

## Faecal excretion of bile acids and neutral steroids on diets differing in type and amount of dietary fat in young healthy persons

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**Abstract.** The effect of different amounts and types of dietary fat on faecal steroid excretion in healthy volunteers was examined. In the first experiment with sixty volunteers four diets which differed in type and amount of dietary fat only were compared during a test-period of 5 weeks. Faeces were collected during 7 days before and at the end of the test period and analysed individually. In the second experiment with thirty-five volunteers, a low fat diet containing 22% of energy as total fat (4% as polyunsaturated fatty acids) was compared with a moderate fat diet (30% of energy as total fat; 11% as polyunsaturated fatty acids) during 13 weeks. Faeces were collected during the first 7 days of the test period and during 7 consecutive days near the end of the test period and pooled per dietary group.

Excretion of faecal neutral steroids and bile acids was not significantly influenced by amounts of total fat ranging from 22% to 40% of daily energy or by differences in polyunsaturated to saturated fatty acids ratio from 0.2 to 1.7. The only exception was the excretion of bile acids in the second experiment which was 0.19 mmol/24 h (75 mg/24 h) higher on the low fat diet than on the moderate fat, high polyunsaturated fat diet ( $P < 0.01$ ).

**Key words.** Faeces, bile acids, neutral steroids, faecal fat, dietary fat, polyunsaturated fatty acids.

### Introduction

Although the effects of dietary fat on serum cholesterol concentrations have been known for over 30 years [1–3] the mode of action of the diet in changing serum cholesterol levels remains somewhat controversial. One of the proposed mechanisms for polyunsaturated fatty acids is an increased excretion of faecal steroids, caused by decreased resorption of cholesterol or increased conversion of hepatic cholesterol into bile acids or both of these mechanisms. However, conflict-

ing results have been published on the effect of changing the type of dietary fat or increasing the amount of dietary fat on faecal steroids.

In the course of two controlled experiments on the effect of diet on serum lipoprotein concentration and composition we have re-examined the effect of different amounts and types of fat on faecal steroid excretion at constant cholesterol intake. Other results of these studies have been published elsewhere [4–6].

### Methods

In both experiments the experimental protocol was fully explained to the participants and informed consent was obtained. The subjects were not offered payment and were free to stop participating at any moment. The design of the first experiment was as follows (Fig. 1).

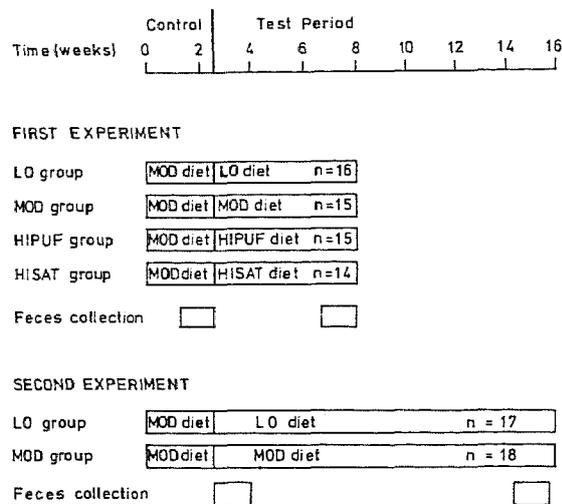
Sixty young healthy student volunteers received a control diet (30% of daily energy as fat; 11% as polyunsaturated fat) for 2.5 weeks. They were then randomized into four subgroups which received test diets as indicated in Fig. 1. The subgroups were stratified for initial serum cholesterol levels, male/female ratio and energy intake. The test period lasted 5 weeks. The second experiment with thirty-five volunteers differed from the first in that only two diets were tested, namely the low fat carbohydrate rich diet (LO) and the 'Dietary Goals' diet (MOD) with 30% of daily energy as fat, 11% as polyunsaturated fat and that the test period was extended to 13 weeks.

In each experiment diets differed between groups in one component only; one group always received the control diet throughout the test period as a check against baseline drift. Throughout the whole experimental period all foodstuffs except for 100 kcal per day were weighed out individually and supplied according to the energy needs of each person. Body weight was measured weekly and energy intake was adjusted when necessary to maintain constant body weight. The subjects were free-living but took one hot meal daily at the department at lunchtime, where they also received a food package for their evening meal and breakfast. The diets were composed of natural foodstuffs. Actual

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**Figure 1.** Experimental design. LO = low fat, high carbohydrate diet (22% of daily energy as fat, 3% of daily energy as polyunsaturated fat). MOD = moderate fat, high polyunsaturated fat diet (30% of daily energy as fat, 11% of daily energy as polyunsaturated fat). HIPUF = high fat, high polyunsaturated fat diet (40% of daily energy as fat, 19% of daily energy as polyunsaturated fat). HISAT = high fat, low polyunsaturated fat diet (39% of daily energy as fat, 3% of daily energy as polyunsaturated fat).

nutrient intake was measured by 7-day weighed food records using Dutch food composition tables [7], and by analysis of duplicate portions [8] for one imaginary person of average energy intake on each diet.

**Diets.** The moderate-fat control diet (MOD) in the first experiment contained 11% of total daily energy as polyunsaturated fatty acids in total, fat provided 30% of daily energy. The polyunsaturated to saturated fatty acid (P/S) ratio was 1.1. The low-fat, low polyunsaturated fatty acids diet (LO) provided 22% of energy as total fat (3% of daily energy as polyunsaturated fatty acids; P/S ratio 0.4), the high-fat, high polyunsaturated fatty acids diet (HIPUF) provided 40% of daily energy intake as total fat (19% of daily energy as polyunsaturated fatty acids; P/S ratio 1.7) and the high-fat, low polyunsaturated fatty acids diet (HISAT) provided 39% of daily energy as total fat (3% of daily energy as polyunsaturated fatty acids; P/S ratio 0.2). In all diets protein provided 13% of daily energy and mono- and disaccharides 23%, while starch made up the total energy balance. Thus the diets with a lower fat content contained more starch but not more sugars. Cholesterol intake was 244, 275, 255 and 210 mg/day for groups LO, MOD, HIPUF and HISAT respectively. Intake of plant sterols was 247, 395, 530 and 270 mg/day and dietary fibre intake was 24, 24 and 25 g/day for groups LO, MOD, HIPUF and HISAT respectively.\*

In the second experiment the moderate fat, high P/S

\* In our earlier publication [4] the values for the plant sterol content of diets HIPUF (rich in polyunsaturated fat) and HISAT (rich in saturated fat) were accidentally interchanged.

ratio control diet and the low fat, low P/S ratio diet of the first experiment were tested again, but this time the test period lasted 13 weeks instead of 5 weeks. Total fat intake and dietary fatty acid composition were equal to the first experiment. Cholesterol intake was 260 and 267 mg/day, dietary fibre intake was 46 and 39 g/day and intake of plant sterols was 269 and 384 mg/day on average for the low and moderate fat diet respectively.

Because the subjects were randomized into the diet groups it can be expected that possible minor disturbing factors, such as consumption of tea [9] were more or less equally present in all diet groups.

**Faeces collection and analysis.** In the first experiment we investigated the effects of various test diets after 4 weeks compared with the preceding control diet; the control group was also reinvestigated so that we could take into account baseline drifts (cf. Fig. 1). Therefore faeces were collected during the last 7 days of the control period and again during the last 7 days of the 5-week test period (Fig. 1) and faeces of each subject was analysed individually.

In the second experiment we investigated whether the acute effect of a low-fat, high-carbohydrate diet on faecal steroid excretion differs from the long-term chronic effect. Faeces were collected during the first 7 days of the test period and during 7 consecutive days near the end of the test period. The moderate fat, high polyunsaturated fat diet again served as a control. Because individual faecal analysis is extremely laborious, in this experiment faeces-pools were made per dietary group by taking 10% of each subject's homogenized, freeze-dried faeces and only pools were analysed.

The stools were usually frozen within 12 h of being passed and stored at  $-40^{\circ}\text{C}$ . Mean transit time through the gut (MTT) was measured using radio-opaque markers [10]. Faecal primary (cholic acid and chenodeoxycholic acid) and secondary (deoxycholic acid and lithocholic acid) bile acids were extracted with glacial acetic acid and toluene and determined by gas-liquid chromatography all according to Evrard & Janssen [11]. Cholesterol and the secondary neutral steroids (coprostanol, (epi)coprostanol and coprostanone) were measured according to Miettinen *et al.* [12] but without prior thin-layer chromatography. Repeated determinations on a control pool of freeze-dried faeces revealed an inter-assay variability of 6.9% (coefficient of variation) for cholesterol, 6.5% for coprostanol and 5% for total bile acids. Faecal fat was measured in the first experiment as fatty acids after saponification [13].

**Statistical evaluation.** For statistical evaluation of results in the first experiment mean changes over the test period were compared between groups, using a two-tailed Student's *t*-test. For the second experiment, only pooled samples were analysed. This allows a good estimate of the change of the group means of steroid excretion under the influence of diet, but not of the

standard deviation of this change. However, the experimental set-up, the type of subjects, the methods and duration of faeces collection and the analytical methodology in the second experiment were all very similar to those in the first experiment and in a previously described dietary experiment [8] from our Department. We therefore estimated the standard deviations for the changes in excretion from these experiments. The mean SD (calculated as the root of the mean variance) was 0.25 mmol/day for the change in bile acid excretion (range: 0.15–0.33;  $n=8$ ) and 0.67 mmol/day for the change in neutral steroid excretion (range 0.41–0.89;  $n=8$ ).

## Results

### Faecal mass and intestinal transit time

Results for mass of faeces passed per day, percentage dry matter, frequency of stools and mean transit time are given in Table 1. There were no differences between the groups in the changes over the test period, whether measured from end of control to end of test period as in the first experiment or from start to end of test period as in the second experiment.

As reported previously [8, 14], there was a large variation in faecal output between subjects; in the control period, when all subjects consumed the control diet rich in polyunsaturated fat but moderate in total fat, faecal wet weight, averaged over a 7-day period ranged from 28 to 246 g/day and the percentage dry

matter from 18% to 38%. Mean transit time through the gut ranged from 13 to 172 h.

### Faecal steroid and fat

Faecal excretion of neutral and acidic steroids and of faecal fat is given in Table 2. There was an increase in the excretion of neutral steroids from the end of the control period to the end of the test period in all four groups in the first experiment. However, the changes in the low fat diet group and in both high fat diet groups were not significantly different from the change in the control group. In the second experiment faecal excretion of neutral steroids increased from the first week to the end of the test period but again the change in the low fat diet group was not significantly different from the change in the moderate fat control group, if we assume that the standard deviations in this experiment were comparable to those in previous studies [8] and the first experiment.

Bile acid excretion (Table 2) was rather constant on all four diets in the first experiment. There was a slight increase on the low fat diet, a slight decrease on the high saturated fat diet and no change on the high polyunsaturated fat diets. The changes in the low fat diet group and in both high fat diet groups were not significantly different from those in the control group.

In the second experiment faecal bile acid excretion was again unchanged on the control diet, but on the low fat diet it was clearly higher at the end of the test period compared with the start of the test period. This

Table 1. The output of faeces and the mean faecal transit time during the two experiments (mean  $\pm$  SD)

	Experiment 1*				Experiment 2†	
	Diet group		Diet group		Diet group	
	LO low fat, low P/S ratio; $n=16$	MOD moderate fat, high P/S ratio; $n=15$	HIPUF high fat, high P/S ratio; $n=15$	HISAT high fat, low P/S ratio; $n=14$	LO low fat, low P/S ratio; $n=17$	MOD moderate fat, high P/S ratio; $n=18$
<b>Wet weight (g/24 h)</b>						
Control period	107 $\pm$ 45	118 $\pm$ 53	109 $\pm$ 49	122 $\pm$ 70	— ‡	— ‡
Start test period	— ‡	— ‡	— ‡	— ‡	151 $\pm$ 71	140 $\pm$ 56
End test period	113 $\pm$ 43	117 $\pm$ 43	123 $\pm$ 65	113 $\pm$ 62	164 $\pm$ 82	160 $\pm$ 74
<b>Dry matter (g/100 g wet weight)</b>						
Control period	24 $\pm$ 4	24 $\pm$ 4	25 $\pm$ 5	25 $\pm$ 6	— ‡	— ‡
Start test period	— ‡	— ‡	— ‡	— ‡	24 $\pm$ 4	24 $\pm$ 3
End test period	26 $\pm$ 5	25 $\pm$ 4	26 $\pm$ 5	27 $\pm$ 6	24 $\pm$ 3	24 $\pm$ 4
<b>Frequency of stools (per 24 h)</b>						
Control period	0.8 $\pm$ 0.3	1.1 $\pm$ 0.4	1.0 $\pm$ 0.6	1.1 $\pm$ 0.5	— ‡	— ‡
Start test period	— ‡	— ‡	— ‡	— ‡	1.0 $\pm$ 0.3	0.9 $\pm$ 0.4
End test period	0.9 $\pm$ 0.3	1.0 $\pm$ 0.4	1.2 $\pm$ 0.5	1.0 $\pm$ 0.5	1.1 $\pm$ 0.4	1.0 $\pm$ 0.3
<b>Mean transit time (h)</b>						
Control period	51 $\pm$ 20	53 $\pm$ 34	62 $\pm$ 45	56 $\pm$ 24	— ‡	— ‡
Start test period	— ‡	— ‡	— ‡	— ‡	59 $\pm$ 28	57 $\pm$ 26
End test period	61 $\pm$ 27	59 $\pm$ 33	67 $\pm$ 41	57 $\pm$ 36	50 $\pm$ 20	46 $\pm$ 26

No changes significantly different from change in control group MOD ( $P \geq 0.25$ ,  $t$ -test).

\* Test period 5 weeks. † Test period 13 weeks. ‡ Not measured.

change was significantly different from the change in the control group, again provided that we made the right estimations of the standard deviations.

Faecal excretion of fat (Table 2) showed an increase in all four groups in the first experiment. The increase in both high-fat diet groups was significantly greater than the change in the control group. However, the differences were small in absolute terms. In the second experiment faecal fat was not measured.

#### *Changes in steroid and fat excretion related to changes in total serum cholesterol concentration*

Mean serum cholesterol concentrations remained stable in groups MOD and HIPUF and increased in groups LO and HISAT, by 0.25 and 0.38 mmol/l respectively [4]. However, as shown in Table 2, group means of faecal neutral steroid excretion increased in all four groups and bile acid excretion was almost unchanged.

Within each diet group, different individuals reacted differently to the diet. In Table 3 the correlations between the changes in steroid excretion and the changes in total serum cholesterol concentration within each group are given. Within the low fat, high carbohydrate group there was a negative correlation between a subject's change in serum cholesterol and his or her change in excretion of bile acids. In general,

however, the correlation between individual changes in serum cholesterol level and faecal steroid excretion within a group was weak and inconsistent.

#### *Faecal concentration of steroids and percentage secondary steroids*

The faecal concentration of neutral steroids at the end of the control period in the first experiment was 15 mmol/kg wet faeces; the faecal concentration of bile acids was 7 mmol/kg wet faeces. The faecal concentration of neutral steroids and bile acids at the start of the test period in the second experiment was 10 and 6 mmol/kg wet faeces respectively. The changes in faecal steroid concentration were small in both experiments and there were no statistically significant differences between the diet groups.

At the end of the control period of the first experiment, 91% of the neutral steroids and 73% of the bile acids in the faeces consisted of secondary steroids, formed from primary steroids by bacterial activity. Changes over the test period in the proportion of secondary products on the various test diets ranged on average from -2 to +4 percentage points for neutral steroids and from -8 to +5 percentage points for bile acids. The changes were not significantly different from changes in the control group MOD.

The results for the second experiment were similar.

**Table 2.** The excretion of neutral steroids and bile acids and of fat during the two experiments (mean  $\pm$  SD). All values are expressed in millimoles per 24 h.\*

	Experiment 1†				Experiment 2‡	
	LO low fat, low P/S ratio; n=16	MOD moderate fat, high P/S ratio; n=15	HIPUF high fat, high P/S ratio; n=15	HISAT high fat, low P/S ratio; n=14	LO low fat, low P/S ratio; n=17	MOD moderate fat, high P/S ratio; n=18
Neutral steroids (mmol/24 h)						
Control period (MOD diet)	1.42 $\pm$ 0.54	1.67 $\pm$ 0.61	1.48 $\pm$ 0.45	1.36 $\pm$ 0.64	— §	— §
Start test period	— §	— §	— §	— §	1.34	1.60
End test period	1.84 $\pm$ 0.60	1.96 $\pm$ 0.63	1.82 $\pm$ 0.78	1.50 $\pm$ 0.74	1.46	1.83
Change	+0.42 $\pm$ 0.44	+0.30 $\pm$ 0.44	+0.34 $\pm$ 0.75	+0.14 $\pm$ 0.89	+0.12	+0.23
Bile acids (mmol/24 h)						
Control period (MOD diet)	0.67 $\pm$ 0.32	0.76 $\pm$ 0.35	0.76 $\pm$ 0.36	0.67 $\pm$ 0.28	— §	— §
Start test period	— §	— §	— §	— §	0.73	0.78
End test period	0.77 $\pm$ 0.29	0.77 $\pm$ 0.30	0.77 $\pm$ 0.34	0.64 $\pm$ 0.26	0.91	0.77
Change	+0.10 $\pm$ 0.21	+0.01 $\pm$ 0.15	+0.02 $\pm$ 0.16	-0.03 $\pm$ 0.19	+0.18¶	-0.01
Fatty acids (mmol/24 h)						
Control period (MOD diet)	5.6 $\pm$ 2.4	6.2 $\pm$ 2.7	6.1 $\pm$ 2.1	4.8 $\pm$ 1.8	— §	— §
Start period	— §	— §	— §	— §	— §	— §
End test period	6.0 $\pm$ 2.8	6.5 $\pm$ 2.5	7.3 $\pm$ 1.8	7.2 $\pm$ 3.3	— §	— §
Change	+0.4 $\pm$ 2.8	+0.3 $\pm$ 1.9	+1.2 $\pm$ 1.4¶	+2.4 $\pm$ 2.7¶	—	—

\* Conversion from SI to traditional units: 1 mmol of neutral steroids (as coprostanol)=389 mg; 1 mmol of bile acids (as deoxycholic acid)=393 mg; 1 mmol of fatty acids (as oleic acid)=283 mg.

† Test period 5 weeks. Start test period not measured. Results of individually analysed faeces.

‡ Test period 13 weeks. Control period not measured. Results of analysis of pooled faeces.

§ Not measured.

¶ Change over test period significantly different from change in control group ( $P < 0.01$ ,  $t$ -test) provided that we made the right estimation of SDs as indicated in the methods-section. In all other cases  $P > 0.15$  ( $t$ -test).

**Table 3.** Correlation coefficient between the change in serum cholesterol level and in faecal bile acid and neutral steroid excretion over the test period per person within the diet groups in the first experiment

	Diet group			
	LO low fat, low P/S ratio; n=16	MOD moderate fat, high P/S ratio; n=15	HIPUF high fat, high P/S ratio; n=15	HISAT high fat, low P/S ratio; n=14
Δ bile acid excretion	-0.52†	-0.04	-0.25	+0.18
Δ neutral steroid excretion	-0.07	+0.10	-0.44†	-0.07

† Significantly different from 0,  $P < 0.05$ .

## Discussion

The present experiments were set up to evaluate the effects of type and amount of dietary fat on serum lipoprotein composition and concentration. One of the possible mechanisms by which dietary fat influences serum cholesterol concentration is by increasing the excretion of faecal steroids. However, we found neither an acute nor a chronic effect of type and amount of dietary fat on faecal excretion of neutral steroids and bile acids in two carefully controlled experiments with large groups of participants and using natural foodstuffs.

### *Replacement of saturated by polyunsaturated fatty acids*

In the first experiment we found no difference in excretion of bile acids and neutral steroids in the faeces between subjects on the high fat, high P/S ratio diet and the high saturated fat diet. These diets contained the same amount of fat and cholesterol, but had P/S ratios of 1.7 and 0.2 respectively.

Table 4, part I, lists studies on the effect on faecal steroid excretion of replacing saturated by polyunsaturated fat. Only controlled studies with healthy subjects consuming natural solid diets are included.

Evaluation of research on the effect of diet on faecal steroid excretion is hampered by the great differences in experimental design, type of diet and type of participants between the various studies. Therefore studies with liquid formula diets were excluded because they usually have a very low fibre content, which could interfere with the results. Studies of patients with disorders of lipid metabolism were also excluded because such patients are known to have a faecal steroid excretion different from healthy controls [15]. The intake of cholesterol and plant sterols are given because these components influence faecal steroid excretion [16–18].

As can be seen from Table 4, replacement of saturated by polyunsaturated fatty acids led to increased bile acid excretion in the experiment of Antonis & Bersohn [19]. Although sterol consumption was not listed, the intake of cholesterol was probably

lower and the intake of plant sterols higher on the polyunsaturated diet. Moore *et al.* [20] found modest increases in bile acid excretion on a high safflower oil diet compared with a butter diet, but the intake of cholesterol and plant sterols was not constant; Nestel *et al.* found no effect of polyunsaturated fatty acids in one experiment [21] and in another experiment they found an increase in bile acid excretion only when the cholesterol content of the diets was high [16]. Replacement of saturated fat by polyunsaturated fat led to a higher excretion of neutral steroids in the above-mentioned experiments, although it cannot be excluded that the analysis of faecal neutral steroids in the experiment of Antonis & Bersohn included some plant sterols. Eneroth *et al.* [22] also found modest increases in faecal neutral steroid excretion on a high corn oil diet compared with a butter diet. One might speculate that this was at least partly due to the higher intake of plant sterols on diets rich in polyunsaturated fat. However, in our first experiment we did not find a higher excretion of neutral steroids on the high fat diet rich in polyunsaturated fat than on the high fat diet rich in saturated fat, despite a higher intake of plant sterols on the diet rich in polyunsaturated fat. In our first experiment the intake of cholesterol was almost identical in all four groups, and uniformly low. According to the study of Nestel *et al.* [16] this might have obscured an effect on bile acid excretion. However, it is not likely that this also explains the absence of a difference in neutral steroid excretion between the high-fat diets HIPUF and HISAT.

### *Replacement of carbohydrates by fat*

Studies on the effect of the proportion of fat in the diet on steroid excretion are rare. The few controlled studies with healthy subjects and regular diets that we are aware of are listed in the second part of Table 4.

Dietary fat elicits the excretion of bile acids into the duodenum, and therefore one might expect that high-fat diets cause an increased loss of bile acids with the faeces. Such an effect on faecal excretion, however, has not been consistently found.

Antonis & Bersohn did find an increased bile acid and neutral steroid excretion when carbohydrates were replaced by polyunsaturated fat, and the reverse was found when polyunsaturated fat was replaced by carbohydrates. However, replacement of carbohydrates by butter led to a decrease of bile acid excretion and a small increase in neutral steroid excretion. Surprisingly, in another phase of the same experiment replacement of butter by carbohydrates

also caused a decreased excretion of bile acids. Cummings *et al.* found an increase in bile acid excretion when the proportion of saturated fat in the diet was increased. However, the intake of cholesterol was also increased.

Our own results do not conform with the suggestion that the faecal excretion of bile acids is proportional to the amount of fat in the diet, be it saturated or polyunsaturated fat. In experiment 1 the bile acid

**Table 4.** Daily faecal steroid excretion in subjects on diets with different amounts and/or types of fat. Data are given only for controlled studies of subjects with normal lipid metabolism consuming natural solid diets.

Authors	No. of participants	Duration	Diet		Faecal excretion			
			Amount of fat (% of energy)	Type of fat	Cholesterol (mg/day)	Plant sterols (mg/day)	Bile acids (mg/day)	Neutral steroids (mg/day)
<b>Part I. Studies on the type of dietary fat</b>								
Antonis & Behrson [19]	9	29 weeks	40	Butter	—*	—*	286	169
	6	8 weeks	40	Sunflower seed oil	—*	—*	524	399
	8	29 weeks	40	Sunflower seed oil	—*	—*	640	463
	5	8 weeks	40	Butter	—*	—*	427	261
Eneroth <i>et al.</i> [22]	6	4 weeks	40–50	Butter	271	0	—*	527
	6	4 weeks	40–50	Corn oil	141	447	—*	614
Moore <i>et al.</i> [20]	5	16 days	40	Butter	347	420	473	486
	5	16 days	40	Safflower oil	197	720	564	580
Nestel <i>et al.</i> [21]	5	4 weeks	45–50	Ruminant saturated	±500	—*	196	607
	5	4 weeks	45–50	Ruminant polyunsaturated	±500	—*	271	681
Nestel <i>et al.</i> [16]	5	19 days	42–44	Dairy fat, beef, lamb, high cholesterol, saturated	789	—*	281	806
	5	23 days	42–44	High cholesterol, polyunsaturated	796	—*	381	1039
	5	23 days	42–44	Low cholesterol, saturated	493	—*	297	674
	5	23 days	42–44	Low cholesterol, polyunsaturated	520	—*	314	847
Nestel <i>et al.</i> [17]	3	28 days	44	Pork saturated	549	—*	231	830
	3	28 days	44	Pork polyunsaturated	535	—*	239	1096
<b>Part II. Studies on the amount of dietary fat</b>								
Antonis & Behrson [19]	9	39 weeks	15	Prison diet	—*	—*	450	184
	9	22 weeks	40	Butter	—*	—*	353	294
	5	8 weeks	40	Butter	—*	—*	427	261
	5	17 weeks	15	Prison diet	—*	—*	351	379
	8	39 weeks	15	Prison diet	—*	—*	539	223
	8	22 weeks	40	Sunflower seed oil	—*	—*	577	636
	6	8 weeks	40	Sunflower seed oil	—*	—*	524	399
	6	17 weeks	15	Prison diet	—*	—*	321	270
Whyte <i>et al.</i> [27]	2	2 weeks	68	Safflower oil	513	423	397	859
	2	2 weeks	10	High sucrose diet	502	—*	513	848
	2	2 weeks	69	Safflower oil	219	383	266	555
	2	2 weeks	5	High sucrose diet	233	375	301	336
	2	2 weeks	10	High sucrose diet	652	20	425	690
	2	2 weeks	68	Saturated fat (lard)	652	20	238	761
Cummings <i>et al.</i> [28]	6	4 weeks	21	Saturated fat	466	—*	140	—*
	6	4 weeks	48	Saturated fat	732	—*	320	—*

\* Data not given.

excretion went up instead of down on the low-fat high-carbohydrate diet, although the effect did not reach the customary requirements for statistical significance (Table 2). In experiment 2 the faecal bile acid excretion in the first week on the low-fat high-carbohydrate diet was about 15% lower than that after 13 weeks on this diet. This is again compatible with a higher steady state excretion of bile acids on the low-fat diet. An alternative explanation is that the replacement of polyunsaturated fat by starch caused an immediate but temporary drop in the bile acid excretion: our data do not allow a choice between these alternatives.

If total serum cholesterol changes are related to changes in steroid excretion one would expect a negative correlation between the two: if serum cholesterol goes up, excretion should go down. This is not what we observed. On the whole, the relation between changes in faecal steroid excretion and changes in serum cholesterol concentration within one diet group was weak in our experiments (Table 3). Thus, differences at the intestinal level apparently do not completely explain the different ways in which serum cholesterol levels of individuals reacted to a given diet.

#### *Faecal steroid concentration and composition*

Populations consuming low-fat diets were reported to have lower concentrations of steroids in their faeces than affluent populations [23, 24]. This is explained most easily by assuming that these low-fat diets have a high content of cereal fibre. This is known to increase faecal bulk, thus diminishing the steroid concentration. In our experiment fibre intake was kept constant within each experiment over the whole range of fat intake; as a result, intestinal transit time, faecal mass and faecal steroid concentration also remained constant. The higher absolute level of faecal wet weight in experiment 2 is explained by the higher overall intake of dietary fibre as compared with experiment 1.

Populations consuming different diets have been found to differ in the extent of bacterial degradation of their faecal steroids [25]. However, in controlled experiments consistent effects of diet on the ratio of primary to secondary bile acids or neutral steroids have not been observed [26]. This agrees with our own results. It could be that much longer periods are necessary to establish a new equilibrium in the gut, or alternatively, that the differences observed between different groups of people are caused by differences other than their dietary habits.

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