 2*n*-6	0·053[a]	0·004
sn-1, 3 16 : 0 high 18 : 2*n*-6	0·062[a]	0·003

[a,b] Mean values with unlike superscript letters are significantly different ($P<0·05$).

was higher while 18 : 0 and dietary cholesterol were lower in the diets providing palm oil compared with those providing lard. Lower serum TC and LDL-cholesterol levels, as well as a lower TC:HDL ratio, were found after feeding the diet containing 16 : 0 in the *sn*-1, 3 positions from palm oil. Differences in fasting serum cholesterol levels compared with the present study could be the attributed ethnicity of the subjects, and/or the type of palm oil administered (refined, bleached palm oil *v.* palmstearin).

In the present study, an explanation for higher fasting TC and TC:HDL ratio after the feeding the diets rich in palmstearin could be due to the higher and lower content of 16 : 0 and 18 : 0 respectively, compared with the diets rich in lard. Despite all attempts to maintain similar dietary fatty acid composition among diets, the diets providing 16 : 0 in the *sn*-1, 3 from palmstearin had on average 3 % more 16 : 0 and 2 % less 18 : 0 than the diets providing *sn*-2 16 : 0 from lard (Table 2). Palmitic acid has been shown to be more cholesterolaemic than 18 : 0 in human dietary interventions (Bonanome & Grundy, 1988). The highest TC concentration was observed in the *sn*-1, 3 16 : 0 low-18 : 2*n*-6 dietary treatment, which contained the highest level of 16 : 0, at 11 % (Table 2). This observation confirms the plasma cholesterol-raising potential of dietary 16 : 0.

The present study presented a novel measure of cholesterolaemia through the assessment of 24 h cholesterol FSR using the ^{2}H incorporation method. Diets providing 16 : 0 in the *sn*-1, 3 positions with a low level of 18 : 2*n*-6 resulted in the lowest non-esterified cholesterol FSR compared with all other diets (Table 4). However, despite lower cholesterol FSR, the *sn*-1, 3 16 : 0 low-18 : 2*n*-6 diet did not have a lower TC or TC:HDL ratio (Table 3). Differences in cholesterol FSR and fasting lipoprotein concentrations suggest that measurement of 24 h cholesterol FSR may not fully explain changes observed in fasting serum TC concentrations. Further, feeding 16 : 0 in the *sn*-2 position at a low intake of 18 : 2*n*-6 may not be metabolically equivalent in terms of the effect on cholesterol synthesis compared with feeding 16 : 0 in the *sn*-1, 3 positions.

Using ^{2}H, it has been shown that the rate of cholesterol synthesis is greater in normal and hyperlipidaemic human subjects fed diets high in 18 : 2*n*-6 *v.* those high in saturated fats (Mazier & Jones, 1991, 1999; Jones *et al.* 1994*a*,*b*, 1998). Plasma TC and LDL-cholesterol concentrations also display an inverse relationship with cholesterol synthesis in response to 18 : 2*n*-6 intake. Jones *et al.* (1994*a*,*b*) studied cholesterol synthesis using ^{2}H incorporation in mildly hypercholesterolaemic patients consuming diets containing 30 % total energy as fat from maize, olive, rapeseed, or rice bran oils, or beef tallow. Higher cholesterol FSR was observed for the diet rich in maize oil, which is naturally abundant in 18 : 2*n*-6, compared with all other diets. The observations of higher cholesterol FSR with 18 : 2*n*-6 consumption have also been seen by Mazier & Jones (1997). In this study, nine young, normocholesterolaemic men were fed diets containing 40 % total energy from fat rich in either safflower-seed oil (rich in 18 : 2*n*-6), olive oil or butter for 21 d. Serum TC concentrations were lowest ($P<0·001$) after consumption of the diet rich in safflower-seed oil, yet cholesterol FSR was highest (Mazier & Jones, 1997). Clandinin *et al.* (1999, 2000) demonstrated that diets fed to normocholesterolaemic subjects containing high and low levels of 16 : 0 from palmolein with high and low levels of 18 : 2*n*-6 had no significant effect of endogenous cholesterol synthesis, suggesting no relationship between 16 : 0 and cholesterolgenesis. However, in these studies, diets that were low in 18 : 2*n*-6 had lower FSR for non-esterified cholesterol than those high in 18 : 2*n*-6. Further, serum TC concentrations were slightly lower with high 18 : 2*n*-6 intake, but these results were not statistically significant. In summary, investigations using ^{2}H incorporation to measure cholesterol FSR suggest that dietary 18 : 2*n*-6 increases cholesterol synthesis while decreasing fasting cholesterol concentrations (Mazier & Jones, 1991). This inverse relationship between circulating cholesterol and cholesterol biosynthesis may be explained by alterations in the hepatic non-esterified cholesterol pool, which is shown to decrease during high 18 : 2*n*-6 feeding (Mazier & Jones, 1999). In response to a smaller hepatic pool of non-esterified cholesterol, LDL-receptors are up regulated and cholesterol synthesis is increased to compensate for the decreased plasma cholesterol concentrations due to increased fractional catabolic rate of LDL (Dietschy *et al.* 1993).

In summary, in the present study, diets providing 16 : 0 in the *sn*-2 position from lard may result in slightly lower fasting cholesterol levels than diets providing 16 : 0 in the *sn*-1, 3 positions from palmstearin, which is probably a result of a lower total dietary intake of 16 : 0. Further, the endogenous synthesis of cholesterol is significantly affected by the dietary level of *n*-6 polyunsaturated fat.

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