Effects of chronic dietary fructose with and without copper supplementation on glycaemic control, adiposity, insulin binding to adipocytes and glomerular basement membrane thickness in normal rats

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Sucrose feeding over a long period has been reported to induce glomerular basement membrane (GBM) thickening and insulin resistance in normal rats. These effects are attributed to the fructose moiety of the sucrose molecule, to Cu deprivation or both. Consequently, our aim was to evaluate the long-term effects of fructose feeding with normal or high amounts of Cu on body weight, plasma lipids, blood glucose regulation, GBM thickening and insulin binding to adipocytes. Four groups of eight Sprague–Dawley rats were fed for 10 weeks on a diet containing 570 g carbohydrate/kg supplied either as starch (S), dextrose (D), fructose (F) or fructose–starch (1:1, w/w; FS), and an adequate amount of Cu (12 μg Cu/g diet). A fifth group was fed on diet F supplemented with 24 μg Cu/g diet (FCu). After 10 weeks the epididymal adipose tissue and kidney weights expressed per 100 g body weight (relative weight) were heaviest in the F and FCu groups (P < 0.0001, ANOVA). The GBM thickness was within the normal range in the five groups but significantly higher in group D (1.95 (SE 0.04) nm and lower in group FS (1.79 (SE 0.02) nm when compared with group S (1.85 (SE 0.03) nm; P < 0.05). Insulin binding to adipocytes (expressed per cell) was lowest in the F and FCu groups, intermediate in groups D and FS and highest in group S (P < 0.05). Fasting plasma insulin level was higher in group F than in the FCu and FS groups (P < 0.05), whereas fasting plasma glucose, total cholesterol and triacylglycerol levels remained within the normal range in all groups. We conclude that in normal rats a 10-week fructose-rich diet with an adequate amount of Cu produced deleterious metabolic effects on adipose tissue, insulin binding to adipocytes, and plasma insulin, but not on GBM thickening even though kidney weight was significantly increased. However, a moderate fructose intake mixed with other sugars did not have adverse effects.

Fructose intake: Copper: Insulin binding: Adipose tissue

Fructose is characterized by a partly insulin-independent metabolism and by a moderate hyperglycaemic and insulinogenic effect in man (Crapo et al. 1982; Bantle et al. 1983). This simple sugar is still often credited with a higher sweetening potency than that of sucrose (Schallenberger, 1963), although this property has recently been questioned (Fontvieille et al. 1989). Because of these advantages and the recent technological advances that have simplified its industrial production, fructose is widely prescribed and used by diabetic subjects (Thorburn et al. 1989a). Fructose utilization has also increased among the general population. However, the metabolic effects of its chronic utilization have not yet been fully established. Fructose was found to increase plasma triacylglycerol levels in predisposed...
individuals and in normal and diabetic rats (Zavaroni et al. 1982; Hallfrisch et al. 1983; Bacon et al. 1984). It has also been found to have inhibitory effects on insulin receptors of blood cells (Beck-Nielsen et al. 1980; Rizkalla et al. 1986), which raises the question of whether or not these effects could also be present in target tissues such as adipocytes.

In addition, fructose was found to increase the thickness of the glomerular basement membrane (GBM) in normal rats (Taylor et al. 1980, 1985). Other authors attributed this undesirable effect to the Cu deficiency in the diets used (Cohen et al. 1982).

Therefore, we decided first to determine whether the chronic consumption of high- or moderate-fructose diets supplemented with an adequate level of Cu produces either beneficial or detrimental consequences, and second, to establish whether doubling the Cu level ameliorates the detrimental effects. The effects of these diets were studied on plasma variables in the kidney (function and morphology) and in the epididymal adipose tissue (cellularity, and insulin binding to adipocytes).

MATERIALS AND METHODS

Experimental protocol

Forty male Sprague-Dawley rats aged 21 d were used. The rats were housed in groups of four in sedentary cages, not allowed to have any form of physical exercise such as running on wheels, and maintained at 24° with a 12 h light–dark cycle. The rats were randomized into five groups. The rats in four groups were fed on a powder diet (synthetic diet no. 210; Factory of Rational Alimentation, Villemoisson/Orge, France) containing 570 g carbohydrate/kg supplied either as corn starch (S; 240 g amylose and 760 g amylopectin/kg), dextrose (D), fructose (F), or fructose–starch (1:1, w/w; FS) and an adequate amount of copper (12 µg Cu/g diet) according to the recommendations of the French National Center for Nutritional and Dietary Research (CNERNA) (Poitier De Courcy et al. 1987). The fifth group was fed on 570 g fructose/kg diet supplemented with twice the amount of Cu (24 µg Cu/g diet; FCu), since it has been reported that diets containing fructose require 2–3 times as much Cu as those fed on starch (Failla et al. 1988). The other components of the powder diet were (g/kg): casein 225, plant and animal fats 55 (P:S ratio 0.67), cellulose 60, minerals 70 and a balanced mixture of vitamins (Poitier De Courcy et al. 1987). Food intake was measured once per day and body weight once per week.

At week 10 the rats were decapitated after an overnight fast. Blood, epididymal fat and kidneys were removed.

Insulin binding study

Epididymal adipocytes were isolated using the method of Rodbell (1964). Isolated adipocytes were incubated in duplicate for 60 min at 24° (Bolinder et al. 1983) in a final concentration of 40 ml/l in a Krebs–Ringer–Bicarbonate buffer (pH 7.4) containing 35 mg bovine serum albumin/ml, 1 mg D-glucose/ml, 0.3 ng mono-125I-(Tyr A14) insulin, and 0–10^4 ng unlabelled insulin/ml. The binding reaction was terminated by adding 10 ml ice-cold saline (9 g NaCl/l). The cells were immediately filtered (Cuatrecasas, 1971) and gently washed with another 10 ml ice-cold saline. The steps of saline addition, filtration and second washing take less than 1 min. The filtrates were removed and counted in a gamma counter. The incubation was done at 24° for 60 min as it has been demonstrated (Bolinder et al. 1983) that insulin degradation is negligible at this temperature, and that a steady state of insulin binding is reached after 40 min and maintained for at least 120 min. All these data were corrected for nonspecific binding, which amounted to about 0.4–0.6% of the total binding, and were expressed as the amount of insulin specifically bound per fat cell. The apparent receptor affinity is the concentration of native insulin required to reduce the...
specific cell-bound fraction of $[^{125}]$insulin by 50% ($ED_{50}$). The receptor number was calculated using Scatchard plots (Scatchard, 1949) in which bound and free radioactive insulin levels were plotted as a function of bound hormone for insulin concentrations from 0.01 to 100 ng/ml. Graphic extrapolation of the curves, to obtain their intercepts with the x axis, gave the receptor number.

The actual volume of the adipocytes in the above suspension was measured in duplicate by centrifugation in hematocrit tubes, at 13400 g for 3 min. The number of adipocytes in the given volume was calculated as total adipocyte fraction volume divided by mean adipocyte volume.

Mean fat-cell size (diameter and volume) was determined using a photographic method according to Lavau et al. (1977); at least 300 cells were photographed for each rat sample. The mean fat-cell volume was calculated with Goldrick's formula (Goldrick, 1967) and the mean fat-cell weight was determined by using triolein density (0.915).

**GBM measurements**

For each rat, three small blocks of kidney cortex were taken and immediately fixed in glutaraldehyde solution (20 g/l), followed by osmium fixation, dehydration and embedding in Vestopol (epoxy-resin; Ladd products, Janning, 92 Vanves, France). Two pictures were selected from each block. The full thickness of the GBM was measured on a strictly perpendicular section on at least ten sites of six capillary loops, giving a total of sixty measurements per rat.

**Biological analysis**

Plasma insulin was measured, in the same batch, by radioimmunoassay (intra-assay reproducibility, 6%) using antiserum raised in guinea pigs (kits CIS International, Gif sur Yvette, France) and a standard curve of rat insulin. Plasma glucose was determined by the glucose oxidase (EC 1.1.3.4) method (Beckman Autoanalyser, Fullerton, CA, USA). Plasma cholesterol (Allain et al. 1974) and triacylglycerols (Bucolo & David, 1973) were also measured.

**Statistical analysis**

Overall comparisons between the different diet groups were made using one-way analysis of variance (ANOVA). When a significant $F$ value was obtained for the ANOVA ($P < 0.05$) the differences between all pairs of means were tested using Fisher's Least Significance Difference (LSD) procedure. Statistical analysis was performed with Statview 5 12+ software (Brainpower Inc., Calabasas, CA, USA). Results are given as mean values with standard errors.

**RESULTS**

**Body, adipose tissue and kidney weight**

As shown in Fig. 1, body weights were significantly different between weeks 3 and 7 (ANOVA, $P < 0.05$). Mean values were highest in the group fed on diet D and lowest in the group fed on diet F. Values for rats fed on diet F were also significantly lower than those of rats fed on diet FCu during weeks 3, 4 and 6. When results were expressed as weight gain (Table 1), the rats fed on diets D and FS nearly always gained more weight than those fed on diets S, F and F/Cu. These differences were clearly significant until week 6, after which this significance started to decline.

Food intake (Table 1) showed a tendency to decrease in group F and S, but an increase in group D. These variations became significantly different at week 6. The total food intake per rat during the 10 weeks of the corresponding diet was significantly higher (ANOVA, $P < 0.005$) in the rats fed on diet D ($1.8 \pm 0.03 \times 10^4$ kJ) than in those fed on diets S
Table 1. Changes in food intake and body weight gain of normal rats given diets containing starch, dextrose, fructose, fructose–starch (1:1, w/w) or fructose with copper* for 10 weeks
(Mean values with their standard errors for eight rats)

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<td>Fructose copper</td>
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<td>12</td>
<td>264</td>
<td>24</td>
<td>274</td>
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<td>251</td>
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<td>NS</td>
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* Significantly different from starch; b significantly different from fructose; c significantly different from fructose copper (P < 0.05).
* For details of diets, see p. 200.
† Weight gain = body weight at the indicated time – initial weight.
Table 2. Body, kidney and epididymal fat-pad weights, energy efficiency, cellularity of fat-pads and fasting plasma creatinine, glucose, insulin and lipid levels of normal rats given diets containing starch, dextrose, fructose, fructose-starch (1:1, w/w) or fructose with copper* for 10 weeks (Mean values with their standard errors for eight rats)

<table>
<thead>
<tr>
<th>Dietary treatment...</th>
<th>Starch</th>
<th>Dextrose</th>
<th>Fructose</th>
<th>Fructose-starch</th>
<th>Fructose copper</th>
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<td></td>
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<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
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<tr>
<td>Body wt (Bw; g)</td>
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<td>6</td>
<td>342</td>
<td>9</td>
<td>330</td>
<td>7</td>
</tr>
<tr>
<td>Energy efficiency†</td>
<td>1.40</td>
<td>0.05</td>
<td>1.38</td>
<td>0.07</td>
<td>1.47</td>
<td>0.04</td>
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<td>Fat-pad wt (g):</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Absolute</td>
<td>3.5</td>
<td>0.2</td>
<td>4.0b</td>
<td>0.2</td>
<td>4.4a,b</td>
<td>0.3</td>
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<tr>
<td>Relative (/100 g Bw)</td>
<td>1.07</td>
<td>0.05</td>
<td>1.18b</td>
<td>0.04</td>
<td>1.31b,c</td>
<td>0.08</td>
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<tr>
<td>Adipocyte number</td>
<td>138</td>
<td>1.3</td>
<td>151</td>
<td>1.4</td>
<td>16.2</td>
<td>0.8</td>
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<td>Adipocyte diameter</td>
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<td>3.4</td>
<td>81.6</td>
<td>2.0</td>
<td>78.9</td>
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<td>Kidney wt (g):</td>
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<tr>
<td>Absolute</td>
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<td>0.04</td>
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<td>0.65</td>
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<td>0.74b,c,d</td>
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<tr>
<td>Plasma creatinine</td>
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<td>64.9</td>
<td>1.9</td>
<td>63.3</td>
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<tr>
<td>(μmol/l)</td>
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<tr>
<td>Plasma glucose</td>
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<td>0.1</td>
<td>5.3</td>
<td>0.1</td>
<td>5.6</td>
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<tr>
<td>(mmol/l)</td>
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<tr>
<td>Plasma insulin</td>
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<td>50.5b,d</td>
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<td>(μl/l)</td>
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<tr>
<td>Plasma cholesterol</td>
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<td>(mmol/l)</td>
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<tr>
<td>Plasma triacylglycerol</td>
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<td>0.15</td>
<td>0.94</td>
<td>0.09</td>
<td>1.10</td>
<td>0.14</td>
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</tbody>
</table>

- * Significantly different from starch; ** significantly different from fructose-starch; † significantly different from dextrose; ‡ significantly different from fructose copper (P < 0.05).
- * For details of diets, see p. 200.
- † Energy efficiency is expressed as weight gain at week 10: total energy intake ratio.
- ‡ Kidney and fat-pads weights represent the weight of the two kidneys or the two fat pads in the eight rats.

(1·6 (se 0·04) × 10⁴ kJ), F (1·6 (se 0·02) × 10⁴ kJ), FS (1·7 (se 0·04) × 10⁴ kJ) and FCu (1·7 (se 0·05) × 10⁴ kJ). However, when the total energy intake was related to weight gain to calculate the energy efficiency, no significant difference was observed among the groups as shown in Table 2.

Fig. 1 and Tables 1 and 2 show that at the end of week 10 neither body weights nor weight gain were different, whereas epididymal fat pads increased in weight in groups F and FCu compared with groups S and FS (ANOVA, P < 0.0004). When the results were expressed per 100 g body weight (relative weight), groups F and FCu remained higher than groups S and FS (ANOVA, P < 0.0001).

Fat deposition in the groups fed on fructose-rich diets was related to an increase (ANOVA, P < 0.05) in the number of adipocytes (groups F and FCu v. group FS) rather than to an increase in cell volume as shown in Table 1.

The kidneys increased in weight in all the fructose-fed groups (F, FCu, FS) compared with group S (ANOVA, P < 0.0001), but only in the high fructose groups (F and FCu) compared with group D. However, when kidney weight was expressed per 100 g body weight, kidneys of rats fed on the high-fructose diets (F, FCu) were heavier than those of groups S, D and FS.
GBM thickness

GBM thickness was strictly within the normal range in all five groups, but significantly higher in group D (1.95 (SE 0.04) nm, ANOVA, P < 0.05) than in groups FS (1.79 (SE 0.02) nm), S (1.85 (SE 0.03) nm), F (1.81 (SE 0.03) nm) and FCu (1.83 (SE 0.04) nm). Therefore, GBM in the groups fed on fructose-rich diets was comparable to the GBM thickness in the control group (S).

Insulin binding to epididymal adipocytes

As shown in Fig. 2a, insulin binding to adipocytes was lower (ANOVA, P < 0.05) in the groups fed on diets F (2.2 (SE 0.3) %/10^6 cells) and FCu (1.9 (SE 0.4) %/10^6 cells) than in those fed on diets S (3.9 (SE 0.4) %/10^6 cells) and D (3.4 (SE 0.34) %/10^6 cells). This was accompanied by a decrease in receptor number (Fig. 2b). There was no correlation between insulin binding to adipocytes and plasma insulin levels.

Plasma variables

Table 1 shows that fasting plasma insulin increased in group F compared with groups FS and FCu (P < 0.05). Plasma glucose, total cholesterol, triacylglycerol and creatinine levels remained within the normal range in all the groups, without any significant difference.

DISCUSSION

A high-fructose diet (570 g/kg) in the present study led to delayed body weight gain and a decrease in food intake during the first 6 weeks, which confirms our previous findings (Rizkalla et al. 1990) and those of Bellomo et al. (1987). However, these differences were no longer present at week 10.
Fig. 2. (a) Insulin binding to adipocytes. Competition inhibition curves, in which the percentage of total radioactivity specifically bound was plotted as a function of insulin concentration, after 10 weeks of starch (--- ), dextrose (--- ), fructose (--- ), fructose-starch (--- ) or fructose copper (--- ) diets (results expressed per 10⁶ cells); (b) Scatchard plots of the competition curves in which bound and free radioactive insulin was plotted as a function of bound hormone for total insulin concentration from 0.01–100 ng/ml. The Scatchard plots are a curve-fitting program based on the negative cooperativity model (De Meyts & Roth, 1975). The maximum insulin binding (%) was significantly lower in groups F and FCu v. groups S and D (ANOVA, *P < 0.05).

At week 10, in all fructose-fed groups, an increase in kidney weight was observed. This finding has often been observed even after a shorter period (Bellomo et al. 1987), or after a similar (Koh et al. 1985) or a longer period (Boot-Handford & Heath, 1981) than that of the present study. This finding could be related to an increase of the glycogen content, since it has been shown that fructose, at least in the liver, increases glycogen synthesis more...
than glucose does (Niewoehner et al. 1984). However, it was the total protein content that increased in the high fructose-fed rats in the present study (results not shown).

The pertinent finding, after 10 weeks of fructose feeding, was the absence of thickening in the GBM, and even a decrease in GBM thickening was found in rats fed on moderate amounts of fructose when compared to those fed on starch or dextrose. Our results are in contradiction with those of Taylor et al. (1985) who demonstrated in young adult Sprague-Dawley rats that a diet containing 550 g fructose/kg (compared with 550 g glucose/kg diet), with 200 g butter/kg produced a thickening in the lamina densa of the GBM after 8 weeks. This thickening further increased after 16 weeks with a considerably expanded mesangium. The absence of any increase in the thickening of GBM in our experiment could be explained by the low-fat content and/or the sufficient amount of Cu (12 µg/g diet) in our experimental diets, which was higher than those recommended by the American Institute of Nutrition (1977). There is no relevant reference in the literature concerning the effect of a low-fat diet on GBM thickness. However, studies over the past 5 years have clearly demonstrated that the type of dietary carbohydrate used affects the consequences of dietary Cu deficiency in young male rats. Recently Failla et al. (1988) reported that rats which were fed on diets containing fructose required 2–3-fold more dietary Cu (2–10 µg Cu/g food instead of 1–5 µg Cu/g) than those fed on starch, in order to maintain control levels of Cu in the tissues and to generate a normal humoral immune response. The observed Cu deficiency from a fructose diet was reported to be a result of impaired Cu absorption through the small intestine and, to a greater extent, to an increase in the utilization of Cu (Fields et al. 1986). Another possible explanation for GBM thickening is that the metabolic pathway of fructose sometimes deviates resulting in sorbitol accumulation which causes cellular damage. Recently Bellomo et al. (1987) and Fields et al. (1990) found that prolonged fructose feeding leads to sorbitol accumulation in the kidneys because of the increased flux of glucose through the polyol pathway. This sorbitol is also a strong tissue chelator of Cu, with which it forms a stable complex. Thus, the presence of high amounts of Cu (12 µg/g) in our experimental diets may have counter-regulated the possible damaging effects of fructose, and the production of sorbitol, with regard to GBM thickening. The high-level Cu supplementation (24 µg/g) had no additional beneficial effects. In other words, there seems to be a level above which Cu has no additional protective effects.

The supplementation with Cu, however, has no protective effects on either epididymal fat-pad weight or on insulin binding to adipocytes. In the present study a high-fructose diet was found to increase the epididymal adipose tissue weight, in spite of the known fructose-induced decrease in adipose tissue lipogenesis (Blakely et al. 1987) and also, to some extent, lipoprotein lipase activity in this tissue. The results we found with a high-fructose diet are in agreement with others (Koh et al. 1985). This increase in weight was due to hyperplasia (increase in the cellularity). A lower amount of fructose (275 g/kg), however, decreased epididymal tissue weight. The mechanism underlying this phenomenon is unknown.

The decrease in insulin binding to adipocytes in rats which were fed on a high- but not a moderate-fructose diet for 10 weeks, as shown in the present study, confirms previous results in non-target cells i.e. erythrocytes and monocytes. Previously we found (Rizkalla et al. 1986) that the presence of small amounts of fructose (as a sole source of carbohydrate) in a very low energy diet (2.1 MJ (500 kcal)/d) given to obese patients inhibits the expected increase in erythrocyte insulin receptors normally induced by this type of diet. This effect was not detected when subjects were fed on equal amounts of glucose or galactose. Furthermore, in normal weight subjects Beck-Nielsen et al. (1980) reported that a 1-week fructose-rich diet (usual spontaneous diet + 250 g fructose/d) decreases insulin binding and insulin sensitivity. Moderate fructose feeding, however, in type 2 diabetic subjects has no
deleterious effects on insulin binding to erythrocytes or adipocytes after 14 d (Crapo et al. 1986) or 2 months (Grigoresco et al. 1988).

The decrease in insulin binding to adipocytes in the present study might contribute to the fructose-induced in vivo insulin resistance reported by others (Reiser & Hallfrisch, 1977; Thorburn et al. 1989b). The mechanisms by which fructose may decrease insulin binding are not well defined. Many hypotheses have been proposed: changes in intracellular ATP and cyclic AMP (Hue, 1975; Thomopoulos et al. 1977; Miller, 1978; Trischitta et al. 1984; Kahn et al. 1985); elevated plasma insulin (Olefsky & Saekow, 1978); or plasma lipids leading to alterations in the physical or chemical nature of membrane lipids (Gould & Ginsberg, 1985). Whatever the mechanism may be, from the present study we cannot confirm if this is due to the observed hyperinsulinaemia, to other factors, or both.

Another surprising result in our study was the absence of hypertriacylglycerolaemia which is in contradiction to the results of most of the authors using Sprague–Dawley rats (Zavaroni et al. 1982; Bacon et al. 1984). This difference in the results might be due to adaptive mechanisms after chronic fructose feeding. Three studies (Vrana et al. 1976; Sheorain et al. 1980; Lombardo et al. 1983) in which female Swiss mice were fed on a 700 g sucrose/kg diet reported an initial rise in serum triacylglycerols, after 3–25 d, that returned to normal values after prolonged exposure (32–45 d). This adaptation could also be true for rats fed on a fructose diet, since previous studies have reported an increase in triacylglycerols after 1–4-week study periods (Zavaroni et al. 1982; Bacon et al. 1984) but not after 9 or 10 weeks (Bellomo et al. 1987). Alternatively, the absence of hypertriacylglycerolaemia in our study might simply be due to the fact that in our experimental design the rats were fasted for about 12 h which might mask the otherwise prolonged postprandial hypertriacylglycerolaemia. In our laboratory (Luo et al. 1992) we found hypertriacylglycerolaemia when rats were studied in the fed state. Our results are consistent with some, but not all, investigators who have found hypertriacylglycerolaemia in rats fasted for no more than 6 h (Sleder et al. 1980; Zavaroni et al. 1982; Bacon et al. 1984) but not in those which have fasted for 12 h (Bellomo et al. 1987). However, Blakely et al. (1987) found hypertriacylglycerolaemia in Wistar rats fasted for 12–14 h after 11 weeks of feeding fructose (270 g/kg diet) which is in contradiction to our results. This difference could be due to the fact that Blakely et al. (1987) used Wistar rats that were housed individually and, thus, were heavier (450 g) at the end of the nutritional period than our rats (350 g) that were Sprague–Dawley rats housed in groups of four. The differences in race and body weight might be implicated in the absence or presence of hypertriacylglycerolaemia.

We conclude first that feeding normal rats on a fructose diet (570 g/kg) containing 12 μg Cu/g diet over 10 weeks resulted in deleterious effects on kidney and epididymal adipose tissue weights, insulin binding to adipocytes and fasting plasma insulin. It did not, however, alter the 12 h fasting plasma glucose or triacylglycerol levels, nor increase GBM thickness. Second, when fructose represented half the amount of carbohydrate in the diet, no adverse effects could be detected. Third, doubling the amount of Cu in the fructose diet brought only a beneficial effect on fasting plasma insulin. Although we cannot extrapolate results in rats to humans, care should be taken when using a high-fructose diet even with adequate Cu levels. Moderate-fructose diets, however, are unlikely to have adverse effects.

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