Age-Related Response to Dietary Fructose in the Rat: Discrepancy in Triglyceride and Apolipoprotein B Synthesis as a Possible Mechanism for Fatty Liver Induction in Adult Rats (43649)

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Abstract. **The effects of fructose feeding on plasma and liver lipids, triglyceride secretion, and plasma apolipoprotein B and their liver mRNA level were studied in young and adult rats. We have shown that the responsiveness of adult rats to dietary fructose differs from that of young rats with regard to body parameters as well biochemical analyses. In young rats, fructose diet causes a coupled induction of liver triglyceride and apolipoprotein B synthesis via increased mRNA level. In adult rats it appears that triglyceride secretion is lower and less inducible by dietary fructose than in young rats. This insufficient export of the excess of synthesized triglycerides may cause fatty liver in adult animals. Reduced adaptation of liver lipoprotein secretion to dietary carbohydrates in adult animals may be explained by the failure to stimulate apolipoprotein B synthesis at the mRNA level in these rats.** [P.S.E.B.M. 1993, Vol 204]

One of the most controversial areas in the literature concerning dietary fructose is the effect of fructose on serum lipids and, in particular, on triglyceride levels (1, 2). Liver is the main site of fructose utilization due to the presence of specific cells and active enzymes that ensure its rapid metabolism (3). Although changes in lipoprotein lipase activity partially account for the changes in blood lipids, hypertriglyceridemia in fructose-fed animals has been essentially related to an increased rate of very low density lipoprotein secretion (1–3).

Age-related differences were observed in plasma and liver lipids and lipogenic enzymes in animals exposed to dietary carbohydrates (4, 5). This raised the possibility that the response to dietary fructose in induction of lipid and lipoprotein synthesis may also differ depending upon the age of animals. Thus, the aim of the present experiment was to compare the effect of fructose-rich diet on plasma and liver lipids and on triglyceride secretion in young and adult rats. Because apolipoprotein B (apoB) is an obligate component of very low density lipoprotein (6), for better understanding of the changes in hepatic lipoprotein synthesis in response to dietary fructose, we also examined whether fructose-rich diet affects plasma apoB concentrations and liver apoB mRNA level.

Materials and Methods

Materials. Dietary components were obtained from L. François (St. Maur, France), except mineral/vitamin mix, which was obtained from UAR (Villemoisson/Orge, France). Restriction enzymes and a random primed DNA labeling 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

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approximately 190 g (2 months old, i.e., young rats) or 580 g (1 year old, i.e., adult rats) were raised for 3 weeks on purified diets containing (as w/w) 20% casein, 5% corn oil, 7% mineral/vitamin mix, 68% wheat starch (starch diet), or 40% fructose/28% wheat starch (fructose diet). Rats were housed in wire-bottomed cages in a temperature-controlled room (22°C) with the dark period from 2000 hr to 0800 hr. Food and water were provided *ad libitum* during the dark period. Food was withdrawn at 0800 hr and the animals were restricted during the light period. The experiments were routinely performed between 0900 hr and 1000 hr. At the end of the experimental period, the animals (six rats per group) were anesthetized with sodium pentobarbital (40 mg/kg). After laparotomy, blood was collected from the abdominal aorta in syringes containing EDTA (1 mg/ml blood) and plasma was obtained by low-speed centrifugation. The liver was excised and portions from the right lobe were immediately plunged into liquid nitrogen, then stored frozen at −70°C until the lipid analysis and RNA extraction were performed. Triglyceride secretion rate was determined as described previously (7) by measuring the increase in plasma triglyceride concentration after an intravenous injection of Triton WR-1339. This study was carried out on different groups of animals (6–10 rats per group) than used for plasma and liver analyses.

Analyses. Triglycerides and cholesterol were determined in plasma by enzymatic procedures (8, 9). Plasma apoB was determined by immunoelectrophoresis assay (10) using anti-rat apoB antibody raised in rabbit and purified rat low density lipoprotein as standard. Liver samples were homogenized and lipids were extracted with chloroform/methanol (2/1, v/v) according to the method described by Folch *et al.* (11). Triglyceride contents were measured in the lipid residue as described previously (12). Total cellular RNA was isolated from the liver samples using the guanidinium/phenol/chloroform method according to Chomczynski and Sacchi (13). 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 RNA by ethidium bromide staining. Aliquots of total RNA were subjected to quantification of mRNA content by dot-blot analysis on nylon filters. Hybridization of immobilized RNA to rat apoB cDNA probe labeled with [α-^{32}P]dATP and washing conditions were described previously (14). 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 (Ultroscan XL; LKB, Bromma, Sweden).

Statistics. Results are given as means ± SE for the numbers of animals specified. Data were analyzed by two-way analysis of variance. When the analysis of variance indicated significant differences ($P < 0.05$), differences between experimental groups were determined by least significant difference at the $P < 0.05$ level of significance.

Results

As shown in Table I, at the end of the experimental period, body weight was similar in both starch- and fructose-fed young rats. In the group of adult rats, there was an increase in the body and liver weights of fructose-fed rats as compared with starch-fed rats. Triglyceride concentrations in plasma were significantly increased in young rats fed fructose compared with young rats fed starch. Adult rats presented higher concentrations of plasma cholesterol than young rats in both starch- and fructose-fed groups. There was no significant effect of dietary fructose on plasma cholesterol concentrations in both young and adult rats. Plasma apoB concentration was significantly increased by dietary fructose in young rats but not in adult animals. Adult rats had significantly higher concentrations of apoB in plasma than did the young rats. Liver triglyceride levels were markedly increased by fructose feeding only in adult rats. There was a significant increase in liver apoB mRNA levels in young rats after fructose diet, while mRNA levels remained relatively constant in adult rats. As shown in Figure 1, triglyceride secretion, studied by the use of Triton, was increased by fructose feeding. Adult rats presented lower triglyceride secretion rates than did young rats fed the same diet.

Discussion

In the present work, we demonstrated that the responsiveness of adult rats to dietary fructose differs from that of young rats with regard to body parameters as well as biochemical analyses. In contrast to young rats, where body weight was not affected by the fructose feeding, adult rats fed fructose diet increased their body weights as compared with the respective starch-fed animals. Also, liver weights increased markedly in adult rats receiving fructose diet. This may be explained, at least in part, by observed high levels of liver lipids in these rats. In agreement with many previous observations (for review, see Refs. 1–3), fructose induces hypertriglyceridemia in young, growing rats. This hypertriglyceridemia reflects an increased rate of triglyceride secretion by the liver. Additionally, we have shown that plasma apoB concentrations and apoB mRNA levels in the liver of young rats were increased by fructose feeding. Thus, it may be concluded that in young rats, fructose diet causes a coupled induction of liver triglyceride synthesis and apoB synthesis via increased mRNA level. In adult rats fed fructose diet, we failed to observe an increase in plasma triglyceride and apoB as compared with young rats. However, adult rats fed starch

Table I. Influence of Fructose Feeding on Studied Plasma and Liver Parameters in Young and Adult Rats[a]

	Young rats		Adult rats		ANOVA[b]
	Starch	Fructose	Starch	Fructose	
Body wt (g)	304 ± 6*	300 ± 6*	588 ± 11†	629 ± 5‡	A, C, A×C
Liver wt (g)	14.3 ± 0.6*	16.7 ± 0.5*	20.5 ± 0.8†	24.7 ± 1.0‡	A, C, A×C
Plasma triglycerides (m*M*)	1.03 ± 0.09*	2.89 ± 0.28†	1.53 ± 0.26*	1.94 ± 0.34*,†	C, A×C
Plasma cholesterol (m*M*)	1.33 ± 0.04*	1.44 ± 0.05*	2.33 ± 0.09†	2.28 ± 0.11†	A
Plasma apoB (mg/100 ml)	14.1 ± 1.0*	22.5 ± 0.3†	22.1 ± 1.7†	22.5 ± 0.5†	A, C, A×C
Liver triglycerides (mg/g wet wt)	12.9 ± 1.1*	15.3 ± 2.7*	13.1 ± 0.9*	28.9 ± 4.8†	A, C, A×C
Liver apoB mRNA (relative level)[c]	1.0 ± 0.2*	2.1 ± 0.3†	0.9 ± 0.3*	1.1 ± 0.2*	A, C

[a] Values are means ± SE of six rats per group. Means in a row with different symbols (*, †, ‡) are significantly different.
[b] Two-way analysis of variance (ANOVA); a *P*-value of at least 0.05 was considered statistically significant for main treatment and interaction given. A, Age; C, carbohydrate.
[c] The mean value of the young starch-fed rats was taken as 1.

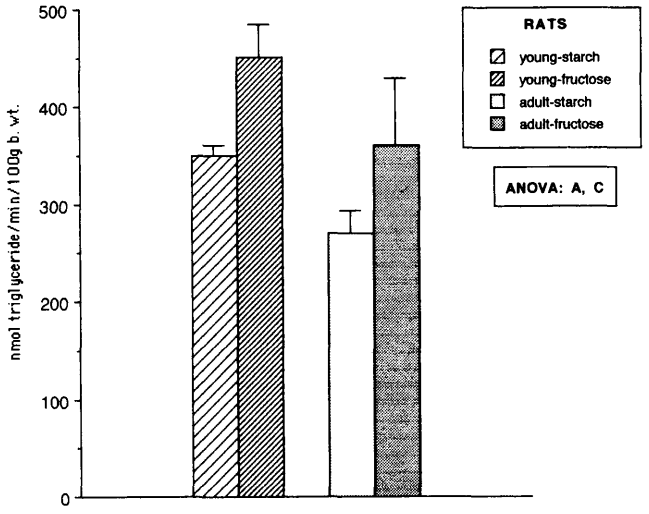

Figure 1. Triglyceride secretion rate in young and adult rats fed starch- or fructose-based diets. Values are means ± SE of 6–10 rats/group. In two-way analysis of variance (ANOVA), a *P*-value of at least 0.05 was statistically significant for main treatment. A, Age; C, carbohydrate.

diet had higher plasma apoB concentrations than young rats receiving the same diet. This may result from less efficient clearance of plasma lipoproteins in older animals, which is in agreement with the higher plasma cholesterol concentration observed in older rats in the present work. Triglyceride secretion was also lower in adult rats than in young ones. In contrast to young rats, fructose feeding in adult animals induced a fatty liver and did not modify plasma apoB concentrations and apoB mRNA levels in the liver. Triglyceride from the liver is secreted in the form of very low density lipoprotein or stored as droplets when the synthetic rate is higher than the secretory rate (6). Since apoB is essential for the secretion of triglyceride-rich lipoproteins, failure in apoB synthesis may impair lipoprotein formation and result in enhanced triglyceride accumulation in the liver (6). Our results suggest that the responsiveness of apoB mRNA to dietary carbohydrate may decrease with age. This may render adult rats more susceptible to fat accumulation in the liver than young, growing rats. As in our recent studies performed on cows, which develop fatty liver as a result of enhanced adipose tissue fat mobilization, a link between high liver triglyceride and low liver apoB mRNA and low plasma apoB levels was reported (15). In the present work, steady state levels of cellular mRNA were measured. Posttranscriptional steps play an important role in apoB synthesis (6). Thus, it would be of interest to assess whether these steps are affected by dietary fructose in relation to the age of animals.

The coinduction by dietary fructose of both lipogenesis and apolipoprotein synthesis is likely to play a role in the increased capacity of hepatocytes to both assemble and secrete triglyceride-rich lipoproteins (16, 17). This coinduction was reported here in young rats but not in adult rats. Interestingly, it has been reported that aging is accompanied by a decrease in activities and mRNA levels of several lipogenic enzymes, both in the basal state and when lipogenesis was induced by carbohydrates (4, 5). It has been discussed (4, 5) that this impaired capability for enzyme induction does not result from a deficiency in hepatic enzyme responsiveness, but results from disturbances in the availability and/or effectiveness of key hormones, i.e., insulin, glucagon, adrenal glucocorticoids, and thyroid hormones. Regulation of rat liver apoB gene expression has also been reported to respond to endocrine signals (17–19). It appears that several proteins implicated in triglyceride synthesis and transport are less inductible by dietary fructose in adult rats than in young animals. Thus, in adult animals, lipoprotein synthesis and secretion appear to be insufficient to export synthesized triglyceride excess.

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