WHOLE-BODY CHOLESTEROL SYNTHESIS AND FECAL NEUTRAL STEROID AND BILE ACID EXCRETION IN MAN ON DIETS HIGH OR LOW IN LINOLEIC ACID

Jan F.C. Glatz, Monique A.M. Berns and Martijn B. Katan

Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen (The Netherlands)

ABSTRACT

In a strictly controlled trial with 23 healthy humans 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 by the sterol balance method. Subjects consumed a mixed natural diet, of which the total fat content was kept constant at 45 energy% but that of polyunsaturated fatty acids (mainly linoleic acid) was 21% for the first three weeks, and then changed to 5% for the following three weeks; the P/S ratios were 1.9 and 0.2, respectively. Cholesterol intake was 41 mg/MJ (about 500 mg/day) during both periods. The contents of cholesterol in the diets and of neutral steroids and bile acids in the feces were analysed by capillary gas-liquid chromatography. The low-P/S diet caused an increase of 25% in serum cholesterol concentration, but the fecal excretion of bile acids and of cholesterol and its microbial metabolites, and hence the rate of cholesterol synthesis, remained unchanged. The steroid composition of the feces was not affected by the change of diets either. These observations indicate that the mechanism by which dietary polyunsaturated fatty acids lower serum cholesterol levels in man does not relate to changes in endogenous synthesis of cholesterol or fecal excretion of steroids.

INTRODUCTION

The concentration of serum cholesterol is markedly affected by the type of dietary fatty acid. In healthy humans replacement of saturated by polyunsaturated fatty acids was found to lower serum cholesterol levels by up to 2 mmol/l (e.g. 1-4). Several mechanisms have been proposed to explain this hypocholesterolemic effect of polyunsaturated fatty acids (5). These include (i) a decreased production of lipoproteins due to a less efficient incorporation into VLDL-triacylglycerols of polyunsaturated fatty acids when compared with saturated fatty acids , and (ii) an increased LDL catabolism, possibly mediated by an altered LDL structure, followed by an increased steroid excretion. An enhanced fecal excretion of neutral steroids and bile acids in subjects changing to a diet enriched in polyunsaturated fatty acids, has been reported (6, 7), but could not be confirmed in dietary trials carried out at this Department (8).

In the present study we determined the effect of dietary fatty acid composition on the rate of production of echangeable 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 constant total

Cholesterol Metabolism in Health and Disease: Studies in the Netherlands, edited by A. C. Beynen, M. J. H. Geelen, M. B. Katan, and J. A. Schouten. Ponsen & Looijen, Wageningen 1985

fat and cholesterol intake. Possible effects of the type of fat on fecal steroid composition were also examined.

METHODS

Subjects

All 23 subjects were healthy volunteers who had earlier participated in trials involving dietary cholesterol (9, 10). Details on he earlier selection of the subjects and their baseline characteristics are described elsewhere (10), and can be taken as representative for the present group. Informed consent was obtained from all participants.

Experimental design

Subjects received a mixed natural diet high in polyunsaturated and low insaturated fatty acids (high P/S diet) for 3 weeks and a diet low in polyunsaturated and high in saturated fatty acids (low P/S diet) for another 3 weeks (Fig. 1).

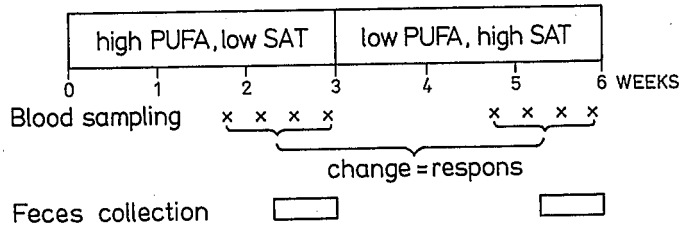

Fig.1. Experimental design. PUFA, polyunsaturated fatty acids (mainly linoleic acid). SAT, saturated fatty acids. Number of subjects, n = 23.

The diets were composed of natural foodstuffs. Actual nutrient composition of the diets was measured by analyses of duplicate portions for one imaginary person of average energy intake on each diet, supplemented with data from the Dutch nutrient data bank "UCV" (Table I).
Blood was sampled in order to establish the effect of dietary fatty acid composition on serum total and HDL cholesterol; results of this part of the study are described elsewhere (Katan, this volume). Feces were collected

Table I. Composition of the diets in the fatty acid experiment.

Nutrient	Unit	High P/S[1] Diet	Low P/S[1] Diet
Protein	% of energy	13	14
Fat	% of energy	45	44
Saturated	% of energy	11	23
Polyunsaturated	% of energy	21	5
Carbohydrates	% of energy	40	39
Alcohol	% of energy	2	2
Cholesterol[2]	mg/MJ	42	40

[1] P/S, Polyunsaturated/saturated fatty acid ratio.
[2] Mean daily intake was about 500 mg.

during the last 5 days of each period. Radio-opaque markers were swallowed by the subjects (20/day), and the recovery of these was used to correct for variations in fecal flow. The stools were frozen as soon as possible after being passed and stored at -20°C. At the end of the dietary trial the fecal collections of each subject and period were combined, homogenized, freeze-dried and again stored at -20 C.

Analysis of diets and feces and determination of whole-body cholesterol synthesis

Cholesterol and plant steroid content of the consumed food (11) and neutral steroid and bile acid contents of the feces (Glatz et al., this volume) were determined by capillary gas-liquid chromatography. Feces of each subject were analysed in duplicate. The inter-assay variability (coefficient of variation) was about 2.5% for dietary cholesterol and less than 5% for the total amount of excreted neutral and acidic steroids. 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 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

When compared with the high P/S diet, the low P/S diet caused an increase of 25% in total serum cholesterol and of 10% in HDL cholesterol (Mn-heparin soluble). Changes of similar magnitude have been found by many other investigators.

Fecal excretion of neutral steroids and bile acids

For both diets large differences among individuals were observed in the total amounts of neutral and acidic steroids excreted (Table II). However, the group means of the excretion of neutral steroids, bile acids and of the sum

Table II. Effect of dietary fatty acid composition on fecal excretion of neutral steroids and bile acids.

Dietary fatty acid levels			Excretion of steroids[1] (mmol/day)		
Polyunsaturated	Saturated	P/S ratio	Neutral steroids[2]	Bile acids	Sum
High	Low	1.9	1.91 ± 0.68 (0.9 – 3.6)	0.74 ± 0.43 (0.2 – 1.8)	2.65 ± 0.99 (1.1 – 5.4)
Low	High	0.2	2.13 ± 0.63 (0.8 – 3.5)	0.77 ± 0.44 (0.2 – 2.0)	2.86 ± 0.90 (1.1 – 4.7)

[1] Corrected for variations in fecal flow. Values represent means ± S.D. of 23 subjects; ranges are given within parentheses.
[2] Cholesterol and its bacterial metabolites.

Table III. Effect of the type of dietary fatty acids on the percentage composition of fecal neutral steroids and of bile acids[1].

Fecal steroid	High P/S diet[2]	Low P/S diet[2]
	(mol/100 mol)	
Neutral		
Cholesterol	15.0 ± 8.7	13.2 ± 10.3
Coprostanol	73.1 ± 9.0	79.1 ± 10.4
Other neutral steroids[3]	11.9 ± 6.7	7.7 ± 5.9
Acidic		
Cholic + chenodeoxycholic acid	3.1 ± 2.7	4.6 ± 6.5
Deoxycholic acid[4]	43.5 ± 11.1	45.3 ± 7.1
Lithocholic acid[5]	39.6 ± 6.0	38.5 ± 5.7
Other bile acids[5]	13.8 ± 5.7	11.6 ± 6.9

1 Values represent the % of the total amount of neutral steroids or of bile acids.
2 P/S, Polyunsaturated/saturated fatty acid ratio.
3 Coprostanol, cholestanol and epicoprostanol.
4 Sum of the 3α- and 3β-isomers.
5 Mainly 12-keto lithocholic and 12-keto isolithocholic acid.

were similar for both experimental periods. In addition, the mean steroid composition of the feces was not different between the high- and low-P/S diet (Table III). Coprostanol was the major neutral steroid present. The primary bile acids cholic and chenodeoxycholic acid made up only 3-5% of the amount of acidic steroids. This latter fraction consisted mainly of deoxycholic and lithocholic acid.Thus, changing the dietary fatty acid composition from P/S ratio 1.9 to 0.2 at a constant fatty acid intake of 45 en% did not affect the fecal excretion of bile acids and of cholesterol and its microbial conversion products.

Whole-body cholesterol synthesis

For each participant the dietary intake of cholesterol was kept constant during the two experimental periods. Among the subjects cholesterol intake ranged from 1 to 1.5 mmol/day, depending on their energy intake. The mean daily fecal excretion of cholesterol and its microbial metabolites ranged from 1 to 5 mmol (Table II). Therefore, both the magnitude of and individual differences in the rate of whole-body cholesterol synthesis are largely determined by the fecal output. Since changing the diet from a high P/S to a low P/S ratio did not affect the fecal steroid excretion (Table II), the rate of cholesterol synthesis also remained unchanged. The mean values for cholesterol synthesis were 1.40 ± 0.79 mmol/day for the high P/S diet and 1.60 ± 0.70 mmol/day for the low P/S diet. The scattergram given in Figure 2 shows that individual values were remarkably constant from one dietary period to another.

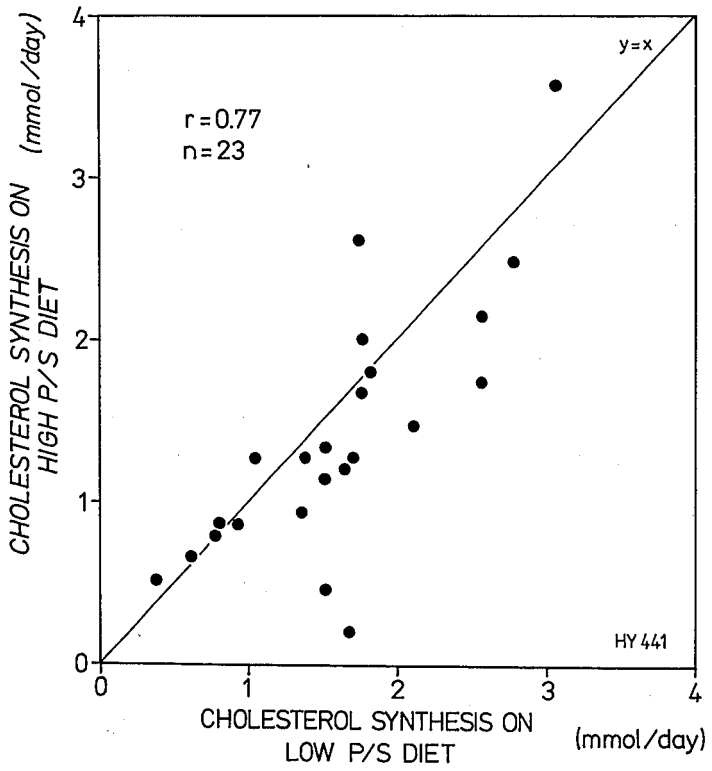

Fig. 2. Relationship between the rate of whole-body cholesterol synthesis on the high-P/S and the low-P/S diet, as measured with the sterol balance method.

DISCUSSION

In earlier dietary studies employing a different design (8) we already observed that the fecal excretion of neutral steroids and bile acids was not significantly influenced by the type of dietary fatty acids. The present results confirm this observation and also demonstrate that the bacterial conversion of steroids in the colon is not affected by replacement of polyunsaturated by saturated fatty acids either. Since the cholesterol intake was kept constant, the rate of whole-body cholesterol synthesis, as determined by the sterol balance method, was also not influenced by the dietary intervention. On the basis of these findings it seems unlikely that a decreased influx of newly synthesized cholesterol or an increased efflux of cholesterol and bile acids from the enterohepatic circulation is the primary cause, or even a secondary feature, of the hypocholesterolemic effect of polyunsaturated fatty acids.

An explanation for the marked effect of the type of fat on the level of serum cholesterol in man must thus be looked for elsewhere. Recently, Beynen and Katan (12) have proposed that polyunsaturated fatty acids, when compared with saturated fatty acids, are preferentially converted by the liver into ketone

bodies, instead of VLDL-triacylglycerols and thus would cause a lowering of serum lipoproteins. Alternatively, saturated fatty acids might cause a "slower" clearance of LDL from plasma by liver receptors, resulting in an expansion of the plasma cholesterol pool. As cholesterol synthesis was not increased, this expansion should be accompanied by either a reduction of the entero-hepatic cholesterol pool or a transient decrease in fecal excretion of cholesterol and its metabolites during the first few days of the low-P/S, high-saturate diet period. Studies in suitable animals models might allow the detailed and prolonged measurements necessary to decide between these various alternatives.

ACKNOWLEDGEMENTS

We thank the volunteers for their excellent cooperation, Frans J.M. Schouten, Geertruida den Engelsman, Ans E.M.F. Soffers and Joke C. Barendse-Van Leeuwen for expert analytical help, Jeanne H.M. de Vries for dietetic assistance, Martie van Essen for help with the data processing and Marcel B.A. van Leuteren for typing the manuscript. This study was supported by the Netherlands Heart Foundation, grants no. 26.003 and 32.015 and an Established Investigatorship to M.B. Katan.

REFERENCES

1. Groen, J., Tjong, K., Kamminga, C.E. and Willebrands, A.F. The influence of nutrition, individuality and some other factors, including various forms of stress, on the serum cholesterol; an experiment of nine months duration in 60 normal human volunteers. Voeding 13, 556 (1952).
2. Keys, A., Anderson, J.T. and Grande, F. Prediction of serum-cholesterol responses of man to changes in fats in the diet. Lancet 2, 959 (1957).
3. Hegsted, D.M., McGandy, R.M., Myers, M.L. and Stare, F.J. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17, 281 (1965).
4. Vergroesen, A.J. Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. Proc. Nutr. Soc. 31, 323 (1972).
5. Paul, R., Ramesha, C.S. and Ganguly, J. On the mechanism of hypocholesterolemic effects of polyunsaturated lipids. Adv. Lipid Res. 17, 155 (1980).
6. Woods, P.D.S., Shioda, R. and Kinsell, L.W. Dietary regulation of cholesterol metabolism. Lancet ii, 604 (1966).
7. Moore, R.B., Anderson, J.T., Taylor, H.L., Keys, A. and Frantz, J.D. Effect of dietary fat on the faecal excretion of cholesterol and its degradation products in man. J. Clin. Invest. 47, 1517 (1968).
8. Brussaard, J.H., Katan, M.B. and Hautvast, J.G.A.J. Faecal excretion of bile acids and neutral steroids on diets differing in type and amount of dietary fat in young healthy persons. Eur. J. Clin. Invest. 13, 115 (1983).
9. Katan, M.B. and Beynen, A.C. Hyper-response to dietary cholesterol in man. Lancet 1, 1213 (1983).
10. Katan, M.B., Beynen, A.C., De Vries, J.H.M. and Nobels, A. Existence of consistent hyper- and hyporesponders to dietary cholesterol in man. Am. J. Epidemiol. (1985), submitted.
11. Jonker, D., Van der Hoek, G.D., Glatz, J.F.C., Homan, C., Posthumus, M.A. and Katan, M.B. Combined determination of free, esterified and glycosilated plant sterols in foods. Lipids, submitted.
12. Beynen, A.C. and Katan, M.B. Why do polyunsaturated fatty acids lower serum cholesterol? Am. J. Clin. Nutr. (1985), in press.