Br. J. Nutr. (1980), 43, 101

The effect of dietary fibre on bile acid metabolism in rats

BY W. G. BRYDON, K. TADESSE AND M. A. EASTWOOD

Wolfson Laboratories, Gastro-Intestinal Unit, Department of Medicine, Western General Hospital, Edinburgh EH4 2XU

AND MARGARET E. LAWSON

Department of Biochemistry, University of Edinburgh, Edinburgh EH8 9AG

(*Received* 18 *June* 1979 – *Accepted* 13 *August* 1979)

1. Forty-eight male rats were fed sequentially for 14 d periods on diets containing different fibre contents.

2. One of the high-fibre diets was a commercial pelleted diet. The other was a low-fibre diet supplemented with 200 g wheat bran/kg.

3. At the end of each feeding period eight rats were killed. Liver microsomal cholesterol 7α-hydroxylase (*EC* 1.14.1.–) activity and bile acid content of small intestine and colon were determined.

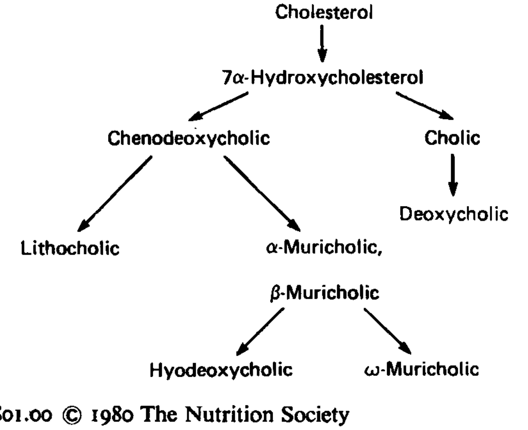
4. The different diets did not significantly alter the total intestinal bile acids, but affected the distribution and qualitative pattern in the colon and small intestine.

5. On the high-fibre diets deoxycholate, and hyodeoxycholate tended to be increased.

6. On the low-fibre diets the α-, β- and ω-muricholic acids tended to be increased.

7. Liver microsomal cholesterol 7α-hydroxylase activity was lower in rats fed on the low-fibre and bran-supplemented low-fibre diets compared with that in rats fed on the commercial pelleted diet.

The effect of dietary fibre on rat bile acid metabolism has been investigated by several groups. The addition of bran to a semi-synthetic diet resulted in an increase in total bile acids in the small intestine of the rat (Eastwood & Boyd, 1967). Gustaffson & Norman (1969) using labelled cholate added to a semi-synthetic, commercial pelleted diet, and a semi-synthetic diet supplemented with 200 g cellulose/kg, fed to rats showed that cholate and certain bile acid metabolites were increased in the small intestine on the commercial pelleted diet, but the addition of cellulose to the semi-synthetic diet had no effect on the small intestinal cholate pool. The total intestinal bile acid pool has been measured in Wistar rats fed on commercial pelleted and semi-synthetic diets (Fisher *et al.* 1976). These authors showed that the total bile acid pool was increased on the commercial pelleted diet, but over-all there was no qualitative change in bile acid pattern. Sacquet *et al.* (1977) demonstrated that re-infecting the colon of germ free rats with heat-resistant bacteria isolated from faeces led to ω-muricholic acid emerging as the major colonic bile acid. The metabolism of bile acids in the rat is as follows:

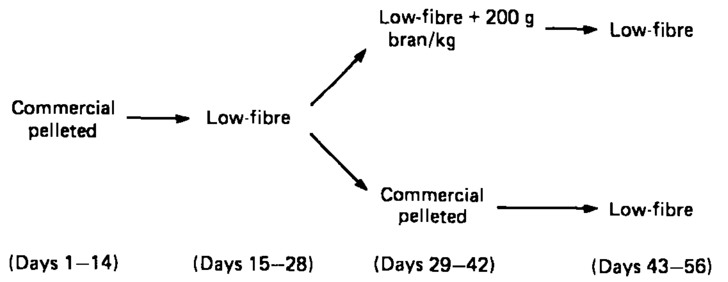


In this study we supplemented a semi-synthetic, low-fibre diet with 200 g wheat bran/kg and compared the pool size and pattern of bile acids in the small intestine and colon separately, with rats fed on a commercial pelleted or semi-synthetic diet alone.

The rate limiting enzyme in the conversion of cholesterol to bile acids in the liver is the microsomal cholesterol 7α-hydroxylase (*EC* 1.14.1.–) (Danielsson *et al.* 1967). This oxygenase is located in the endoplasmic reticulum and is sensitive to changes in the enterohepatic circulation of bile acids. Liver microsomal cholesterol 7α-hydroxylase activity was measured during the different feeding regimens.

MATERIALS AND METHODS

Forty-eight mature male rats of Wistar strain were used in this study. The animals weighed approximately 200 g at the start of the experiment, and received dietary regimens of different fibre composition in a specific sequence, each feeding period lasting two weeks. The feeding sequence was as follows:



Details of the diets given are shown in Table 1. Eight rats were killed at the end of each period. The contents of the small intestine and colon were washed out separately with distilled water, freeze-dried and weighed. A segment of liver was removed, weighed and stored immediately at −20° for liver microsomal cholesterol 7α-hydroxylase assay (Mitton *et al.* 1971). Bile acids were measured according to Brydon *et al.* (1979), methyl acetate derivatives being separated by gas–liquid chromatography on 30 g SE 30/kg. Recoveries of bile acids using this procedure were determined by analyses of intestinal contents for bile acids before and after the addition of known amounts of bile acids. The precision of the method was determined by the analysis of the same sample in two separate analytical batches.

RESULTS

Recovery of bile acids from intestinal contents. Values obtained (%) were lithocolic 98, deoxycholic 100, chenodeoxycholic acid 97, hyodeoxycholic acid 100, cholic acid 98, Na taurocholate 77, Na glycocholate 82.

Precision (*bile acids*). The coefficients of variation of results for total bile acids based on the repeat analysis of samples were small intestine 7·9 (*n* 10, range 16–58 μmol) colon 12·9 (*n* 10, range 7–63 μmol).

Bile acids in small intestine and colon during different dietary regimes. The results are shown in Tables 2 and 3. The total intestinal bile acids expressed either per rat or per kg body-weight were not significantly altered by diets of different fibre content. Total bile acids in the small intestine were significantly higher on the pelleted diets and bran supplemented diet than on the low-fibre diet ($P < 0·001$). Conversely, total bile acids in the colon were significantly lower during the high-fibre diet periods ($P < 0·001$).

The pattern of bile acids in the small intestine showed less hyodeoxycholic acid and more ω-muricholic acid during the low-fibre feeding periods ($P < 0·005$).

Table 1. *Nutritional composition (g/kg edible substances) of different dietary regimens*

	Diet		
Nutrient	Commercial pelleted	Low-fibre	Low-fibre + bran 200 g/kg*
Carbohydrate	600	646	610
Protein	190	202	191
Fat	20	18	24
Acid detergent fibre†	59	21	41
Lignin	12	4	10
Moisture	100	95	97
Others	30	18	37

* American Association of Cereal Chemists standardized wheat bran.

† Van Soest (1963).

Table 2. *Composition of bile acids in the small intestine and dried weight of small intestinal contents of rats given diets with different fibre content*

(Mean values and 1 standard deviation for eight rats/group)

	Dietary sequence*											
	Commercial pelleted		Low-fibre		Low-fibre + bran		Low-fibre		Commercial pelleted		Low-fibre	
Day of experiment . . .	1–14		15–28		29–42		43–56		29–42		43–56	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bile acids:												
(μmol/rat)	39·0	9·8	18·8	7·9	45·0	9·7	28·7	9·9	51·3	13·9	29·5	7·5
μmol/kg body-weight	197	38	72	25	147	24	82	22	179	67	88	24
Bile acids (mmol/mol):												
Cholic	484	33	452	104	499	50	444	68	464	29	451	73
Chenodeoxycholic	97	27	75	31	75	12	51	15	95	27	43	19
Deoxycholic	15	2	14	4	11	5	14	6	18	6	26	15
Hyodeoxycholic	59	18	5	7	77	34	30	20	67	15	20	6
α-Muricholic	117	29	165	44	113	15	129	35	115	35	125	40
β-Muricholic	206	43	212	37	193	49	222	33	210	45	230	31
ω-Muricholic	23	10	79	23	32	11	111	33	31	19	104	26
Small intestinal dried contents (g)	0·78	0·13	0·18	0·07	0·57	0·10	0·50	0·15	1·14	0·38	0·45	0·07

* For details of diet, see Table 1.

In the colon, on changing to the low-fibre diet on the first occasion, there was a significant increase in the relative proportions of cholic, chenodeoxycholic and all muricholic acids, and a significant decrease in the relative amounts of lithocholic and hyodeoxycholic acids. On the second change from the high-fibre (pelleted diet) to the low-fibre diet, the changes in muricholic acid remained significant, with an even greater proportion of ω-muricholic acid being present. When changing from the bran-supplemented diet to the low fibre diet, qualitative changes were less marked, only ω-muricholic acid being significantly different ($P < 0·05$).

Liver microsomal cholesterol 7α-hydroxylase activity. The results are shown in Table 4.

Table 3. *Composition of bile acids in the colon and dried weight of colonic contents of rats given diets with different fibre content*

(Mean values and 1 standard deviation for eight rats/group)

Dietary sequence*	Commercial pelleted		Low-fibre		Low-fibre+bran		Low-fibre		Commercial pelleted		Low-fibre	
Day of experiment ...	1–14		15–28		29–42		43–56		29–42		43–56	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bile acids:												
(μmol/rat)	7·8	4·2	39·8	18·0	13·1	8·8	24·0	7·8	14·2	8·0	30·7	11·9
μmol/kg body-weight	38	20	152	61	42	26	70	19	43	24	95	44
Bile acids (mmol/mol):												
Cholic	79	21	163	53	107	46	62	39	138	79	51	25
Chenodeoxycholic	13	10	24	8	27	17	16	18	27	21	9	4
Deoxycholic	217	24	35	13	95	39	102	28	187	50	107	32
Lithocholic	116	38	11	7	36	26	41	40	105	49	20	9
Hyodeoxycholic	268	52	25	6	137	88	84	91	259	36	32	22
α-Muricholic	27	16	114	20	52	31	47	13	19	13	62	16
β-Muricholic	104	48	273	67	184	94	140	73	92	35	271	93
ω-Muricholic	196	92	355	101	362	74	507	136	171	91	453	86
Colonic dried contents (g)	0·98	0·25	1·28	0·30	1·52	0·50	1·65	0·41	1·60	0·73	1·57	0·41

* For details of diet, see Table 1.

Table 4. *Liver microsomal cholesterol 7α-hydroxylase activity (% conversion of ^{14}C cholesterol/60 mins) in rats given diets with different fibre contents*

(Mean values and 1 standard deviation; no. of rats/group given in parentheses)

Dietary sequence ...	Commercial pelleted	Low-fibre	Low-fibre+bran	Low-fibre	Commercial pelleted	Low-fibre
Day of experiment	1–14	15–28	29–42	43–56	29–42	43–56
	4·9±0·5 (8)	2·7±1·2 (8)	3·1±0·9 (4)	3·5±0·2 (4)	4·3±1·2 (4)	3·6±0·6 (4)

Cholesterol 7α-hydroxylase activity was significantly reduced on changing from the pelleted to the low-fibre diet, and also significantly increased on returning to the pelleted diet ($P < 0·05$). There was no significant increase on supplementing the low-fibre diet with bran.

DISCUSSION

The type and distribution of bile acids along the intestinal tract is a complex function of diet, colonic bacteria, enterohepatic circulation, and intestinal motility.

In this study the total intestinal bile acid pool was not altered by the different dietary regimens, but the distribution of bile acids between small intestine and colon was changed. The qualitative pattern of bile acids in the colon was altered such that muricholic acids increased on the low-fibre diet.

Gustaffson & Norman (1969), using rats fed on commercial pelleted, semi-synthetic and semi-synthetic plus cellulose diets, showed a decrease in the cholate pool in the small intestine on both semi-synthetic and cellulose supplemented semi-synthetic diets compared with commercial pelleted diets. Fisher *et al.* (1976) and Sacquet *et al.* (1976) have observed an increased total bile acid pool on changing from the semi-synthetic diet to the pelleted diet. Eastwood & Boyd (1967) showed that the addition of bran to a semi-synthetic diet increased the bile acid pool in the small intestine. Thus a smaller bile acid pool in the small intestine on the low-fibre diet is a consistent finding. This may be due to the differences in absorption from the small intestine during the different feeding regimens. The bile acids may be trapped within the fibre matrix on the high-fibre diets.

In this study, the bile acid content of the colon on the high-fibre diets was consistently lower than that on low-fibre diets. Gustaffson & Norman (1969) using carmine indicator, have shown transit time to be increased on a low-fibre diet. The increase in colonic transit time with a low-fibre diet may, in part, account for the larger pool of bile acids in the colon.

Both diet and bacteria have been shown to influence the qualitative pattern of colonic bile acids. Sacquet *et al.* (1977) have shown that the re-introduction of a selective group of bacteria into the colon of the germ free rat can lead to ω-muricholic acid becoming the major faecal bile acid. Wostmann *et al.* (1977) have shown that 'steam-sterilized' lactose-containing casein starch diets fed to rats greatly increases the ω-muricholic:hyodeoxycholic acid value in faeces. In the present experiment the muricholic acid : hyodeoxycholic acid value in the colon was greatly increased when changing from a pelleted to a low-fibre diet. This was partially reversed by adding bran to a low-fibre diet; however the muricholic acid content of the bran-supplemented, low-fibre diet group is still much higher than in pellet-fed rats. This may have been due to differences in the total fibre content of the two diets (see Table 1).

Uchida *et al.* (1978) have demonstrated that when the relative proportions of each bile acid in the intestinal contents and intestinal walls are compared, α-muricholic, β-muricholic and cholic acids are higher in the walls than in the contents. This suggests greater selective absorption of these acids from colon and small intestine.

It has been demonstrated here that the distribution of bile acids along the intestine is altered by the fibre content of the diet. This effect may be due to trapping of bile acids in the fibre matrix and alterations in the intestinal transit time.

A simple hypothesis is presented: on a pelleted diet, α- and β-muricholic acids are converted to both hyodeoxycholic and ω-muricholic acids in the colon, and there is little absorption of these bile acids from the caecum. On the low-fibre diet conversion to hyodeoxycholic acid is greatly reduced but conversion to ω-muricholic acid continues. Since one of the metabolic pathways has been blocked, and colonic transit time increased due to fibre reduction, α- and β-muricholic acids accumulate, and recirculate from the caecum to the liver. These acids may inhibit over-all bile acid synthesis.

The addition of bran to the semi-synthetic diet caused an increase in the small intestinal pool and a decrease in the colonic pool but had little effect on the pattern of bile acids in the colon. This finding suggests that the effect of bran on bile acids is mainly of a physical nature, altering the distribution of bile acids along the intestine.

The authors wish to thank Professor G. S. Boyd for helpful discussions in the preparation of this paper, Vitamins Inc., Chicago and American Association of Cereal Chemists for supplying bran, and the World Health Organisation for assistance with a fellowship (K.T.).

REFERENCES

Brydon, W. G., Tadesse, K., Smith, D. M. & Eastwood, M. A. (1979). *J. Chromatog.* **172**, 450.
Danielsson, H., Einarsson, K. & Johansson, G. (1967). *Eur. J. Biochem.* **2**, 44.
Eastwood, M. A. & Boyd, G. S. (1967). *Biochim. biophys. Acta* **152**, 159.
Fisher, M. A., Kakis, G. & Youseff, I. M. (1976). *Lipids* **11**, 93.
Gustaffson, B. E. & Norman, A. (1969). *Br. J. Nutr.* **23**, 429.

Mitton, J. R., Scholan, N. A. & Boyd, G. S. (1971). *Eur. J. Biochem.* **20**, 569.
Sacquet, E., Leprince, C., Riottot, M., Mejean, C. & Leglise, P. (1977). *C.r. hebd. Séanc. Acad. Sci., Paris*, **284**, 557.
Sacquet, E., Mejean, C., Leprince, C., Riottot, M. & Raibaud, P. (1976). *Annls Nutr. Aliment.* **30**, 603.
Uchida, K., Okuno, I., Takase, H., Nomura, Y., Kadowaki, M. & Takeuchi, N. (1978). *Lipids* **13**, 42.
Van Soest, P. J. (1963). *J. Assoc. Off. Agric. Chem.* **46**, 829.
Wostmann, B. S., Beaver, M., Chang, I. & Madsen, D. (1977). *Am. J. clin. Nutr.* **30**, 1999.

Printed in Great Britain