Fructose Intake Increases Hyperlipidemia and Modifies Apolipoprotein Expression in Apolipoprotein AI-CIII-AIV Transgenic Mice

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ABSTRACT Fructose intake has increased steadily during the past two decades. The objective of this study was to determine the effect of fructose intake on lipid metabolism in apolipoprotein (apo) AI-CIII-AIV transgenic (Tg) mice that have severe hypertriglyceridemia and moderate hypercholesterolemia. Tg and control mice were fed for 9 mo a commercial nonpurified diet and had free access to water or 250 g/L fructose solution. In Tg mice, fructose intake increased triglycerides and cholesterol but did not induce insulin resistance. There were no differences in human hepatic apo AI and apo CIII mRNA levels in fructose-fed mice compared with untreated mice, but apo AIV mRNA was greater, indicating a differential expression of the apo AI and apo AIV genes in response to dietary perturbations. Interestingly, the plasma concentration of the three human apolipoproteins was enhanced in fructose-fed Tg mice compared with untreated Tg mice. Our data suggest that long-term fructose consumption had strong adverse effects in this hyperlipidemic mouse model. J. Nutr. 132: 918–923, 2002.

KEY WORDS: • fructose • transgenic mice • apolipoprotein gene expression.

Cardiovascular disease is the main cause of death in Western countries. Its development is determined by genetic and environmental factors. The diet is the most important external determinant of plasma lipids and can easily be modified. Thus, a diet rich in saturated fat is classically associated with a higher prevalence of cardiovascular disease. However, other nonlipid components of diet can have atherosclerotic effects. High fructose diets enhance hepatic secretion of VLDL and may decrease its plasma clearance, which frequently results in modest hypercholesterolemia and hypertriglyceridemia (1–4). Furthermore, fructose feeding has frequently been used to induce hyperinsulinemia, euglycemia and insulin-resistance (IR) in rats (5,6). However, this was not observed in the only mouse model studied to date. Indeed, Merat et al. (7) found that a diet rich in fructose resulted in a marked hypercholesterolemia, but did not induce IR in LDL receptor–deficient mice (LDL−/−). However, these mice developed more extensive atherosclerosis than the fat-fed LDLR−/− mice. This atherosclerotic increase was not accompanied by an increase in advanced glycation end products or oxidation-specific epitopes in the atherosclerotic lesions, indicating that other mechanisms such as a decrease in reverse cholesterol transport or an elevated blood pressure may be involved.

Fructose intake has increased steadily during the past two decades due to its introduction as sweetener in pharmaceuticals and in mainstream food applications such as carbonated beverages, canned fruits, jams, jellies and dairy products. This has led to an increased interest in the study of diets rich in this nutrient.

The apolipoprotein (apo) AI-CIII-AIV gene cluster is involved in lipid metabolism and atherosclerosis (8,9). Overexpression of apo CIII in mice causes hypertriglyceridemia and induces atherogenesis (10,11), whereas overexpression of apo AI or apo AIV, the main components of HDL, protects against atherosclerosis (12–15). Expression of the three genes is controlled by a series of elements that are distributed throughout and extend beyond the cluster. These elements seem to coordinate regulate the transcription of all three genes (16–18). We recently generated transgenic (Tg) mice for the human apo AI-CIII-AIV gene cluster using a 33-kb genomic fragment containing the three genes and flanking sequences (19). In these mice, the three genes of the cluster are expressed in hepatic and intestinal tissues. Transgenic mice exhibit gross hypertriglyceridemia and accumulation of apo B48–rich lipoproteins of d < 1.006 kg/L, but are protected against atherosclerosis. They are therefore an adequate model with which to study the effect of different nutrients on the expression of the three human genes.

The purpose of this study was to investigate in vivo the consequences of long-term fructose consumption on lipid metabolism and in human apo AI, CIII and AIV gene expression.
MATERIALS AND METHODS

Animal models. Male Tg mice for the human gene cluster apo AI-CIII-AIV (line 12) generated in our laboratory (19) were used. The Tg line was established and maintained in the C57BL/6 genetic background (Charles River, Saint Aubin les Elbeuf, France). Nontransgenic littermates were used as controls. Mice were housed in a temperature-controlled room with alternating 12-h light (0700–1900 h) and dark periods (1900–0700 h). The mice consumed water ad libitum, and a cholesterol-free nonpurified diet containing (g/kg) the following: protein, 214; lipid, 51; carbohydrate, 517; and sucrose and fructose <5 (Standard diet A03, UAR, Toulouse, France). At 2 mo of age, the nonfructose groups were provided with tap water and the fructose groups with a 250 g/L fructose solution. The calculated average daily intake of fructose per mouse was 1.5 g. For 9 mo, control and Tg mice were allowed free access to the food and water or sugar solution. All procedures involving animal handling and care were conducted in accordance with Pasteur Institute Guidelines for Husbandry of Laboratory Mice.

RNA isolation and Northern blot experiments. After overnight food deprivation, mice were killed by cervical dislocation and tissues (liver and small intestine) were removed and frozen (−80°C). Total RNA was extracted with RNA-Plus (Quantum, Illkirch, France) and samples were purified, separated by electrophoresis (20 μg total RNA/ lane) on a 1% agarose gel containing formaldehyde, blotted onto Hybond-N+ membrane (Amerham Pharmacia Biotech, Saclay, France) and hybridized in quickHyb solution (Stratagene, La Jolla, CA) with 32P-labeled DNA probes for each human apolipoprotein, as previously reported (19). The human ubiquitin probe (Clontech Laboratories, Palo Alto, CA) was used as a control. Hybridization was carried out according to the QuickHyb Hybridization Solution instruction manual (Stratagene). Autoradiographs were scanned with NIH Image program. Signals were normalized according to the ubiquitin signal. The results were analyzed with the Statview computer program. Differences were tested by ANOVA, and P <0.05 was considered significant.

Apolipoprotein quantification. Human apo A-I and C-III concentrations in plasma were quantified by gel agarose immunoelectrophoresis with highly specific polyclonal antibodies (Hydragels SEBIA, Issy-les-Moulineaux, France); mouse apoproteins did not cross-react at detectable levels. Human apo A-IV plasma concentration was determined using an antibody-sandwich ELISA assay. Apo B48 and apo B100 were quantified in different lipoprotein fractions. They were separated by SDS-PAGE in 4–15% gradient polyacrylamide PhastGels (Amersham Pharmacia Biotech). Gels were silver stained, scanned and analyzed using a videodensitometer scanner and NIH image software; results were normalized by total protein concentration in each fraction. Protein concentrations were determined using a Micro BCA Protein Assay Kit (Pierce, Rockford, IL). Analyses were repeated three times for each different group of mice.

Glucose, insulin and lipid determination. Blood from mice deprived of food for 8 h was obtained by retroorbital bleeding under isoflurane anesthesia, before the sugar supply, and once a month during the treatment. Plasma triglycerides and cholesterol levels were determined enzymatically using commercial kits (Peridochrom Tri- glycerides GPO-PAP and Cholesterol CHOD-PAP, Boehringer Mannheim, Mannheim, Germany). All assays were calibrated using appropriate dilutions of pooled human serum, Precinorm L (Boehringer Mannheim). Plasma glucose concentration was also determined enzymatically using a GOD-PAP method (Boehringer Mannheim). Plasma insulin was measured by RIA (kit ref. Insulin CT, ORIS, GiF sur Yvette, France).

Lipoprotein analysis. Lipoproteins were separated into d < 1.006, d = 1.006–1.019 and d = 1.019–1.063 kg/L fractions by sequential isopycnic ultracentrifugation using KBr to adjust densities. Pooled plasma samples (equal aliquots of plasma from each group of mice) were used. Each centrifugation was carried out for 2 or 2.5 h at 435,000 × g at 15°C in a fixed-angle rotor as described (20). Plasma lipoprotein distribution was also assayed using a fast protein liquid chromatography system (FPLC; Pharmacia, Sweden) with one Superose 6 and one Superose 12 HR column (Pharmacia, Sweden). The columns were equilibrated with PBS-EDTA buffer containing 1.5 mM NaF. The absorbance of the eluate was monitored at 280 nm. In each analysis, 200 μL of pooled plasma was injected and eluted at a constant flow rate of 12 mL/h. Fractions of 0.5 mL were collected in which we determined the distribution of the human apolipoproteins after quantification with the same methods used for whole-plasma samples. These experiments were carried out 4 mo after the beginning of the treatment.

Statistical analysis. Statistical analysis was done using the StatView program (Berkeley, CA). Data were expressed as means ± SD. Two-way ANOVA was used to test the significant genetic effects, fructose effects and their interaction on plasma lipids, glucose concentration, final weight and final insulin concentration. Fisher’s protected least significant difference was used as a post-hoc test. One-way ANOVA was applied to analyze differences in human apolipoprotein concentration and in human apolipoprotein mRNA levels. All analyzed variables except triglycerides were normally distributed as assessed by the Kolmogorov-Smirnov test. Triglyceride concentrations were logarithmically transformed to achieve an approximately normal distribution, and ANOVA was then applied to the transformed values. Differences were considered significant at P <0.05. Values are means ± SD.

RESULTS

The weight of mice in all groups increased significantly during the study period (data not shown). Fructose increased final weight in both Tg and control mice (Table 1). Plasma

TABLE 1

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Fluid</th>
<th>Final weight</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Glucose</th>
<th>Final insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>pmol/L</td>
<td>(10−3)</td>
</tr>
<tr>
<td>Control</td>
<td>Water</td>
<td>32 ± 3.3c</td>
<td>1.99 ± 0.39c</td>
<td>0.85 ± 0.2c</td>
<td>9.23 ± 2.22</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>39 ± 5.1a</td>
<td>2.49 ± 0.57c</td>
<td>0.99 ± 0.18c</td>
<td>11.8 ± 3.73</td>
<td>4.1 ± 1.9</td>
</tr>
<tr>
<td>Tg</td>
<td>Water</td>
<td>37 ± 4.0b</td>
<td>3.19 ± 1.5b</td>
<td>7.4 ± 5.51b</td>
<td>10.1 ± 2.17</td>
<td>3.5 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>41 ± 6.1a</td>
<td>4.53 ± 1.89a</td>
<td>13.33 ± 8.84a</td>
<td>10.6 ± 2.61</td>
<td>3.9 ± 1.4</td>
</tr>
<tr>
<td>Two-way</td>
<td>Genetic effect</td>
<td>NS3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Fructose effect</td>
<td>0.0008</td>
<td>0.0008</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P-values</td>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>0.004</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Data are means ± SD, n = 7.
2 Values in each column with different letters differ, P < 0.05.
3 Average values throughout the study.
4 Data logarithmically transformed before ANOVA.
5 NS, nonsignificant, P ≥ 0.05.
glucose and insulin concentrations were not affected by fructose or genetic group (Table 1). Plasma cholesterol and triglyceride concentrations were significantly higher in Tg than in control mice and an increase due to fructose treatment was observed only in Tg mice (Table 1).

Lipid distribution among plasma lipoproteins was determined after 4 mo of treatment. This period was chosen because beyond this point, no further changes occurred in triglyceride and cholesterol levels. The addition of fructose to the drinking water increased triglyceride concentrations in Tg mice in the d < 1.006 and d = 1.006–1.019 kg/L fractions and cholesterol in the d < 1.006, d = 1.006–1.019 and d = 1.019–1.063 kg/L fractions (Table 2). In control mice, only cholesterol concentration was also enhanced in the d = 1.019–1.063 kg/L fraction, corresponding to LDL (Table 2). In Tg mice, fructose augmented the apo B100 particles in the three lipoprotein fractions, whereas in control mice, fructose increased apo B48 particles mainly in the d = 1.019–1.063 kg/L fraction. In Tg mice, fructose augmented the apo B100 particles in the three lipoprotein fractions, whereas in control mice consuming fructose, apo B100 particles were also increased, but principally in the d = 1.019–1.063 kg/L fraction (Table 2). In conclusion, fructose ingestion in apo A-I-CIII-AIV Tg mice increased cholesterol and triglyceride levels mainly in VLDL particles containing apo B48 and apo B100, and increased LDL cholesterol in control mice.

To determine the effects of fructose on intestinal and hepatic human apo AI, apo CIII and apo AIV gene expression, we measured the abundance of the corresponding mRNA species in individual organs of untreated and fructose-treated Tg mice (Table 3). Hepatic human apo AIV mRNA abundance was enhanced 320% by fructose, whereas the levels of intestinal human apo AIV mRNA were 63% lower. The abundance of human hepatic apo AI and apo CIII mRNA was not affected by fructose (Table 3). The plasma concentration of the three human apolipoproteins was greater in fructose-fed Tg mice than in untreated mice (Fig. 1). The greatest increase (threefold) was in the human apo AIV plasma concentration, consistent with the threefold augmentation of the corresponding apolipoprotein mRNA concentration in liver.

The distribution of human apolipoproteins in size subclasses of lipoproteins was analyzed by gel filtration chromatography performed on pooled plasma from transgenic mice consuming water or fructose (Fig. 2). Results confirmed the increased concentrations of the three apolipoproteins found in plasma due to fructose treatment. In mice fed both diets, apo A-I was present principally in HDL, but also in small lipid-poor particles; interestingly apo A-IV and apo C-III were also present in this size range. Apo C-III levels were not enhanced in VLDL particles of Tg mice that drank the fructose solution.

### DISCUSSION

The purpose of this study was to investigate the consequences of long-term fructose consumption on lipid metabolism and human apolipoprotein expression in a Tg mouse model that presents severe hypertriglyceridemia and moderate hypercholesterolemia. In this model, fructose increased triglycerides and cholesterol but did not induce IR. The difference between Tg and control mice, in which plasma triglyceride and cholesterol concentrations were not modified, may have been due to the fact that Tg mice are hyperlipidemic, and this can amplify the diet response. Recently, Bantle et al. (21) demonstrated that a fructose diet increases triglycerides in men. They also observed a transient increase in LDL cholesterol that did not persist. However, they could not exclude the possibility that fructose has adverse effects on fasting plasma cholesterol, effects that their study did not have the power to

### TABLE 2

<table>
<thead>
<tr>
<th>Density, kg/L</th>
<th>Triglycerides, mmol/L</th>
<th>Cholesterol, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Water</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.21 ± 0.03</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Tg</td>
<td>Water</td>
<td>3.67 ± 0.48</td>
</tr>
<tr>
<td>Fructose</td>
<td>8.30 ± 1.08</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td>Two-way</td>
<td>Genetic effect</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Fructose effect</td>
<td>0.007</td>
</tr>
<tr>
<td>P-values</td>
<td>Interaction</td>
<td>0.016</td>
</tr>
</tbody>
</table>

| Control      | Water | 15 ± 2 | 1 ± 1 | 55 ± 7 | 10 ± 1 | 1 ± 0.1 | 173 ± 23 |
| Fructose     | 46 ± 6 | 10 ± 1 | 212 ± 28 | 20 ± 2.6 | 18 ± 2.4 | 593 ± 77 |
| Tg           | Water | 248 ± 32 | 37 ± 5 | 237 ± 31 | 104 ± 14 | 15 ± 1.9 | 250 ± 33 |
| Fructose     | 468 ± 61 | 134 ± 18 | 482 ± 63 | 416 ± 54 | 105 ± 13 | 513 ± 67 |
| Two-way      | Genetic effect | <0.0001 | <0.0001 | 0.036 | <0.0001 | 0.01 | NS |
| ANOVA        | Fructose effect | 0.005 | 0.006 | 0.002 | 0.003 | 0.002 | 0.001 |
| P-values     | Interaction | 0.023 | 0.008 | NS | 0.004 | NS | NS |

1 Values are mean ± SD, n = 3 pools of 7 mice.
2 Values in each column with different letters differ, P < 0.05.
3 Data logarithmically transformed before ANOVA.
4 A.U., arbitrary units.
5 NS, nonsignificant, P ≥ 0.05.
Because their analysis was performed in healthy subjects, the results observed in the apo A1-CIII-AIV Tg mice in the present study suggest that the effect of fructose on hyperlipidemic subjects may be more dramatic. Hyperlipidemia in humans has been related to genetic variations in the apo A1-CIII-AIV gene cluster (22, 23) and to mutations in the apo CIII gene (24). Therefore, it would be interesting to study the effect of fructose in these groups.

The mechanism by which fructose increases triglycerides may be due to the stimulation of triacylglycerol synthesis, as had been previously described in rats (4, 25). This is consistent with the fact that we observed an increase in apo B48 and apo B100 levels due to fructose. The apo A1-CIII-AIV Tg mice are characterized by an increase of \( \frac{d}{H_1} \) 1.006 kg/L apo B48-rich particles when fed a standard diet (19). However, after fructose treatment, the accumulation of particles in this fraction also rich in apo B100 indicate that they have a possible hepatic origin. We hypothesize that the apo B48 increase observed is related to the fact that in mice, a high percentage of apo B mRNA from liver is subject to splicing (26). In control mice, the particles accumulated are rich in apo B100 and have a \( \frac{d}{H_1} \) 1.019–1.063 kg/L, corresponding to LDL. Thus, the differences between Tg and control mice can be due to the higher expression of apo CIII in the Tg mice, which inhibits triglyceride hydrolysis mediated by lipoprotein lipase, resulting in a greater accumulation of triglyceride-rich lipoproteins.

We did not observe any difference in final weight due to the fructose treatment between the control and the Tg mice. However, at the end of the study, the fructose-treated Tg mice had a large fat accumulation in the sternum that was not observed in Tg mice that consumed water (data not shown). The plasma glucose concentration was unaffected in Tg and control mice throughout the study period and we did not observe any difference in insulin levels among the different groups of mice at the end of the study. These results suggest that, in contrast to those described in rats (5, 6), fructose does not induce IR in mice. In a unique murine model previously subjected to a fructose-rich diet, Merat et al. (7) obtained similar results, i.e., a fructose diet did not enhance IR in LDLR\(^{-/-}\) mice. However, after consuming a Western-type diet, these mice develop IR. Interestingly, mice fed this diet gained much more weight than those fed a fructose-rich diet. In the same study, the authors analyzed the extent of atherosclerosis in mice fed both diets and observed that the fructose-fed group had a significantly greater extent of atherosclerosis than the Western-diet group (7). We have also analyzed atherosclerotic formation in our model, but we did not find any lesions in either Tg or control mice that consumed fructose, suggesting that the cholesterol increase observed due to this diet is not sufficient to produce lesions in mice with a C57Bl/6 genetic background.

We have also examined the regulation of the human apo A1-CIII-AIV gene cluster expression in response to fructose. The Tg mouse model used was generated using a 33-kb human genomic fragment containing the entire cluster and its 5' and 3' flanking regions. These mice provide an interesting model for studies of the regulation of the three genes in combination. We observed a threefold increase in hepatic human apo AIV mRNA abundance after the fructose treatment; in contrast,
the levels of intestinal human apo AIV mRNA decreased 63%. In addition, we did not find a difference in human apo AI and apo CIII mRNA levels in liver. In the intestine, no expression of the two human genes was observed. However, the concentration of the three human apolipoproteins in total plasma as well as in the subfractions isolated by FPLC, were greater in fructose-treated Tg mice than in untreated Tg mice. The main increases were observed for apo AIV. Interestingly, FPLC analysis did not show major changes in the distribution of human apolipoproteins in Tg mice consuming fructose. Several studies in animals have demonstrated differential expression of the apo AI and apo AIV genes in response to dietary perturbations. Apo AIV, but not apo AI mRNA abundance in enterocytes of rats increased after a fat bolus (27). Interestingly, we have recently demonstrated that after a cholesterol-rich diet, apo AI-CIII-AIV Tg mice had increased intestinal human apo AI, apo CIII and apo AIV mRNAs. This was accompanied by an augmentation in the concentration of the corresponding proteins in plasma, but particularly in that of apo AIV (28). These results were consistent with previous observations that apo AIV plasma concentration in rats and in humans was enhanced after a fat- or cholesterol-rich diet (29,30). We have no experimental explanation to account for the decreased human apo AIV mRNA level in the intestine after the fructose treatment. We hypothesize that the addition of fructose to the drinking water induces a satiety effect that could reduce food consumption (hence lipid intake), without modifying total energy intake.

In this report, we demonstrated that fructose increases principally human apo AIV gene expression in liver at the transcriptional level. Because fructose increases hepatic triglyceride synthesis and secretion, our study suggests an association between apo AIV gene transcription and hepatic lipogenesis. Although the function of apo AIV in the context of cellular lipoprotein assembly and secretion is unknown, several lines of evidence have already suggested a strong association between apo AIV gene expression and triglyceride synthesis and secretion (31–35). Similarly, the hepatic apo AIV and triglyceride increase have been observed in rats fed a sucrose-rich diet (36). Because sucrose is composed of glucose and fructose, the latter observation can be explained by the presence of fructose in the diet.

In conclusion, the present study demonstrates that fructose administered as a 250 g/L drinking solution increases triglyceride and cholesterol concentrations in apo AI-CIII-AIV Tg mice. This is associated with an increased abundance of human hepatic apo AIV mRNA and of the plasma apo AIV concentration. To test the atherogenic effect of fructose, further experiments must be performed using the apo AI-CIII-AIV Tg mice but in an apo E-deficient background.

ACKNOWLEDGMENTS

We thank Anne Tailleux for assistance in determining plasma insulin levels, and Pascale Vienne for assistance in statistical analysis. We also thank David Elfant and Georges Cohen for help in preparing the manuscript.

LITERATURE CITED


