Dietary Fructans, but Not Cellulose, Decrease Triglyceride Accumulation in the Liver of Obese Zucker fa/fa Rats

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ABSTRACT This study was designed to compare the effects of dietary supplementation with nondigestible carbohydrates, differing in fermentability by colonic bacteria, on hepatic steatosis in growing obese Zucker rats. Male Zucker fa/fa rats were divided into three groups: a control group that received the basal diet, a fructan group that received 10 g highly fermented Synergy 1/100 g diet and a cellulose group that received 10 g poorly fermented Vivapur Microcrystalline cellulose/100 g diet. Rats consuming fructan had a lower energy intake, a lower body weight and less triacylglycerol accumulation in the liver as assessed in vivo by nuclear magnetic resonance (NMR) spectroscopy, and ex vivo by biochemical and histochemical analysis compared with the control and/or cellulose groups. The high fermentation of fructans compared with cellulose was reflected by greater cecal contents and by a twofold greater propionate concentration in the portal vein of rats fed fructan compared with those fed cellulose. By measuring the capacity of hepatocytes isolated from liver of Zucker rats to synthesize triglycerides or total lipids from different precursors, we showed that propionate, at the concentrations measured in the portal vein of rats treated with fructan, selectively decreased the incorporation of acetate into total lipids, a phenomenon that could contribute, along with the lower energy intake, to less triglyceride accumulation in the liver of obese Zucker rats fed dietary fructans. J. Nutr. 132: 967–973, 2002.

KEY WORDS: fructan • cellulose • rats • hepatic steatosis

Obesity, type 2 diabetes mellitus and hyperlipidemia are often linked to modifications of hepatic fatty acid metabolism, leading to the accumulation of triacylglycerols (TAG) in the liver, defined as steatosis (1,2). Fatty liver, even if it is an asymptomatic perturbation, is believed to be the setting for a more severe and progressive disorder, namely, nonalcoholic steatosis hepatitis (3,4). The diagnosis of steatosis is based mainly on liver biopsy, which is an invasive and constraining technique because no clear clinical serum biochemical markers have been validated. No predictable correlation exists between abnormalities of liver enzymes and histologic lesions (4). Although ultrasound diagnosis is often used as a useful technique to evaluate the presence of a fatty liver, this method does not provide quantitative measurements and is not always adequately sensitive; 1H nuclear magnetic resonance (NMR) spectroscopy has been proposed as a suitable tool for grading the liver fat content in animals (5–8) and in humans (9–13). In experimental animals, a close correlation was found between hepatic triglyceride content measured in vivo by 1H NMR spectroscopy and chemically by liver biopsy (7). Several experimental models of hepatic steatosis exist including the Zucker fa/fa rat. It is characterized by a modified leptin receptor and exhibits a metabolic syndrome similar to that in overweight humans, namely, hyperphagia, hypertriglyceridemia and hepatic steatosis, associated with a moderate hyperglycemia and insulinemia (14–16). This model may be used to understand the biochemical mechanisms underlying the excessive accumulation of TAG in tissues, but may also serve to test for the putative effect of food components that could help control steatosis. We found that the addition of 100 g fructan/kg basal diet protected male Wistar rats from liver TAG accumulation induced by fructose (17) and that it lessened hepatic steatosis in Zucker fa/fa rats (18).

Fructans are naturally occurring components of many vegetables, such as onions, garlic and artichokes. Some fructans may be obtained by enzymatic hydrolysis of chicory inulin; they are used by the food industry largely as fat or sugar replacers and as functional nutrients (19). Once ingested, due to the β-linkage between fructosyl moieties, they escape enzymatic hydrolysis by eukaryotic amylases and saccharidases, but are fermented largely in the ceco-colon by bacterial strains exhibiting fructosidase activities (20,21). The fermentation of these fructans produces short-chain fatty acids (SCFA), namely, acetate, propionate and butyrate. Butyrate is metabolized by colonocytes, whereas acetate and propionate reach the liver through the portal vein (22,23).
How could such nondigestible carbohydrates have an influence on liver TAG accumulation? Could 1H NMR spectroscopy be useful in assessing the effect of nondigestible carbohydrates (NDG) on hepatic steatosis in vivo? Is the fermentation of NDG necessary to observe any effect on lipid metabolism? Is the high concentration of propionate observed in the portal vein be useful in assessing the effect of nondigestible carbohydrates (NDG) on hepatic steatosis in vivo? Is the high concentration of propionate observed in the portal vein be useful in assessing the effect of nondigestible carbohydrates (NDG) on hepatic steatosis in vivo?

In vivo study. Male obese Zucker fa/fa rats weighing 330–350 g were purchased from Ifla Credo (Les Oncins, France) and housed in a temperature and light-controlled room. They consumed standard diet AO4 (Villemoisson-sur-Orge) and water ad libitum. For in situ perfusion of the liver, rats were anesthetized with intraperitoneal injection of sodium pentobarbitral solution (Nembutal, 60 mg/kg body). The hepatocytes were isolated from fed rats, according to the standard procedure described by Krack et al. (31).

Analytical procedures

In vivo study. Serum TAG, total cholesterol, nonesterified fatty acids (NEFA) and glucose were measured using kits coupling enzymatic reactions and spectrophotometric detection of end products (Triglycerides ESPAS, phospholipids-PAP, Cholesterol PAP and Glucose PAP, Elitech, Brussels, Belgium). The same kits were used for liver lipids analysis, after a chloroform/methanol extraction according to Folch et al. (32). SCFA (acetate, propionate and butyrate) in the portal vein were measured in collaboration with Prof. C. Remésy (Laboratoire des Maladies Métaboliques, INRA de Clermont Ferrand/Thieix, France). SCFA were measured using a gas-liquid chromatography after ethanolic extraction as described by Remésy and Demigné (33).

In vitro study. Hepatocytes were incubated at a density of 105 cells/cm² in Williams’ E medium supplemented with t-glutamine (2 mM/L), bovine serum albumin (1 g/L), insulin (100 μg/mL) and propionate (0.3–0.6 mM/L). Incubations were carried out at 37°C under an atmosphere of O2/CO2 (95:5%) in a shaking water-bath (100 oscillations/min). The reaction was started by adding 2 mM/L [1-14C]-acetate (7.4 Bq/mmol) or 0.2 mM/L [1-14C]-palmitate (7.4 Bq/mmol). At 60-min intervals, samples were removed from the suspension and cells were sedimented by centrifugation. Lipid synthesis was measured by quantifying the incorporation of 14C-labeled precursors into intracellular and extracellular 14C-labeled lipids, as previously described by Krack et al. (34). The results were expressed in nanomoles of precursor incorporated into lipids per milligram protein (35). Cellular lysis during incubation was estimated by measuring lactate dehydrogenase leakage from the cells, as described by Krack et al. (31).

Nuclear magnetic resonance spectroscopy. Rats were anesthetized with pentobarbitral and then immobilized in a birdcage coil for ~20 min. All spectroscopy and imaging experiments were performed with a 4.7 T (200 MHz, 1H) Bruker Biospec (Bruker, Karlsruhe, Germany) imager equipped with a 40-cm i.d. and a 12-cm shielded gradient hardware (rise time, 80 μs; maximum gradient strength, 200 mT/m). Images were obtained in a linearly polarized “birdcage” transmitter–receiver coil, 70-mm i.d. Images and spectra were processed via an in-house program based on the IDL 5.3 (Interactive Display Language, IDL, Boulder, CO) development software running on a PC-Pentium computer. Water and lipid peaks areas were calculated by fitting the spectrum. Hepatic TAG content was estimated by comparing the signal integrated intensity of fat peak with the signal intensity of water. Two transverse slice images through the liver were used for voxel placement, and localized spectra were obtained at two different positions in each slice, i.e., in the right and left lateral lobes.

Statistical analysis. All statistical analyses were performed with the SPSS program for Windows system (SPSS, Chicago, IL). Data are presented as means ± SEM except in Figure 5. To compare the time course of the effect of oligofructose feeding on body weight and food intake, two-way ANOVA was performed. A one-way ANOVA followed by Scheffé’s test was applied for statistical evaluation of treat-
RESULTS

Body weight increased steadily with time but it was significantly lower in fructan- than in cellulose-fed rats from wk 5 until the end of the treatment at wk 8 (Fig. 1). Total weight gain was significantly less in fructan-fed rats (226 ± 5 g) than in control (260 ± 7 g; P = 0.017) and cellulose (275 ± 8 g; P = 0.001) groups.

Food consumption (P = 0.12) and energy intake (P = 0.91) did not differ between the cellulose-fed and control rats (Table 1). The fructan group ate the same amount of food per day as controls but total energy intake was lower in fructan-fed than in control and cellulose-fed rats (Table 1).

Cellulose or fructan addition in the diet did not affect postprandial or postabsorptive concentrations of TAG, NEFA or glucose in the serum from the tail vein during the treatment or from the tail, portal and cava veins at the end of the treatment (data not shown). Portal vein insulin tended (P = 0.08) to be greater in rats fed fructan (4872 ± 348 pmol/L) than in controls (3306 ± 348 pmol/L).

The voxels localization for NMR spectroscopy and representative magnetic resonance spectra from Lean or fa/fa obese Zucker rats are shown in Figure 2A and B, respectively. No fat signal was found in the spectrum from the control lean Zucker rats, whereas a high signal intensity, indicating hepatic steatosis, was observed in fa/fa Zucker rats fed control and cellulose diets. Fat signals were present in the spectra of all fa/fa Zucker rats but were clearly lower in the fructan group. The fat content relative to water (weighted proton density of fat relative to water, mol/mol in a given volume) was lower in fructan-fed rats compared with the other groups (Fig. 3).

After 8 wk of dietary treatment, liver weight was less in fructan-fed than in cellulose-fed or control rats (Table 2). Compared with the Lean Zucker rats, the histological examination of the liver tissue (Fig. 4) from the fa/fa obese Zucker rats revealed an altered liver structure with a huge macrovacuolar steatosis disseminated from the portal space to the centrolobular vein. This was present in control and cellulose-treated rats. In fructan-treated rats, enlarged hepatocytes with micro- and macrovacuoles were scarce and the number of Sudan Red positive cells was much lower than in other groups because only small irregular positive foci were disseminated in the median and centrolobular zone of the liver. The biochemical quantification of TAG in the liver was consistent with the histochemical analysis because the hepatic TAG level was 48% lower in the fructan-fed than in the cellulose-fed group (Table 2). The activities of key enzymes controlling fatty acid synthesis (fatty acid synthase; malic enzyme; ATP citrate lyase) and fatty acid esterification (phosphatidate phosphohydrolase) were not modified by the treatment (data not shown).

Cecal contents of fructan-fed rats (4.29 ± 0.36 g) were greater than in controls (2.87 ± 0.18 g) (P = 0.003) or cellulose-fed rats (3.48 ± 0.19 g) (P = 0.09).

Portal vein acetate concentration was not affected by dietary treatment and the concentration of butyrate tended to be greater than in controls after fructan treatment (P = 0.1) (Fig. 5). The concentration of propionate was measurable (limit of detection 0.1 mmol/L) in the serum of the portal vein of 3 of 6 rats in the control group, and in 2 of 6 rats in the cellulose group. The level of propionate was higher than 0.1 mmol/L in all portal sera obtained from fructan-treated rats. It tended to be greater in rats fed fructan than in controls (P = 0.08) (Fig. 5).

Because the higher concentration of propionate measured in the serum of the portal vein of fructan-treated rats was 0.6 mmol/L and the mean 0.3 mmol/L, we assessed the influence of propionate at those concentrations (0.3 and 0.6 mmol/L) on fatty acid synthesis in obese Zucker rat hepatocytes (Table 3). The addition of propionate to the medium did not modify either the capacity for esterification or secretion of 14C-palmitate. In Zucker rats, propionate, at both concentrations (0.3 and 0.6 mmol/L), generally decreased (0.05–0.07) by ~30% the incorporation of acetate into total lipids.

DISCUSSION

Fatty liver is often linked to an excess of fat mass, and would result from an increase in total energy and carbohydrate intake in humans. There is evidence that food restriction and a lower body weight are accompanied by an improvement of fatty liver in obese adults and children (36–39). However, long-term maintenance of a low energy regimen by obese people is difficult; thus the discovery of nutrients that would ameliorate liver hepatic steatosis is of interest.

We showed recently that the addition for 10 wk of fructans obtained from enzymatic hydrolysis of chicory root inulin decreases body weight and TAG accumulation in hepatic steatosis. The results presented here show that dietary fructans also decrease the amount of TAG in liver.

TABLE 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Cellulose</th>
<th>Fructan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g</td>
<td>24.1 ± 0.7ab</td>
<td>26.3 ± 0.7a</td>
<td>22.5 ± 0.7b</td>
</tr>
<tr>
<td>Energy intake,2</td>
<td>334 ± 10a</td>
<td>328 ± 9a</td>
<td>292 ± 9b</td>
</tr>
<tr>
<td>kj</td>
<td>79.9 ± 2.5a</td>
<td>78.5 ± 2.1a</td>
<td>69.9 ± 2.2b</td>
</tr>
<tr>
<td>kcal</td>
<td>24.1 ± 0.7ab</td>
<td>26.3 ± 0.7a</td>
<td>22.5 ± 0.7b</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 7. Means in a row without a common letter differ, P < 0.05.
2 Data were calculated from the mean amount of food ingested per rat per week.
tissue of obese Zucker fa/fa rats (18). In the present study, we confirmed this effect and showed that the lessened steatosis is present after 6 wk as assessed by using NMR spectroscopy. TAG accumulation was assessed by three different techniques, i.e., NMR spectroscopy, histochemical detection of fat and biochemical quantification of lipids in the liver tissue, which are all very different in their underlying principles. The three techniques showed a good protective effect of fructan consumption on liver steatosis. A lack of protection by cellulose was also evident. NMR spectroscopy did not show any difference between cellulose-fed and control rats; however, the histological analysis, performed 2 wk later, suggested a more pronounced steatosis in cellulose-fed rats, compared with controls; the higher liver TAG content in cellulose-fed rats, assessed by biochemical analysis, was not significant ($P = 0.7$). A different repartition of lipids inside a lobule could explain the discrepancies between the histological images and (semi)-quantitative technique, such as imagery and biochemistry. The fact that NMR spectroscopy was performed 2 wk before histological analysis could also be involved in the different interpretation. Further studies should address the intertechnique comparison of liver TAG accumulation. Control and cellulose-fed rats ingested the same amount of energy, grew to the same extent and developed similar fat mass (data not shown).

**FIGURE 2** Nuclear magnetic resonance (NMR) spectroscopy of the liver of Zucker rats fed a control diet or that diet enriched with cellulose or fructan. (A) Images of fatty liver in control obese Zucker rat: squares indicate the voxel placement for recording the localized $^1$H spectrum. (B) Typical in vivo localized $^1$H spectrum from (A) a control obese Zucker rat liver, (B) a cellulose-fed obese Zucker rat liver, (C) a fructan-fed obese Zucker rat liver and (D) a control lean Zucker rat liver.

**FIGURE 3** Liver fat abundance measured by nuclear magnetic resonance (NMR) spectroscopy in Zucker rats fed a control diet or that diet enriched with cellulose or fructan. Values with different letters differ, $P < 0.05$.

**TABLE 2** Hepatic lipids in obese Zucker rats fed a control diet or that diet supplemented with cellulose or fructan

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Cellulose</th>
<th>Fructan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>394 ± 9a</td>
<td>405 ± 11a</td>
<td>346 ± 6b</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>18.3 ± 0.4a</td>
<td>20.0 ± 1.3a</td>
<td>14.8 ± 0.6b</td>
</tr>
<tr>
<td>g/100 g body</td>
<td>4.8 ± 0.1b</td>
<td>4.9 ± 0.3</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Liver TAG, nmol/mg protein</td>
<td>452 ± 72ab</td>
<td>548 ± 96a</td>
<td>286 ± 48b</td>
</tr>
</tbody>
</table>

1 Values are the means ± SEM, n = 5–7 rats. Means that do not share a letter in a row differ, $P < 0.05$. 
shown), whereas fructan-fed rats, which did not compensate for the lower energy value of the diet by increasing their food intake, had a body weight gain ~50 g less than the other groups at the end of the study. Because some investigators have recently shown by indirect calorimetry that microcrystalline cellulose enhanced the velocity of carbohydrate absorption, whereas fermentable fiber such as resistant starch or pectin had a slowing effect (40), we assessed serum glucose and insulin levels in the different groups. No significant modifications of either postprandial (portal or peripheral) glucose concentrations occurred, but the portal concentration of insulin was increased by 47% in fructan-fed rats compared with the control group. What could be the consequences of such modifications of TAG metabolism? Glucose, together with insulin, is an important key regulatory nutrient, involved in carbohydrate-induced lipogenic gene expression (41). The lower TAG concentration in the liver of fructan-fed rats occurred without any effect on the activity of key enzymes of lipogenesis. Insulin also plays a role in TAG hepatic secretion because when perfused into the portal vein, it increases VLDL secretion in fed rats (42). The higher portal concentration of insulin in obese rats fed fructan could thus promote TAG secretion and thus avoid TAG accumulation in the tissue (18). However, no modification of serum TAG in the postprandial or postabsorptive state occurred in the present study.

Because the protective effect of NDG is found in highly fermentable fructans but not in poorly fermentable cellulose, the hypothesis of a role for the end products of carbohydrate fermentation in the modification of hepatic TAG synthesis

FIGURE 4 Histological analysis of liver from Zucker rats fed a control diet or that diet supplemented with cellulose or fructan. Panels (A)–(D) were stained with hematoxylin and eosin and panels (E)–(H) with Sudan Red 7b for lipid detection. (A) Normal trabecular structure of the liver lobule with homogeneously stained hepatocytes from Lean Zucker rats fed control diet. Seriously altered liver structure with considerably enlarged hepatocytes with micro- and macrovacuoles dispersed throughout the cytoplasm of hepatocytes from a fa/fa obese Zucker rat fed the control (B) or cellulose (C) diets. (D) Rare vacuoles are dispersed on the periphery of the perportal area in the liver of a fructan-fed fa/fa obese Zucker rat. (E) Lean Zucker rat liver sections exhibited a normal and Sudan Red–free pattern of liver parenchyme. Numerous Sudan Red positive micro- and macrovacuoles are dispersed in the hepatocytes of fa/fa obese Zucker rats fed the control (F) or cellulose (G) diets. (H) Some Sudan Red positive in the liver lobule of a fa/fa Zucker rat fed the fructan diet. Panels (A)–(D) = 1475 μm; panels (E)–(H) = 3690 μm.

FIGURE 5 Concentration of short-chain fatty acids (SCFA) in the portal serum of obese Zucker fa/fa rats fed a control diet or that diet enriched with cellulose or fructan for 8 wk. Individual points are shown and mean values are indicated as lines.
has been tested. SCFA and lactate are produced in the cecum of fructan-fed rats due to the fermentation by lactic acid-producing bacteria; cecal lactate concentration decreased after 2 wk of treatment, whereas total SCFA (particularly butyrate) concentrations increased (43). Butyrate is generally considered to be the preferential fuel for colonocytes, and it thus less available for other tissues than are acetate and propionate (44). Butyrate thus generally appears as a minor SCFA in the portal or peripheral blood. In the present study, we showed that butyrate concentration in the portal vein of obese Zucker rats was not negligible, reaching concentrations near 0.25 mmol/L in control- and cellulose-fed rats, and 0.5 mmol/L in some rats fed fructans. One putative explanation could be that fatty Zucker rats have an impairment of the β-oxidation of butyrate, although no studies have clearly shown this effect in colonocytes (45). We have shown that fructan-feeding tended to increase (P = 0.08) the propionate concentration in the portal vein. Could it be implicated in the modulation of lipid metabolism? Even if hexoses (e.g., glucose, fructose) generally are considered to be the physiologic substrates for fatty acid synthesis, acetate, which may be produced by fermentation in the colon, is used mainly as a substrate for both cholesterol and fatty acid synthesis. The fact that acetate could constitute a lipogenic and cholesterogenic substrate in the liver is supported by the fact that lactulose, a nondigestible polyol that is largely fermented into acetate in the colon, leads to an increase in LDL-cholesterol and TAG in the serum when added to the diet of rats (46), and acetate in the liver is mainly lipogenic and cholesterogenic rather than oxidized because only acetyl-CoA synthetase I (mitochondrial activity) is mainly lipogenic and cholesterogenic rather than oxidized because only acetyl-CoA synthetase II (mitochondrial activity) is generally inhibiting the cholesterogenic and lipogenic effects of acetate reaching the liver (47). The relevance of the anticholesterogenic effect of propionate coming from NDG fermentation in obese rats in vivo is supported by the fact that propionate instillation in hind gut decreased the hepatic cholesterol pool (49). One interesting approach would be to analyze in parallel not only TAG but also cholesterol metabolism in the liver of obese Zucker rats treated with fructan. However, in a previous study, fructan treatment did not modify portal or peripheral total serum cholesterol concentration in obese rats (18) and we have observed that the hepatic concentration of total cholesterol in obese Zucker rats is much less than that of lean rats whatever the treatment (Daubioul C., unpublished results).

The interaction between carbohydrate fermentation pattern and lipid metabolism has also been proposed for several fermentable fibers, such as class 2 resistant starches (50) and whole flours from oats, rice, barley or wheat (51). They are able to decrease hepatic lipid content (sometimes with effects on serum lipids) and together are characterized by fermentations high in propionic acids.

We cannot extrapolate directly to humans the data obtained in rats because several variables (dose of fructans, fecal flora, etiology of steatosis) differ. However, some effects shown in rats consuming 10% dietary fructans for several weeks, such as an increase in calcium absorption or a decrease in triglyceridemia, where also shown in humans ingesting lower doses (8–15 g fructan/d) (18). If confirmed in humans, the beneficial effects of fermentable NDG on liver steatosis could be useful in overweight people, and could be proven by using a noninvasive technique such as 1H NMR spectroscopy.

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LITERATURE CITED


