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Dietary saturated fatty acids increase cholesterol synthesis and fecal steroid excretion in healthy men and women

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Abstract. In a strictly controlled 6-week trial with 47 healthy volunteers we have determined the effect of replacement of polyunsaturated by saturated fatty acids on the fecal steroid excretion and on the rate of whole body cholesterol synthesis, as measured both by the sterol balance method and by the concentration of the cholesterol precursor lathosterol in serum. Subjects were fed mixed natural diets, of which the total fat content was kept constant at 45% energy. Consumption of polyunsaturated fatty acids, mainly linoleic acid, was 21% energy for the first 3-week period (P:S ratio 1·9), and 5% of energy (P:S ratio 0·2) for the next 3-week period, or vice versa. Cholesterol intake as determined by analysis of duplicate diets was 41 mg MJ^{-1} (about 500 mg day^{-1}) during both periods. Feces were collected for 5 days at the end of both periods. The steroid composition of the feces was not affected by the change of diets. The fecal excretion of neutral steroids was significantly higher on the low P:S high-saturated-fat ($2{\cdot}25 \pm 0{\cdot}68$ mmol day^{-1}) than on the high P:S high-linoleic-acid diet ($2{\cdot}00 \pm 0{\cdot}69$ mmol day^{-1}; $P < 0{\cdot}01$). The excretion of bile acids was similar ($0{\cdot}77 \pm 0{\cdot}40$ and $0{\cdot}79 \pm 0{\cdot}41$ mmol day^{-1}, respectively). The cholesterol balance and the rate of cholesterol synthesis were higher during the low P:S ($1{\cdot}86 \pm 0{\cdot}83$ mmol day^{-1}) than during the high P:S period ($1{\cdot}55 \pm 0{\cdot}85$ mmol day^{-1}; $P < 0{\cdot}01$). The ratio of lathosterol to cholesterol in serum was $0{\cdot}86 \pm 0{\cdot}33$ μmol $mmol^{-1}$ on the high- and $1{\cdot}07 \pm 0{\cdot}39$ μmol $mmol^{-1}$ on the low P:S diet ($P < 0{\cdot}01$). Thus, both the balance and the cholesterol precursor method suggested that saturated fatty acids stimulate whole-body cholesterol synthesis.

Keywords. Cholesterol, cholesterol synthesis, dietary fatty acids, fecal steroids, unsaturated fatty acids.

Abbreviation

P:S ratio, ratio of polyunsaturated to saturated fatty acids.

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Introduction

Replacement of saturated by polyunsaturated fatty acids is the single most powerful dietary intervention to lower plasma total cholesterol concentrations [1]. Several mechanisms have been proposed to explain the hypocholesterolaemic effect of polyunsaturated relative to saturated fatty acids. These include a decreased production of lipoproteins due to a less efficient incorporation into VLDL-triacylglycerols of polyunsaturated than of saturated fatty acids [2,3], possibly coupled with a preferential conversion by the liver of polyunsaturated fatty acids into ketone bodies instead of VLDL-triacylglycerols [4], and an increased LDL catabolism, mediated by either an increased receptor activity or an altered LDL structure [5,6]. An increase in receptor activity might be triggered by a shift of cholesterol in liver cells from a metabolically active pool into storage as cholesteryl esters, as proposed by Dietschy and colleagues [7]. A higher rate of catabolism of LDL might also lead to an increased fecal excretion of cholesterol and its acidic and neutral metabolics. An enhanced fecal excretion of neutral steroids and bile acids in subjects changing to a diet enriched in polyunsaturated fatty acids has indeed been reported [8–11], but could not be confirmed in other experiments [12,13,14] including our own [15]. In the present study we determined the effect of dietary fatty acid composition on the rate of production of exchangeable cholesterol by the whole body at constant cholesterol intake in healthy humans, during a trial that involved drastic changes in dietary fatty acid composition at a constant total fat and cholesterol intake. Both the classical balance technique [12] and the cholesterol precursor method [16,17] were used to estimate synthesis rates.

Subjects and methods

Subjects

Fifty-four healthy normolipemic volunteers, all from the general population living in or near Wageningen, The Netherlands, entered this study. They consisted of two groups of 27 subjects each. Group Norm-egg

Table 1. Baseline characteristics (mean ± SD) of subjects. Group Norm-egg consisted largely of university students and staff, with a normal level of egg consumption. Group Hab-egg consisted of middle-aged citizens from the Wageningen region with a high habitual egg intake

	Group Norm-egg	Group Hab-egg
Number of subjects (men/women)	23 (14/9)	24 (10/14)
Age at entry (years)	34 ± 13	54 ± 13
Height (cm)*		
men	183 ± 5	177 ± 5
women	169 ± 4	168 ± 6
all	178 ± 9	172 ± 7
Weight (kg)†		
men	76·4 ± 6·7	83·8 ± 12·4
women	63·6 ± 6·9	66·6 ± 12·1
all	71·4 ± 9·5	73·7 ± 15·2
Body mass index (kg m^{-2})		
men	22·7 ± 1·8	25·9 ± 2·8
women	22·3 ± 2·6	23·4 ± 3·9
all	22·5 ± 2·2	24·4 ± 3·8
Serum total cholesterol (mmol l^{-1})	5·01 ± 0·75	6·29 ± 1·24
Serum HDL cholesterol (mmol l^{-1})	1·36 ± 0·25	1·48 ± 0·37
Serum triacylglycerols (mmol l^{-1})	1·12 ± 0·55	1·51 ± 0·86

* Standing height was measured without shoes; † weights were measured to the nearest 0·1 kg, after breakfast, and without shoes, sweaters, jackets, etc.

consisted of 11 women and 16 men who had participated in three earlier well-controlled studies on the responsiveness of serum cholesterol to dietary cholesterol [18]. Group Hab-egg consisted of 16 women and 11 men, who had originally been selected for their habitual consumption of at least one egg per day. They had previously participated in two or three trials on the effect of dietary cholesterol on serum lipids [19,20,21]. The congruence between subjects' serum cholesterol responses in the present trial and in previous trials has been reported elsewhere [22]. Data of four subjects from group Norm-egg and three from group Hab-egg were rejected prior to data analysis because of drop-out, illness, weight loss, poor dietary adherence, or combinations of these factors. Baseline characteristics for the remaining subjects in group Norm-egg (nine women and 14 men) and group Hab-egg (14 women and 10 men) are given in Table 1. The aim and the design of the experiment were extensively explained to the participants and informed consent was obtained. The study was approved by the Medical-Ethical Committee of the Department of Human Nutrition.

Study design and diets

Groups Norm-egg and Hab-egg were studied simultaneously, in a cross-over design. Subjects in group Norm-egg first received a mixed natural high-fat diet high in polyunsaturated and low in saturated fatty acids (high P:S diet) for 3 weeks, and then changed to a diet with the same high fat content, but now low in polyunsaturated and high in saturated fatty acids (low P:S diet), for another 3 weeks. For the subjects in group Hab-egg the order of these diets was reversed

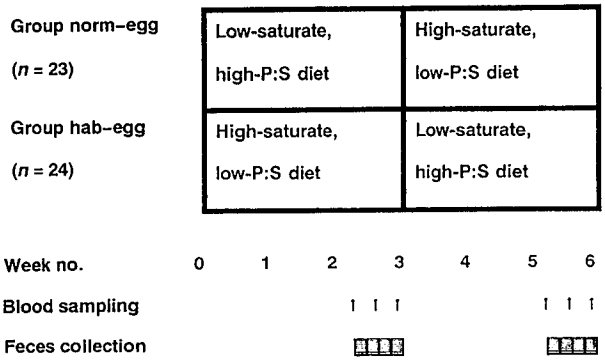

Figure 1. Design of the experiment. Arrows indicate the days on which fasting blood samples were taken. Feces were collected during the last 5 days of each experimental period. P:S, polyunsaturated/saturated fatty acids ratio.

(Fig. 1). The diets were composed of natural foodstuffs and formulated so that the composition of dietary fatty acids was the only variable. Actual nutrient intake was assessed by analysis of duplicate portions for one imaginary person of average energy intake on each diet as previously described [22] (Table 2). The high fibre intake was due largely to wholewheat bread, which was partly used as a vehicle for the dietary fats [22,23]. Dependent on energy needs, subjects received between four and 10 slices per day, which provided 10 to 26 g of dietary fibre.

The diets were formulated at 15 levels of energy intake, ranging from 7·7 to 16·1 MJ day^{-1}, and provided daily at the Department (group Norm-egg) or delivered at the subject's home three times a week (group Hab-egg) similar to previous studies [18,21].

Table 2. Composition of the high and low P:S diets*

	Group Norm-egg ($n=23$)		Group Hab-egg ($n=24$)	
Dietary component	High P:S	Low P:S	High P:S	Low P:S
Energy (MJ day^{-1})	12·5±3·0	12·4±2·7	10·8±2·3	10·6±2·1
(kcal day^{-1})	2980±717	2961±646	2577±550	2532±502
Energy % from:				
protein	13·1	14·3	13·2	14·0
total fat	45·3	44·4	44·6	44·6
saturated	11·0	23·3	10·2	23·4
monounsaturated	11·6	14·1	11·8	14·5
polyunsaturated†	20·9	5·3	20·8	5·1
carbohydrates	39·4	39·4	40·4	39·4
polysaccharides	18·1	19·1	18·4	18·8
Alcohol	2·2	2·0	1·9	2·0
P:S ratio	1·9	0·2	2·0	0·2
Dietary fibre (g day^{-1})	42·7	39·6	37·6	37·3
Cholesterol				
(mg MJ^{-1})	40	40	44	40
(mg day^{-1})	494	492	471	420
Campesterol				
(mg MJ^{-1})	6	3	5	3
(mg day^{-1})	70	38	59	31
Stigmasterol				
(mg MJ^{-1})	4	2	4	2
(mg day^{-1})	50	24	44	19
Sitosterol				
(mg MJ^{-1})	32	14	31	13
(mg day^{-1})	398	171	339	135
Other sterols				
(mg MJ^{-1})	22	8	26	10
(mg day^{-1})	271	102	283	104

* The nutrient density of the diets was kept constant over the range of energy intakes; absolute intakes per day thus represent averages. Intakes (±SD for energy intake) were calculated from analyses of duplicate portions collected throughout the experiment, supplemented with data on cholesterol- and fat-free items (1 MJ (239 kcal) per day) selected by the subjects and noted in their diaries. Overall plant sterol concentration of these items (in mg MJ^{-1}) was assumed to be the same as in the duplicate portions; † mainly linoleic acid, C18:2 ($n-6$).

Body weights were checked to the nearest 0·1 kg twice a week and energy intake was adjusted when necessary. Mean (±SD) weight changes between the high P:S and the low P:S period were insignificant; they amounted to −0·28±0·54 kg for the subjects in group Norm-egg and to 0·43±0·84 kg for those in group Hab-egg. Good adherence to the diets was also reflected by observed changes in the linoleic/oleic acid ratio of serum cholesteryl esters [23], measured in individual pools of the two serum samples obtained at the end of each dietary period. Changing from the high P:S to the low P:S diet caused the mean (±SD) linoleic/oleic acid ratio to decrease from 6·5±0·9 to 3·5±0·4 in group Norm-egg, while the reverse dietary manipulation in group Hab-egg caused this ratio to increase from 2·9±0·4 to 5·5±0·9. Data for individual fatty acids are available elsewhere [23].

Blood was sampled after an overnight fast on 3 of the last 8 days of each dietary period, and serum stored at −20°C. Feces were collected during the last 5 days of each period. Twenty radio-opaque polyethylene rings were ingested by the subjects each day for 10 days prior to the fecal collection period and throughout the 5 days of fecal collection, and the recovery of these markers was used to correct for variations in fecal flow [24]. The mean recovery (±SD) for all 47 subjects amounted to 100·1±15·0% on the high P:S diet and to 105·1±17·8% on the low P:S diet. Mean transit time through the gut could also be measured, because on the day before each feces collection period subjects were given markers of a smaller diameter (3 instead of 4·5 mm). The stools were frozen as soon as possible after being passed, and stored at −20°C. At the end of the dietary trial the feces of each subject were pooled per period, homogenized, freeze-dried, and again stored at −20°C.

Analytical methods

Serum total lathosterol (free plus esterified) was assayed by capillary gas chromatography, as pre-

Table 3. The output of feces and the mean intestinal transit time in healthy volunteers fed high- and low P:S diets

	Group Norm-egg ($n=23$)		Group Hab-egg ($n=24$)	
	High P:S	Low P:S	High P:S	Low P:S
Wet weight (g day^{-1})*	178±78	162±82	167±58	153±70
Dry matter (g 100 g^{-1} wet weight)	26·0±5·0	26·0±5·7	22·3±3·6	22·8±4·7
Frequency of stools* (per 24 h)	1·2±0·5	1·3±0·6	1·3±0·3	1·3±0·4
Mean intestinal transit time (h)	70·3±47·8	65·8±43·5	59·3±33·3	58·8±35·8

Values represent means±SD for the indicated number of subjects. * Actual values, not corrected for fecal flow (*cf.* Methods).

viously described [25]. Protein, fat, fatty acids and carbohydrates were determined in the duplicate portions, as described [26]. Cholesterol and plant steroid content of the consumed food [27] and neutral steroid and bile acid contents of the feces [28,29] were determined by narrow-bore capillary gas-liquid chromatography. Feces of each subject were analysed in duplicate. Peaks in the gas chromatograms of neutral and acidic steroids were identified and their homogeneity confirmed by subjecting various representative fecal samples to gas-liquid chromatography-mass spectrometry and comparing the fragmentation patterns with those of known standards, as described in detail elsewhere [28]. In our procedure, 20 different neutral steroids and 22 bile acids, among which five iso- and six oxo-bile acids could be identified. Recovery of added pure steroids was 98·1+5·0% for coprostanol and 97·4+7·3% for cholesterol (means±SD of six determinations). Recovery of radioactivity from feces of a patient injected with radioactive deoxycholic acid amounted to 94±8%, and that of another patient injected with chenodeoxycholic acid to 89±12% (means±SD for four fecal samples each. Repeated determinations on three duplicate diet samples and on a control pool of freeze-dried human feces revealed an interassay variability (coefficient of variation) of about 2·5% for dietary cholesterol, of 2·3% for endogenous fecal neutral steroids, and of 4·4% for bile acids. The rate of whole-body cholesterol synthesis was calculated as the net steroid balance, i.e. the sum of the excreted endogenous steroids and bile acids minus the cholesterol intake. In this approach small amounts of cholesterol and its metabolites that may be excreted through the skin or in the urine are neglected, but generally these can be left out of consideration when the effect of changes in the diet are studied.

Results

Fecal mass and intestinal transit time

The group means of the mass of feces passed per day, the frequency of stools, and the mean intestinal transit time did not change significantly from one dietary period to another (Table 3). There were, however, large variations between subjects in these parameters, similar to previous findings [15]. The mean total daily output of dry matter was slightly higher on the high P:S than on the low P:S diet.

Fecal steroid excretion

The amounts of neutral steroids excreted per day were higher ($P<0·01$) on the low P:S high-saturated-fat diet than on the high P:S high-linoleic-acid diet, while the excretion of bile acids was similar on both diets (Table 4). The fecal excretion of plant steroids was higher on the high P:S diet than on the low P:S diet, because of differences in intake. The recovery of dietary plant sterols in the feces was satisfactory (Table 4).

The mean composition of the fecal steroids was not different between the high P:S and the low P:S diet periods, except for a higher content of coprostanone on the high P:S diet, at the expense of coprostanol (Table 5). Of the neutral steroids 13–17% was present as cholesterol or cholesterol sulphate and the remainder as secondary steroids (mainly coprostanol), formed from cholesterol by bacterial activity in the colon. The primary bile acids cholic and chenodeoxycholic acid made up only 3–5% of the acidic steroids; the remainder consisted mainly of the bacterial metabolites deoxycholic and (iso)lithocholic acid. The fecal steroid composition agrees with data for healthy humans reported by other investigators [30].

Cholesterol balance

The dietary intake of cholesterol relative to calories was kept constant for all participants and during both experimental periods. Absolute cholesterol intakes (Table 4) ranged from 0·7 to 2·1 mmol day^{-1} (270–800 mg day^{-1}), depending on energy intake (Table 2). The mean daily fecal excretion of cholesterol and its metabolites (Table 5) ranged from 1·1 to 5·5 mmol (428–2140 mg in terms of coprostanol). The cholesterol balance was higher ($P<0·01$) on the low P:S high-saturated-fat diet than on the high P:S diet. Individual values for the cholesterol balance were remarkably constant from one dietary period to another. When the cholesterol balance was expressed as mmol day^{-1} kg^{-1} body weight, the correlation

Table 4. Effect of dietary fatty acid composition on fecal excretion of neutral steroids and bile acids, and on cholesterol balance (means ± SD; ranges in parentheses)

	Group Norm-egg ($n=23$)		Group Hab-egg ($n=24$)		All ($n=47$)	
	High P:S	Low P:S	High P:S	Low P:S	High P:S	Low P:S
			(mmol day^{-1})			
Neutral steroids†	1·91 ± 0·68* (0·9–3·6)	2·13 ± 0·63 (0·8–3·5)	2·09 ± 0·70* (1·1–3·8)	2·37 ± 0·72 (1·3–4·5)	2·00 + 0·69‡ (0·9–3·8)	2·25 + 0·68 (0·8–4·5)
Bile acids	0·74 ± 0·43 (0·2–1·8)	0·77 ± 0·44 (0·2–2·0)	0·84 ± 0·40 (0·2–1·8)	0·77 ± 0·36 (0·2–1·6)	0·79 + 0·41 (0·2–1·8)	0·77 + 0·40 (0·2–2·0)
Sum (cholesterol plus metabolites)	2·65 ± 0·99 (1·1–5·4)	2·90 ± 0·90 (1·1–4·7)	2·93 ± 0·96 (1·5–5·1)	3·13 ± 0·98 (1·8–5·5)	2·79 + 0·98* (1·1–5·4)	3·02 + 0·94 (1·2–5·5)
Cholesterol intake	1·28 ± 0·37 (0·7–1·8)	1·27 ± 0·30 (0·8–1·6)	1·22 ± 0·31 (0·8–2·1)	1·09 ± 0·24 (0·7–1·7)	1·24 + 0·33 (0·6–2·1)	1·17 ± 0·28 (0·7–1·7)
Cholesterol balance	1·37 ± 0·79 (0·2–3·6)	1·63 ± 0·70 (0·4–3·1)	1·71 ± 0·90‡ (0·6–4·0)	2·04 ± 0·90 (0·8–4·0)	1·55 + 0·85† (0·2–4·0)	1·86 ± 0·83 (0·4–4·0)
Sitosterol						
Excretion§	0·86 ± 0·17	0·59 ± 0·08	0·72 ± 0·20	0·46 ± 0·13	0·78 ± 0·20	0·52 ± 0·12
Intake	0·96	0·41	0·82	0·33	0·89	0·37
Stigmasterol						
Excretion¶	0·12 ± 0·03	0·08 ± 0·01	0·11 ± 0·03	0·07 ± 0·02	0·11 ± 0·03	0·07 ± 0·02
Intake	0·12	0·06	0·11	0·05	0·11	0·05

* Significantly different from the low P:S diet by paired t-test, $P<0·01$; † cholesterol and its bacterial metabolites; ‡ significantly different from the low P:S diet by paired t-test, $P<0·05$; § as ethylcoprostanol + sitosterol + ethylcoprostanon + sitostanol, in order of amounts; ¶ as ethylcoprostenol + stigmasterol + stigmastenol + ethylcoprostenon, in order of amounts.

Table 5. Effect of the type of dietary fatty acids on the percentage composition of fecal neutral steroids and bile acids in healthy volunteers (mean ± SD)

	Group Norm-egg ($n=23$)		Group Hab-egg ($n=24$)	
Fecal steroid	High P:S	Low P:S	High P:S	Low P:S
		(mol 100 mol^{-1})		
Neutral steroids				
cholesterol	11·6 ± 6·7	10·1 ± 7·9	13·4 ± 9·0	11·9 ± 8·8
cholesterol sulfate	3·4 ± 1·3	3·1 ± 1·4	3·4 ± 1·1	3·0 ± 1·0
coprostanol*	73·1 ± 9·0‡	79·1 ± 10·4	66·3 ± 10·9‡	73·9 ± 11·6
coprostanone	9·7 ± 3·2‡	6·0 ± 3·5	15·1 ± 9·2‡	9·6 ± 8·6
cholestanol	2·2 ± 0·6	1·7 ± 0·7	1·8 ± 0·6	1·6 ± 0·5
Sum	100	100	100	100
Acidic steroids				
cholic acid	1·7 ± 1·6	1·6 ± 1·3	2·2 ± 1·3	1·8 ± 1·2
iso-chenodeoxycholic acid	3·9 ± 2·2	3·7 ± 2·4	4·0 ± 2·0	3·9 ± 2·3
chenodeoxycholic acid	1·4 ± 1·0	1·3 ± 1·0	1·9 ± 1·1	1·7 ± 1·2
iso-deoxycholic acid	8·2 ± 5·4	8·2 ± 4·9	9·7 ± 4·0	9·2 ± 3·6
deoxycholic acid	37·1 ± 6·9	37·9 ± 7·0	35·2 ± 6·1	34·0 ± 7·1
iso-lithocholic acid	15·5 ± 3·9	15·3 ± 3·3	16·9 ± 4·8	16·7 ± 4·9
lithocholic acid	24·0 ± 5·4	23·1 ± 5·5	21·0 ± 4·9	22·8 ± 4·3
oxo bile acids†	8·2 ± 4·7	8·9 ± 5·3	9·1 ± 3·6	9·9 ± 4·1
Sum	100	100	100	100

* Including epicoprostanol; † mainly 12-oxo isolithocholic and 12-oxo lithocholic acid, and traces of 7-oxo deoxycholic and 7-oxo lithocholic acid; ‡ significantly different from value on the low P:S diet, $P<0·05$.

coefficients were r=0·75 for group Norm-egg and r=0·51 for group Hab-egg. Thus the correlation was independent of body mass. Although the mean fecal steroid excretion and cholesterol balance were 30–50% higher for men than for women, the diet-induced changes were similar for the two sexes.

For the participants in group Norm-egg, the cholesterol balance had also been measured 2 years earlier, both on a low-cholesterol (106 mg day^{-1}) and on a high-cholesterol (636 mg day^{-1}) diet, at a total fat intake of 41% of energy and a constant dietary P:S ratio of 0·2 [24]. The average cholesterol balance at that time amounted to $2·36 \pm 0·86$ mmol day^{-1} in the low-cholesterol period and to $1·95 \pm 0·76$ mmol day^{-1} in the high-cholesterol period (means $\pm$ SD, $n=23$). The mean individual values for the cholesterol balance found in that previous experiment (mean per subject of the values on the low- and the high-cholesterol diet) and in the present fatty acid experiment (mean of the values on the low- and high P:S diet) were strongly correlated ($r=0·84$, $n=23$; $P<0·01$). Thus, the differences in cholesterol balance among our subjects appear to be quite stable, and much larger than the changes induced within individuals by manipulation cholesterol intake or dietary P:S ratio over the range habitually consumed.

Serum lathosterol

For both groups, the ratio of Δ-7 lathosterol to cholesterol in serum was significantly higher on the low P:S, high-saturated-fat diet than on the high P:S diet. For all 47 participants combined, the ratio was $0·86 \pm 0·33$ μmol $mmol^{-1}$ on the high and $1·07 \pm 0·39$ on the low P:S diet (95% confidence interval for the difference, 0·14 to 0·27, $P<0·01$). The absolute lathosterol concentration was $4·30 \pm 1·84$ μmol l^{-1} on the high and $6·35 \pm 2·45$ on the low P:S diet (95% confidence interval for the difference, 1·64 to 2·45, $P<0·01$).

Discussion

There is considerable controversy as to the mechanism by which saturated fatty acids elevate and polyunsaturated fatty acids lower plasma cholesterol. Dietschy, Spady and coworkers have developed an elegant hamster model in which dietary fatty acids were found to affect LDL cholesterol levels through changes in the activity of the hepatic LDL receptor [6,7]. The liver enzyme that converts free cholesterol into cholesteryl esters has a lower affinity for saturated than for unsaturated fatty acids. As a result, hepatic cholesteryl ester content falls when hamsters are fed saturated fatty acids [7]. It is conceivable that at the same time the amount of free cholesterol increases in certain hepatocyte compartments, including the putative regulatory pool which controls the expression of the LDL receptor [7]. This leads to down-regulation of the receptor, accumulation of LDL in plasma, and increased formation of LDL from its plasma precursor VLDL [31,32]. Such a mechanism does not require net sterol balance to change when LDL cholesterol concentrations in plasma change [7].

We studied the effects of a marked change in dietary fatty acid composition on sterol excretion and whole-body cholesterol balance in two large groups of healthy subjects who were fed controlled natural solid diets differing only in type of fat. Fat provided 45% of daily calories; although high, this is still below the 75th percentile of fat intake in middle-aged men and women in North-America [33], and thus well within the range of normal intakes.

Changing the dietary P:S ratio from about 2·0 to 0·2 at a constant intake of total fat and cholesterol increased the mean rate of whole-body cholesterol synthesis. This result is at variance with the results of some earlier studies which found either no change or an enhanced fecal excretion of neutral steroids and bile acids upon increasing the polyunsaturated fat content of the diet [8–11]. It is conceivable that in a few of these latter studies the analysis of fecal steroids included some plant steroids. Plant sterols are abundant in polyunsaturated vegetable oils, and before the advent of capillary gas-liquid chromatography, separation of fecal metabolites of plant sterols from endogenous steroids was sometimes difficult to achieve. Alternatively, there might be a transient increase in fecal steroid excretion after a rise of the dietary P:S ratio, as suggested by Nestel *et al.* [11]. However, such a temporary increase in the loss of cholesterol from the body cannot explain the permanent fall in plasma LDL induced by diets high in polyunsaturated and low in saturated fatty acids. This is illustrated by the case of LDL apheresis: after removal of an appreciable amount of LDL from the blood stream of a hypercholesterolaemic patient, LDL levels show a temporary fall followed by a return to starting levels. In fact, there is no *a priori* reason why even a permanent increase in fecal steroid loss should be coupled with a decrease in plasma LDL levels. Steroid output must necessarily reflect total body cholesterol synthesis, as long as intake is constant; when cholesterol synthesis rises, output will also rise, and vice versa. However, a rise in synthesis may either occur secondary to increased steroid loss induced by e.g. treatment with bile acid binding resins, which lowers LDL, or the increase in synthesis may be primary, the enhancement of fecal steroid excretion being a secondary consequence. The latter may be the case in obesity, where both cholesterol synthesis and serum cholesterol are elevated [34].

The observation that serum lathosterol and the lathosterol/cholesterol ratio were increased significantly on the high-saturated-fat diet provides independent evidence that saturated fatty acids stimulate the body to synthesize cholesterol. Lathosterol is a precursor in the biosynthesis of cholesterol, and its concentration in serum, either in absolute terms or relative to cholesterol, is a valid index of whole body cholesterol synthesis [16,17]; the ratio to cholesterol is preferred as

an indicator because it eliminates the influence of the number of lipoprotein particles on the lathosterol concentration [17].

It has been shown that saturated fat consumption stimulates VLDL and LDL production and turnover in man [35,36] probably because of an increased lipoprotein output by the liver [37]; for a review see [38]. If one assumes that the increased output of lipoproteins requires an enhanced *de novo* synthesis of cholesterol by the liver then our data fit in with these observations, and together they would provide an explanation for the cholesterol-elevating effect of saturated fatty acids.

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