Substituting Honey for Refined Carbohydrates Protects Rats from Hypertriglyceridemic and Prooxidative Effects of Fructose

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ABSTRACT Recent findings indicate that a high fructose diet has a prooxidant effect in rats compared with a starch diet. Because honey is rich in fructose, the aim of this study was to assess the effect of substituting honey for refined carbohydrates on lipid metabolism and oxidative stress. Rats were fed for 2 wk purified diets containing 65 g/100 g carbohydrates as wheat starch or a combination of fructose and glucose or a honey-based diet prepared by substituting honey for refined carbohydrates (n = 9/group). The same amount of fructose was provided by the honey and fructose diets. The hypertriglyceridemic effect of fructose was not observed when fructose was provided by honey. Compared with those fed starch, fructose-fed rats had a lower plasma α-tocopherol level, higher plasma nitrite and nitrate (NOx) levels and were less protected from lipid peroxidation as indicated by heart homogenate TBARS concentration. Compared with those fed fructose, honey-fed rats had a higher plasma α-tocopherol level, a higher α-tocopherol/triacylglycerol ratio, lower plasma NOx concentrations and a lower susceptibility of heart to lipid peroxidation. Further studies are required to identify the mechanisms underlying the antioxidant effect of honey but the data suggest a potential nutritional benefit of substituting honey for fructose in the diet. J. Nutr. 132: 3379–3382, 2002.

KEY WORDS: • high fructose diet • honey • oxidative stress • rats • free radicals

Because starch has been a major component of the diet for a long time, the amount of starch consumed has been considered safe and adequate. Conversely, before industrialization, fructose, provided mainly by honey, was a carbohydrate with a minor role in the diet. Fructose is a monosaccharide that exists in foods as a simple sugar and as a component of the disaccharide, sucrose, which consists of one molecule of glucose and one of fructose. In a relatively short period of time, dietary consumption of fructose has increased several fold above the amount present in natural foods because of the use of high fructose corn sweeteners and of sucrose in manufactured foods (1,2). Although there is little evidence that modest amounts of fructose have detrimental effects on carbohydrate and lipid metabolism, larger doses have been associated with numerous metabolic abnormalities in laboratory animals and humans, suggesting that high fructose consumption adversely affects health (3,4). Rats fed high sucrose and high fructose diets displayed metabolic changes observed in syndrome X, a disorder in which insulin resistance, hypertension, dyslipidemia and a high incidence of cardiovascular diseases are described (5).

The mechanisms underlying the detrimental consequences of a high fructose diet are not clear (6). Recent studies strongly suggest that oxidative stress occurs in rats fed a high fructose diet. Detrimental effects of fructose are enhanced when antioxidant defenses are decreased (7) or when free radical production is increased (8), whereas vitamin E supplementation improves insulin sensitivity in high fructose–fed rats (9). Moreover, recent findings indicate that a high fructose diet has a prooxidant effect in rats compared with a starch-based diet (10,11). Female rats are less susceptible than males; thus, estrogens, due to their antioxidant capacity, may explain the gender difference in the prooxidant effect of fructose (12). Fructose in the diet can also be provided by honey. However, honey has been shown to contain other carbohydrates and a variety of micronutrients, several of which are antioxidants (13,14). The aim of this study was to assess the effect of substituting honey for refined carbohydrates on lipid metabolism and oxidative stress. Rats were fed similar purified diets containing starch, honey or a combination of fructose and glucose.

MATERIALS AND METHODS

Materials. Triglycerides were measured using the PAP 150 Bio-merieux kit (Charbonnière-les-Bains, France). Other chemicals were from Sigma Chemical (St. Louis, MO). Honey was purchased from a local supplier (Ceyrat, France). Analysis of the flower sources was performed by Laboratoire France-Miel (Mouchard, France) and showed that the honey used in the present study was multifloral, containing dominant pollens from bramble and wild white clover. The composition of the honey, including the major carbohydrates, is listed in Table 1.

Animals and diets. The rats were maintained and handled according to the recommendations of the INRA Ethics Committee, in accordance with decree no. 87–848. Weaning male Wistar rats (IFFA-CREDO; L’Arbresle, France), 3 wk old, weighing 61 ± 2 g (mean ± SEM) were randomly divided into wheat starch, fructose or honey groups (n = 9/group). They were housed two per cage (wire bottomed) in a temperature-controlled room (22°C) with a 12-h light-dark cycle and fed for 2 wk the appropriate diets (Table 1). The purified diets contained 65 g/100 g carbohydrate as starch or a combination of fructose and glucose. The honey-based diet was obtained by substituting honey for refined carbohydrate (65 g/100 g dry matter) so that the same quantity of fructose was provided by the...
honey- and fructose-based diets. Diet and distilled water were consumed ad libitum.

**Sample collection.** At the time of sampling (0900 h), rats were weighed and anesthetized with sodium pentobarbital (40 mg/kg body, intraperitoneally). Blood was collected from the abdominal aorta into heparinized tubes. The plasma obtained after low speed centrifugation (2000 × g for 15 min) was stored at −80°C for biochemical analysis. The cecum was removed and weighed. The heart was rapidly repositioned in heparinized tubes. The plasma obtained after low speed centrifugation (2000 × g for 15 min) was stored at −80°C until the lipid peroxidation assay was performed.

**Plasma analysis.** Plasma vitamin E was assayed by reversed-phase HPLC (HPLC apparatus; Kontron series 400; Kontron, St. Quentin Yvelines, France) using a hexane extract. Briefly, α-tocopherol acetate was added to samples as an internal standard; they were then extracted twice with hexane after ethanol precipitation of the proteins. This extract was evaporated to dryness under N2, dissolved in ethanol/methylene chloride (65:35, v/v) and injected onto a C18 column (Nucleosil, 250 mm, i.d. 46 mm, 5-μm particle size). Pure methanol, at a flow rate of 2 mL/min eluted α-tocopherol in 5.0 min and tocopherol acetate in 6.3 min. The compounds were detected by UV light (292 nm), then quantified by an external standard calibration using standard solutions under daily control. Triacylglycerol (TG)\(^2\) concentrations were determined in plasma by enzymatic procedures using a commercial kit. Nitrite + nitrate, degradation products of nitric oxide (NOx), were measured in deproteinized plasma. We used the Griess reaction procedure as previously described (15). Briefly, after protein precipitation of the samples and reduction of nitrate to nitrite with nitrate reductase, nitrite was quantified colorimetrically at 450 nm. Standards were made by serial dilutions of sodium nitrite. Plasma glucose concentration was measured colorimetrically according to Bergmeyer et al. (16). Plasma fructose concentration was measured by the same method, after incubation with glucose oxidase.

**Tissue susceptibility to peroxidation.** The susceptibility of heart to peroxidation was determined in tissue homogenates after lipid peroxidation was induced in tissue homogenates after lipid peroxidation was induced with FeSO₄ (2 μmol/L)-ascorbate (50 μmol/L) for 30 min in a water bath at 37°C, using a standard of 1,1,3,3-tetramethoxypropane (17).

**Statistical analysis.** Statistical analyses were performed using the GraphPad Instat software package (GraphPad, San Diego, CA). Results were expressed as means ± SEM. All data were subjected to one-way ANOVA, followed by the Student-Newman-Keuls test to determine differences (\(P < 0.05\)) among the dietary groups.

**RESULTS**

There were no differences in body weight or plasma glucose concentration among the groups. Plasma fructose concentrations in rats fed fructose- and honey-based diets did not differ but were greater than in those fed the starch-based diet. Rats fed honey had greater relative cecum weights than those in the other groups (Table 2). Plasma TG concentration was greater and that of α-tocopherol lower in the fructose-fed group than in the other two groups. The α-tocopherol/TG ratio was lower in rats fed the fructose and honey diets compared with those fed the starch diet, and the decrease was greater in rats fed the fructose diet. Plasma NOx concentrations were greater in the fructose-fed group than in the other two groups. After exposure of heart homogenates to iron-induced lipid peroxidation, thiobarbituric acid-reactive substances (TBARS) were significantly higher (+58%) in rats fed the fructose diet compared with the other two groups (Table 3).

**DISCUSSION**

Under normal conditions, fructose, absorbed from the intestine, is extracted from the liver and quickly phosphorylated by fructokinase. Plasma fructose was very low in rats fed the starch diet but increased due to fructose ingestion from either the fructose- or honey-based diet. Honey is thought to produce a lower glycemic response than the same quantity of fructose or sucrose although there is little scientific evidence to substantiate this view (18). In the present experiment, there was no significant effect of carbohydrate on blood glucose level in the postprandial state. On the other hand, the effect of sugar on postprandial lipemia is well known (19). The present experiment confirmed the hypertriglyceridemic reaction to dietary fructose, either as a monosaccharide or a component of the disaccharide sucrose, previously reported by many investigators (20,21) and demonstrated that dietary fructose increased plasma TG to twice the level in rats fed the starch diet. Fructose-induced hypertriglyceridemia is a result of enhanced lipogenesis, overproduction of VLDL triglycerides and decreased peripheral catabolism (22). Increased gene expression of several enzymes including acetyl-CoA-carboxylase (23) and fatty acid synthase (24) are responsible for enhanced synthesis of TG in the liver. On the other hand, fructose

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>Fructose</th>
<th>Honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>650</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fructose</td>
<td>0</td>
<td>340</td>
<td>0</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
<td>310</td>
<td>0</td>
</tr>
<tr>
<td>Honey(^2)</td>
<td>0</td>
<td>0</td>
<td>650</td>
</tr>
<tr>
<td>Alphacel</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^1\) Sources of ingredients: casein and wheat starch (Louis François, Saint-Maur, France) corn oil, fructose, glucose, and DL-methionine (Sigma Chemical, St. Louis, MO), mineral AIN-76 mix, vitamin-76A mix (39) and choline bitartrate (ICN Biomedicals, Orsay, France).

\(^2\) Fructose- and honey-based diets provided the same quantity of fructose (340 g/kg). Honey-based diet provided 274 g/kg glucose and 36 g/kg other components. We substituted those components (99.2% carbohydrates) by glucose in the fructose-based diet.

\(^3\) Composition of honey (g/kg dry matter basis): fructose, 523; glucose, 421; mannose, 32; sucrose, 2; other carbohydrate (including FOS), 21.7; other components (including minerals, vitamin, polyphenols), 0.3.

**TABLE 2**

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Fructose</th>
<th>Honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>159 ± 4</td>
<td>158 ± 3</td>
<td>165 ± 3</td>
</tr>
<tr>
<td>Cereals, g/100 g body</td>
<td>1.15 ± 0.06(^b)</td>
<td>1.22 ± 0.06(^b)</td>
<td>1.53 ± 0.06(^a)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>8.9 ± 0.4</td>
<td>8.9 ± 0.4</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>Fructose, mmol/L</td>
<td>0.09 ± 0.01(^b)</td>
<td>0.14 ± 0.01(^a)</td>
<td>0.16 ± 0.01(^a)</td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as means ± SEM, \(n = 9\). Means in a row with superscripts not sharing a letter differ, \(P < 0.05\).
feeding may decrease lipoprotein lipase activity (25). It is noteworthy that substituting honey for refined carbohydrates provided protection against the hypertriglyceridemic effect of fructose in rats.

Honey is a biological product with a very complex chemical composition. It is composed primarily of fructose and glucose but also contains ~3 to 4% fructooligosaccharides (FOS) (26). Even if the quantity of FOS was relatively low in the honey diet, it may have had metabolic effects. These carbohydrates are not hydrolyzed in the small intestine but are degraded in the cecum by the intestinal microflora (27). This was reflected in the slight but significant increase in the relative weight of the cecum of rats fed the honey diet. In addition, honey has been reported to influence the microflora (28), which may be beneficial for gut functions and lead to improvements in the general health and lipid metabolism. However, honey has been reported to contain ~181 substances (29) and it is not known whether factors other than FOS may affect the microbes.

The hypotriglyceridemic effect of fermentable carbohydrates has been described in both humans and animals (30). This effect appears to be due mainly to reduced secretion of VLDL particles from the liver and to be associated with reduced gene expressions and activities of lipogenic enzymes (31). Dietary fermentable carbohydrates may delay carbohydrate absorption, leading to decreased lipogenic activity in the liver (31). Fermentable carbohydrates also modulate the concentration of intestinal peptides that regulate postprandial insulin release and have direct insulin-like actions on lipid metabolism (32). Because the FOS content in the honey diet was much lower than that used in experimental studies in rats that evaluated the effect of FOS on lipid metabolism (~10%) (31), further studies are required to assess the contribution of FOS to the lipid-lowering effect of honey and to determine whether other constituents are involved.

Compared with starch, the plasma TG-raising effect of fructose was accompanied by lower plasma α-tocopherol levels. Because vitamin E normally acts as an antioxidant, low plasma vitamin E likely results from increased vitamin E utilization (33). Fructose-fed rats were also less protected against lipid peroxidation as shown by TBARS in heart tissue homogenates. Moreover, fructose-fed rats were characterized by a higher plasma NOx level, suggesting greater nitric oxide production (11). The present experiment agrees with previous studies showing that short-term consumption of a high sucrose diet negatively affects the balance between free radical production and antioxidant defense, increasing lipid susceptibility to peroxidation and suggesting that the fructose moiety of the sucrose molecules was responsible for the prooxidant effect (10). There are several possible pathways by which a diet rich in sucrose may alter cellular metabolism which in turn may accelerate oxidative stress. The increased oxidative stress could be due to oxygen free radical production and/or decreased protection by nonenzymatic or enzymatic antioxidants (10). Moreover, the susceptibility of tissues to oxidative stress can depend on alterations in lipid composition. Another possibility is that fructose induces an accumulation of advanced glycation end-products and that oxidative degradation of fructose adducts leads to production of free radicals (34). Because plasma glucose concentration did not differ between the fructose- and honey-fed groups and because of the relatively short duration of the experiment, it is unlikely that glycation reactions are involved in the prooxidant effect of fructose. Which of the possible pathways of free radical generation occurs in response to a high fructose diet is still unclear.

Several studies suggest that honey may be used as a healthy alternative to sugar in many products and thereby serve as a supplementary source of antioxidants (35,36). Honey has been found recently to be more effective than traditional preservatives (BHT or α-tocopherol) in preventing meat oxidation (36). Honey was also found to decrease human LDL oxidation in vitro (35). The present experiment also clearly identified an enhanced antioxidant capacity in rats fed the honey-based diet compared with rats fed the fructose diet, as shown by higher plasma α-tocopherol level, a higher α-tocopherol/TG ratio and decreased susceptibility of heart lipids to peroxidation. Moreover, plasma NOx concentration was lower in the honey group compared with the fructose-fed rats. Nitric oxide can be viewed as a radical, and it has been reported that nitric oxide molecules are scavenged directly by flavonoids (37). Hence, because fruits and vegetables are especially good sources of antioxidants, honey, which originates from plant nectar, has also been reported to be rich in polyphenolic compounds (38). However, even if the present experiment did not allow a determination of the components involved, the antioxidant potential of honey could explain in part its protective effect against the prooxidant effect of fructose.

Increased consumption of any purified energy source without accompanying food components containing complex carbohydrates and micronutrients can lead to an unbalanced diet and an altered metabolism, which can have chronic health consequences. Results of the present experiment do not allow identification of the constituents responsible for the beneficial effects of honey and future studies are required to compare the effects of honey of different origins and compositions. However, on the basis of the triglyceride level and oxidative stress parameters, the present experiment suggests a potential nutritional benefit to substituting honey for fructose in the diet.

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