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Are heterozygous carriers for hereditary fructose intolerance predisposed to metabolic disturbances when exposed to fructose?

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ABSTRACT

Background: High fructose intake causes hepatic insulin resistance and increases postprandial blood glucose, lactate, triglyceride, and uric acid concentrations. Uric acid may contribute to insulin resistance and dyslipidemia in the general population. In patients with hereditary fructose intolerance, fructose consumption is associated with acute hypoglycemia, renal tubular acidosis, and hyperuricemia.

Objective: We investigated whether asymptomatic carriers for hereditary fructose intolerance (HFI) would have a higher sensitivity to adverse effects of fructose than would the general population.

Design: Eight subjects heterozygous for HFI (hHFI; 4 men, 4 women) and 8 control subjects received a low-fructose diet for 7 d and on the eighth day ingested a test meal, calculated to provide 25% of the basal energy requirement, containing ¹³C-labeled fructose (0.35 g/kg), glucose (0.35 g/kg), protein (0.21 g/kg), and lipid (0.22 g/kg). Glucose rate of appearance (GRa, calculated with [6,6-²H₂]glucose), fructose, net carbohydrate, and lipid oxidation, and plasma triglyceride, uric acid, and lactate concentrations were monitored over 6 h postprandially.

Results: Postprandial GRa, fructose, net carbohydrate, and lipid oxidation, and plasma lactate and triglyceride concentrations were not significantly different between the 2 groups. Postprandial plasma uric acid increased by 7.2% compared with fasting values in hHFI subjects ($P < 0.01$), but not in control subjects (-1.1% , ns).

Conclusions: Heterozygous carriers of hereditary fructose intolerance had no significant alteration of postprandial fructose metabolism compared with control subjects. They did, however, show a postprandial increase in plasma uric acid concentration that was not observed in control subjects in responses to ingestion of a modest amount of fructose. This trial was registered at the US Clinical Trials Registry as NCT02979106. *Am J Clin Nutr* 2018;108:1–8.

Keywords: fructose, hereditary fructose intolerance, uric acid, plasma triglyceride concentration

INTRODUCTION

There is increasing concern that a high fructose intake may be directly involved in the development of obesity, diabetes mellitus, dyslipidemia, gout, and high blood pressure (1, 2). This proposal is mainly supported by animal studies (3), but there are nonetheless robust observations in humans that fructose overfeeding impairs hepatic insulin sensitivity, increases de novo lipogenesis and VLDL secretion, and causes hypertriglyceridemia and ectopic lipid deposition in the liver and skeletal muscle (4–7). Sucrose and high fructose corn syrup, which both contain nearly isomolar amounts of glucose and fructose, are the major sources of dietary fructose. Concerns about the health effects of these sugars have recently led several health organizations to propose that consumption of free sugars should not exceed 5% (8) or 10% (9) of total energy intake (10, 11).

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Supplemental Tables 1 and 2 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: ALDOB, aldolase B; B, effect of baseline; BW, body weight; C, effect of condition; Cr, creatine; Crt, creatinine; Fe, fractional excretion; FOX, fructose oxidation; GAA, guanidinoacetate; GC-MS, gas chromatography-mass spectrometry; GRa, glucose rate of appearance; HFI, hereditary fructose intolerance; hHFI, heterozygous for hereditary fructose intolerance; NEFA, nonesterified free fatty acid; T, effect of time; T × C, time and condition interaction.

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A high fructose intake also increases blood lactate and uric acid concentrations (12, 13). The latter is often increased in patients with metabolic syndrome, and has been previously proposed as one of its diagnostic criteria (14). Fructose-induced hepatic phosphate depletion, leading to defective ATP recycling, increased ATP hydrolysis up to adenosine, and enhanced purine degradation, is often proposed as the main factor responsible for increased uric acid production (15). Recently it has been proposed that hyperuricemia may be not only a consequence of fructose metabolism, but also a key mediator in some of the adverse cardiometabolic effects of fructose (16–18). However, the underlying mechanisms remain incompletely elucidated (19). It has been proposed that uric acid may contribute to insulin resistance by impairing endothelium-dependent vasodilation (16), promoting proinflammatory effects (17) and dyslipidemia by activating de novo lipogenesis (18).

These consequences of fructose overconsumption may be even more marked in individuals with hereditary alterations in fructose metabolism. Indeed, individuals with hereditary fructose intolerance (HFI), owing to biallelic mutations in the gene coding for aldolase B (*ALDOB*), may develop acute, life-threatening manifestations when exposed to even minute amounts of fructose. In such individuals, administration of small amounts of fructose (or sorbitol, which is a precursor for endogenous fructose synthesis) will cause acute ATP depletion in hepatocytes and proximal kidney tubule cells owing to rapid phosphorylation of fructose by fructokinase and accumulation of intracellular fructose-1-phosphate. The energy crisis thus elicited is responsible for acute hypoglycemia, renal tubular acidosis, and hyperuricemia (20–25). Chronic consumption of small amounts of fructose also causes fatty liver and renal tubular dysfunction in HFI individuals (26). No genotype-phenotype correlations have been identified for HFI; clinical severity and extent of organ damage appear to depend on individual nutritional habits. HFI prevalence in central Europe is estimated to be 1:26,100 (27). Heterozygous carriers of the *ALDOB* mutation are therefore quite common in the general population, with a predicted frequency ranging between 1:55 and 1:120 (28). Few studies have examined the effect of fructose ingestion in subjects heterozygous for HFI (hHFI). Heterozygous carriers are generally considered to have normal fructose metabolism since a ~50% level of *ALDOB* activity is presumed to be sufficient for adequate function. In contradiction with this postulate, however, heterozygous carriers were reported to have enhanced uric acid responses to large intravenous and/or oral fructose loads (29, 30). We therefore hypothesized that heterozygous carriers may also have mild defects of fructose metabolism and/or a larger increase in cardiometabolic risk factors than the normal population after ingestion of moderate amounts of fructose.

METHODS

Subjects

Eight hHFI subjects (4 men, 4 women) and 8 control subjects (4 men, 4 women) were included in the study. The hHFI subjects were parents of children with HFI followed in the Department of Medical Genetics at the University Hospital of Liège/CHU Sart Tilman, Liège. The *ALDOB* genotype was established in heterozygous subjects as a part of the routine assessment and follow-up, and for genetic counseling purposes. Control

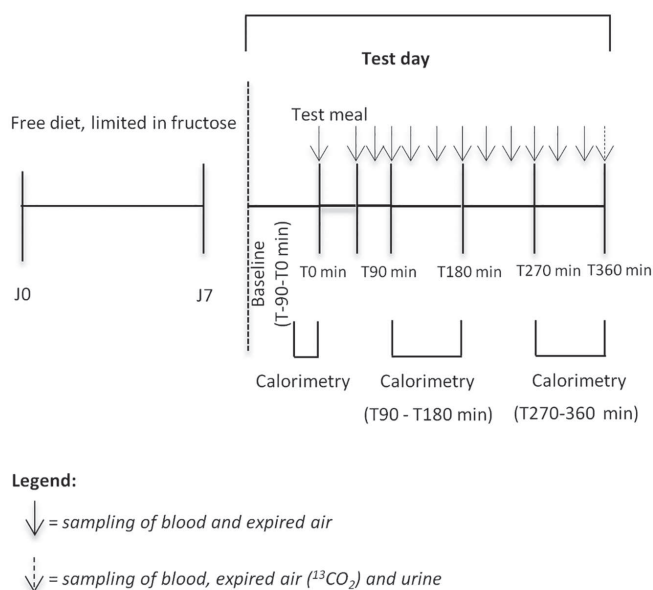


FIGURE 1 Experimental setting of the metabolic test. Each subject was studied on one occasion, after 7 d on a low-fructose diet (<10 g/d). On day 8, overnight fasted subjects remained on a bed to assess their postprandial response to a mixed meal (0.35 g glucose/kg, 0.35 g fructose/kg, 0.21 g protein/kg, and 0.22 g lipid/kg). Dietary fructose was labeled with 1% [$^{13}\text{C}_6$] fructose, and plasma glucose metabolism was measured with [6,6- $^2\text{H}_2$]glucose. An indirect calorimetry period and baseline plasma samples were obtained prior to meal ingestion, and repeated sequentially thereafter (for details, see Methods). T, time point.

subjects were recruited in the general population and were genotyped in the Center for Molecular Diseases (Switzerland) to ensure that they had no *ALDOB* mutation. The subjects were currently not taking any medication, and had no history of diabetes, dyslipidemia, or renal insufficiency. Before inclusion, they underwent a physical examination to ensure that they were in good physical health. The experimental protocol was approved by the Ethics Committee of the University Hospital of Liège (Belgium) and Lausanne (Switzerland). All participants provided written informed consent. This trial was registered at the US Clinical Trials Registry as NCT02979106.

Study design

Each subject was studied on a single occasion of 8 d. During the first 7 d, the subjects were asked to consume a low-fructose diet (<10 g/d, counseled by a registered dietitian). The subjects then reported to the Clinical Research Center on day 8 for a metabolic test in which the response to a fructose-containing mixed meal was assessed (Figure 1).

Metabolic test

On day 8, subjects reported to the Clinical Research Center of CHU Sart Tilman, Liège at 0700 after a 12-h fast. Upon their arrival, their weight was recorded and their body composition was measured using bioelectrical impedance analysis (Imp Df50, ImpediMed). The subjects were asked to empty their bladder, and the urine passed was discarded. Urine was thereafter collected from 0800 ($t = 0$ min) to the end of the test at 1600 ($t = 480$ min). After subjects had been transferred to a bed, a catheter was inserted into a vein of the right forearm for blood sampling.

Patency was maintained by a slow infusion of 0.9% NaCl (150 mL over 6 h). No additional drink was allowed during the test. Another catheter was inserted into a vein of the left arm and was used for the administration of a primed continuous infusion of [6,6-²H₂]glucose [bolus, 2 mg/kg body weight (BW), and continuous infusion, 0.03 mg · kg BW⁻¹ · min⁻¹; Cambridge Isotope Laboratories] throughout the test. Subjects were studied for 6 h after the ingestion of a controlled-weight maintenance meal containing (means ± SDs) 0.35 ± 0.02 g fructose/kg BW (labeled with 1% [U-¹³C₆]fructose), 0.35 ± 0.02 g glucose/kg BW, 0.21 ± 0.01 g protein/kg BW and 0.22 ± 0.03 g lipid/kg BW. This meal was calculated to provide 25% of resting 24-h energy requirements, calculated by using the Harris-Benedict equation and a correction factor of 1.1 to account for dietary thermogenesis (31). Blood and breath samples were collected at baseline (*t* = baseline), immediately after catheter insertion, before the test meal (*t* = 0 min), and every 30 min after the test meal until *t* = 360 min. Blood pressure was measured at baseline using an automatic blood pressure device (Omron 907, Omron). Energy expenditure and net substrate oxidation rates were monitored for 90 min under fasting conditions (baseline; *t* = -90 min to *t* = 0 min) and for 180 min after ingestion of the test meal at 2 separate times (*t* = 90 min to *t* = 180 min) and *t* = 270 min to *t* = 360 min) by indirect calorimetry (Deltatrac II, Datex Instrument).

Analytic procedures

Appropriate informed consent for genetic testing was obtained from all individuals. Blood samples were collected on EDTA and genomic DNA was extracted from blood leukocytes according to standard protocols. The 8 coding exons of the *ALDOB* gene (GenBank accession NM_000035.3, Ensembl ENST00000374855), as well as the intron-exon boundaries, were amplified from genomic DNA by polymerase chain reaction (using the Qiagen TaqPCR MasterMix, and *ALDOB* specific primers—available upon request) and directly sequenced by Sanger sequencing on a ABI3500 sequencer according to the manufacturer's procedure (Applied Biosystems/LifeTechnologies).

Plasma [6,6-²H₂]glucose isotopic enrichment and plasma and urinary fructose concentrations were measured by gas chromatography-mass spectrometry (GC-MS). Plasma [6,6-²H₂]glucose enrichment was measured on pentaacetyl derivatives, using GC-MS in chemical ionization mode with selective monitoring of *m/z* 333 and *m/z* 331 as described previously (32). After the addition of 69 nmol D-[1,2,3-¹³C₃]fructose as an internal standard, 250-μL plasma samples were deproteinized by ZnSO₄-Ba(OH)₂, partially purified over anion- and cation-exchange resins, dried, and derivatized with acetic anhydride and pyridine. Samples were then dried under a stream of nitrogen and resuspended in 60 μL of ethyl acetate. To 250 μL of urine, 23 nmol of internal standard solution (D-[1,2,3-¹³C₃]fructose) and 55 μL of urease (26 units/mL, recombinant from *Canavalia ensiformis*; jack bean, Sigma) were vortex-mixed and centrifuged for 10 min at 14,000 × *g* and incubated for 60 min at 37°C. Then 800 μL of cold methanol was added, and the samples were again vortex-mixed and centrifuged. Next, 500 μL of the supernatant was transferred into a glass vial and dried under reduced pressure. Urinary samples were then derivatized and analyzed as described previously (33). Plasma and urinary fructose were analyzed by GC-MS (Agilent Technologies) in electron impact mode,

with selected monitoring of *m/z* 275 and *m/z* 277. The fructose concentration in samples was determined from the ratio of *m/z* 277 to *m/z* 275 by means of an unlabeled pure fructose standard curve. After deproteinization with sulfosalicylic acid, plasma amino acids were separated and quantified by ion exchange chromatography with post-column ninhydrin derivatization using a JLC-500/V AminoTac amino acid analyzer (Jeol Ltd).

Plasma glucose, lactate, triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, uric acid, creatinine (Cr), nonesterified fatty acids (NEFAs), urea and urinary urea were measured using enzymatic methods (RX Monza analyser, Randox Laboratories Ltd). Commercial radioimmunoassay kits were used for the determination of plasma insulin and glucagon. Creatine (Cr) and guanidinoacetate (GAA) determination in plasma was performed by liquid chromatography/MS-MS, as described elsewhere (34). Briefly, D₃-Cr and [¹³C₂]GAA (CDN Isotopes) were added to plasma samples as internal standards, and Cr and GAA were purified by microsolid phase extraction (Oasis MCX μElution Plate, Waters). Separation of Cr and GAA was achieved on an ACQUITY UPLC BEH HILIC silica column (Waters) using an H₂O-acetonitrile gradient. The column effluent was monitored using a Triple Quadrupole TSQ Quantum Discovery (ThermoFisher) equipped with an electrospray interface. Samples were analyzed in positive ionization mode using the selected reaction monitoring mode.

Calculations

Plasma glucose rate of appearance (GRa) and disposal were calculated from glucose concentration and [6,6-²H₂]glucose isotopic enrichment using the nonsteady state equation of Steele as modified by DeBodo et al. (35), using a volume of distribution for glucose of 0.2 times BW and a pool fraction of 0.75.

[¹³C]Fructose oxidation (FOX) was calculated over 30 min periods as:

$$\text{FOX} = (180 \times {}^{13}\text{CO}_2 \text{ IE} \times \text{VCO}_2) / ({}^{13}\text{C-fructose IE}_{(\text{meal})} \times 22.29 \times 6 \times 0.8) (\text{g/min}) \quad (1)$$

where ¹³CO₂ IE is breath CO₂ isotopic enrichment (atom% excess), VCO₂ is carbon dioxide production in the breath (L/min), [¹³C]fructose IE_(meal) is the amount of ¹³C-labeled fructose in the meal (mol% excess), 180 is the molecular weight of fructose, 22.29 is the volume occupied by 1 mol of CO₂ under laboratory conditions (L), 6 is the number of carbons in a fructose molecule and 0.8 is the recovery factor of ¹³CO₂ in breath (36).

The area under the FOX curve was then calculated to determine the total fructose oxidation over 6 h (grams), and the nonoxidative fructose disposal (grams) was obtained by subtracting the total FOX from the fructose load (grams). Similarly, net glycogen synthesis was obtained by subtracting the total carbohydrate oxidation (accounting for the net oxidation of endogenous and exogenous carbohydrates) from the glucose and fructose intake (grams).

The fractional excretion (Fe) of sodium (Fe-Na) was calculated as:

$$\text{Fe-Na} (\%) = ([(\text{P}_{\text{Cr}} \times \text{U}_{\text{Na}}) / (\text{P}_{\text{Na}} \times \text{U}_{\text{Cr}})]) \times 100 \quad (2)$$

where $P_{\text{Cr}} =$ plasma Cr (μmol/L), $U_{\text{Na}} =$ urinary sodium (μmol/L), $P_{\text{Na}} =$ plasma sodium (μmol/L) and $U_{\text{Cr}} =$ urinary Cr (μmol/L). A similar calculation was done to calculate the fractional excretion of urea (Fe-Urea) and of uric acid (Fe-Uric acid).

Cr clearance (CL_{Cr}) was measured directly by collecting a 6-h urine sample and then drawing a blood sample, and was calculated as:

$$CL_{\text{Cr}} = (U_{\text{Cr}} \times \text{Vol}/\text{Time}) / (P_{\text{Cr}}/1000) \quad (3)$$

where $U_{\text{Cr}} =$ urine Cr (μmol/L), Vol = volume collected (mL), time = collection time (min) and $P_{\text{Cr}} =$ plasma Cr (μmol/L).

Statistics

Based on previous data obtained in our laboratory with similar methodology (37), we calculated that a sample size of 8 subjects/group would be appropriate ($1 - \beta$: 80%; $\alpha = 0.05$) to detect an ~20% difference in mean GRa between hHFI and control subjects after fructose loading. First, all the variables (expressed as mean \pm SD) were visually inspected, then distribution normality and homoscedasticity were assessed with the use of Shapiro-Wilk and Bartlett's tests, and data were log-transformed when appropriate (plasma insulin, uric acid, ornithine and citrulline). Baseline patients' characteristics, mean GRa, and carbohydrate and lipid oxidations were determined using an unpaired Student's *t* test. The significance of changes over time was determined by mixed-models analysis, with fixed effects of time (T) and condition (C), and random effects for subject-specific intercepts and slopes. The time and condition interaction ($T \times C$) and baseline (B) effects were included in the models each time that model goodness of fit was improved. Linear regression was checked using Pearson's coefficient. Analyses were performed with R, version 3.0.3, and the level of significance was set as 2-tailed $P = 0.05$.

RESULTS

Subjects' characteristics and mutation analysis

At inclusion, control and hHFI subjects did not differ in age, weight, BMI, percentage of body fat, blood pressure, or heart rate (Table 1). Absence of mutation in the *ALDOB* (OMIM #229,600) coding sequence was confirmed in control subjects ($n = 8$). Heterozygosity for the most common pathogenic variants in the *ALDOB* gene was identified in the 8 hHFI subjects (c.448G > C, p.Ala150Pro; ExAC frequency 0.2% in 7 subjects and the c.548T > C, p.Leu183Pro pathogenic variant in one subject).

Carbohydrate and lipid metabolisms

Baseline GRa, net carbohydrate and lipid oxidation, plasma carbohydrate, lipids and hormone concentrations were not different in fasted hHFI and control subjects (all $P > 0.05$). GRa increased during the first 120 min after meal ingestion, then returned to fasting values, similarly in both hHFI and control subjects (kinetic data not shown; T effect: $P < 0.05$; $T \times C$ effect: $P > 0.05$). Energy expenditure and carbohydrate oxidation increased, and lipid oxidation decreased, in both groups

TABLE 1

Baseline clinical characterization of study subjects¹

Characteristics	Control subjects	hHFI subjects
Age, y	37.25 \pm 5.12	36.38 \pm 6.70
Weight, kg	73.51 \pm 16.66	74.36 \pm 18.21
BMI, kg/m ²	24.32 \pm 3.84	24.92 \pm 4.39
Body fat, %	23.50 \pm 4.84	28.14 \pm 6.53
Lean body mass, %	76.38 \pm 4.84	71.89 \pm 6.50
Systolic BP, mm Hg	121.46 \pm 15.93	121.56 \pm 16.07
Diastolic BP, mm Hg	75.34 \pm 9.08	75.64 \pm 10.43
Heart rate, beats/min	63.13 \pm 8.75	63.14 \pm 11.11

¹Values presented as means \pm SDs ($n = 8$ subjects/group). Distribution normality and homoscedasticity were visually inspected and checked using Shapiro-Wilk and Bartlett's tests. Changes were assessed by a Student's unpaired *t* test. Control and hHFI subjects showed no significant difference in anthropometric variables (all $P > 0.05$). BP, blood pressure; hHFI, heterozygous for hereditary fructose intolerance.

(all T effects: $P < 0.05$; all $T \times C$ effects: $P > 0.05$). Over the 6 h following the test meal, 36.8% \pm 4.05% (9.4 \pm 1.9 g/6 h) and 38.8% \pm 6.0% (9.5 \pm 2.0 g/6 h) of the ingested fructose were oxidized to CO₂ in control and hHFI subjects, respectively ($P > 0.05$). The remaining 63.2% \pm 4.1% (16.2 \pm 3.4 g) and 61.2% \pm 6.0% (15.2 \pm 4.1 g) were disposed of nonoxidatively ($P > 0.05$). Net glycogen synthesis was also not different between control and hHFI subjects (23.7 \pm 15.0 compared with 17.9 \pm 5.1 g; $P = 0.32$) (Table 2).

After ingestion of the fructose-containing mixed meal, systemic fructose concentration increased to a peak of between 200 and 250 μmol/L after 60 min, then decreased similarly in hHFI and control subjects (Figure 2A; T effect: $P < 0.01$; $T \times C$ effect: $P = 0.60$). Plasma lactate, glucose, and insulin concentrations followed similar postprandial patterns, with no difference between conditions (Figure 2B–D; all T effects: $P < 0.05$; all $T \times C$ effects: $P > 0.05$). Plasma glucagon was not significantly altered by meal ingestion (data not shown; all $P > 0.05$). NEFA concentrations decreased (Figure 2E; T effect: $P < 0.01$; $T \times C$ effect: $P = 0.35$) and total triglycerides concentrations increased over time (Figure 2F; T effect: $P < 0.01$;

TABLE 2

Substrate kinetics over 6 h after ingestion of a fructose-containing mixed meal in hHFI and control subjects¹

Characteristics	Control subjects	hHFI subjects
Fructose intake, g	25.6 \pm 5.0	24.7 \pm 5.2
FOX, g/6 h	9.4 \pm 1.9	9.5 \pm 2.0
NOFD, g/6 h	16.2 \pm 3.4	15.2 \pm 4.1
Total carbohydrate oxidation, g/6 h	27.6 \pm 13.0	31.5 \pm 10.7
Net glycogen synthesis, g/6 h	23.7 \pm 15.0	17.9 \pm 5.1
GRa, mg \cdot kg ⁻¹ \cdot min ⁻¹	2.2 \pm 0.2	2.4 \pm 0.2
Lipids oxidation, g/6 h	22.9 \pm 6.9	20.5 \pm 6.1
Protein oxidation, g/6 h	16.6 \pm 6.4	19.7 \pm 7.0
Energy expenditure, kcal/6 h	383.0 \pm 77.9	389.4 \pm 67.7

¹Values presented as means \pm SDs ($n = 8$ subjects/group). Distribution normality and homoscedasticity were visually inspected and checked using Shapiro-Wilk and Bartlett's tests. Changes were assessed by a Student's unpaired *t* test. Control and hHFI subjects showed no significant difference in substrate kinetics (all $P > 0.05$). FOX, fructose oxidation; GRa, glucose rate of appearance; hHFI, heterozygous for hereditary fructose intolerance; NOFD, nonoxidative fructose disposal.

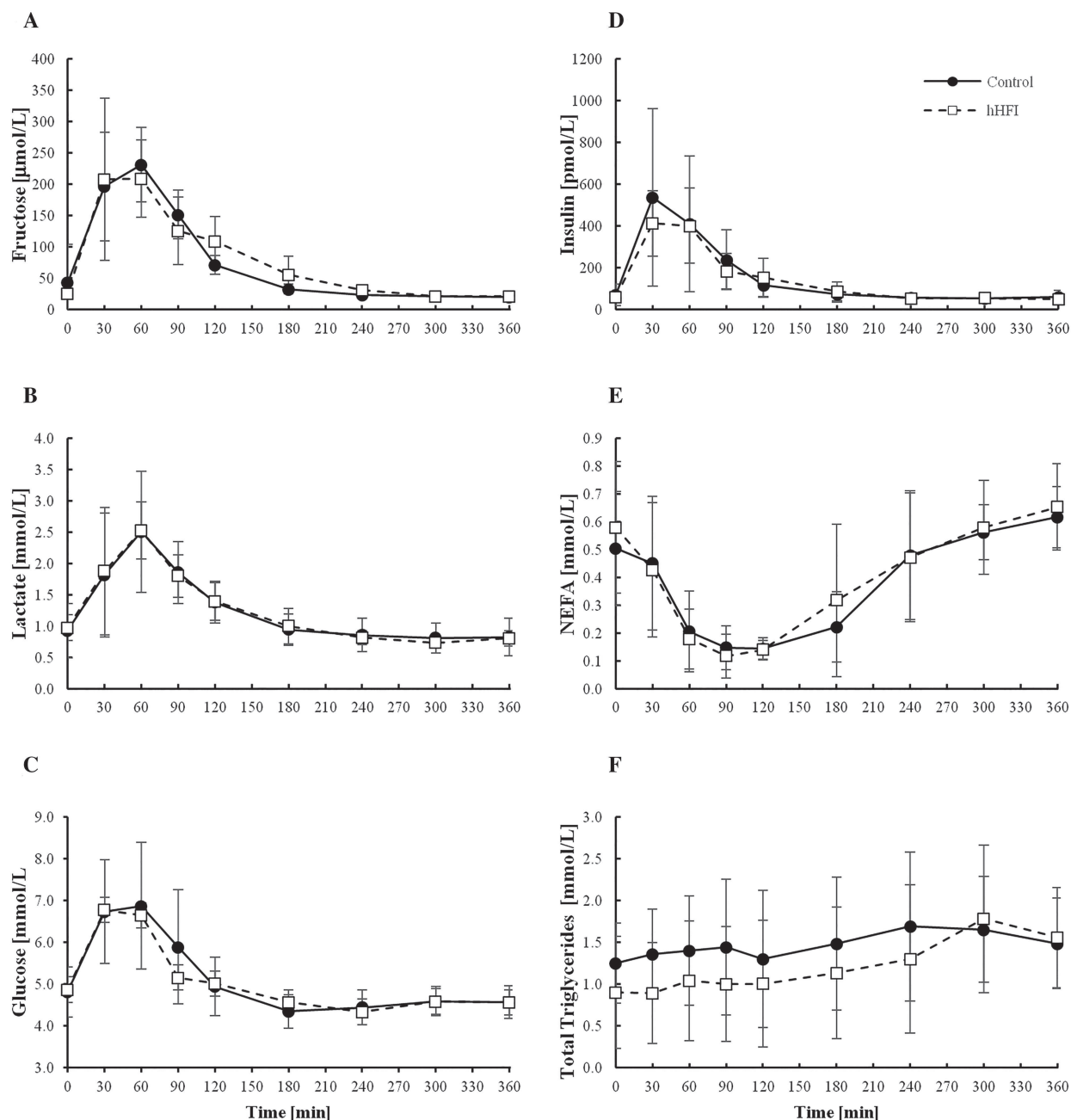


FIGURE 2 Plasma substrates and insulin concentrations over 6 h after ingestion of a fructose-containing mixed meal in hHFI and control subjects. Changes over time in plasma fructose (A), lactate (B), glucose (C), insulin (D), NEFA (E), and TG (F) concentrations after ingestion of a fructose-containing mixed meal (time = 0 min). Values are means \pm SDs ($n = 8$ subjects/group). Distribution normality and homoscedasticity were visually inspected and checked using Shapiro-Wilk and Bartlett's tests. Insulin values were log-transformed for analyses. Changes over time were assessed by mixed-model analysis, with time and condition as fixed effects. The $T \times C$ interaction and baseline values were included as covariates each time that model goodness of fit was improved. Time-paired contrasts were used to determine differences between conditions. There was no significant difference between conditions over time ($T \times C$ interaction: all $P > 0.05$). hHFI, heterozygous for hereditary fructose intolerance; NEFA, nonesterified fatty acids; $T \times C$, time-by-condition; TG, triglycerides.

$T \times C$ effect: $P = 0.48$) similarly in hHFI and control subjects. Total- and LDL-cholesterol concentrations were also not different between the 2 groups (data not shown; all $P > 0.05$).

Blood and urinary uric acid, creatinine, amino acids, and related metabolites

Baseline plasma uric acid ($P = 0.43$) and urea ($P = 0.36$) concentrations and urinary indexes (**Supplemental Table 1**; all $P > 0.05$) were highly variable among subjects and were

similar in hHFI and control subjects. Interestingly, the plasma Cr concentration was significantly lower ($P = 0.04$), whereas the plasma glycine was higher ($P = 0.04$) and arginine tended to be higher ($P = 0.06$), in fasted hHFI subjects compared with control subjects. Other amino acids measured in the fasted state did not differ between groups (**Supplemental Table 2**). Plasma Cr (38.1 ± 13.5 compared with 29.5 ± 9.9 $\mu\text{mol/L}$; $P > 0.05$) and guanidinoacetate (2.3 ± 0.3 compared with 2.1 ± 0.6 $\mu\text{mol/L}$; $P > 0.05$) also showed no statistical difference at baseline (data not shown; all $P > 0.05$).

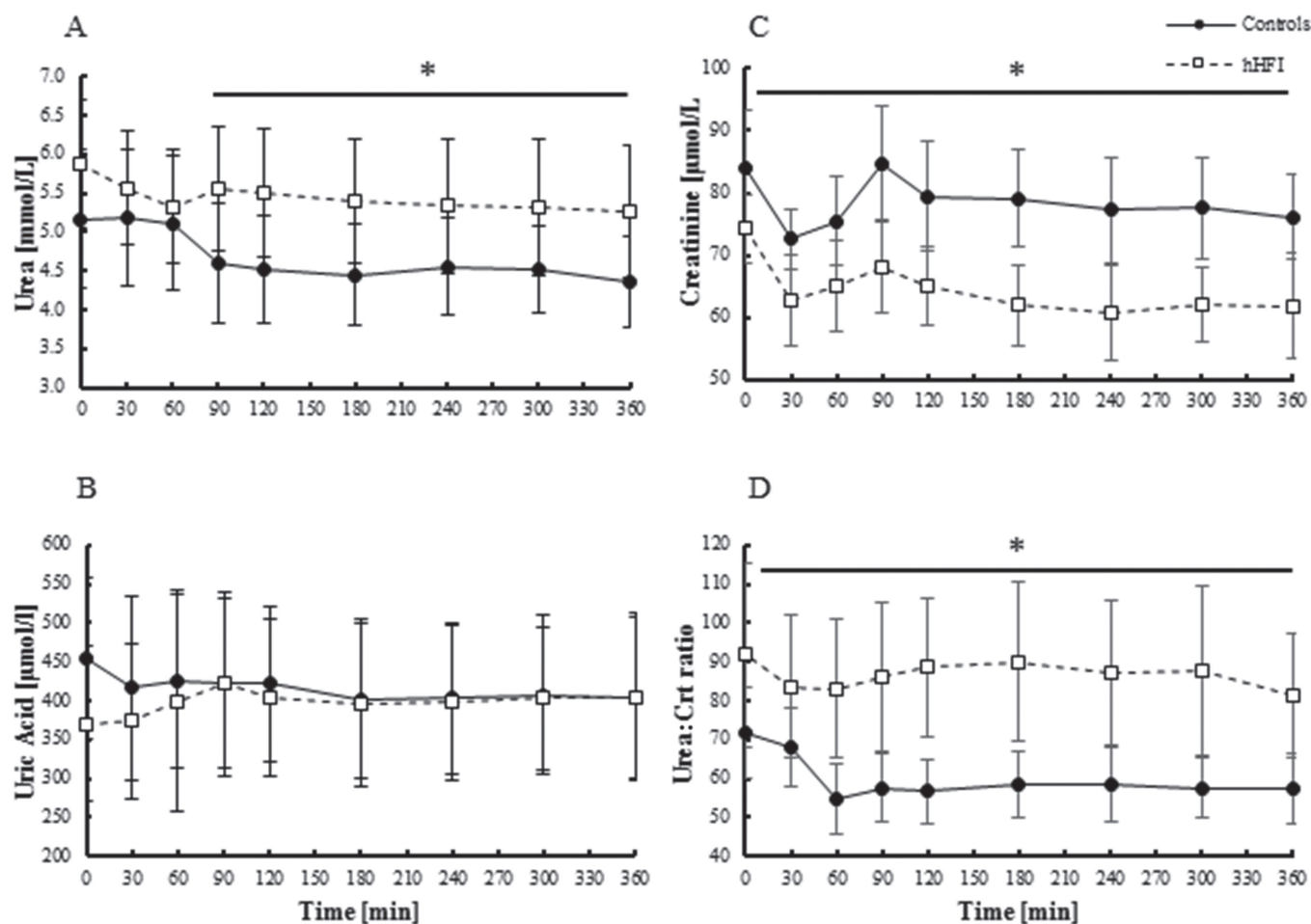


FIGURE 3 Plasma urea, uric acid, and Cr concentrations over 6 h after ingestion of a fructose-containing mixed meal in hHFI and control subjects. Changes over time in plasma urea (A), uric acid (B), and Cr (C) concentrations after ingestion of a fructose-containing mixed meal (time = 0 min), and urea:Cr ratio (D). Values are means \pm SDs ($n = 8$ subjects/group). Distribution normality and homoscedasticity were visually inspected and checked using Shapiro-Wilk and Bartlett's tests. Uric acid values were log-transformed for analyses. Changes over time were assessed by mixed-model analysis, with time and condition as fixed effects. The $T \times C$ interaction and baseline values were included as covariates each time that model goodness of fit was improved. Time-paired contrasts were used to determine differences between conditions. The linear relation between covariates was determined using Pearson's test (coefficients indicated). Plasma urea and uric acid were not different at baseline (both $P > 0.05$), but were subsequently significantly higher in hHFI subjects than in control subjects (both $T \times C$ interaction: $P < 0.05$). Plasma creatinine was lower at baseline in hHFI subjects than in control subjects ($P = 0.04$), but then evolved similarly in both conditions ($T \times C$ interaction effect: $P = 0.25$; baseline effect: $P < 0.01$). Plasma urea:Cr ratio was higher at baseline and after fructose ingestion. *, hHFI and control subjects significantly different ($P < 0.05$). Cr, creatinine; hHFI, heterozygous for hereditary fructose intolerance; NEFA, nonesterified fatty acids; $T \times C$, time-by-condition; TG, triglycerides.

Postprandial plasma uric acid increased significantly above fasting concentrations in hHFI subjects but not in control subjects (Figure 3B: T effect: $P = 0.29$; $T \times C$ effect: $P = 0.03$). The average increase was +7.2% of preprandial hHFI values ($P < 0.01$) compared with -1.1% in control subjects ($P = 0.16$). Ingestion of the mixed meal decreased plasma urea concentrations only in control subjects (Figure 3A: T effect: $P < 0.01$; $T \times C$ effect: $P < 0.01$). No effect on Cr concentrations was shown (Figure 3C: T effect: $P = 0.13$; $T \times C$ effect: $P = 0.25$). However, the urea:Cr ratio was significantly higher in hHFI subjects in both plasma (Figure 3D: T effect: $P < 0.01$; $T \times C$ effect: $P < 0.01$) and urine (data not shown; $P < 0.05$). There was no statistical difference between hHFI and control subjects for postprandial plasma and urinary amino acids (Supplemental Table 2; all $T \times C$ effects: $P > 0.05$) or Cr and GAA concentrations (data not shown; all $T \times C$ effects:

$P > 0.05$). There was no significant change in urinary indexes (Supplemental Table 1).

DISCUSSION

Given the high dietary intake of fructose in many affluent countries and North American populations (38), as well as the recognition that fructose may be associated with specific adverse metabolic effects such as dyslipidemia, hepatic steatosis, and hepatic insulin resistance, we wondered whether the possibly deleterious effects of dietary fructose may be enhanced in hHFI individuals compared with the general population. To our knowledge, this study provides the first detailed assessment of postprandial responses to a mixed meal containing fructose in carriers of one mutated *ALDOB* allele. A relatively small fructose load (~25 g) was chosen to replicate levels of

fructose intake commonly observed in the general population. In addition, fructose was incorporated into a liquid mixed meal together with isocaloric amounts of glucose in order to obtain a fructose:glucose ratio comparable to that of sucrose or high fructose corn syrup (39).

We had hypothesized that, compared with control counterparts, hHFI subjects would present an increased sensitivity to fructose-induced metabolic alterations, leading to dysregulation of glucose and lipid homeostasis. Postprandial systemic GRa was selected as our primary outcome since this variable provides a reliable estimate of overall postprandial glucose homeostasis. However, GRa measured after ingestion of a fructose-containing meal was not different in hHFI and control subjects. We also monitored fructose metabolism with the use of ^{13}C -labeled fructose, and observed that fructose oxidation and nonoxidative fructose disposal were not different in hHFI and control subjects. This observation is consistent with previous reports that hHFI subjects have normal splanchnic fructose uptake unless the dose administered is very high (40, 41). We next turned our attention to markers of postprandial glucose and lipid homeostasis. There was no difference in postprandial net carbohydrate oxidation, nor in blood glucose and triglyceride concentrations. Our results therefore do not support the hypothesis that hHFI subjects may be more prone to fructose-induced dysregulation of glucose homeostasis than the general population, at least with moderate (25 g) fructose loads.

However, an analysis of exploratory outcomes suggested that hHFI subjects may have an increased sensitivity to fructose-induced hyperuricemia. Ingestion of the fructose-containing meal increased significantly plasma uric acid concentrations in hHFI subjects, but had no such effect in control subjects. Over 30 y ago, Oberhaensli et al. (29) had already reported an enhanced uric acid response to a 50-g fructose load in hHFI subjects, and had suggested that heterozygous carriers of *ALDOB* mutations may be at increased risk of gout. The uric acid concentrations we observed were markedly lower than those reported by Oberhaensli et al.; however, this is probably explained by the lower fructose dose used in our protocol. Fructose has long been known to cause an acute increase in uric acid concentration when given in large doses over a short period, i.e., with intravenous administration or large oral fructose loads (42). Under such conditions, the higher activity of fructokinase in regard to that of *ALDOB* causes a transient intracellular accumulation of fructose-1-phosphate, together with ATP depletion and purine catabolism (43). However, ingestion of mixed meals containing fructose, or lower fructose loads, does not seem to elicit such an acute increase in uric acid concentration in normal subjects (44). Our observation that hHFI subjects had a modest yet significant increase in postprandial uric acid concentrations in response to such a low fructose load suggests that their reduced levels of *ALDOB* make them more sensitive to fructose-induced transient ATP depletion.

Since renal proximal tubular cells metabolize fructose, and since acute proximal tubule dysfunction is a hallmark of acute and chronic reactions to fructose in patients with HFI, we also considered the possibility that fructose administration may acutely affect renal processes. We did not, however, observe any urinary indexes of tubular dysfunction (Fe-Na, Fe-Urea, pH, and glucose). An exploratory analysis of plasma and urine amino acid concentrations was included to search for possible indications of altered tubular amino acid reabsorption since this is

a prominent effect of fructose in *ALDOB*-deficient HFI patients. However, hHFI subjects had no increase in urine amino acid excretion after fructose loading. A careful analysis nonetheless revealed a slight increase in the baseline glycine concentration and a trend for an increase in arginine concentration, together with an increased urea:Crt ratio. This, together with low Crt, made us suspect that hHFI subjects may have some defect in Cr metabolism. Endogenous Cr is synthesized from arginine and glycine in the liver, which is converted to GAA via the successive action of arginine:glycine amidinotransferase and guanidinoacetate methyltransferase (45–47). To further investigate Cr biosynthesis, Cr and its precursor GAA were measured in plasma and urine. However, there was no significant difference between the hHFI and control groups. Furthermore, there are no reports in the literature of a defect in Cr synthesis associated with HFI. We also reassessed the data of HFI patients followed at our clinics and did not find any biochemical evidence for such a defect. We further considered the possibility that it may be explained by major differences in the diet compositions of the hHFI and control subjects. This appears unlikely, however, since all participants were on the same controlled, low-fructose diet over the week preceding the experiments. Furthermore, we recontacted all hHFI participants a posteriori, and obtained a 3-d dietary diary from 7 of them. Analysis of these diaries indicated that fructose accounted for $6.31\% \pm 2.0\%$ of their total energy intake, i.e., close to the average consumption of a normal population (48). We can therefore discard the hypothesis that the difference was because of the hHFI subjects spontaneously consuming a low-fructose diet. In the absence of additional metabolic alterations, we therefore conclude that these small alterations may be fortuitous.

Our study has some limitations which must be pointed out. First, we used a fructose-containing mixed test meal challenge instead of a large pure fructose load, and cannot discard the hypothesis that a larger load of fructose would have been required to exceed the catalytic activity of the remaining *ALDOB* in heterozygotes. This choice was, however, made in order to meet the recommendation of a recent NIH panel position paper to study the effects of fructose relevant to real nutrition, i.e., together with other macronutrients and equimolar amounts of glucose (39). Second, our study evaluated only the acute effect of a single fructose load in subjects who had been on a low-fructose diet for the previous 7 d. This design had been chosen to search for signs of altered fructose metabolism (i.e., slower fructose oxidation, uric acid production), but may not be optimal to search for increased sensitivity to the long-term metabolic effects of fructose. An experimental design including an exposure to dietary fructose over several days may be needed to document differences between hHFI and control subjects.

In conclusion, hHFI subjects had no major alteration of postprandial glucose homeostasis or of fructose metabolism after ingestion of a fructose-containing meal. They did, however, have a slight but significant increase in uric acid concentrations after ingestion of a single fructose-containing mixed meal, which suggests that their reduced *ALDOB* expression is associated with subtle metabolic changes.

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LT, LM-C, OB, CR, and NP: provided essential materials; CT, F-GD, KD, RR, OB, LM-C, and J-PDB: analyzed the data; CT, KD, F-GD, J-PDB, RR, NP, LT, LB, FB, and OB: wrote the paper; CT: had primary responsibility for final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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