Dietary Oligofructose Modifies the Impact of Fructose on Hepatic Triacylglycerol Metabolism

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The aim was to investigate if chronic feeding with oligofructose (OFS), a nondigestible fructan that decreases triacylglycerol-very–low-density lipoproteins (TAG-VLDLs) in the serum of rats by reducing hepatic de novo lipogenesis, could counteract the impact of fructose on TAG metabolism. Male Wistar rats fed a standard diet supplemented or not with 10% OFS for 30 days received either tap water or a 10% fructose drinking solution for 48 hours. TAG, phospholipids (PLs), cholesterol, and free fatty acids were assayed both in serum and in liver. Fatty acid de novo synthesis, esterification, and β-oxidation were assessed in the liver by measuring the activity of key enzymes: fatty acid synthase (FAS), phosphatidate phosphohydrolase (PAP), glycerol-3-phosphate acyltransferase (GPAT), and carnitine palmitoyltransferase-I (CPT-I), respectively. The acute load of fructose increased (1) both liver and serum TAG without affecting other lipids, and (2) de novo fatty acid synthesis and esterification, through induction of FAS and PAP without affecting CPT-I. Long-term feeding with OFS protected rats against liver TAG accumulation induced by fructose. The lower lipogenic capacity of the liver could be the key event in this protection, since even after the fructose load FAS activity remained significantly lower in OFS-fed rats. However, despite its protective effect on the liver, OFS was not able to prevent fructose-induced hypertriglyceridemia, suggesting that OFS feeding could not counteract the fructose-induced defect in TAG-VLDL clearance.

SEVERAL STUDIES in human subjects report that diets containing high amounts of saccharose and fructose may, under some circumstances, lead to hypertriglyceridemia.[1] The triacylglycerol (TAG)-raising effect of saccharose has usually been attributed to its content in fructose. Fructose promotes hepatic synthesis of TAGs and their release into the plasma in the form of very–low-density lipoproteins (VLDLs). Different mechanisms have been proposed to explain the impact of an acute load of fructose on TAG metabolism: an increase in de novo fatty acid synthesis, a higher availability of nonesterified fatty acids (NEFAs) released by adipose tissue, and/or a shift of fatty acid from β-oxidation toward the esterification pathway. Besides such effects on liver lipid metabolism, other investigators have also suggested a reduced hydrolysis of TAG-rich lipoproteins as an important factor in fructose-induced hypertriglyceridemia.[1-3]

The concomitant intake of other nutrients may influence the effects of carbohydrates like fructose on plasma TAG concentrations, including the type of fat consumed (saturated or polyunsaturated), the proportion of carbohydrate, and the dietary fiber content of the diet.[1]

We have recently shown that the addition of oligofructose (OFS) as a dietary fiber in the diet of rats decreases the concentration and hepatic release of VLDL-TAG.[4,5] This phenomenon is due to a decrease in hepatic TAG synthesis, resulting mainly from a lower rate of lipogenesis; the latter correlates with a decrease in fatty acid synthase (FAS) activity, the last key enzyme of the lipogenic pathway.[6,7]

In the present study, we tested the hypothesis that the modifications in lipid metabolism resulting from long-term feeding with OFS could counteract the effect of an acute fructose load on TAG metabolism in the rat.

MATERIALS AND METHODS

Chemicals

Raftilose P_{95} (Raffinerie Tirlemontoise, Tirlemont, Belgium) was used as the OFS source. It is obtained by enzymatic hydrolysis of chicory inulin and is a mixture of $(glucose)_1$-fructosyloligomers (64%) and homooligomers of fructose (36%) with a mean degree of polymerization of 4.8.

Enzymes and coenzymes were purchased from Boehringer (Mannheim, Germany). All other chemicals were of the purest analytical grade available from Sigma Chemicals (St Louis, MO).

Animals and Diets

Male Wistar rats (ICOPS-WY IOPS; Iffa Credo, Les Oncins, France) initially weighing about 120 g were housed in individual cages on a 12-hour dark/light cycle. One group was fed ad libitum a powdered chow diet obtained from a commercial source (AO_4; UAR, Villemoisson-sur-Orge, France). The other group received the same diet containing 10% Raftilose P_{95}. All had free access to tap water. After 30 days of treatment, half the animals per group received fructose in their drinking water (10% wt/vol) 48 hours before killing, whereas the other animals were kept on tap water throughout the treatment. After anesthesia with Nembutal (pentobarbital, 60 mg/kg body weight; Sanofi, Brussels, Belgium), blood was collected from the descending vena cava and the liver was excised and clamped immediately in liquid nitrogen.

Analytical Procedures

TAG, phospholipid (PL), total cholesterol, and NEFA levels were measured in serum using enzymatic kits (Sopar-Biochem, Brussels, Belgium). The same procedure was performed for liver lipid composition analysis after chloroform-methanol extraction.[8] The measurement of carnitine palmitoyltransferase-I (CPT-I) activity was performed on liver homogenates using the fluorometric assay described by Schäfer et al.[9] FAS activity was assayed on liver cytosolic fractions according to the method used by Linn,[10] and Mg^{2+}-dependent phosphatidate phosphohydrolase (PAP) and glyc-

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erol-3-phosphate acyltransferase (GPAT) activities were measured in liver microsomes.[11,12] For all enzymes, the results are expressed as milliunits per milligram of protein. One unit is defined as the amount of enzyme needed to catalyze the transformation of 1 μmol substrate/min.

The glycerol-3-phosphate level was measured spectrophotometrically on neutralized clamped-liver extracts.[13] Proteins were assayed by the method of Lowry et al[14] using bovine serum albumin as standard.

Statistical Analysis

Results are expressed as the mean ± SEM (10 rats per group). Statistical analysis was performed with Student's *t* test. The level of significance was set at P less than .05.

RESULTS

By comparing the results obtained in rats fed a basal diet enriched with 10% OFS versus rats fed the standard diet, before fructose supplementation, we confirmed the results obtained previously, namely that OFS decreases TAG and PL levels both in the serum and the liver. Glycerol-3-phosphate content was significantly higher in the liver of OFS-fed rats, but the free fatty acid level was not significantly affected by such treatment (Table 1). OFS feeding did not modify the activity of the key enzymes of fatty acid esterification (PAP) or β-oxidation (CPT-I). However, it significantly decreased FAS activity, thus indicating a lower lipogenic capacity (Table 2).

Half the animals from both groups received fructose at a concentration of 10% in the drinking water for 48 hours. The total fructose intake was similar in both groups (10.0 ± 1.0 and 9.1 ± 0.8 g in non–OFS-fed and OFS-fed groups, respectively). In the present experiment, a fructose load of about 10 g within 48 hours increased the fructose concentration to 0.534 ± 0.064 and 0.608 ± 0.05 mmol/L in the portal vein of control and OFS-fed rats, respectively.

Table 1. Effect of Dietary OFS and Fructose on Lipid Content

	Tap Water		Fructose	
Lipid	OFS⁻	OFS⁺	OFS⁻	OFS⁺
Serum (mmol/L)				
TAGs	1.94 ± 0.13	1.18 ± 0.01‡	2.69 ± 0.33§	3.29 ± 0.35¶
PLs	2.10 ± 0.08	1.74 ± 0.06†	2.21 ± 0.19	2.17 ± 0.07¶
Total cholesterol	1.77 ± 0.11	1.69 ± 0.10	1.73 ± 0.10	1.53 ± 0.07
Free fatty acids	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.01	0.09 ± 0.01
Liver (nmol/mg protein)				
TAGs	58.3 ± 4.7	44.3 ± 5.2*	72.8 ± 5.2§	59.5 ± 4.1*§
PLs	123.5 ± 4.8	108.7 ± 3.5*	130.7 ± 6.6	135.3 ± 3.3‖
Total cholesterol	34.9 ± 1.8	31.7 ± 1.4	30.9 ± 1.3	29.1 ± 1.4
Free fatty acids	12.9 ± 2.5	17.6 ± 2.9	14.3 ± 2.4	16.8 ± 1.6
Glycerol-3-phosphate	1.44 ± 0.14	2.14 ± 0.24*	1.35 ± 0.07	1.66 ± 0.12*§

NOTE. Results are the mean ± SEM of 10 rats (comparisons by Student's *t* test).

Abbreviations: OFS⁻, without OFS; OFS⁺, with OFS.

*P < .05, †P < .01, ‡P < .001: OFS+ *v* OFS−.

§P < .05, ‖P < .01, ¶P < .001: fructose *v* tap water.

Table 2. Effect of Dietary OFS and Fructose on the Activity of Key Enzymes in Lipogenesis (FAS), Esterification (PAP), and β-Oxidation (CPT-I) in the Liver

	Tap Water		Fructose	
Enzyme	OFS⁻	OFS⁺	OFS⁻	OFS⁺
FAS	63.7 ± 4.8	37.3 ± 3.4*	131.8 ± 5.1†	109 ± 6.0*†
PAP	4.77 ± 0.45	4.45 ± 0.43	11.10 ± 0.59†	11.20 ± 0.16†
CPT-I	24.3 ± 1.8	22.4 ± 1.4	21.5 ± 1.4	23.3 ± 1.8

NOTE. Enzyme activities (mU/mg protein) are the mean ± SEM of 10 or 5 rats for FAS or PAP and CPT-I, respectively.

*P < .01, OFS⁺ *v* OFS⁻.

†P < .01, fructose *v* tap water.

After the fructose load, the level of serum TAG increased and was even higher in OFS-fed than in standard diet–fed rats. However, fructose did not modify PL, total cholesterol, or NEFA content in standard diet–fed rats, but slightly increased PL levels in OFS-fed rats. Even if fructose increased the TAG level in the liver of animals from both groups, the amount of liver TAG remained significantly lower in OFS-fed rats receiving fructose, and in those animals reached values similar to the levels measured in control rats (receiving standard diet and tap water only). Fructose significantly decreased glycerol-3-phosphate content in the liver of OFS-fed rats, but did not modify this parameter in rats fed the basal diet (Table 1).

Table 2 lists the liver activity of some key enzymes involved in TAG metabolism. FAS activity was strongly induced by fructose, but remained significantly lower in OFS-fed rats compared with animals receiving the standard diet. In contrast, CPT-I activity was not affected by fructose or OFS feeding. Mg^{2+}-dependent PAP activity in liver microsomes was strongly increased in rats receiving fructose, and to the same extent in OFS-supplemented and standard diet–fed rats. In addition, the activity of GPAT was also measured in the present experiment, and was similar in the liver of rats receiving fructose: 1.77 ± 0.14 versus 1.67 ± 0.09 mU/mg protein in rats fed the standard and OFS-supplemented diets, respectively.

DISCUSSION

The hypertriglyceridemic effect of fructose has been widely attributed to an overproduction of hepatic TAG-VLDL.[1,15-16] The effect of fructose on hepatic TAG synthesis and secretion may result from a shift of fatty acids from β-oxidation to esterification, or can be mediated by activation of lipogenesis.

It has now been firmly established that the regulatory site for controlling the balance between fatty acid oxidation and esterification lies in the oxidative pathway, at the step catalyzed by CPT-I.[17] The liver activity of this enzyme was not affected by fructose feeding, suggesting that the changes in liver TAG content were not mediated through this enzyme.

This latter result is not in agreement with the studies reporting a direct antiketogenic effect of fructose on the liver.[18-20] Most of these experiments were performed in the

starved state, and it is unclear whether malonyl coenzyme A inhibition of CPT-I can explain these results. Moreover, this antiketogenic effect of fructose is nearly always due to its inhibition of NEFA mobilization from adipose tissue.[21] Indeed, in the present experiment, we showed that fructose slightly but nonsignificantly decreased serum NEFA concentrations in rats.

The activity of PAP is known to be of regulatory importance in TAG synthesis.[22] Moreover, several groups found a close positive correlation between PAP activity in the liver and plasma TAG levels. Skorve et al[23] showed that sulfur-substituted fatty acid analogs that reduce plasma and hepatic TAG levels in normal rats also decrease hepatic PAP activity. In contrast, acute feeding with fructose increases the hepatic activity of PAP, enhancing the liver capacity to synthesize and secrete TAG.[24] This PAP induction was observed in the present experiment in both groups of rats receiving fructose.

We have previously shown that OFS supplementation in a standard diet significantly decreases GPAT activity in the liver of rats.[7] However, the activity of this key enzyme catalyzing the first reaction of the esterification pathway, as well as PAP activity, were similar in the liver of control and OFS-fed rats receiving fructose. This led us to postulate that the decrease in TAG and PL contents after OFS feeding could be due to a decreased availability of de novo synthesized fatty acids rather than to a reduced esterification. Similar conclusions have been proposed by Stals et al,[25] who suggested that the rate of TAG synthesis is controlled by the fatty acid supply.

Declercq et al[13] have also suggested that the level of glycerol-3-phosphate could act as a regulator of the rate of liver fatty acid esterification. In the present experiment, we show that hepatic glycerol-3-phosphate content is not affected by fructose (in rats receiving the basal diet, at least). These results are in accordance with a previous study by Zakim and Herman,[26] who demonstrated that physiological concentrations of fructose do not increase liver glycerol-3-phosphate concentration in rats.

Fructose is considered an excellent lipogenic precursor,[1] but it is also able to induce lipogenic enzymes.[27,28] This induction of lipogenic enzymes may be estimated by measuring FAS activity, the last enzyme complex involved in de novo fatty acid synthesis. FAS activity is directly dependent on the protein level, since no covalent or allosteric mechanisms have been described until now.[29]

In the present experiment, we show that FAS activity is strongly induced by fructose. The impact of fructose on liver and serum TAG could then result from an increased availability of de novo synthesized fatty acids, together with an increase in esterification capacity through activation of key enzyme activity.

In a standard diet, OFS feeding decreases TAG-VLDL by reducing de novo fatty acid synthesis in the liver, mainly through modulation of FAS activity.[6,7] In the present study, we report that even after the fructose load, FAS activity remains significantly lower in OFS-fed rats. In contrast, the activity of key enzymes involved in either fatty acid β-oxidation or esterification was similar in OFS-supplemented and standard diet–fed rats after the fructose load. Moreover, OFS feeding did not modify free fatty acid serum concentrations, thus suggesting that adipose tissue lipolytic activity is not involved in the decrease in TAG observed after OFS ingestion. We may thus conclude that the lower lipogenic capacity (illustrated by the lower FAS activity) could be the key event that allows OFS to protect rats against liver TAG accumulation after a fructose load.

Despite its protective effect on the liver, OFS is not able to prevent fructose-induced hyperlipidemia. Triglyceridemia results from both TAG-VLDL hepatic output and the catabolism of TAG-rich lipoproteins.

An enhanced TAG secretion as VLDL to explain the hypertriglyceridemia observed in OFS-fed and fructose-fed rats is not supported, and is even counteracted, by our data. FAS activity is lower in the liver of OFS-fed rats, thus suggesting a lower lipogenic capacity. Arbeeny et al[30] and Sparks and Sparks[31] have recently demonstrated that TAG-VLDL secretion capacity is directly linked to de novo fatty acid synthesis. This led us to postulate that OFS feeding, through inhibition of lipogenesis, decreases the TAG-VLDL secretion capacity of the liver. This hypothesis is supported by our previous studies, in which we showed that OFS feeding decreased the capacity of hepatocytes to secrete TAG synthesized from ^{14}C-acetate or ^{14}C-palmitate.[5-7]

It is also possible that TAG-VLDL removal was impaired in OFS-fed and fructose-fed rats. Indeed, fructose has been shown to decrease TAG-VLDL clearance.[1,32] No data relative to an effect of OFS on TAG-rich lipoprotein catabolism have been obtained so far.

In conclusion, we have demonstrated that OFS feeding, through its modulation of de novo lipogenesis, may act as a functional food, ie, one with the ability to protect against toxic effects, namely liver lipid accumulation, induced by other nutrients (fructose) that are eaten concomitantly. The relevance of such interaction comes from the fact that fructose and OFS are already associated in some foodstuffs, both as saccharose substitutes.

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