Plasma lipoproteins and apolipoproteins B and E in rats fed a high-fiber fructose-based diet

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This study was conducted as an attempt to provide additional informations on the effects of high-fiber diets on plasma lipoprotein metabolism. For this purpose, male Wistar rats were fed control or sugar-beet fiber diets for 3 weeks. High-fiber-diet–fed rats presented lower plasma triglyceride and cholesterol concentrations than fiber-free-diet–fed animals. The lipid-lowering effect of dietary fiber is associated with a decrease of triglycerides in all lipoprotein fractions and with a decrease of cholesterol in very low density lipoproteins and high density lipoproteins. Very low density lipoproteins isolated from fiber-fed animals contained significantly fewer triglycerides and more proteins compared with fiber-free-diet–fed rats. Mean size of very low density lipoprotein particles was smaller in fiber-fed rats compared with control rats. Plasma apolipoprotein B concentration did not differ significantly between both groups studied, but fiber feeding decreased plasma apolipoprotein E concentration. Similar levels of apolipoprotein B and of apolipoprotein E mRNAs were found in the livers in these two groups. In short, this work provides an unequivocal demonstration of lipid-lowering effect of a sugar-beet diet, which is associated with marked modifications in lipoprotein distribution and characteristics. (J. Nutr. Biochem. 5:499–503, 1994.)

Keywords: dietary fiber; lipoproteins; apolipoproteins; VLDL; triglycerides; rat

Introduction

The hypolipidemic effects of some dietary fibers have frequently been reported in humans and experimental animals.[1,2] Water-soluble fibers have been described as especially effective cholesterol-lowering agents.[1,2] However, less information is available concerning the changes in lipoprotein and apolipoprotein metabolism induced by fiber-containing diets. Recent studies from this laboratory indicate that diets rich in fermentable carbohydrates significantly decrease not only plasma cholesterol, but also plasma triglycerides in rat.[3–5]

Very low density lipoprotein (VLDL) is a major triglyceride secretory product of the liver. The rate of VLDL secretion influences the plasma concentration of most lipoprotein classes. Two major apolipoproteins, apo B and apo E, are closely related to the VLDL secretion and catabolism.[6] Thus, the purpose of this study was to determine plasma lipoprotein distribution, VLDL composition, apo B and apo E concentrations and mRNA levels in the liver in response to a high-fiber diet. Because it has been well documented that feeding high levels of sucrose or fructose causes an increase in plasma triglycerides and liver lipogenesis,[7] we used a low-fat, fructose-based diet to maximize the proportion of plasma lipoproteins originated from the liver.

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Methods and materials

Materials

Sugar-beet fiber preparation was obtained from Sofalia (Paris, France). Other dietary components were from L. François (St. Maur, France) except mineral/vitamin mix, which was obtained from U.A.R. (Villemoisson/Orge, France). Restriction enzymes, random primed DNA labeling kit, and nick translation kit were purchased from Boehringer (Mannheim, Germany). [α-^{32}P]dATP (3,000 Ci/mmol), Nylon Highbond N+ and Hyperfilm-βmax were purchased from Radiochemical Centre (Amersham, Bucks, UK). All chemicals were of analytical grade.

Animals and diets

Male Wistar rats weighing approximately 190 g were raised for 3 weeks on low-fat, fructose-based diets containing either 30% wheat

starch (fiber-free diet) or 30% sugar-beet fiber (high-fiber-diet) (*Table 1*). Rats were housed two per cage in wire-bottomed cages in a temperature-controlled room (22° C) with the dark period from 20:00 hr to 08:00 hr. Food and water were allowed ad libitum during the dark period. Food was withdrawn at 08:00 hr and animals were restricted during the light period.

Blood and tissue sampling

At the end of the experimental period, rats were sampled at 08:00 hr after maximal food consumption during the dark period. All animals were anesthetized with sodium pentobarbital (40 mg/kg), and after laparotomy, blood was withdrawn into syringes containing EDTA (1 mg/mL blood) from the abdominal aorta, and plasma was obtained by low-speed centrifugation. Liver was excised and portions were taken for lipid analysis and RNA extraction. Liver samples were immediately plunged into liquid nitrogen, then stored frozen at −70° C until the assays were performed.

Lipoprotein isolation

Lipoprotein isolation was performed on unfrozen plasma. Prior to the separation of lipoproteins, NaN_3 (0.02%), merthiolate (0.005%), and Na_2EDTA (0.04%) were added to each sample. Samples were overlaid with 0.15 M NaCl–0.01% EDTA (d = 1.006 g/mL), and chylomicrons were recovered following centrifugation for 30 min at 12,000*g*. VLDL (d < 1.006 g/mL), low density lipoprotein (LDL) (1.006 to 1.050 g/mL), and high density lipoprotein (HDL) (1.050 to 1.21 g/mL) were isolated by sequential preparative ultracentrifugation, after the density was adjusted with solid KBr, as previously described.[4]

Electron microscopy of VLDL

VLDL fraction was examined in a Philips EM 400 electron microscope (Philips Gloeilampenfabrieken, Eindhoven, The Netherlands) after negatively staining (2% sodium phosphotungstate at pH 7.4), as described by Forte and Nordhausen.[8] The mean diameter of particles and the frequency distribution of their size were determined by measuring 200 free-standing particles on micrographs.

Biochemical analyses

Triglycerides, total cholesterol, and phospholipids were determined in plasma and lipoprotein fractions by enzymatic procedures.[9-11] Protein content of isolated lipoproteins was determined by a modified Lowry method.[12] Bovine serum albumin, fraction V, was used as a standard. Plasma apo B and apo E were assayed by radial immunodiffusion[13] by using specific antibodies raised in sheep. Liver samples were homogenized and lipids were extracted with chloroform/methanol (2/1, vol/vol) according to the method described by Folch et al.[14] Triglyceride and cholesterol contents were measured in the lipid residue as previously described.[4]

Table 1 Composition of diets (g/100g)

	Fiber-free	High-fiber
Fructose	41	41
Wheat starch	30	—
Sugar-beet fiber*	—	30
Casein, vitamin free	20	20
Corn oil	2	2
Vitamin/mineral mixture	7	7

*Sugar-beet fiber preparation contains 75% dietary fiber (21% cellulose, 33% hemicellulose, 19% pectin, 2% lignin), 10% moisture, 7% protein, 4% sugar, and 4% ash.

Detection of the mRNAs in liver extracts

Total cellular RNA was isolated from liver tissue using the guanidinium/phenol/chloroform method according to Chomczynski and Sacchi.[15] RNA was quantitated by measuring the absorbance at 260 nm. Its integrity was systematically assessed by agarose-gel electrophoresis and visualization of 18S and 28S ribosomal RNAs by ethidium bromide staining. Aliquots of total RNA were subjected to quantification of mRNA content by dot blot analysis on nylon filters. Rat apo B and apo E cDNA clones (kindly provided by P. Cardot, URA CNRS, Paris, France) were used to detect specific mRNAs. The presence of an equal amount of total RNA was checked by hybridizing the filters with mouse β-actin cDNA probe as control.[16] Hybridization of immobilized RNA to cDNA probes labeled with [α-^{32}P]dATP and washing conditions were previously described.[16] 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 using a laser densitometer (Ultroscan XL, LKB, Uppsala, Sweden).

Statistics

All values were expressed as the mean ± standard error (SEM). Student's two-tail *t* test was used to assess differences between groups. *P* values lower than 0.05 were considered to be significant.

Results

As shown in *Table 2*, at the end of the experimental period the body weight of rats fed a high-fiber diet was not significantly different from rats fed fiber-free diet; however, differences in body weight could have been masked by the increased content of material in the digestive tract of sugar-beet fiber-fed rats as shown in our previous work.[3] The liver weight was reduced in high-fiber-diet–fed rats. Consumption of experimental diets was approximately the same in both groups studied. High-fiber-diet–fed rats presented lower plasma triglyceride and cholesterol concentrations than fiber-free-diet–fed animals. The liver triglyceride content was reduced in fiber-fed rats compared with fiber-free-diet–fed rats, while liver cholesterol level was unaffected by the diet. The patterns of triglyceride and total cholesterol distribution among plasma lipoproteins in the two groups

Table 2 Effect of dietary fiber on animals and on plasma and liver lipid concentrations

Diet	Fiber-free	High-fiber
Final body weight, g	298 ± 6	284 ± 8
Food intake, g/day†	26.8 ± 1.1	26.5 ± 1.2
Liver weight, g	16.2 ± 0.7	14.1 ± 0.6*
Plasma triglycerides, mmol/L	2.94 ± 0.34	1.78 ± 0.19**
Plasma total cholesterol, mmol/L	1.65 ± 0.08	1.16 ± 0.05***
Liver triglycerides, μmol/g wet wt	30.9 ± 2.3	17.8 ± 1.2***
Liver total cholesterol, μmol/g wet wt	13.7 ± 0.5	12.9 ± 0.5

Means ± SEM of 10 rats per group; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†Food per day was measured during the last week of experiment.

of animals are shown in *Figure 1*. The lipid-lowering effect of dietary fiber is associated with decrease of triglycerides in all lipoprotein fractions and with decrease of cholesterol in VLDL and HDL. VLDL isolated from fiber-fed animals contained significantly fewer triglycerides and more proteins compared with fiber-free-diet–fed rats (*Table 3*). Study of the size of VLDL has shown smaller mean diameter of particles in fiber-fed rats compared with control rats (*Table 3*). Size distribution of VLDL particles has shown the disappearance of the population of the largest VLDL in the fiber-fed rats (*Figure 2*). Plasma apo B concentration did not significantly differ between both groups, but fiber feeding decreased plasma apo E level (*Table 4*). Similar levels of liver apo B and apo E mRNAs were found in these two groups (*Table 4*).

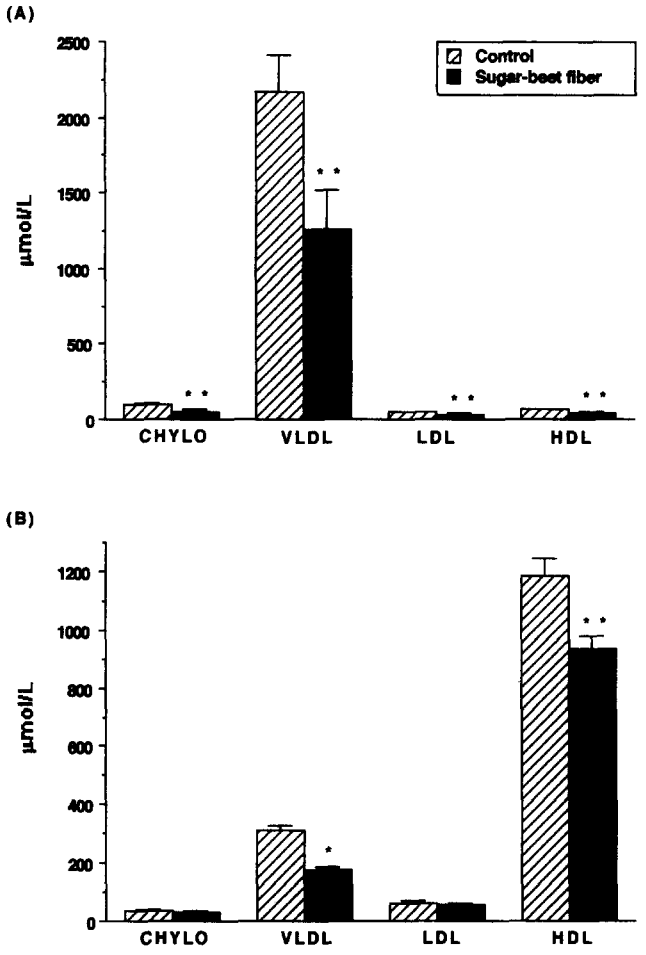

Figure 1 Plasma lipoprotein triglyceride (A) and cholesterol (B) concentrations in control and sugar-beet fiber fed rats (means ± SEM of six rats per group; *$P < 0.05$; **$P < 0.01$).

Table 3 Effect of dietary fiber on chemical composition and mean diameter of VLDL.

Diet	Fiber-free	High-fiber
	% weight†	
Triglycerides	72.4 ± 0.6	68.2 ± 1.7*
Total cholesterol	4.7 ± 0.4	5.1 ± 0.2
Phospholipids	13.2 ± 0.3	13.6 ± 0.2
Proteins	9.7 ± 0.6	13.1 ± 0.7**
	nm‡	
Mean particle diameter	58.6 ± 1.4	44.4 ± 1.2***

Means ± SEM.
†6 samples, ‡5 samples.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

The biologic properties of sugar-beet fiber, which is rich in pectin and hemicelluloses, have recently been investigated

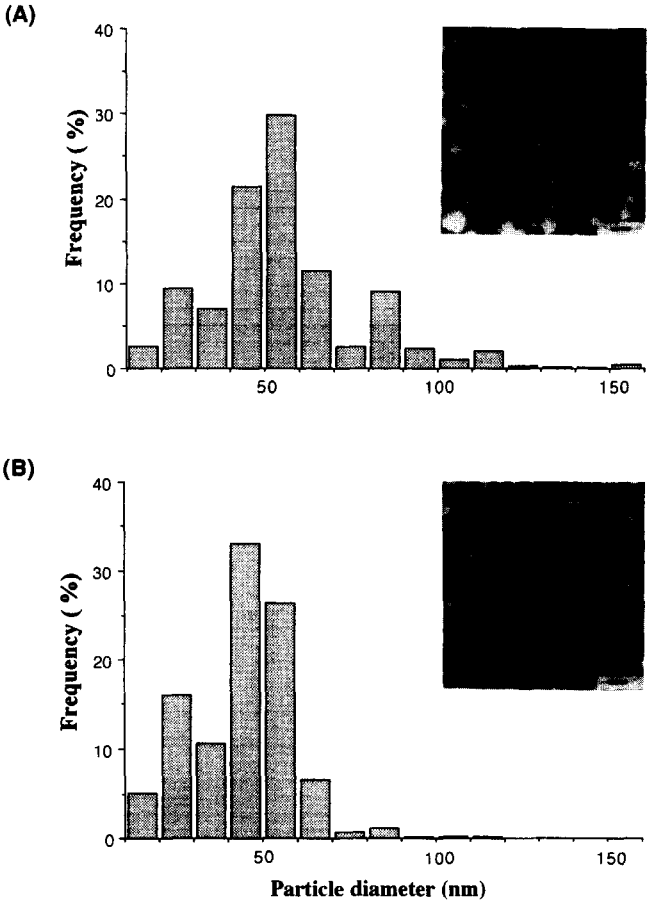

Figure 2 Electron photomicrographs of negatively stained VLDL and frequency distribution of VLDL particle diameters from control (A) and sugar-beet-fiber–fed (B) rats.

Table 4 Effect of dietary fiber on apo B and apo E plasma concentrations and on their mRNA levels in the liver.

Diet	Fiber-free	High-fiber
Plasma apo B, *g/L*†	0.25 ± 0.01	0.23 ± 0.03
Plasma apo E, *g/L*†	0.14 ± 0.01	0.06 ± 0.01*
Liver apo B mRNA, *au*‡,§	1.00 ± 0.10	1.11 ± 0.06
Liver apo E mRNA, *au*‡,§	1.00 ± 0.07	0.94 ± 0.08

Means ± SEM.
†10 samples, ‡8 samples, §arbitrary units. Values for fiber-free diet fed rats were taken as 1.
*$P < 0.001$.

by Johnson et al.[17] This fiber combines some of the useful biologic characteristics of insoluble and soluble dietary fibers.[17] Striking changes in the lipid concentrations and lipoprotein profiles were seen in rats fed this fiber. The marked hypocholesterolemic effect of the fiber-enriched diet is in agreement with our previous work[3] and that of Johnson et al.[17] This response to dietary fiber mainly resides in HDL fraction. It has been previously demonstrated in our laboratory using other high-fiber diets[4,5] that HDL-1 fraction appears to be particularly affected by such diets. HDL-1 contains a high proportion of apo E. This apolipoprotein plays a major role in the reverse transport of cholesterol as a recognition signal for the removal of lipoproteins.[18] Recently Nishina et al.[19] also found a decrease in plasma apo E concentration in pectin-fed rats. This decrease in plasma apo E and apo E-containing lipoproteins may be explained in great part by an increase in their removal because an increased catabolic rate of lipoproteins via apo B/apo E receptors has been demonstrated in fiber-diet–fed animals.[4,20] However, a decrease in apo E synthesis seems not to be involved in such a modification in plasma concentration of this apolipoprotein. Indeed, in the present study we have not shown any modification in hepatic apo E mRNA level in the fiber-fed rats, and in a previous study carried out on Zucker rats fed a high-fiber diet for 3 months, we observed an increase in liver apo E mRNA level.[21]

Interestingly, sugar-beet fiber has a marked hypotriglyceridemic effect in rats fed sucrose-rich, low-fat and thus highly lipogenic diet. The very low dietary lipid content has limited effect on postprandial lipemia as monitored by the low level of chylomicra (*Figure 1*). Thus, a possible explanation for hypotriglyceridemic effect of sugar-beet fiber containing diet might be a decreased liver lipogenesis and triglyceride secretion, as reported in our earlier work.[3] However, conflicting data are obtained concerning the effects of various dietary fibers on plasma triglyceride levels, liver lipogenesis, and triglyceride secretion.[3] The extent of these effects may depend on the type and the quantity of fiber used, as well as on the time of adaptation. In addition to the hypotriglyceridemic effect, we observed in the present study an influence of the fiber-diet on plasma VLDL level and characteristics, i.e., decreased triglyceride to protein ratio and smaller particle size. This observation indicates that there is a lower triglyceride charge by the VLDL particle. This effect of dietary fiber appears to be opposite to that observed in subjects fed high carbohydrate diets in which the size and triglyceride content of VLDL were increased.[22] Changes in VLDL size and/or composition can profoundly modulate the VLDL-triglyceride catabolic rate.[23,24] To what extent the observed modifications induced by high-fiber-diet feeding may result in modifications in the fate of VLDL remains to be determined. Nevertheless, we could not demonstrate significant changes in plasma apo B concentration and in hepatic apo B mRNA level. Similar results were obtained recently by Redard et al.[25] with oat bran-fed and psyllium-fed rats. Because apo B synthesis appears to be regulated mainly at the posttranslational level[26] a modification in apo B synthesis is not completely excluded in this model.

In conclusion, this work provides an unequivocal demonstration of the lipid-lowering effect of a sugar-beet-rich diet. This lipid-lowering effect is associated with marked modifications in lipoprotein distribution and characteristics. Further studies will be conducted to elucidate the metabolic consequences of such modifications.

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