

## Consuming Fructose-sweetened Beverages Increases Body Adiposity in Mice

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### Abstract

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**Objective:** The marked increase in the prevalence of obesity in the United States has recently been attributed to the increased fructose consumption. To determine if and how fructose might promote obesity in an animal model, we measured body composition, energy intake, energy expenditure, substrate oxidation, and several endocrine parameters related to energy homeostasis in mice consuming fructose.

**Research Methods and Procedures:** We compared the effects of ad libitum access to fructose (15% solution in water), sucrose (10%, popular soft drink), and artificial sweetener (0% calories, popular diet soft drink) on adipogenesis and energy metabolism in mice.

**Results:** Exposure to fructose water increased adiposity, whereas increased fat mass after consumption of soft drinks

or diet soft drinks did not reach statistical significance ( $n = 9$  each group). Total intake of energy was unaltered, because mice proportionally reduced their caloric intake from chow. There was a trend toward reduced energy expenditure and increased respiratory quotient, albeit not significant, in the fructose group. Furthermore, fructose produced a hepatic lipid accumulation with a characteristic pericentral pattern.

**Discussion:** These data are compatible with the conclusion that a high intake of fructose selectively enhances adipogenesis, possibly through a shift of substrate use to lipogenesis.

**Key words:** fructose, soft drink, energy balance, energy expenditure, rodent

### Introduction

Over the last decade, obesity has been increasingly recognized as a global health threat and is associated with a number of diseases and comorbidities including insulin resistance, diabetes mellitus, hypertension, coronary artery disease, and some types of cancer. The current scientific view proposes that an interaction of a variable, but widespread, polygenetic predisposition, combined with environmental influences such as an abundant availability of low-cost, highly palatable nutrients and an increasingly sedentary lifestyle, is responsible for the marked increase in the prevalence of obesity and its sequels (1–3).

Consumption of soft drinks, in particular carbonated beverages, has increased markedly in the past two to three decades, and they are now the most popular refreshments among much of the world's population. Most soft drinks are sweetened with sugars containing a high proportion of fructose (4). Whether sucrose (50% fructose) or high fructose corn syrup (usually ~55% fructose) is used as the sweetening agent, the fructose content of beverages sweetened with sugars ranges from 7% to 15% by weight (5). The resulting per capita increase of fructose consumption has occurred simultaneously with the dramatic raise in the prevalence of obesity in the United States and worldwide (1,6).

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Free fructose or fructose derived from the enzymatic cleavage of sucrose is absorbed primarily in the jejunum and transferred (7) through the apical membrane of the epithelial cell into the portal circulation (8). When it arrives at the liver, fructose is phosphorylated by the enzyme fructokinase to fructose-1-phosphate, which can be converted to glycerol-3-phosphate for the synthesis of glycerol (9) or metabolized to acetyl-CoA and incorporated into fatty acids through de novo lipogenesis. In fact, fructose is converted in the liver to fatty acids at a greater rate than glucose (10), because, unlike fructose, glucose flux into glycolysis is regulated at the level of phosphofructokinase and is subject to feedback inhibition by ATP and citrate when hepatocellular energy status is high (11). The fructose that does not enter into the lipogenic pathway is either anaerobically metabolized to lactate and released or can be indirectly incorporated into glycogen. A small proportion of carbon from ingested fructose is converted into glucose by the liver and enters the general circulation. The preferential entry of fructose into lipogenesis is likely to contribute to the effects of fructose to induce hyperlipidemia and, in particular, a marked increase of postprandial triglyceride levels (12,13).

Importantly, with respect to energy homeostasis, fructose, unlike glucose, does not directly stimulate insulin secretion (14) because pancreatic  $\beta$  cells have very low levels of the glucose transporter GLUT5 (15). Insulin is an important direct signal to the central nervous system in the long-term regulation of energy balance (16,17), and, in addition, insulin indirectly affects energy balance through its effects on the production of at least two other hormones. Insulin increases leptin production in adipose tissue indirectly through regulation of adipocyte glucose metabolism (18,19), and there is evidence that insulin, in combination with glucose, is involved in the postprandial suppression of ghrelin secretion (20,21). Ghrelin is a peptide hormone produced by the stomach that stimulates food intake and increases adiposity in rodents (22–24). Accordingly, fructose consumption with three meals over the course of a day in humans not only reduces meal-induced insulin responses but also results in a reduction of leptin production over a 24-hour period, a blunting of the diurnal leptin amplitude, and an attenuation of the suppression of ghrelin concentrations 1 to 2 hours after each meal (13).

Although there are a few studies examining the effects of fructose consumption on body weight and body fat mass, these reports are primarily descriptive, and results are conflicting. Some authors report an increase in body weight associated with fructose consumption (25,26), whereas others report no significant impact of fructose consumption on body weight and fat mass (27). To determine if and how fructose might promote obesity in an animal model, we measured body composition, energy intake, energy expenditure, substrate oxidation, and several endocrine param-

eters related to energy homeostasis in mice consuming fructose. Fructose was provided either as the free sugar or in combination with glucose (as sucrose) in a decarbonated popular soft drink beverage (with water and noncaloric soft drink controls).

## Research Methods and Procedures

### *Animals and Study Protocol*

Studies were performed in 3-month-old male adult NMRI mice (Charles River, Sulzfeld, Germany) with a mean starting body weight of  $39.2 \pm 0.4$  grams. Mice were singly housed at a temperature of  $22^\circ\text{C}$ , with a 12:12-hour light-dark cycle (lights on at 6:00 AM), and were fed ad libitum standard laboratory chow and water (control group) or the specific beverage being studied. Before study onset, mice were assigned to one of four groups ( $n = 8$  to  $9$ ), matching mean body weight among the groups. Water, fructose dissolved in water [15% D(-) fructose, 61.5 kcal/100 mL; 4981.2 ROTH], a sucrose-sweetened soft drink (~10% sucrose, 41.7 kcal/100 mL; European version of a very popular soft drink), or a noncaloric “diet” soft drink (sweetened with sodium cyclamate, aspartame, sodium saccharine, 1.2 kcal/100 mL; European version of a very popular diet soft drink) was provided for 73 days (referred to as water control group, fructose group, soft drink group, and diet soft drink group, respectively). Carbon dioxide was removed from the beverages by stirring before filling the drinking bottles. The concentration of fructose dissolved in water in the fructose group was chosen as 15% to imitate the highest amount of fructose in U.S. brands of fructose-sweetened soft drinks, which are higher than the European soft drinks because of the use of high fructose corn syrup instead of sucrose.

To quantify the amount of beverage lost during handling of the bottles throughout the study, control bottles for each treatment group were handled identically in the absence of a test animal, and data were corrected for losses caused by differences in viscosity and leakage of the study beverages. A standard diet (grain-based, containing vegetable fat) was accessible ad libitum and contained 2826 kcal/kg energy (23.5% protein, 11.8% vegetable fat, 64.7% nitrogen-free extract, mono- and disaccharides negligible; maintenance diet for rats and mice; 1324; Altromin, Lage, Germany). All experiments were performed in accordance with the guidelines of the ethics committee of the Ministry of Agriculture, Nutrition, and Forestry (State of Brandenburg, Germany) and were approved by that same ministry.

### *Measurements*

Body weight, body composition with quantitative nuclear magnetic resonance (28,29), and food intake of all mice in each study group were measured three times per week throughout the study. Blood glucose was measured every

second week in blood samples collected from the tail vein, using a MediSense Precision Q-I-D glucose meter (Abbott Laboratories, Abbott Park, IL). All measurements were taken at the same time-points in all animals throughout the study. On Day 64 of the study, indirect calorimetry was performed in all studied mice using a 16-cage equal flow OXYMAX calorimeter (Columbus Instruments, Columbus, OH) as described earlier (30). The calorimetry measurements were performed 24 hours after an acclimation period of 24 hours.

After 70 days of the study (16- to 18-week-old mice), a glucose tolerance test was performed. Animals were fasted overnight for 18 hours and injected intraperitoneally with 1 g/kg body weight D-glucose (20%, solved in water). Blood was obtained from the tail vein for blood glucose determination at time-point 0 and at 10, 30, 60, 120, and 180 minutes after glucose injection. Blood glucose was measured using a MediSense Precision Q-I-D glucose meter (Abbott). On Day 73, the animals were killed, and the livers were removed, cleaned, weighed, and frozen at  $-80^{\circ}\text{C}$ . Serum samples were collected and immediately frozen at  $-80^{\circ}\text{C}$  for measurement of insulin, adiponectin, and ghrelin.

### Assays, Histology, and Data Analysis

Serum insulin levels were determined by ELISA for rat insulin using a mouse insulin standard (INSKR020 and INSSM021; CrystalChem, Chicago, IL) according to the instructions of the manufacturer as previously described (31). Serum adiponectin levels were determined with the mouse adiponectin RIA kit (MADP-60HK; LINCO Research, St. Charles, MO). Total plasma ghrelin was measured without an extraction step using a commercial RIA (Phoenix Peptide, Phoenix, AZ) as described previously (13).

Liver tissue was obtained from the central lobe of each animal and immediately frozen, and sections were imbedded in tissue blocks. For pathohistological evaluation of the liver morphology, liver samples were subjected to hematoxylin-eosin staining. The intra- and extracellular fat deposits were shown by SUDAN II staining of frozen tissue sections (32).

Data are expressed as mean  $\pm$  SE. Area under glucose curves were calculated as trapezoidal. Treatment effect was estimated using general linear models with and without repeated measurement design. Dunnett's pairwise multiple comparison Student's *t* test was performed to compare the means of the treatment groups against the mean of the water group (control group). Paired samples Student's *t* test was used to compare body weight and body composition at the beginning and after study period. All statistical analyses were performed using SPSS 11.5 (SPSS, Chicago, IL).

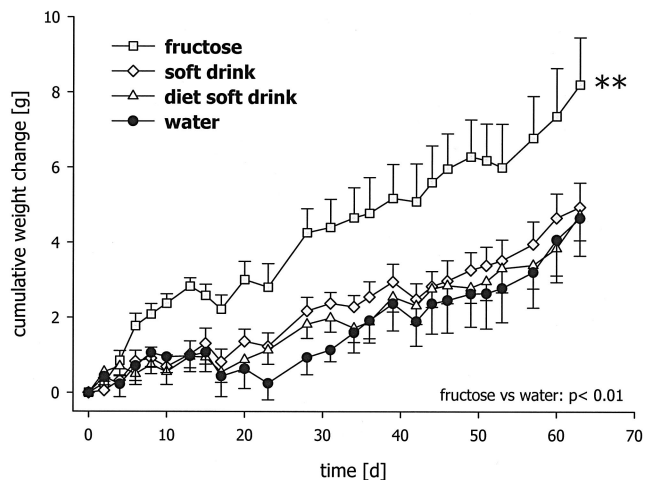


Figure 1: Cumulative change in body weight. The average body weight of the fructose group significantly increased ( $p = 0.003$ ,  $n = 8$  to  $9$ ). Average body weight of the soft drink group or diet soft drink group increased but did not differ from average body weight of the water control group ( $p = 0.98$ ,  $n = 8$  to  $9$ ). The increase in body weight seems to depend on the fructose concentration (15% in fructose group, 5% in soft drink group/10% sucrose). \*\*  $p < 0.01$ .

## Results

### Effect of Fructose on Body Weight

To analyze the effect of fructose on the development of body weight, we determined the body weight of NMRI mice that had access to water, to fructose dissolved in water (15%), to a sucrose-sweetened soft drink, and to a noncaloric soft drink (Figure 1). All four groups of mice significantly gained body weight during the study period (fructose:  $n = 9$ ,  $p < 0.001$ ; soft drink:  $n = 9$ ,  $p < 0.001$ ; diet soft drink:  $n = 9$ ,  $p < 0.001$ ; water:  $n = 8$ ,  $p = 0.002$ ). Body weight of the fructose group increased to a significantly greater extent compared with any of the other three groups ( $p \leq 0.003$ ). Body weight in the soft drink group and the diet soft drink group did not change significantly compared with the water control group (Figure 1; Table 1).

### Effect of Fructose on Body Composition

As shown in Figure 2, A and B, all four groups of mice significantly gained body fat during the study period (fructose:  $n = 9$ ,  $p = 0.001$ ; soft drink:  $n = 9$ ,  $p < 0.001$ ; diet soft drink:  $n = 9$ ,  $p = 0.001$ ; water:  $n = 8$ ,  $p = 0.008$ ). Body fat of mice in the fructose group increased to a significantly greater extent compared with any of the other groups ( $p = 0.02$ ). Body fat of the soft drink group showed a strong trend but did not increase significantly ( $p = 0.791$ ) compared with the water control group. Body fat of the diet soft drink group did not change significantly compared with the water control group (Figure 2, A and B; Table 1). These

**Table 1.** The effect of fructose on the development of body weight

Group	Water control (n = 9)	Fructose (n = 9)	Soft drink (n = 9)	Diet soft drink (n = 9)
Body weight				
Body weight 0-time	39.34 ± 0.81	39.67 ± 0.69	39.03 ± 0.76	38.84 ± 0.76
Body weight final	43.99 ± 1.53	47.88 ± 1.38*	43.98 ± 0.74	43.58 ± 1.26
Cumulative weight change (g)	+4.65 ± 1.0	+8.21 ± 1.25**	+4.94 ± 0.65	+4.73 ± 0.68
Cumulative weight change (%)	11.71 ± 2.40	20.79 ± 3.10**	12.82 ± 1.85	12.10 ± 1.59
Body composition				
Cumulative change in body fat (%)	+5.41 ± 1.46	+10.53 ± 2.49*	+7.88 ± 0.98	+7.41 ± 1.63
Cumulative change in lean mass (%)	-3.13 ± 0.86	-5.65 ± 1.40	-3.94 ± 0.76	-4.42 ± 1.11
Cumulative caloric intake				
Total (kcal)	990.5 ± 36.7	1045.3 ± 25.1	1066.2 ± 32.6	1051.7 ± 34.6
From food (kcal)	990.5 ± 36.7	836.4 ± 28.6**	918.0 ± 27.7	1048.4 ± 34.6
From drinking (kcal)	—	208.9 ± 8.7	148.3 ± 6.3	3.3 ± 0.2
Hormones				
Insulin (pg/mL)	2655.23 ± 422.14	2744.04 ± 268.40	2771.70 ± 301.29	5728.25 ± 1346.24*
Adiponectin (µg/mL per g)	0.815 ± 0.106	0.650 ± 0.071	0.777 ± 0.097	0.846 ± 0.124
Ghrelin (pg/mL)	796.5 ± 101.6	926.0 ± 229.3	786.3 ± 94.4	671.4 ± 127.3
Calorimetry				
TEE dark (kcal/h per g)	0.0130 ± 0.001	0.0121 ± 0.0003	0.0121 ± 0.0006	0.0122 ± 0.0005
RQ dark (VCO <sub>2</sub> /VO <sub>2</sub> )	0.956 ± 0.048	1.043 ± 0.014	1.116 ± 0.032**	0.988 ± 0.047
Liver				
Liver weight absolute (g)	1.90 ± 0.06	2.23 ± 0.09**	1.91 ± 0.03	1.98 ± 0.07
Liver weight relative (%)	4.511 ± 0.120	4.857 ± 0.164	4.499 ± 0.066	4.633 ± 0.125

Values are shown as the mean ± SEM.

\*  $p < 0.05$  compared to water control group.

\*\*  $p < 0.01$  compared to water control group.

results clearly show that increased body weight induced by fructose was caused by increased body fat.

During the study period, all four groups of mice significantly gained lean mass (fructose:  $n = 9$ ,  $p < 0.001$ ; soft drink:  $n = 9$ ,  $p = 0.004$ ; diet soft drink:  $n = 9$ ,  $p = 0.001$ ; water:  $n = 8$ ,  $p = 0.002$ ). Expressed as percentage of total body mass, however, all four groups tended to lose lean mass (Figure 2, C and D; Table 1). Lean mass was slightly but significantly higher in the fructose group than in the other three groups, suggesting that fructose also altered the growth of muscles.

#### **Effect of Beverages on Overall Caloric Intake**

To study whether the higher body weight of the fructose group was the result of an increase in caloric intake, we determined the cumulative caloric intake from food alone and from food plus beverage. There were no significant differences among the four groups regarding the total ca-

loric intake (food + fluid), although all treatment groups showed a trend toward higher cumulative overall caloric intake compared with the water group ( $p \geq 0.62$ ,  $n = 8$  to 9; Figure 3, A and C; Table 1).

In contrast, in the fructose group, the cumulative caloric intake from food during the study period was significantly lower compared with the water control group ( $p = 0.009$ ,  $n = 8$  to 9). Total food calorie intake of the soft drink group did not differ significantly in comparison with the water control group ( $p = 0.291$ ,  $n = 8$  to 9). The diet soft drink group showed a trend toward higher cumulative caloric intake from food compared with the water control group, but this effect was not significant ( $p = 0.622$ ,  $n = 8$  to 9; Figure 3, B and C; Table 1). These data show that additional calories provided as beverage were compensated by a respective decrease in food intake and that increased body weight induced by fructose was not the result of increased energy intake.

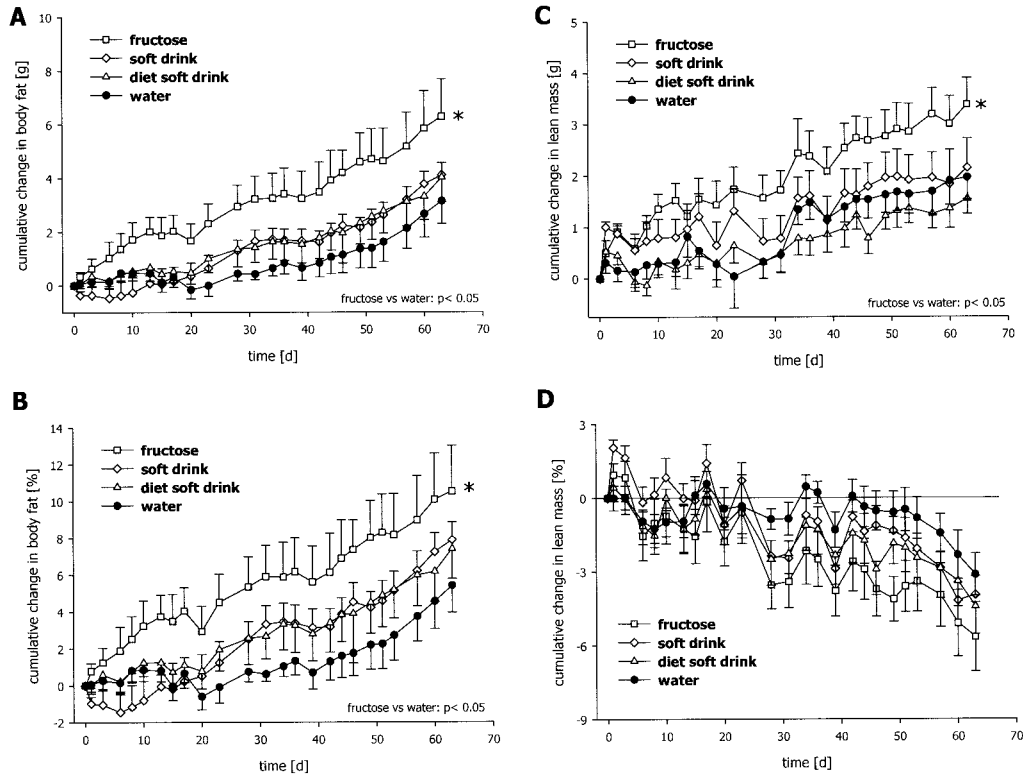


Figure 2: (A and B) Cumulative change in body fat (measured by nuclear magnetic resonance body composition analyzer). The average body fat of the fructose group increased impressively ( $p = 0.02$ ,  $n = 8$  to 9). The average body fat of the soft drink group and the diet soft drink group increased but did not differ from average body fat of the water control group ( $p = 0.7$ ,  $n = 8$  to 9). (C and D) Cumulative change in lean mass. The average lean mass of the fructose group significantly increased ( $p < 0.05$  compared with water group,  $n = 8$  to 9). The average lean mass of the soft drink group and the diet soft drink group increased but did not differ from the average lean mass of the water control group ( $p > 0.05$ ,  $n = 8$  to 9). \*  $p < 0.05$ .

### Effect of Fructose on Energy Expenditure and Respiratory Quotient

Because no difference in total energy intake was detected for the fructose group, we examined energy expenditure by indirect calorimetry. As shown in Figure 4A, energy expenditure did not differ significantly among the study groups during treatment with fructose/soft drinks. However, all treatment groups showed a trend toward decreased energy expenditure per gram of body weight during the dark phase compared with the water control group ( $p \geq 0.628$ ,  $n = 8$  to 9; Table 1).

Respiratory quotient (RQ)<sup>1</sup> during the dark phase in the soft drink group was significantly higher compared with the water control group ( $p = 0.003$ ,  $n = 8$  to 9; Figure 4B). The RQ during the dark phase (12 hours) in mice in the fructose group showed a trend toward an increase but did not differ significantly compared with the water control group ( $p = 0.131$ ,  $n = 8$  to 9). RQ during the dark phase in the diet soft

drink group did not differ significantly compared with the water control group ( $p = 0.13$ ,  $n = 8$  to 9). No changes in spontaneous locomotor activity were found among mice exposed to fructose water, sucrose-containing soft drinks, diet soft drinks, or water (quantified in a separate study using implanted transponders; data not shown).

### Effect of Fructose on Blood Glucose, Glucose Tolerance, and Insulin, Adiponectin, and Ghrelin Plasma Levels

We next analyzed the effect of fructose supplementation on glucose homeostasis and on hormones involved in the regulation of energy homeostasis (Figure 5). Blood glucose was within the normal range, and no significant changes compared with the water control group were observed. (fructose:  $n = 9$ ,  $p = 0.440$ ; soft drink:  $n = 9$ ,  $p = 0.514$ ; diet soft drink:  $n = 9$ ,  $p = 0.281$ ; Figure 5A).

**Glucose Tolerance Test.** Blood glucose levels of the fructose group during the glucose tolerance test appeared to be the highest and to stay up for the longest period of time; however, this effect was not significant ( $1999.09 \pm 202.48$

<sup>1</sup> Nonstandard abbreviations: RQ, respiratory quotient; TEE, total energy expenditure.

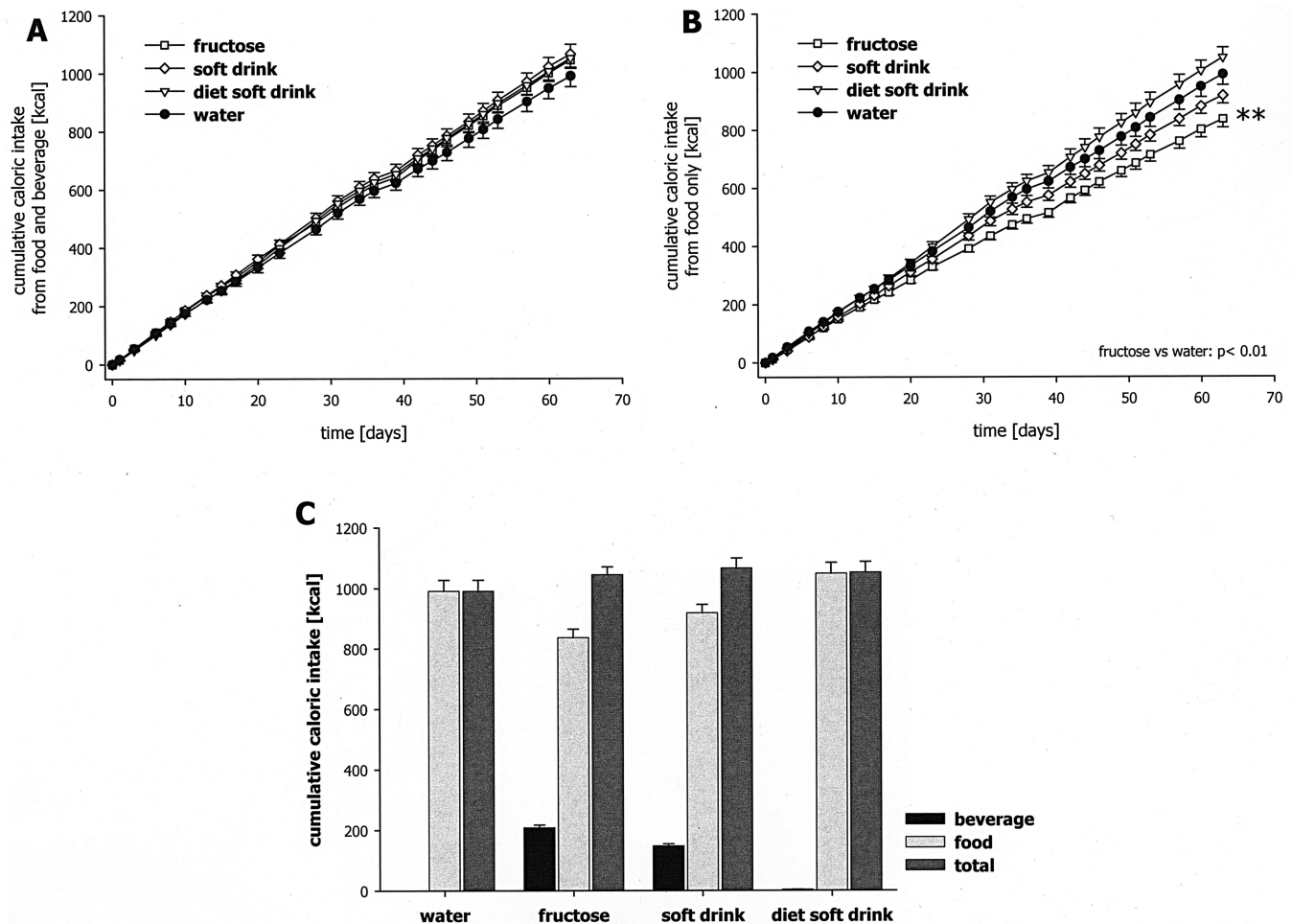


Figure 3: (A and C) Cumulative total caloric intake (food + beverage). There were no significant differences among the four groups regarding total caloric intake ( $p > 0.05$ ,  $n = 8$  to  $9$ ). (B and C) Cumulative total food caloric intake. The cumulative caloric intake from food in the fructose group was lower compared with the water control group ( $p = 0.009$ ,  $n = 8$  to  $9$ ). The cumulative caloric intake from food in the soft drink group and the diet soft drink group were not different from the water control group ( $p > 0.05$ ,  $n = 8$  to  $9$ ). \*\*  $p < 0.01$ .

area under the curve,  $n = 7$ ,  $p = 0.354$ ) compared with the water control group ( $1785.23 \pm 59.74$  area under the curve,  $n = 8$ ). Glucose tolerance of the soft drink group and the diet soft drink group did not differ significantly from the water control group ( $p \geq 0.978$ ,  $n = 8$  to  $9$ ; Figure 5B; glucose sampling between 9:00 AM and 12:00 PM).

**Plasma Insulin.** Plasma insulin levels of the diet soft drink group were significantly higher compared with the water control group ( $p = 0.02$ ,  $n = 8$  to  $9$ ). Insulin levels of the fructose group or soft drink group did not differ significantly compared with the water control group ( $p \geq 0.999$ ,  $n = 8$  to  $9$ ; Figure 5C; Table 1).

**Plasma Adiponectin.** Plasma adiponectin levels did not differ significantly in any of the four groups at the end of the study period ( $p \geq 0.534$ ,  $n = 8$  to  $9$ ; Figure 5D; Table 1).

**Plasma Ghrelin.** Plasma ghrelin levels were not significantly different among any of the four groups at the end of

the study period ( $p \geq 0.877$ ,  $n = 8$  to  $9$ ; Figure 5E; Table 1). These data indicate that fructose supplementation leads to an impaired glucose tolerance but does not influence the levels of insulin, adiponectin, and ghrelin.

#### Effect of Fructose Supplementation on Liver Histology

Because fructose is metabolized in the liver, we studied the histology of the livers of all animal groups (Figure 6). Liver weights of the fructose group were found to be significantly increased at the end of the study compared with the water control group ( $p = 0.006$ ,  $n = 8$  to  $9$ ; Table 1). Liver weights of the soft drink group and the diet soft drink group did not differ significantly from the water control group ( $p \geq 0.8$ ,  $n = 8$  to  $9$ ). When corrected for changes in body weight, however, (relative) liver weight did not differ among any of the four groups ( $p \geq 0.1$ ,  $n = 8$  to  $9$ ). Interestingly, histological evaluation showed increased he-

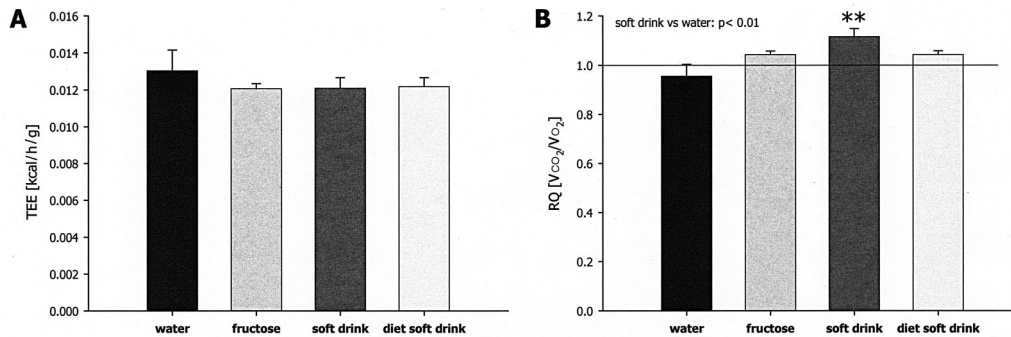


Figure 4: (A) TEE dark phase (6:00 PM to 6:00 AM) per gram of body weight (A). The average TEE of the fructose group, the soft drink group, and the diet soft drink group showed a trend toward lower levels but did not significantly differ from average TEE of the water control group ( $p \geq 0.628$ ,  $n = 8$  to  $9$ ). (B) RQ dark phase (6:00 PM to 6:00 AM). The average RQ of all treatment groups tended to be higher compared with the water group; however, only the soft drink group differed significantly ( $p = 0.003$ ,  $n = 8$  to  $9$ ). Because this group's drink contained sucrose (fructose:glucose = 1:1), an increased RQ in the soft drink group could reflect a glucose-induced decrease of fat oxidation. \*\* $p < 0.01$ .

patic storage of lipids in the fructose group (Figure 6A) and in the soft drink group (Figure 6B) but did not show increases in the diet soft drink group (Figure 6C) compared with the water control group (Figure 6D), indicating that fructose treatment increases lipogenesis.

## Discussion

A recent review examined the published literature from human and animals studies investigating the potential role of fructose in the etiology of obesity and metabolic disease (e.g., insulin resistance and hyperlipidemia) and also proposed that an increase in fructose consumption may be linked to the increased prevalence of obesity over the past two to three decades (33). Another recent paper reviewed the epidemiological evidence between an increasing consumption of fructose and the rapidly rising incidence of obesity (34). New evidence suggests that exposure of school children to soft drinks promotes obesity, whereas removal of these fructose-sweetened beverages from their diet prevents further increases in the incidence of overweight and obesity (35). A causal relationship between dietary fructose and obesity and the precise pathophysiological mechanisms have not been identified. However, a new study investigating the impact of fructose consumption on the endocrine systems involved in the long-term regulation of body weight in human subjects has shown that consuming fructose-sweetened, compared with glucose-sweetened, beverages with meals leads to reduced insulin secretion and leptin production and an attenuated suppression of ghrelin (13). These data may provide an endocrinologic explanation for dietary fructose's contribution to increased energy intake, weight gain, and obesity.

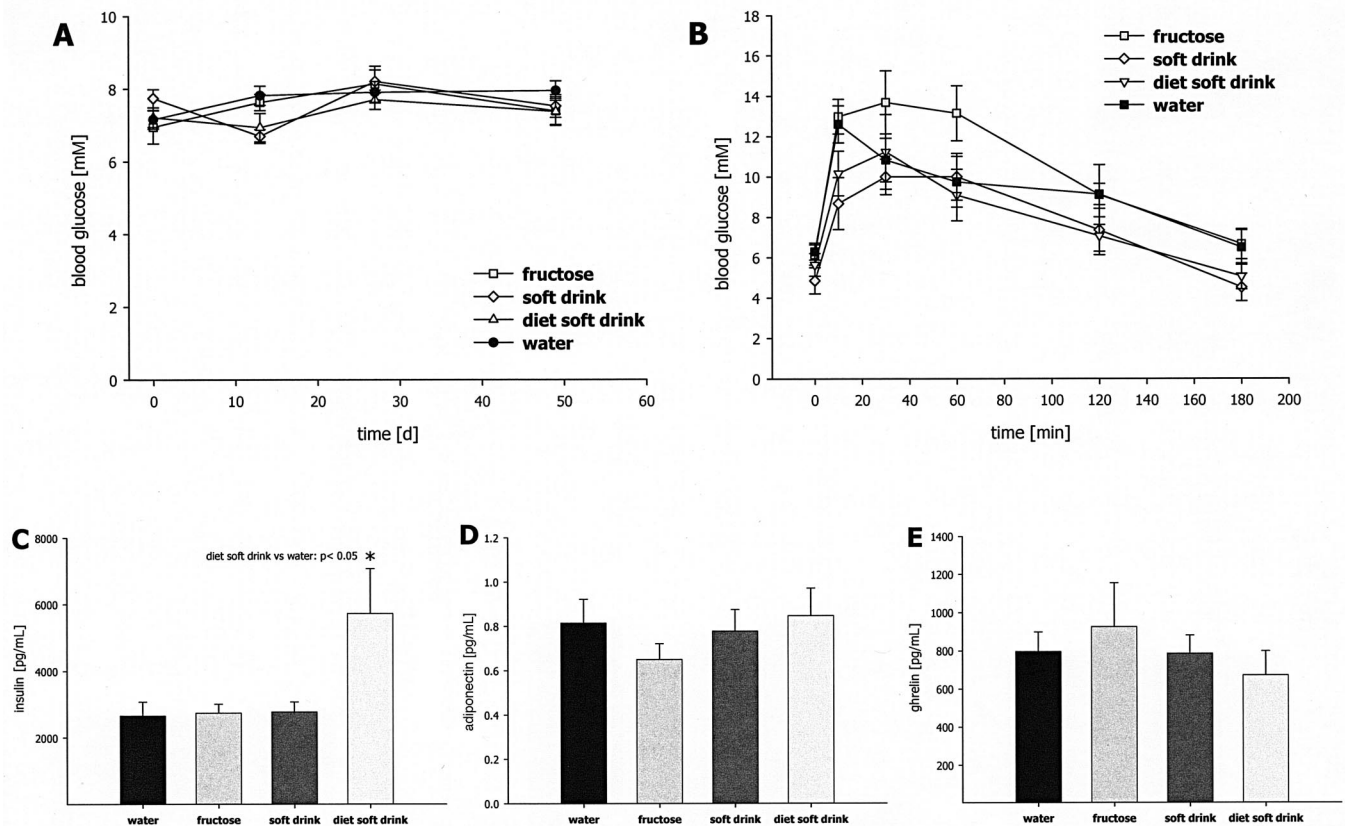
In this study, providing mice with fructose-sweetened beverages resulted in a substantial increase in body weight

without increasing overall caloric intake. Using noninvasive nuclear magnetic resonance technology in conscious mice (28,29), we were able to further define this change in body weight as an increase in fat mass. In agreement with an overall increase in adiposity, we also observed evidence of early hepatic steatosis in mice exposed to fructose-sweetened beverages. While the biochemical mechanisms leading to such nonalcoholic fatty liver will be examined in future studies, its pure pathohistological aspect impressively underlines the detrimental metabolic impact of dietary fructose.

While it seemed most likely that the observed effects on body weight and fat mass would result from an increase of energy intake, our data suggest that the additional calories provided by the beverages were compensated by a respective decrease in food intake. The fact that overall caloric intake did not significantly change despite the additional calories from fructose-sweetened beverages may indicate an interaction between a fructose-sensing system and the regulatory mechanisms governing food intake (Figure 3). This finding seems particularly interesting because fructose does not readily cross the blood-brain barrier.

The decrease in ad libitum food intake in rodents triggered by chronic exposure to fructose-sweetened beverages might provide an elegant tool to further study the identity of pathways essential for sugar sensing, i.e., using relevant gene-disrupted mouse models.

Because the overall amount of calories ingested was not different among the treatment groups in our study, other mechanisms relevant for energy balance must be responsible for the increased adiposity in mice exposed to dietary fructose. Despite the tendency toward decreased total energy expenditure (TEE), no signs of a decrease in thermogenesis to an extent that could explain the significant increase of fat mass were found. This discrepancy may be



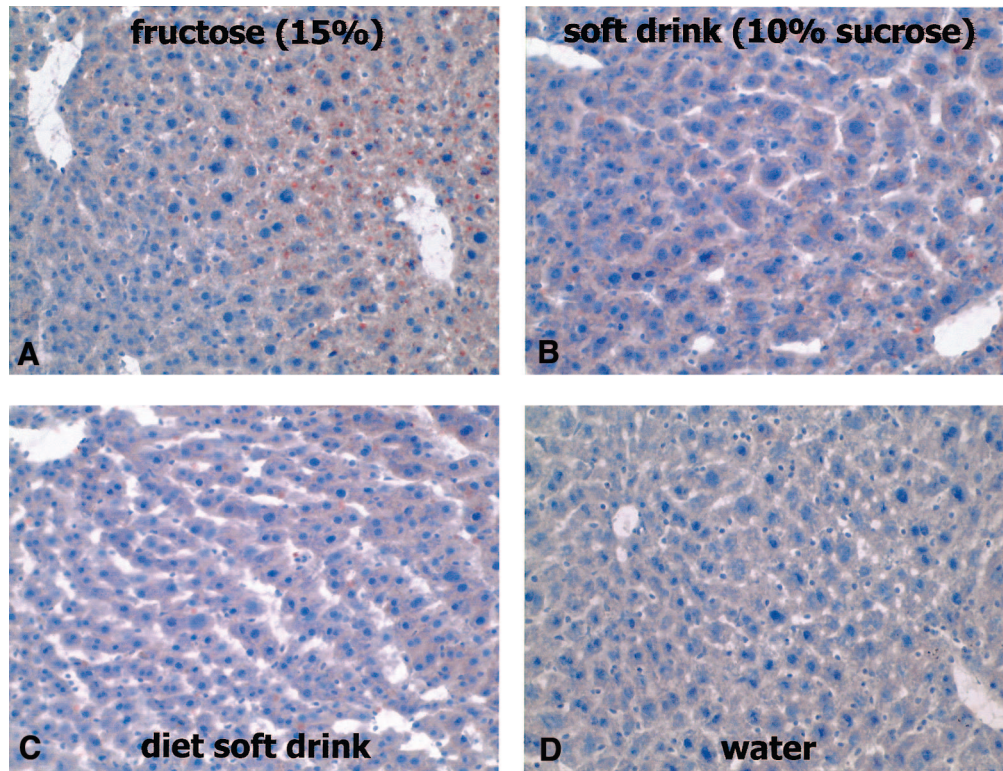
**Figure 5:** (A) Blood glucose levels. The average blood glucose levels did not differ among the four groups ( $p > 0.05$ ,  $n = 8$  to  $9$ ). (B) Glucose tolerance. The area under the curve of the blood glucose levels after glucose injection did not differ among the four groups ( $p > 0.05$ ,  $n = 8$  to  $9$ ), although the fructose group reached the highest blood glucose levels. (C) Serum insulin levels. The average serum insulin level of the diet soft drink group was significantly higher compared with the water group ( $p = 0.02$ ,  $n = 8$  to  $9$ ). The average serum insulin levels of the fructose group and the soft drink group did not differ from the average serum insulin levels of the water control group ( $p > 0.05$ ,  $n = 8$  to  $9$ ). (D) Serum adiponectin levels. The average serum adiponectin levels did not differ among the four groups, although we observed a trend toward lower adiponectin levels in the soft drink group. (E) Serum ghrelin levels. The average serum ghrelin levels did not differ among the four groups ( $p > 0.05$ ,  $n = 8$  to  $9$ ). \*  $p < 0.05$ .

caused by two technical issues. One problem is that the measurement of energy expenditure using indirect calorimetry in rodents is less sensitive as a method than most studies in the field of energy balance and obesity research would require. While a chronic change in energy expenditure of, e.g., 3% to 4%, may have significant impact on energy storage and fat mass, the lower detection limit available in instruments for indirect calorimetry in rodents may not reach levels  $<5\%$ . The second possibility is that our failure to detect a significantly lower energy expenditure in mice exposed to fructose-sweetened beverages is caused by our study design. We measured energy expenditure at the end of the 2-month treatment period, because we aimed to avoid interference of the calorimetry and the associated environmental changes and adaptation processes with our feeding study. However, the putative changes in energy expenditure and thermogenesis may occur predominantly in the acute phases of an exposure to dietary fructose and may

be not any more significant in a later state, when fat mass already is increased and compensation mechanisms are chronically activated.

In addition, there are several other examples in the field of rodent obesity research where, despite solid changes in fat mass, no significant changes in food intake or energy expenditure could be detected (22,36). Undetected mechanisms of metabolic nutrition partitioning or assimilation of ingested macronutrients may offer one explanation for these as well as for the fructose-induced changes in energy balance. This explanation would be in agreement with our reported finding of an increase in RQ, which may reflect decreased fat oxidation rates and modified nutrition partitioning.

We exposed mice to three different beverages in comparison with water: fructose-sweetened water (15% to mimic the highest high fructose corn syrup concentration of U.S. soft drinks), a popular European soft drink (because this study was performed at the German Institute of Human



*Figure 6:* Liver pathology. The histological study shows increased storage of fat in the liver of the (A) fructose group compared with (B) the soft drink group, (C) the diet soft drink group, and (D) the water control group (6 days).

Nutrition, Postdam, Germany) containing 10% sucrose, which translates into 5% fructose, and a popular diet soft drink (which did not contain fructose or any calories). While our data indicate a dose-dependent effect of fructose on body fat mass compared with control mice with access to water only, we observed a tendency in the soft drink group toward increased body fat mass. In the soft drink group, ad libitum food intake was not decreased but slightly increased (Figure 3B), an observation that might offer one potential explanation.

It has been reported that exposure to dietary fructose enhances insulin resistance and decreases insulin sensitivity (37–39). In our study, mice exposed to fructose-sweetened beverages did not exhibit an increase in plasma insulin levels or blood glucose. A glucose tolerance test performed at the end of the 2-month treatment period did not reveal significant changes in insulin sensitivity; however, the fructose group achieved the highest glucose concentration for the longest period of time (Figure 5B). Other studies have shown a comparable lack of increased insulin levels, together with more or less significant glucose intolerance (25,40). In the diet soft drink group, we were again surprised by an unexpected finding: the diet soft drink used seemed to powerfully stimulate insulin secretion *in vivo* in mice (Figure 5C). On the other hand, this increase in insulin levels has been reported earlier and is believed to be caused

by a direct effect of the artificial sweetener cyclamate (41). Nevertheless, it remains to be evaluated why such a pronounced increase in serum insulin does not affect body weight in mice, especially because epidemiologic evidence has previously linked artificial sweeteners to obesity in women (42), although most intervention studies do not support these findings (43).

The view of physicians and diabetes and nutrition specialists on fructose as a dietary component has changed over the last years based on results of recent experimental and clinical studies. Only a decade ago, it was thought that diabetic patients would benefit from consuming fructose instead of glucose, because fructose intake did not trigger an acute insulin response comparable to that after glucose intake (11,44). This postprandial insulin response is now understood as a physiological and rather necessary mechanism, which is lacking after fructose consumption, and should not be confused with chronic hyperinsulinemia, reflecting insulin resistance. In addition, Kelley et al. (40) recently reported that consumption of dietary fructose might promote dyslipidemia in diabetic patients and other individuals at risk.

We and others have shown in clinical studies that fructose-sweetened beverages, compared with glucose-sweetened beverages, in combination with standardized meals trigger an impressively differential response in terms of

peripherally circulating hormones involved in the regulation of energy homeostasis. Dietary fructose not only reduces acutely circulating insulin and leptin levels but also attenuates postprandial suppression of ghrelin and modifies profiles of glucose-dependent insulinotropic peptide and glucagon-like peptide-1 in healthy volunteers (13).

Based on these clinical findings, it is possible that the fructose-induced increase in fat mass in this rodent study is mediated through neuroendocrine systems regulating energy balance, including modified hormone secretion patterns (13,14,19,27,33,45,46). Therefore, we also measured peripherally circulating levels of ghrelin (as a hormone that induces a positive energy balance and adiposity) and adiponectin (as a hormone associated with insulin sensitivity and energy balance) at the end of the study. However, no significant differences between fructose-treated and control groups were observed, which might be because of the time-point at which these hormones were studied.

In summary, dietary fructose consumed with beverages promotes adiposity and the risk for nonalcoholic fatty liver disease in mice. In view of the impressive rise in worldwide fructose consumption over the last two decades, fructose is, therefore, likely to represent one causal factor for the rapidly increasing number of obese patients. This may be based, in part, on the increasing per capita consumption of dietary fructose from popular carbonated soft drinks.

To our knowledge, this study shows for the first time prospectively that a causal relationship between exposure to fructose-sweetened beverages and an increase in fat mass exists. We also report that the fructose-induced increase in fat mass is mainly based not on an increased amount of ingested calories but more likely on the specific energy and sugar metabolism of dietary fructose. Studies to uncover the exact pathophysiological and biochemical mechanisms responsible for the observed adiposity-promoting effects are underway and may allow a better understanding of crucial molecular interactions between macronutrients and the regulatory mechanisms governing energy balance.

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