Dietary Oligofructose Lessens Hepatic Steatosis, but Does Not Prevent Hypertriglyceridemia in Obese Zucker Rats

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ABSTRACT We studied the influence of oligofructose (OFS), a nondigestible fructan, on lipid metabolism in obese fa/fa Zucker rats. The addition of 10 g/100 g OFS to the diet slowed the increase in body weight without modifying serum triglycerides or glucose concentrations after 7 wk of treatment. However, an oral load of 2 g glucose and 5 g corn oil/kg body weight increased triglyceridemia more in OFS-fed rats than in control rats. After 10 wk, OFS decreased the hepatic concentration of triglycerides 57% relative to controls. The less severe steatosis was confirmed by histologic analysis. Among the key enzymes involved in fatty acid synthesis and esterification, only malic enzyme activity was significantly lower in OFS-fed rats than in controls. The epididymal fat mass was significantly lower in OFS-fed rats. In conclusion, dietary enrichment with OFS can counteract both the fat mass development and the hepatic steatosis that occur in obese Zucker rats. Future studies will be designed to clarify in obese animals the influence of dietary OFS on postprandial triglyceridemia, which is an important variable associated with the development of atherosclerosis in humans, and to analyze the biochemical mechanism underlying the "hepatoprotective" effect of OFS.

KEY WORDS: • lipogenesis • triglycerides • fructans • liver • rats • oligofructose

An increase in the hepatic synthesis and production of triglycerides (TAG) is frequently associated with insulin resistance and obesity in humans. This metabolic disturbance often leads to the accumulation of TAG in the liver, defined as steatosis, but also contributes to the hypertriglyceridemia that occurs in such patients (Bacon et al. 1994). Experimental data support the hypothesis that an increase in postprandial TAG and lipoprotein remodeling is associated with atheroma deposition and coronary events in hyperlipidemic, diabetic and obese subjects, but the relationships among dietary intake, postprandial response and pathologic events remain to be clarified (Lairon 1996). A well-established association exists between chronic hyperinsulinemia and hypertriglyceridemia. Insulin appears to be a key hormone in the regulation of TAG-VLDL secretion. Its influence is dependent on the nutritional state, i.e., insulin stimulates TAG secretion by perfused liver of fed rats, but inhibits it in the liver from food-deprived or insulin-deficient rats (Zammitt et al. 1999). Studies have been conducted to identify specific nutrients that would contribute to the regulation of lipid homeostasis in obesity. Interestingly, some dietary components that completely escape digestion can modulate serum lipid concentrations. Oat bran, through its interaction with bile acid metabolism, is an effective hypocholesterolemic agent (Lia et al. 1997). In addition to their effects in the gastrointestinal tract, several nondigestible but fermentable carbohydrates have been shown to exert systemic effects by modifying lipid metabolism. Resistant starch, for example, lowers plasma cholesterol and TAG concentrations in hamster or rats (Levrat et al. 1996, Trautwein et al. 1999, Younes et al. 1995). The modulation of lipoprotein profile (decrease in LDL cholesterol and increase in HDL cholesterol) by resistant starch was also shown in hypercholesterolemic patients; this effect seems dependent on the activity of colonic microflora (Jenkins et al. 1999). In previous studies, we demonstrated that the addition of oligofructose (OFS) to the diet of male Wistar rats decreased serum and hepatic TAG levels (Delzenne and Kok 1998, Kok et al. 1996).

OFS is a nondigestible oligomer of β-D-fructose, obtained by enzymatic hydrolysis of inulin extracted from chicory root; these fructans are also present in vegetables such as onions, artichoke, asparagus and garlic. OFS is highly fermented in the cecocolon, in both human and rodents, leading to the selective proliferation of bifidobacteria [for review, see Roberfroid and Delzenne (1998)]. In rats, the addition of OFS to a standard diet enriched in saccharose decreases both serum and hepatic TAG levels (Agheli et al. 1998, Kok et al. 1996). This phenomenon is due mainly to a decrease in the expression and activity of glycerol.

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3 To whom correspondence should be addressed.
4 Abbreviations used: ATPCL, adenosine triphosphate citrate lyase; FAS, fatty acid synthase; ME, malic enzyme; NIDDM, noninsulin-dependent diabetes mellitus; OFS, oligofructose; PAP, phosphatidate phosphohydrolase; PL, phospholipids; RT-PCR, reverse transcriptase-polymerase chain reaction; TAG, triacylglycerol.

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key lipogenic enzymes such as fatty acid synthase (FAS) (Delzenne and Kok 1998). The hypotriglyceremic effect of OFS has also been observed in rats and hamsters fed a fat- and cholesterol-rich diet; such effects could be attributed to extra-hepatic events such as the modulation of bile acid metabolism or an increase in the catabolism of triglyceride-rich lipoproteins in the circulation (Kok et al. 1998b, Trautwein et al. 1998).

Could fructans like OFS, through their influence on lipid homeostasis, be considered as "functional foods" beneficial for human health? Several papers support their beneficial influence on serum lipids in humans (Jackson et al. 1999), but a recent study did not show any influence of fructans on circulating TAG in patients with type 2 diabetes (Alles et al. 1999). Analysis of the effect of dietary supplementation with fructans in models mimicking metabolic alterations occurring in obese/non-insulin-dependent diabetes mellitus (NIDDM) patients is therefore important. This study was designed to challenge the metabolic effects of supplementing OFS to a diet of obese male Zucker rats with a genetic deficiency in the leptin receptor, leading to insulin resistance and preferential utilization of carbohydrates toward fatty acid storage as TAG in the liver and adipose tissue.

### MATERIALS AND METHODS

#### Animals

Genetically obese Zucker (fa/fa) male rats, weighing 140 ± 10 g at the beginning of the experiment, were obtained from Iffa Credo (Les Oncins, France) 5 wk after birth. All animals received care in compliance with institutional guidelines from the NIH (NRC 1985). Rats were housed in individual cages in a room with temperature control and an automatic light:dark cycle (lights on from 0700 to 1900 h). Rats were randomly assigned to one of the two groups. Control rats were given free access to a powdered nonpurified standard diet obtained from a commercial source (UAR AO4, UAR Villemoisson-sur-Orge, France). This diet contained the following (g/100 g dry weight): 19% protein (consisting of an equivalent mix of soy and fish proteins); 70% total carbohydrates obtained from corn, wheat, barley and bran (containing 3% saccharose and 5% cellulose); 3.2% total lipids; 7.3% minerals and vitamins; 0.32% methionine; and 0.04% choline. OFS-fed rats were fed the same diet containing 100 g raffinose P95/kg. The energy value for the control and OFS diet was 13.86 and 13.08 kJ/g, respectively. Body weight and food intake were monitored two times per week. Serum metabolites were measured during the experiment in rats in the postprandial state, i.e., at 0900 h and after 9 h food deprivation in the afternoon, at 1700 h.

After 8 wk of treatment, an oral glucose and lipid tolerance test was performed according to the procedure described by Iritani et al. 1999. Food was removed 24 h before the test; the rats were fed by gavage a glucose load (2 g/kg body) followed by a lipid load (5 g corn oil/kg). Corn oil was commercially available and the fatty acid composition (g/100 g fatty acids) was as follows: myristic acid 0.6; palmitic acid 2.2; palmitoleic acid 0.3; oleic acid 8.1; linoleic acid 47.8; and linolenic acid 1.5. Glucose, triglyceride, and insulin concentrations in plasma were determined using blood obtained from the tail vein.

After 10 wk of treatment, at 0900 h (corresponding to the postprandial period), all rats were anesthetized with pentobarbitonal (60 mg/kg body). Blood was collected from the cava and portal veins. Some liver samples were clipped immediately in liquid nitrogen and kept at −80°C. Some pieces were frozen in isopentane, then cut cryostatically for histochemical fat staining with Sudan 7b. Some liver specimens were fixed in 10% formalin, embedded in paraffin and cut with a microtome for hematoxylin and eosin staining.

#### Chemicals

Raffinose P95 (Raffinerie Tirlemontoise, Tienen, Belgium), a mixture of glucosyl-(fructosyl), fructose and (fructosyl)n-, fructose with an average degree of polymerization of 4–8, was used as the fructose source.

#### Analytical procedures

Triglycerides, phospholipids, total cholesterol and glucose concentrations were measured in the serum with the use of kits coupling enzymatic reaction and spectrophotometric detection of reaction end products (Triglycerides ESFAS, Phospholipids-PAP, Cholesterol PAP and Glucose PAP, Elitech, Brussels, Belgium). The same kits were used for liver lipid analysis, after a chloroform-methanol extraction according to Folch et al. (1957).

Insulin concentrations were determined on frozen plasma samples using a RIA kit from CIS bio international (Insulin-CT, Oris group, Gif-sur-Yvette, France).

Fatty acid synthase (FAS) activity was measured according to the method of Linn (1981). ATP citrate lyase (ATPCL) and malic enzyme (ME) activities were measured in the cytosolic fractions according to Takeda et al. (1969) and Hsu and Lardy (1969), respectively. The esterification pathway was assessed by measuring phosphatidate phosphohydrolase (PAP) activity as described by Cheng and Saggerson (1978). Protein concentration was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Total RNA was isolated from frozen liver (200 mg) by using the RNAgent total RNA isolation system (Promega, Leiden, Netherlands). The quantification of FAS mRNA was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) as described in the RT-PCR system kit (Promega, Leiden, Netherlands). Amplification was carried out under standard cycling conditions. Specific primer pairs were as follows: 1) FAS, sense 5'-CCAGGCTGTGGAACACAGTGTGGAACAC-3' and anti sense 5'-CCAGGCTGTGGAACACAGTGTGGAACAC-3'; and 2) β-actin, sense 5'-GTCAGCCGCGCTGCGTTACAG-3' and anti sense 5'-GTCAGCCGCGCTGCGTTACAG-3'. Cycle numbers used for each primer pair were adjusted to ensure linear amplification. Reaction products were separated on 1.8% agarose gel in TAE buffer (2 mol/L Tris base, 5.7% acetic acid; 0.05 EDTA, pH 8) containing 0.1 g/L ethidium bromide. RT-PCR products were visualized under short-wave UV light and photographed. Photographs were scanned and the density of the bands was calculated using the program Image Master (Pharmacia Biotech Benelux, Roosendaal, The Netherlands). The FAS/β-actin mRNA ratio was used as a relative estimate of FAS mRNA abundance.

#### Statistical analysis

Data are presented as means ± SEM. Data were analyzed by two-way ANOVA when testing for the interaction of time and treatment. Student’s t-test was applied to compare results at one particular time point. StatView 312+ (Abacus Concepts, Berkeley, CA) was used as software. The level of significance was set at P < 0.05.

### RESULTS

Body weight at the beginning of the feeding period did not differ in control and OFS-fed rats (Fig. 1A). Body weight gradually increased with time but it was significantly lower in OFS than in control rats (P < 0.05) after 4 wk of treatment. At the end of the study, body weight was ±10% lower in OFS-fed than in control rats (Table 1).

Food intake (Fig. 1B) was significantly lower in OFS than in control rats mainly at the beginning of the study; the difference between groups gradually disappeared with time from wk 4.

Serum triglyceride concentrations increased gradually with time during the study in both the control and OFS-fed rats. In the postprandial state, TAG levels started at 0.71 ± 0.04 mmol/L and reached 2.95 ± 0.37 mmol/L in control rats within 7 wk of treatment. They increased from 0.68 ± 0.10 to 3.43 ± 0.33 mmol/L in OFS-fed rats. In food-deprived rats, TAG concentrations reached 2.72 ± 0.29 and 3.09 ± 0.35 mmol/L in control and OFS-fed rats, respectively. No significant differences in glycemia were observed between groups when measured in the postprandial or food-deprived states (data not shown).

After 8 wk of treatment, we evaluated the putative effect of OFS on glycemia and triglyceridemia, after an oral load in glucose and lipids, in rats that had been deprived of food for 24 h. Within 1 h after gavage, triglyceridemia decreased in...
Macroscopic analysis of the organs revealed an enlargement of the cecum in OFS-fed rats; The cecal content weight (control, 4.05 ± 0.59 g; OFS-fed rats, 8.79 ± 0.75 g) and the weight of cecal tissue (control, 1.34 ± 0.12 g; OFS-fed rats, 2.10 ± 0.15 g) were significantly greater in OFS-fed than in control rats (P < 0.005). The epidymidal adipose tissue weight was 16% lower in OFS-fed rats (8.46 ± 0.32 g) compared with controls (10.08 ± 0.40 g) (P = 0.01).

Liver weight was 20% lower in OFS-fed rats than in controls (P = 0.002) (Table 1). Hepatic triglyceride concentration was 57% lower in OFS-fed rats compared with control rats (P = 0.04), and hepatic PL concentration was also lower (P = 0.04). This was confirmed by histologic examination of the liver (Fig. 3). Fat staining performed on cryostat section in controls (Fig. 3A) showed numerous micro- and macrovacuolar Sudan 7B positive hepatocytes, which were present mainly in the midzonal, partially in the periportal area of the hepatic lobule. Fewer fat positive cells were found in the liver of OFS-treated rats (Fig. 3B); these cells were dispersed mainly in the periphery of the midzonal area. This difference in fatty degeneration of hepatocytes between Zucker rats fed control and OFS-containing diets, was also visible after hematoxylin and eosin staining (white vacuoles) (Fig. 3C and D).

The activities of three key lipogenic enzymes (FAS, ATPCL and ME) were measured in liver (Table 1), as was that of PAP, a key enzyme controlling fatty acid esterification. Only malic enzyme activity was significantly lower in OFS-fed rats than in controls. FAS mRNA levels did not differ between groups.

## DISCUSSION

Obesity and the insulin resistance occurring in NIDDM are often related to disturbances of lipid metabolism that lead to an increase in hepatic and serum TAG concentrations, which are involved in the development of liver disorders (steatosis and steatohepatitis) and cardiovascular diseases (Boulangé et al. 1981, Diehl 1999, Furnell and Brunzell 1997). Our previous studies used nonobese rats; other results obtained in hypertriglyceremic nonobese patients suggest that the supplementa-
tion of diets with some specific nutrients such as nondigestible fructans could be helpful in the control of TAG metabolism (Delzenne and Kok 1999, Fiordaliso et al. 1995, Jackson et al. 1999, Kok et al. 1996 and 1998a and 1998b). Our work was designed to analyze the putative influence of dietary supplementation with a nondigestible fructan, oligofructose, in obese \textit{fa/f} Zucker rats, which develop hypertriglyceridemia associated with muscle insulin resistance and obesity (Penicaud et al. 1987). The addition of 10% OFS to the standard diet of male Zucker rats slowed the increase in body weight, an effect that was not shown in previous experiments performed in nonobese rats receiving the same dose of OFS (Delzenne and Kok 1998, Fiordaliso et al. 1995). This effect was significant after 4 wk of treatment, whereas the inhibitory effect of OFS on food and energy intake was significant during the first few weeks of treatment only. At the end of the treatment (10 wk), body weight was \(\pm\)10% lower in OFS than in controls rats. Interestingly, a significantly lower epididymal fat mass was observed in OFS-fed rats.

The influence of OFS on lipemia and glycemia in Zucker rats varied with the duration of the treatment. In contrast to the results obtained in rats fed OFS in either standard or lipid-rich diets, in which a lower triglyceridemia was observed from wk 1 of treatment (Kok et al. 1996 and 1998b), serum TAG were not modified by OFS treatment in either the postprandial or food-deprived state. A glucose and lipid load test was performed after 8 wk of treatment. The increase in triglyceridemia occurring 2 h after an oral load of glucose and corn oil was significantly higher in OFS-fed than in controls rats, suggesting either a higher capacity to absorb lipids from the intestinal tract and/or a greater secretion of endogenous VLDL from the liver. The putative promoting effect of OFS on lipid absorption is disturbing because it is generally accepted that dietary fiber promotes the fecal excretion of bile acids and thus interferes with the absorption of dietary lipids. However, some investigators have obtained results suggesting that the intestinal activity of lipase and the secretion of bile acids, two processes participating in TAG digestion, are higher in rats fed a diet containing fermentable fibers such as guar gum or pectin (Favier et al. 1997, Foreman and Scheemann 1982, Levrat et al. 2000). Could such a mechanism also occur in OFS-fed rats? The question remains open. What about a putative effect on VLDL secretion? This question will be discussed below.

Knowing the putative atherogenic role of postprandial hypertriglyceridemia in humans, it is essential to clarify the mechanism and the putative relevance in humans of the higher triglyceridemia observed in OFS-treated rats after a fat load. After 10 wk of treatment, serum TAG and cholesterol levels, measured in the cava and portal veins, were similar in control and OFS-fed rats. However, OFS feeding led to a dramatic decrease in the concentration of TAG in liver. This phenomenon, which reduced the relative liver weight, was confirmed by histologic analysis. In Zucker rats fed the standard diet, accumulation of lipid vacuoles, stained by Sudan 7b, was observed mainly in the midzonal area and partially in the periportal zone of the liver lobule, as previously shown by Koneru et al. (1995). The phenomenon was much less dramatic in the liver of rats fed OFS, in which only rare enlarged fat-containing hepatocytes were dispersed, mainly in the periphery of the midzonal area.

Obese Zucker rats develop hepatic steatosis due to excessive synthesis and storage of TAG in the liver (Fukuda et al. 1982). Therefore, we measured the activities of key enzymes involved in fatty acid synthesis (ME, ATPCL and FAS) and esterification (PAP). The only significant effect was a lower activity of ME, which provides NADPH for fatty acid elongation by FAS complex in OFS-fed rats, whereas the other enzyme activities and FAS mRNA were not different. This effect differed from the huge decrease in lipogenic enzyme mRNA and activities observed in the liver of OFS-fed Wistar rats (Delzenne and Kok 1999). Glucose and insulin are important regulators of

\[\text{FIGURE 2} \quad \text{Effect of oligofructose (OFS) on (A) the triglyceridemia (TG), (B) glycemia and (C) insulinemia of rats given a load of glucose and corn oil (0.2 and 0.5 g/100 g body weight, respectively). Values are means \(\pm\)SEM, } n = 5 \text{ (control) or } n = 6 \text{ (OFS). Two-way ANOVA indicated a significant difference between control and OFS-fed rats in triglyceridemic response only } (P < 0.05).\]
fatty acid synthesis because they coordinately promote lipogenic enzyme gene expression (Girard et al. 1997). Postprandial insulin was significantly lower in nonobese rats fed fructan-enriched diets, in which hepatic lipogenesis was strongly inhibited (Kok et al. 1998b). In the present study, we did not detect any modifications of glycemia during OFS treatment, but a lower serum glucose concentration was observed in the portal and cava veins at the end of the study in OFS-fed rats. This effect was accompanied by a higher concentration of insulin. Although the role of insulin as a regulator of VLDL secretion is controversial, several studies support its role as a promoter of VLDL secretion from liver tissue in vivo and in vitro (Zammitt 1996). A higher secretion of VLDL in Zucker rats treated with OFS could explain the lower TAG accumulation in the liver. This hypothesis remains to be tested. De novo lipogenesis is not the sole source of fatty acids for TAG synthesis in the liver. In Zucker rats, the large fat mass releases nonesterified fatty acids that reach the liver. We have shown that the epididymal fat mass is lower in OFS-fed rats, but the postprandial and post-food deprivation concentrations of nonesterified fatty acids in the serum was not modified by the treatment, at least until wk 5 (data not shown).

The mechanism by which such nondigestible carbohydrate such as oligofructose may modulate systemic lipid metabolism remains unknown. It could be related to the lower food and energy intakes at the beginning of the treatment, or to OFS fermentation in the cecocolon. A possible role of the microflora in providing a hepatotrophic factor or in modifying their bioavailability would be interesting to test. On the other hand, short-chain carboxylic acids such as acetate and propionate are end products of bacterial fermentation of fructans, which reach the liver through the portal vein. Some in vitro studies indicate that both acetate and propionate have antagonistic effects on lipid metabolism (acetate is a lipogenic substrate, whereas propionate is an effective inhibitor of lipid synthesis in isolated hepatocytes) (Demigné et al. 1995 and 1999).

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Portal vein</th>
<th>Cava vein</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>OFS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>10.73 ± 0.65</td>
<td>7.16 ± 1.62*</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>6379 ± 1207</td>
<td>13,448 ± 2759</td>
</tr>
<tr>
<td>Phospholipids, mmol/L</td>
<td>4.09 ± 0.19</td>
<td>3.63 ± 0.16</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>2.31 ± 0.24</td>
<td>2.31 ± 0.37</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.78 ± 0.43</td>
<td>4.54 ± 0.24</td>
</tr>
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1 Values are the means ± SEM, n = 5 (control) or 6 (OFS); *significantly different from control, P < 0.05.
Their putative role as regulators of TAG synthesis and secretion in the liver of obese Zucker rats remains to be evaluated.

In conclusion, this study suggests that the metabolic response to dietary fructans is different in obese Zucker rats than in other animal models. They do not have the hypertriglyceridemia observed in numerous studies of animals and humans (Roberfroid and Delzenne 1998). This study suggests that fructans do not counteract the hyperlipidemia associated with insulin resistance due to a leptin-receptor defect. This could explain why some authors failed to find a hypotriglyceridemic effect of fructans in diabetic patients (Alles et al. 1999). However, we found that dietary fructan supplementation counteracted both fat mass development and the hepatic steatosis that occur in obese Zucker rats. The putative influence of dietary fructans on body weight and fat mass, if confirmed in humans, would constitute an interesting tool in the control of obesity. A recent paper reported that in severely obese people (body mass index >45 kg/m²), 86% were found to have steatosis (Marceau et al. 1999). The pathologic consequences of TAG accumulation in the liver are not well understood, but more and more studies suggest that fatty liver is more sensitive to viral infection (hepatitis C), ischemia, or toxic compounds (Hourigan et al. 1999, Koneru et al. 1995). Future studies will be designed to analyze the mechanism of the “hepatoprotective” effect of dietary fructans in obese animals to clarify its putative usefulness in the control of the hepatic alterations linked to obesity in humans.

LITERATURE CITED


