Volume 284, number 1, 63–65 FEBS 09803 June 1991

ADONIS 001457939100512W

Apolipoprotein B gene expression in rat intestine

The effect of dietary fiber

Andrzej Mazur, Catherine Felgines, Fatiha Nassir, Dominique Bayle, Elyett Gueux, Christian Rémésy, Yves Rayssiguier and Philippe Cardot

Laboratoire des Maladies Métaboliques, Institut National de la Recherche Agronomique, Theix, 63122 Ceyrat, France

Received 10 April 1991

The effect of the dietary fiber on apo B mRNA level was studied in the intestine of rats that were fed either fiber-free or high-fiber (30% sugar-beet fiber) low-fat diets for 3 weeks. The fiber diet studied does not affect jejunal apo B mRNA levels but decreases the level of ileal apo B mRNA. In the rat cecum, in both fiber-free and fiber groups, we failed to detect the apo B mRNA. The test fiber diet feeding markedly increased fecal bile salt and cholesterol excretions. We suggest that dietary fiber can modify apo B expression in the intestine. The increased fecal bile salt excretion might be involved in such a modification.

Apolipoprotein B; mRNA level; Dietary fiber; Intestine; Bile salt

1. INTRODUCTION

The liver and the small intestine are major contributors of plasma lipoproteins. Both tissues synthesize apolipoprotein (apo) B as an obligate component of triglyceride-rich lipoproteins [1]. Scarce knowledge is available regarding the effects of dietary manipulations on apo B synthesis in the intestine. In previous studies it has been reported that intestinal apo B synthesis in the rat is unaltered by dietary triglyceride intake but is modified in response to biliary lipid availability [2–4]. These authors [2,3] demonstrated that bile diversion decreases intestinal apo B synthesis and that bile salt replacement was effective in a dose- and structure-dependent manner in re-expressing intestinal apo B synthesis after bile diversion. It was concluded that biliary lipid flux plays an important role in the regulation of intestinal apo B metabolism. This regulation appears independent in jejunal and ileal enterocytes.

It is well known that one of the mechanisms involved in the hypocholesterolemic effect of certain dietary fibers is neutral steroid and bile salt sequestration and in turn their increased fecal excretion [5]. Bile salts are known to interact with the metabolism of cholesterol and lipoproteins in several ways [6]. They influence the absorption of cholesterol and triglyceride by the intestine and may regulate the inflow of chylomicrons and intestinal VLDL into the circulation. This raises the interesting possibility that dietary fibers, which bind bile salts and enhance bile salt excretion, affect the regulation of the triglyceride-rich lipoprotein and apo B synthesis. In this context, the experiments reported here were designed to examine the effects of prolonged high-fiber, low-fat diet feeding on the intestinal apo B gene expression at the level of mRNA.

Correspondence address: A. Mazur, Laboratoire des Maladies Métaboliques, INRA, Theix, 63122 Ceyrat, France. Fax: (33) (73) 624450.

2. EXPERIMENTAL

Sugar-beet fiber preparation (75% dietary fiber: 21% cellulose, 33% hemicellulose, 19% pectin, 2% lignin) was obtained from Sofalia (Paris, France). Other dietary components were obtained from L. François (St. Maur, France) except mineral/vitamin mix, which was obtained from UAR (Villemoisson/Orge, France). Restriction enzymes, random primed DNA labeling kit and nick translation kit were purchased from Boehringer (Mannheim, Germany). [α-^{32}P]dATP (3000 Ci/mmol), nylon Highbond N$^+$ and Hyperfilm-βmax were purchased from Radiochemical Centre (Amersham, Bucks., UK). Purified hydroxysteroid dehydrogenase (EC 1.1.1.50) was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade.

Male Wistar rats weighing approximately 190 g were raised for 3 weeks on purified diets containing (as wt/wt) 20% casein, 2% corn oil, 7% mineral/vitamin mix, 41% fructose, and 30% wheat starch (fiber-free diet) or 30% sugar-beet fiber (fiber diet). Rats were housed in wire-bottomed cages in a temperature-controlled room (22°C) with the dark period from 20:00 h to 08:00 h. Food and water were allowed ad libitum during the dark period. Food was withdrawn at 08:00 h and the animals were restricted during the light period. During the last week of the experiment food intake was recorded and feces collected daily. Feces samples were stored at −20°C before being freeze-dried and pulverised. The experiments were routinely performed between 08:00 h and 10:00 h. At the end of the experimental period the body weights of rats fed a fiber diet were not significantly different from rats fed a fiber-free diet (282 ± 7 g vs 298 ± 8 g). Consumption of experimental diets was approximately the same in both groups studied (25.9 ± 1.1 g/day and 25.5 ± 1.2 g/day, for fiber and fiber-free diet fed rats, respectively). Fiber diet fed rats presented lower plasma

cholesterol concentrations than fiber-free diet fed animals (44 ± 2 mg/100 ml vs 64 ± 3 mg/100 ml). Studies of biliary secretion and sampling of mucosa were performed on separate groups of animals. Food intake, weight gain and plasma cholesterol concentrations were not different between the same diet groups in both experiments.

At the end of the experimental period the animals were anesthetized with sodium pentobarbital (40 mg/kg). After laparotomy, the proximal intestine (jejunum), distal intestine (ileum) and cecum were removed and rinsed with ice-cold 0.9% NaCl. The mucosa was scraped off and RNA extraction was performed immediately. Total cellular RNA was isolated from mucosal scrapings using the guanidinium/phenol/chloroform method according to Chomczynski and Sacchi [7]. RNA was quantitated by measuring the absorbance at 260 nm. Its integrity was systematically assessed by agarose-gel electrophoresis, and visualization of 18 S and 28 S ribosomal RNAs by ethidium bromide staining. Aliquots of total RNA were subjected to Northern blot analysis and quantification of mRNA content by dot blot analysis on nylon filters. Hybridization of immobilized RNA to rat apo B and mouse β-actin cDNA probes labeled with [α-^{32}P]dATP and washing conditions have been previously described [8,9]. The filters were blotted dry and autoradiography was performed with intensifying screens at −70°C. Quantification of the relative amounts of specific mRNA was performed by densitometric analysis of the hybridization signal by using a laser densitometer (Ultrascan XL, LKB, Sweden).

Rats were anesthetized with pentobarbital before the introduction of a PE 10 catheter into the bile duct. Animals were maintained at 37°C and bile samples were collected for 30 min in pre-weighed tubes. Samples were stored at −20°C until required for analysis. Bile flow was determined gravimetrically, assuming a density of 1 mg/ml. Following the bile collection, livers were excised and weighed.

Portions of the bile and feces samples were either extracted with ethanol for bile acids determination using 3-hydroxysteroid dehydrogenase [10] or with chloroform/methanol (2:1) for cholesterol and lipid phosphorous analysis [11,12].

Student's t-test [13] was used to assess differences between fiber-free and fiber diet fed groups or between intestinal segments.

3. RESULTS AND DISCUSSION

Northern-blot analysis of intestinal apo B mRNA size distribution showed a similar picture to that found in liver (Fig. 1). A polydisperse population was demonstrated with a distinct band at ~14 kb. It has been suggested by Davidson et al. [3] that this polydisperse population may result from partial degradation of high molecular weight apo B mRNA even though the RNA was shown to be intact by agarose gel electrophoresis and ethidium bromide staining.

The results in Table I indicate that the diet studied did not affect jejunal apo B mRNA level but decreased the level of ileal apo B mRNA. In the rat cecum, in both fiber-free and fiber groups, we failed to detect the apo B mRNA.

The fiber diet used did not affect bile flow nor biliary salt secretion; therefore, cholesterol and phospholipid secretions were increased compared to fiber-free diet fed animals (Table II). The fiber diet studied markedly increased fecal bile salt and cholesterol excretions (Table II).

It has recently been demonstrated, in biliary drainage and bile restoration experiments, that bile salts can modulate apo B synthesis in rat intestine [2,3]. However, these previous short-term experiments carried out on bile diverted rats failed to demonstrate the modifications in apo B mRNA levels despite the marked effect on apo B synthesis. In the present study carried out on the rats that were fed a fiber diet for 3 weeks we observe a decrease in the apo B mRNA in the ileal mucosa. In this experiment we have shown that the dietary fiber used markedly increases fecal bile salt excretion. Thus it may be hypothesized that the decrease in the intestinal apo B synthesis after biliary diversion [2,3] and the decrease in the ileal apo B mRNA level shown in this work may have a similar basis. It is unlikely that the observed decrease in the apo B mRNA in the ileum was substantially and directly influenced by the modification of lipid absorption since the low-fat diets were used. Furthermore, when a low-fat diet is fed to rats, fat absorption is generally thought to be completed in the jejunum. Moreover, it has been shown that dietary lipids have no significant effect on apo B synthesis in the intestine [2,4]. Apparent increases in apolipoprotein secretion following lipid feeding may reflect mobilization of apolipoprotein from the intracellular pool rather than alterations in apolipoprotein synthesis [4].

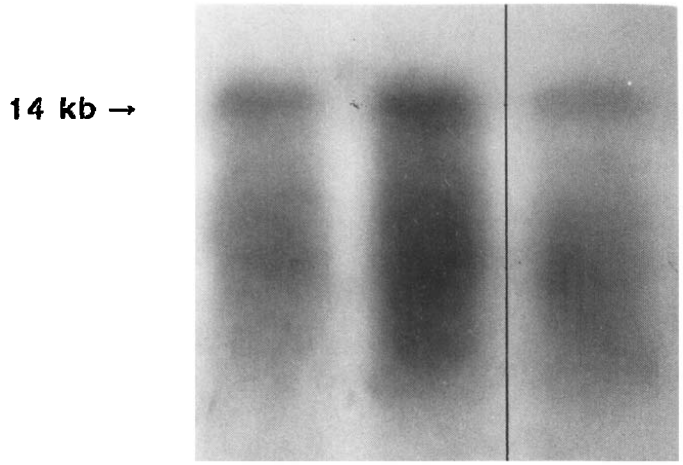

Fig. 1. Northern blot of intestinal and hepatic apo B mRNA. RNAs were hybridized to apo B probe as described in section 2.

The mechanisms by which bile salts affect apo B synthesis are not well known, however they may include a direct effect of bile salt uptake or an effect mediated by facilitated uptake of other lumenal lipid components [2,3]. The work of Davidson et al. [3] suggests that lumenal fatty acid uptake is a key regulatory event in mediating re-expression of intestinal apo B synthesis and that lumenal phospholipid flux may play an important role in the above mechanism. Biliary lipid flux may maintain threshold levels of microsomal triglyceride and in turn mediate apo B synthesis.

The ileum plays an important role in the uptake of bile salts. Also, in the rat the ileum is relatively defective

Table I

Effect of experimental diets on intestinal apo B mRNA levels

Diet	Fiber-free	Sugar-beet fiber	*P* value
Jejunum	1.00 ± 0.07	1.11 ± 0.08	>0.05
Ileum	0.97 ± 0.09	0.72 ± 0.05	<0.025
P value	>0.05	<0.001	

$\bar{x}$ ± SEM; 10–12 determinations per mean. Results are expressed as apo B/actin mRNA ratio and are normalized to the mean apo B mRNA level taking the mean value of the fiber-free rat jejunum as 1.

Table II

Effect of experimental diets on bile flow, biliary lipid outputs and fecal excretion of bile acids and cholesterol

Diet	Fiber-free	Sugar-beet fiber	*P* value
Biliary output			
Bile flow (μl/g liver/min)	1.2 ± 0.1	1.3 ± 0.1	>0.05
Bile salts (nmol/g liver/min)	43.6 ± 2.9	43.5 ± 1.7	>0.05
Phospholipids (nmol/g liver/min)	3.6 ± 0.4	5.8 ± 0.4	<0.001
Cholesterol (nmol/g liver/min)	1.1 ± 0.1	1.6 ± 0.1	<0.005
Fecal excretion			
Feces dry weight (g/day)	1.4 ± 0.1	3.1 ± 0.1	<0.001
Bile salts (μmol/day)	10.5 ± 0.5	16.7 ± 0.8	<0.001
Cholesterol (μmol/day)	14.2 ± 0.8	40.1 ± 2.5	<0.001

$\bar{x}$ ± SEM; 12 determinations per mean.

in the triglyceride-rich lipoprotein synthesis [14]. Taken together it may be conceivable that the impact of impaired lumenal bile salt availability on lipid metabolism is more pronounced in the lower intestine. The observation of Davidson et al. [3] that re-induction of apo B synthesis in the ileum was incomplete after bile salt replacement supports the above hypothesis.

In short, the obtained results suggest that some dietary fibers may play a role in the regulation of apo B synthesis in the intestine. However, contrasting effects of various dietary fibers and bile salts binding resins (i.e. cholestyramine) on bile acid secretion, fecal excretion and composition have been shown [15,16]. Therefore, it must be emphasized that use of other dietary fibers or drugs which interact with bile salt metabolism may have contrasting effects on the regulation of apo B synthesis to the fiber used in this study. To what extent the regulation of apo B synthesis in the intestine by the dietary fiber is affected by changes in bile salt metabolism remains to be elucidated. Various other factors could be involved in the effect of dietary fiber on intestinal lipoprotein synthesis, i.e. delayed and decreased digestion and absorption of fat, proliferative modifications, and volatile fatty acid metabolism. Experiments are currently in progress to explain the role of various dietary factors on the regulation of apo B synthesis in the intestine.

Acknowledgements: We thank A. Bellanger and P. Rémésy for their excellent technical assistance, and A. MacQuaid for assistance in the preparation of the manuscript.

REFERENCES

[1] Kane, J.P. (1983) Ann. Rev. Physiol. 45, 637–650.
[2] Davidson, N.O., Kollmer, M.E. and Glickman, R.M. (1986) J. Lipid Res. 27, 30–39.
[3] Davidson, N.O., Drewek, M.J., Gordon, J.I. and Elovson, J. (1988) J. Clin. Invest. 82, 300–308.
[4] Magun, A.M., Mish, B. and Glickman, R.M. (1988) J. Lipid Res. 29, 1107–1116.
[5] Kritchevsky, D. (1988) Annu. Rev. Nutr. 8, 301–328.
[6] Angelin, B. and Einarson, K. (1986) in: Bile Acids and Atherosclerosis (Grundy, S.M. ed.) vol. 15, pp. 41–66, Raven press, New York.
[7] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[8] Mangeney, M., Cardot, P., Lyonnet, S., Coupe, C., Benarous, R., Munnich, A., Girard, J., Chambaz, J. and Bereziat, G. (1989) Eur. J. Biochem. 181, 225–230.
[9] Ribeiro, A., Mangeney, M., Cardot, P., Loriette, C., Rayssiguier, Y., Chambaz, J. and Bereziat, G. (1991) Eur. J. Biochem. 196, 499–507.
[10] Talalay, P. (1960) Methods Biochem. Anal. 8, 119–143.
[11] Zlatkis, A., Zak, B. and Boyle, A.J. (1953) J. Lab. Clin. Med. 41, 486–492.
[12] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[13] Snedecor, G.W. and Cochran, W.G. (1984) Méthodes statistiques, 6ème edition, ACTA, Paris.
[14] Wu, A.L., Bennet-Clark, S. and Holt, P.R. (1980) Am. J. Clin. Nutr. 33, 582–589.
[15] Lafont, H., Lairon, D., Vigne, J.L., Chanussot, F., Chabert, C., Portugal, H., Pauli, A.M., Crotte, C. and Hauton, J.C. (1985) J. Nutr. 115, 849–855.
[16] Ide, T., Horii, M., Yamamoto, T. and Kawashima, K. (1990) Lipids 25, 335–340.