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Contribution of galactose and fructose to glucose homeostasis

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Abstract

To determine the contributions of galactose and fructose to glucose formation, 6 subjects (26 ± 2 years old; body mass index, 22.4 ± 0.2 kg/m^2) (mean ± SE) were studied during fasting conditions. Three subjects received a primed constant intravenous infusion of [6,6-$^2H_2$]glucose for 3 hours followed by oral bolus ingestion of galactose labeled to 2% with [U-^{13}C]galactose (0.72 g/kg); the other 3 subjects received a primed constant intravenous infusion of [6,6-$^2H_2$]glucose followed by either a bolus ingestion of fructose alone (0.72 g/kg) (labeled to 2% with [U-^{13}C]fructose) or coingestion of fructose (labeled with [U-^{13}C]fructose) (0.72 g/kg) and unlabeled glucose (0.72 g/kg). Four hours after ingestion, subjects received 1 mg of glucagon intravenously to stimulate glycogenolysis. When galactose was ingested alone, the area under the curve (AUC) of [$^{13}C_6$]glucose and [$^{13}C_3$]glucose was 7.28 ± 0.39 and 3.52 ± 0.05 mmol/L per 4 hours, respectively. When [U-^{13}C]fructose was ingested with unlabeled fructose or unlabeled fructose plus glucose, no [$^{13}C_6$]glucose was detected in plasma. The AUC of [$^{13}C_3$]glucose after fructose and fructose plus glucose ingestion was 20.21 ± 2.41 and 6.25 ± 0.34 mmol/L per 4 hours, respectively. Comparing the AUC for the $^{13}C_3$ vs $^{13}C_6$ enrichments, 67% of oral galactose enters the systemic circulation via a direct route and 33% via an indirect route. In contrast, fructose only enters the systemic circulation via the indirect route. Finally, when ingested alone, fructose and galactose contribute little to glycogen synthesis. After the coingestion of fructose and glucose with the resultant insulin response from the glucose, fructose is a significant contributor to glycogen synthesis.

1. Background

Fructose and galactose are the predominant nonglucose carbohydrates consumed on a daily basis by children and adolescents in the form of lactose and sucrose. Galactose is a unique dietary sugar in that its exclusive source is milk lactose. In infants, who are exclusively breast-fed, galactose alone provides approximately 20% of their total caloric intake.

It has long been assumed that galactose consumption is associated with first-pass hepatic clearance, and intravenously administered labeled galactose has been used as a test of hepatic function [1,2]. After lactose consumption, systemic plasma galactose concentrations in the fed newborn and in adults increase from 0 to 25 μmol/L to 200 to 500 μmol/L [3], as compared with plasma glucose concentration, which increases from 5 to 8 mmol/L after glucose consumption. Ingestion of 0.5 g/kg of galactose alone increases the plasma glucose by approximately 1 mmol/L (ie, ~20%), whereas plasma concentrations of galactose increase to approximately 2 mmol/L [4]. Similar results were obtained by Sunehag and Haymond [5] who reported in healthy subjects peak plasma galactose concentrations of approximately 2.3 mmol/L after ingesting galactose at a rate of 33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Under these conditions, galactose becomes the nearly exclusive carbon source for glucose production, although the absolute rate of glucose production remains essentially unchanged [5].

Fructose constitutes an important source of carbohydrate intake in Western societies, with adults consuming approximately 100 g/d in the form of sucrose plus fructose [6,7]. In rats, 55% of ingested fructose is taken up by the liver on a first-pass basis. Similarly, in humans, 50% of intravenously administered fructose is taken up by the liver [8]. In the liver, fructose is phosphorylated to fructose-1-phosphate by fructokinase; aldolase B of the liver reversibly splits fructose-1-phosphate into glyceraldehyde and dihydroxyacetone-phosphate, a member of the glycolysis sequence;

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then glyceraldehyde is phosphorylated to glyceraldehyde-3-phosphate by the action of the triokinase, another intermediate of the glycolytic pathway [9]. When fructose is coingested with glucose, the absorption of fructose is thought to be enhanced, suggesting an important role of the ingested sugars in the regulation of this process [10]. The conversion of fructose to glucose has been estimated to be from 20% to 100% in humans after oral, intravenous, or intragastric administration of labeled fructose [11-16]. Recently, Gopher et al [13] challenged the traditionally held pathway by which fructose is thought to be metabolized. Using high-resolution nuclear magnetic resonance (NMR) spectroscopy, they reported a direct conversion of approximately 50% of [U-^{13}C]fructose into [U-^{13}C]glucose in healthy children after an oral dose of [U-^{13}C]fructose, suggesting a direct conversion pathway not previously recognized in humans.

The present studies were undertaken to determine (1) the contribution of galactose or fructose to glucose formation by the direct pathway (hexose→glucose) and the indirect pathway (hexose→3 carbon substrates→glucose) and (2) if the coingestion of glucose with fructose modifies the contribution of fructose to glucose formation.

2. Materials and methods

2.1. Tracers

[U-^{13}C]galactose (99 atom % ^{13}C), [U-^{13}C]fructose (99 atom % ^{13}C), [6,6-$^{2}H_2$]glucose (99 atom % ^{2}H), and [1-^{2}H] glucose (99 atom % ^{2}H) were obtained from Cambridge Isotope Laboratories (Andover, MA). The labeled compounds were tested for sterility and pyrogenicity by the company and the investigation pharmacy at Texas Children's Hospital, Houston, TX, and dissolved in 0.45% saline. The solution was subsequently filtered through a 0.2-μm Millipore (Bedford, MA) filter into sterile syringes. Sterile isotope solutions were prepared less than 48 hours before study and maintained at 4°C until used.

2.2. Study design

The protocol was approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine in Houston, TX. Informed consent was obtained from each subject.

2.2.1. Subjects

Six healthy adult volunteers were recruited (Table 1). All were free of any identified diseases as assessed by a medical history, physical examination, and normal screening laboratory studies including hemoglobin, plasma glucose, aspartate transaminase, alanine transaminase, bilirubin, lactate dehydrogenase, and a negative pregnancy test in all women with child-bearing potential. Three subjects participated in protocol A, whereas 3 additional subjects were studied on 2 occasions in protocols B and C.

Table 1
Subjects

Protocol A					
Subject	Age (y)	Sex	Weight (kg)	Height (cm)	BMI (kg/m^2)
1	24	M	71	177	22.7
2	34	F	68	171	23.1
3	29	M	68	174	22.5
	29 ± 3		69 ± 1	174 ± 2	22.7 ± 0.2

Protocols B and C					
Subject[a]	Age (y)	Sex	Weight (kg)	Height (cm)	BMI (kg/m^2)
1	26	F	55	157	22.3
2	18	M	66	175	21.6
3	30	F	61	160	23.8
	25 ± 4		61 ± 3	164 ± 6	22.6 ± 0.7

Mean ± SE. Protocols: A: galactose bolus; B: fructose bolus; C: fructose + glucose bolus.

[a] The same subjects participated in both protocols.

2.3. Protocol design

The subjects were admitted to the Metabolic Research Unit at the Children's Nutrition Research Center the evening before study. During the week before each study, the subjects consumed a diet consisting of approximately 50% carbohydrate, 15% protein, and 35% fat, as instructed by the dietician. At 5:00 PM on the evening of admission, 2 intravenous catheters were introduced in the subject's antecubital fossa or forearm vein under EMLA (AstraZeneca Pharmaceuticals, Wayne, PA) cream analgesia: one for isotope infusion and the other for blood sampling. Subjects were fed a supper meal of 10 kcal/kg (same composition as mentioned above) at 6:00 PM and were subsequently fasting, except for water ad libitum overnight.

At 6:00 AM on the day of the study (−180 minutes), a baseline blood sample (3 mL) was obtained; and the subjects received primed constant infusions of [6,6-$^{2}H_2$] glucose (10 μmol · kg^{-1}, 0.16 μmol · kg^{-1} · min^{-1}) for a total of 8 hours to measure the rate of appearance (Ra) of glucose into the plasma space (protocols A, B, and C). Blood samples were obtained at specific time points (−120, −60, −45, −30, −15, and 0 minute[s]). Beginning at zero time (9:00 AM), the subjects consumed a drink bolus (0.72 g/kg) of galactose labeled to 2% with [U-^{13}C] galactose (protocol A), a drink bolus (0.72 g/kg) of fructose labeled to 2% with [U-^{13}C]fructose (protocol B), or a single bolus of fructose labeled to 2% with [U ^{13}C]fructose (0.72 g/kg) and unlabeled glucose (0.72 g/kg) (a total of 1.44 g/kg) (protocol C). Blood samples were obtained at 15, 30, 45, 60, 80, 90, 100, 110, 120, 150, 180, 210, 220, 230, and 240 minutes. Four hours after the ingestion of the labeled carbohydrate drink and directly after the 240-minute blood sample, subjects were given an intravenous bolus (1.0 mg) of glucagon to stimulate glycogenolysis in an attempt to estimate the release of glucose from glycogen

that was labeled from the ingestion of the labeled galactose (protocol A), fructose (protocol B), or fructose with unlabeled glucose (protocol C). Additional blood samples were at 250, 260, 270, 285, and 300 minutes.

2.4. Analytical methods

2.4.1. Plasma analyses

Glucose concentrations were determined using a glucose and lactate analyzer (Model 2700 Select; Yellow Springs Instruments, Yellow Springs, OH). Galactose concentrations were determined using a glucose and lactate analyzer (Model 2300 Stat$^+$, Yellow Springs Instruments) retrofitted to read galactose. Plasma insulin concentrations were measured using commercially available radioimmunoassay kits (Linco Research, St Charles, MO).

The pentaacetate derivatives of glucose and galactose were prepared, and the glucose and galactose isotopomers were determined by gas chromatography–mass spectrometry (GCMS) as previously described [17]. The glucose was analyzed using positive chemical ionization with methane as the reactant gas with selective monitoring of *m*/*z* 331 to 337, reflecting unlabeled glucose and glucose molecules labeled with ^{13}C in 1 to 6 of its carbons.

The acetyl-pentafluorobenzyl derivative of lactate was prepared as described previously [18]. The method was modified as follows: The pentafluorobenzyl derivative was acetylated by the addition of a mole excess of 2:1 acetic anhydride/pyridine (Aldrich, Milwaukee, WI). The ^{13}C enrichments in lactate were analyzed by GCMS (HP 6890 GC coupled with HP 5973 MSD; Hewlett-Packard, Palo Alto, CA); an HP 5 column (25 m × 0.25 mm × 0.25 μm) was used. The conditions for the GCMS lactate analysis were as follows: injector, 250° (splitless injection); temperature program—initial temperature of 70°C for 1 minute, ramp at 15°C/min up to 320°C, followed by a 2-minute hold. Negative chemical ionization with methane as the reagent gas and selective monitoring of *m*/*z* 131 to 134 was performed. The coefficient of variation of the tracer analysis methods (GCMS, IR/MS, etc) in our laboratory is less than 2% and often less than 1%. [19].

2.5. Calculations

The mole ratios of M + 2 and M + 6 of glucose were calculated from the mass to charge ratios using daily standard curves of [6,6-$^2H_2$]glucose and [U-^{13}C]glucose, respectively. Furthermore, the M + 2 isotopomer of glucose was corrected for the contribution from M + 1 glucose using standard curve based on the M + 2 molar ratio obtained from the [1-^2H]glucose standard curve. The enrichment of each isotopomer of glucose (ie, M + 1, M + 2, M + 3, and M + 6) was calculated as the isotopomer divided by the sum of all the glucose isotopomers [20].

The Ra of glucose into the systemic circulation was calculated using the average enrichments of M + 2 between −45 and 0 minute(s) and at each time point throughout the study using the following equation:

$$\text{Total Ra} = \left[\left(E_i/E_p\right) - 1\right] \cdot i\left(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\right) \quad (1)$$

where Ra is the rate of appearance; E_i and E_p are the isotopic enrichments in the infusate and the plasma, respectively; and i is the rate of infusion of the tracer.

2.6. Area under the curve

These studies were designed to determine relative metabolic pathways. True steady states were not achieved to use steady-state equations. The number of subjects is small because of the tremendous cost of the isotopes. Therefore, to determine the relative contributions of galactose and fructose to glucose production, we analyzed the postingestion data as area under the curve (AUC). The AUCs were calculated using the trapezoidal method: AUC = $((C_1 + C_2)/2) \times (t_2 - t_1)$, where C_1 and C_2 represent the concentrations of the variable being calculated and t_2 and t_1 are the corresponding time points.

For each protocol, the change from baseline (ie, after subtraction of baseline values) in plasma glucose and insulin concentrations, plasma enrichments of [$^{13}C_3$]glucose and [$^{13}C_6$]glucose, and the plasma concentrations of [$^{13}C_3$]glucose and [$^{13}C_6$]glucose were calculated between 0 and 240 minutes (time of meal ingestion and absorption) and after glucagon injection, that is, between 240 and 300 minutes (after subtraction of the 240-minute values).

2.7. Direct and indirect pathways

After the ingestion of the uniformly labeled galactose or fructose, the ratio of the AUC of the plasma [$^{13}C_3$]glucose/([$^{13}C_3$]glucose + [$^{13}C_6$]glucose) enrichments or concentrations provides an estimate of the rates of entry of the labeled fructose or galactose into the plasma glucose pool via the indirect pathway. Conversely, the ratio of the AUC of the plasma [$^{13}C_6$]glucose/([$^{13}C_3$]glucose + [$^{13}C_6$]glucose) enrichments or concentrations provides an estimate of the rates of entry of the labeled fructose or galactose into the plasma glucose pool via the direct pathway.

Similarly, the relative amounts of labeled carbon from the uniformly labeled galactose and fructose that were stored in glycogen via the direct and indirect pathways were calculated using the AUC data after the glucagon infusion.

2.8. Statistics

All data are presented as mean ± SE. Data were compared using analysis of variance (ANOVA) followed by Student-Newman-Keuls test as post hoc analysis, with a level of significance of P less than .05.

3. Results

3.1. Baseline glucose, insulin, and glucose Ra

The mean (±SE) baseline (−60 to 0 minute[s]) plasma glucose concentrations were 4.8 ± 0.3, 4.4 ± 0.1, and 4.4 ± 0.4 mmol/L in protocols A, B, and C, respectively; and the baseline plasma insulin concentrations were 5.3 ± 1.2, 7.1 ± 2.1, and 6.9 ± 2.1 μU/mL, respectively. The steady-state baseline glucose Ra was 11.5 ± 0.9, 13.2 ± 1.2, and 12.3 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in protocol A, B, and C, respectively. No differences were observed among these values.

3.2. Plasma galactose concentrations

The plasma galactose concentrations were 0.07 ± 0.01, 2.57 ± 0.53, 0.73 ± 0.26, 0.16 ± 0.04, 0.10 ± 0.03, and 0.07 ± 0.01 mmol/L at 0, 1, 2, 3, 4, and 5 hours, respectively, in protocol A.

3.3. Plasma enrichments of [$^{13}C_6$]glucose and [$^{13}C_3$]glucose

The enrichment values of [$^{13}C_6$]glucose and [$^{13}C_3$]glucose for protocol A, B, and C are shown in Table 2. After ingestion of [U-$^{13}C_6$]galactose, the enrichment of both [$^{13}C_6$]glucose and [$^{13}C_3$]glucose increased. However, only the enrichment of [$^{13}C_3$]glucose increased after the ingestion of [U-$^{13}C_6$]fructose (Table 2).

Table 2
Glucose isotopomers enrichment values (%)

	[$^{13}C_6$]glucose				
Protocol	1 h	2 h	3 h	4 h	5 h
A	0.52 ± 0.05	0.82 ± 0.06	0.81 ± 0.01	0.65 ± 0.03	0.59 ± 0.03
B	0	0	0	0	0
C	0	0	0	0	0
	[$^{13}C_3$]glucose				
Protocol	1 h	2 h	3 h	4 h	5 h
A	0.36 ± 0.02	0.49 ± 0.03	0.59 ± 0.05	0.60 ± 0.01	0.51 ± 0.01
B	1.86 ± 0.03	2.38 ± 0.08	2.43 ± 0.18	1.87 ± 0.23	1.42 ± 0.29
C	0.49 ± 0.04	0.65 ± 0.01	0.82 ± 0.02	1.02 ± 0.09	1.09 ± 0.04

Mean ± SE. Protocols: A: galactose bolus; B: fructose bolus; C: fructose + glucose bolus.

3.4. AUC analysis

3.4.1. Glucose

The ΔAUCs (mean ± SE) for glucose for protocols A, B, and C were 10 ± 18, 27 ± 48, and 194 ± 113 mmol/L per 4 hours, respectively. After glucagon, the ΔAUCs for glucose were 107 ± 44, 82 ± 37, and 80 ± 40 mmol/L per 1 hour for

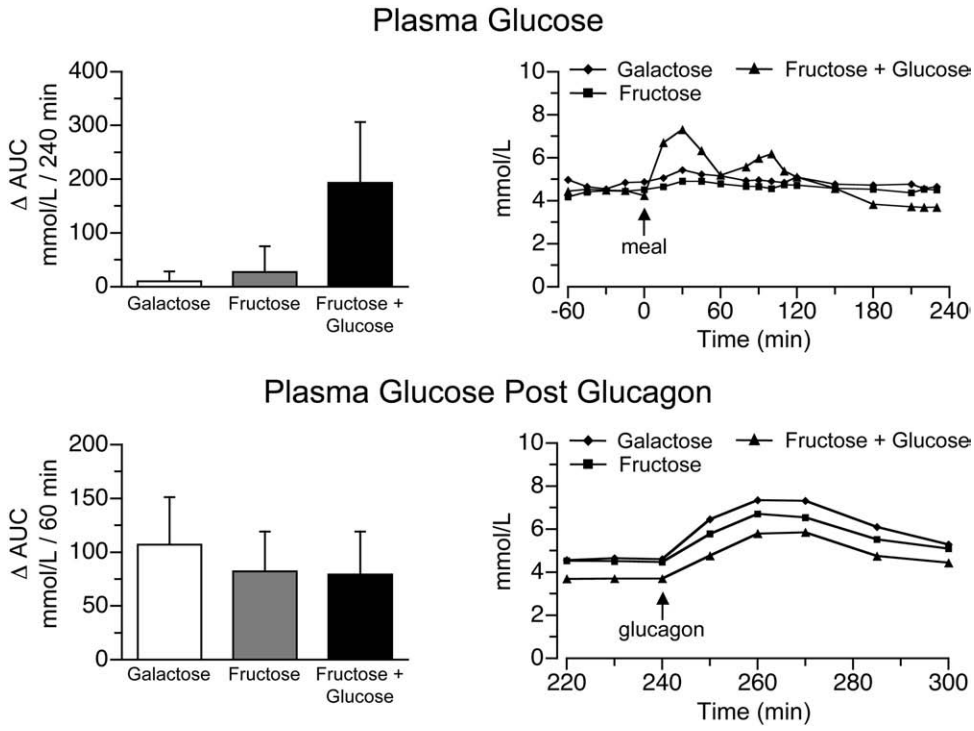

Fig. 1. Plasma glucose concentration. Left upper panel, ΔAUC for plasma glucose concentrations (mmol/L)/240 minutes (0-240 minutes after subtraction of baseline values). Left bottom panel, ΔAUC for plasma glucose concentrations (mmol/L)/60 minutes after the glucagon infusion (240-300 minutes after subtraction of the preglucagon 240-minute values). Right upper panel, Average values of the plasma glucose concentration (mmol/L), 0 minute represents the time of the drink ingestion. Right bottom panel, Average values of the plasma glucose concentration after the glucagon infusion (mmol/L) (240-300 minutes); 240 minutes represents the time of the glucagon administration. Values represent mean ± SE for n = 3.

protocols A, B, and C, respectively. No difference was observed among the groups for either value (Fig. 1).

3.4.2. Insulin

The ΔAUCs for insulin for protocols A, B, and C were 1.37 ± 0.34, 1.03 ± 0.40, and 6.28 ± 0.30 mU/mL per 4 hours. No difference was observed between the galactose alone and fructose alone, but fructose + glucose was greater ($P < .05$) than either of the other two. After glucagon, the ΔAUCs for insulin were similar among the 3 groups (1.02 ± 0.21, 1.52 ± 0.63, and 1.13 ± 0.54 mU/mL per 1 hour for protocols A, B, and C, respectively; not significant) (Fig. 2).

3.4.3. Glucose Ra

The ΔAUCs for glucose Ra for protocols A, B, and C were −0.11 ± 0.09, −0.37 ± 0.18, and 1.74 ± 0.39 mmol · kg^{-1} per 4 hours. No difference was observed between the galactose alone and fructose alone, but fructose + glucose was greater ($P < .05$) than either of the other two. After glucagon, the ΔAUCs for glucose Ra were not different among the groups (0.16 ± 0.07, 0.17 ± 0.02, and 0.06 ± 0.02 mmol · kg^{-1} per 1 hour for protocols A, B, and C, respectively) (Fig. 3).

3.4.4. [$^{13}C_6$]glucose

The ΔAUC for the plasma concentration of [$^{13}C_6$]glucose after the bolus ingestion of [$^{13}C_6$]galactose was 7.28 ± 0.39 mmol/L per 4 hours, whereas that after [$^{13}C_6$]fructose was 0 mmol/L per 4 hours whether glucose was included in the meal or not (Fig. 4). After glucagon, the ΔAUC for plasma concentrations of [$^{13}C_6$]glucose increased in 2 of the 3 subjects after galactose bolus ingestion, with a mean value of 0.72 ± 0.44 mmol/L per 1 hour; but in 1 subject, no change was observed. No [$^{13}C_6$]glucose was observed following ingestion of [$^{13}C_6$]fructose, with or without glucose. The AUC values for the plasma enrichments of [$^{13}C_6$]glucose are included in Table 3.

3.4.5. [$^{13}C_3$]glucose

The ΔAUC for the plasma concentration of [$^{13}C_3$]glucose after the bolus ingestion of galactose was 3.52 ± 0.05 mmol/L per 4 hours (Fig. 5), whereas that after fructose alone (protocol B) or fructose plus glucose (protocol C) was 20.21 ± 2.41 and 6.25 ± 0.34 mmol/L per 4 hours, respectively ($P < .05$). Differences ($P < .05$) existed for the fructose bolus group when compared with the others. Over the 1 hour after glucagon, the ΔAUCs for the plasma concentration of [$^{13}C_3$]glucose were 0.35 ± 0.21,

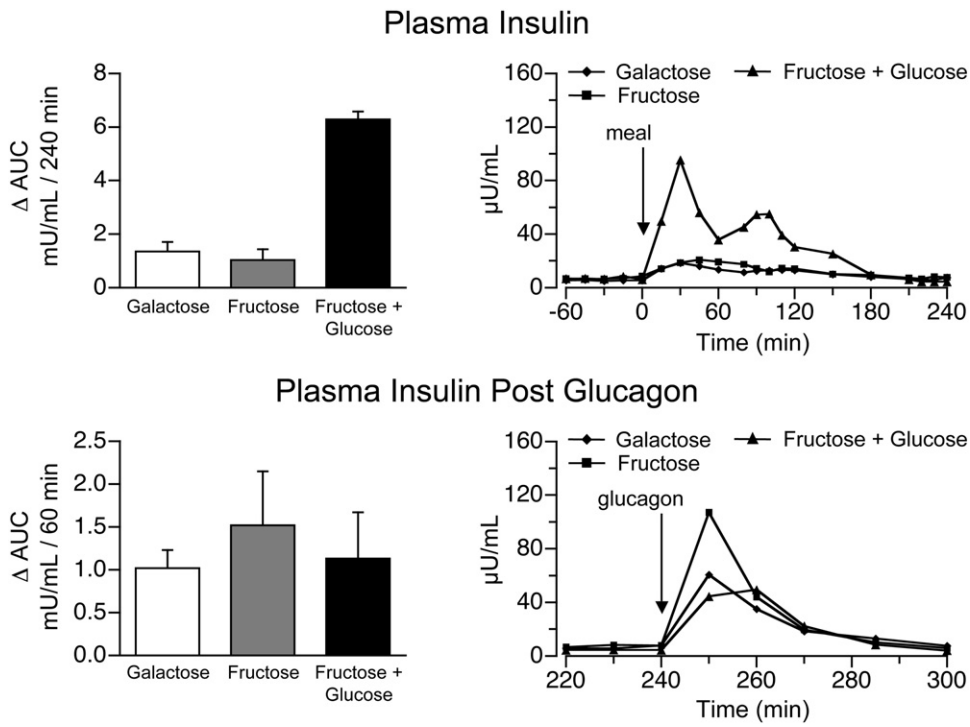

Fig. 2. Plasma insulin concentration. Left upper panel, ΔAUC for plasma insulin concentrations (mU/mL)/240 minutes (0-240 minutes after subtraction of baseline values). ¶P less than .05, fructose + glucose vs galactose and fructose bolus by ANOVA. Left bottom panel, ΔAUC for plasma insulin concentrations (mU/mL)/60 minutes after the glucagon infusion (240-300 minutes after subtraction of the preglucagon 240-minute value). Right upper panel, Average values of the plasma insulin concentration (μU/mL); 0 minute represents the time of the drink ingestion. Right bottom panel, Average values of the plasma insulin concentration after the glucagon infusion (μU/mL) (240-300 minutes); 240 minutes represents the time of the glucagon administration. Values represent mean ± SE for n = 3.

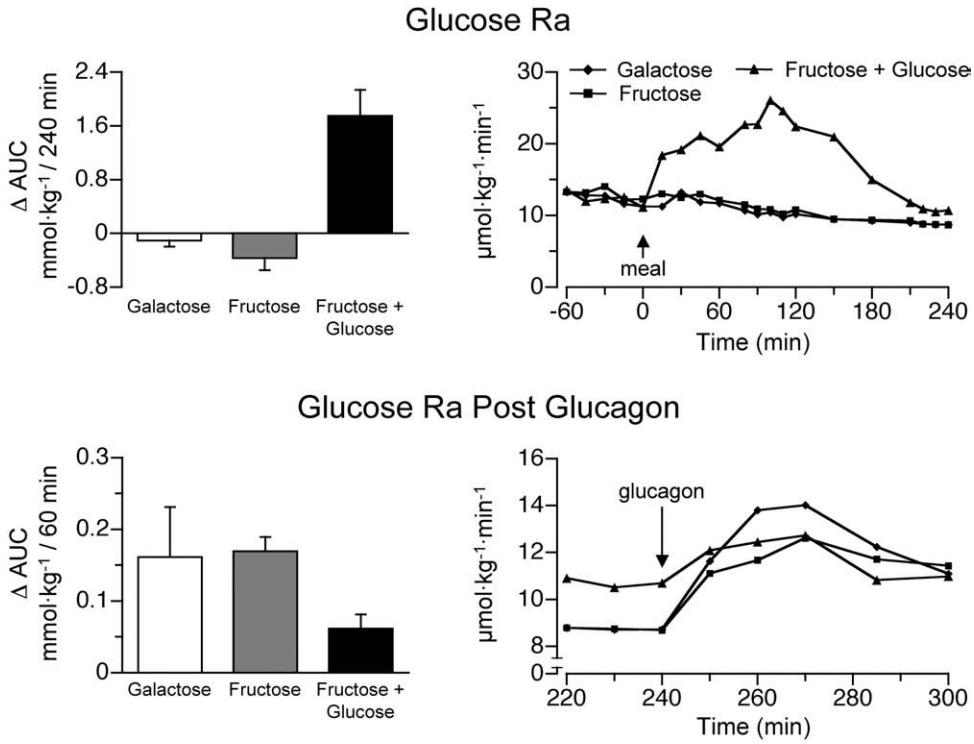

Fig. 3. Glucose Ra. Left upper panel, ΔAUC for glucose Ra (mmol · kg^{-1})/240 minutes (0-240 minutes after subtraction of baseline values). ¶*P* less than .05, fructose + glucose vs galactose and fructose bolus by ANOVA. Left bottom panel, ΔAUC for glucose Ra (mmol · kg^{-1})/60 minutes after the glucagon infusion (240-300 minutes after subtraction of the preglucagon 240-minute value). Right upper panel, Average values of the glucose Ra (μmol · kg^{-1} · min^{-1}); 0 minute represents the time of the drink ingestion. Right bottom panel, Average values of glucose Ra (μmol · kg^{-1} · min^{-1}) after the glucagon infusion (240-300 minutes); 240 minutes represents the time of the glucagon administration. Values represent mean ± SE for n = 3.

0.39 ± 0.40, and 1.18 ± 0.56 mmol/L per 1 hour for protocols A, B, and C, respectively.

There were no significant differences among the groups. The AUC values for the plasma enrichments of [$^{13}C_3$] glucose are included in Table 3.

3.5. Direct and indirect pathways

The fraction of galactose entering the glucose pool by the direct pathway ($^{13}C_6/^{13}C_6 + {}^{13}C_3$) was 0.67, and that by the indirect pathway ($^{13}C_3/^{13}C_6 + {}^{13}C_3$) was 0.33. Conversely, all of the labeled fructose entered the plasma as $^{13}C_3$ regardless of the coingestion of glucose.

4. Discussion

We previously demonstrated that, during ingestion of galactose at 33 μmol · kg^{-1} · min^{-1}, the splanchnic uptake of galactose was saturable at about 15 μmol · kg^{-1} · min^{-1} and glucose production was derived almost exclusively from the conversion of galactose to glucose without increase in the absolute rate of glucose production. In that study, only [1-^{13}C]galactose was used; and therefore, we could not distinguish the contributions of galactose to glucose formation via the direct and indirect pathways, respectively. The use of [U-^{13}C]galactose in the current study enabled us to quantify both pathways. The results of study A (galactose alone) confirm our previous finding that the majority of ingested galactose is converted to glucose without increase in the rate of glucose production, indicating a compensatory reduction in glucose production from other substrates.

No enrichment of [$^{13}C_6$]glucose was observed in plasma after ingestion of [U-^{13}C]fructose (essentially zero enrichment of glucose isotopomers M + 6) (Fig. 4). Thus, the present study clearly demonstrates that no direct conversion of fructose into glucose (fructose-1-phosphate to fructose-1,6-bisphosphate) occurs. Only M + 3 glucose was observed in both studies using [U-^{13}C]fructose (ingestion of fructose alone and fructose + glucose, respectively) (Fig. 5). In addition, the ΔAUC for [$^{13}C_3$]glucose was significantly higher during ingestion of fructose alone (Fig. 5) compared with ingestion of fructose plus glucose. It was not possible to determine how much of the label was derived from direct conversion of the triose phosphates to glucose vs via lactate and pyruvate because the fructose isotopomers (ie, M + 3 fructose) were too low to be accurately measured by GCMS.

Gopher et al [13] addressed the metabolic pathways of fructose conversion to glucose using ^{13}C NMR measurement of plasma [^{13}C]glucose isotopomers. Eight control infants and 3 children with hereditary fructose intolerance received a constant nasogastric infusion of D-[U-^{13}C]fructose. The investigators reported that direct conversion of fructose by

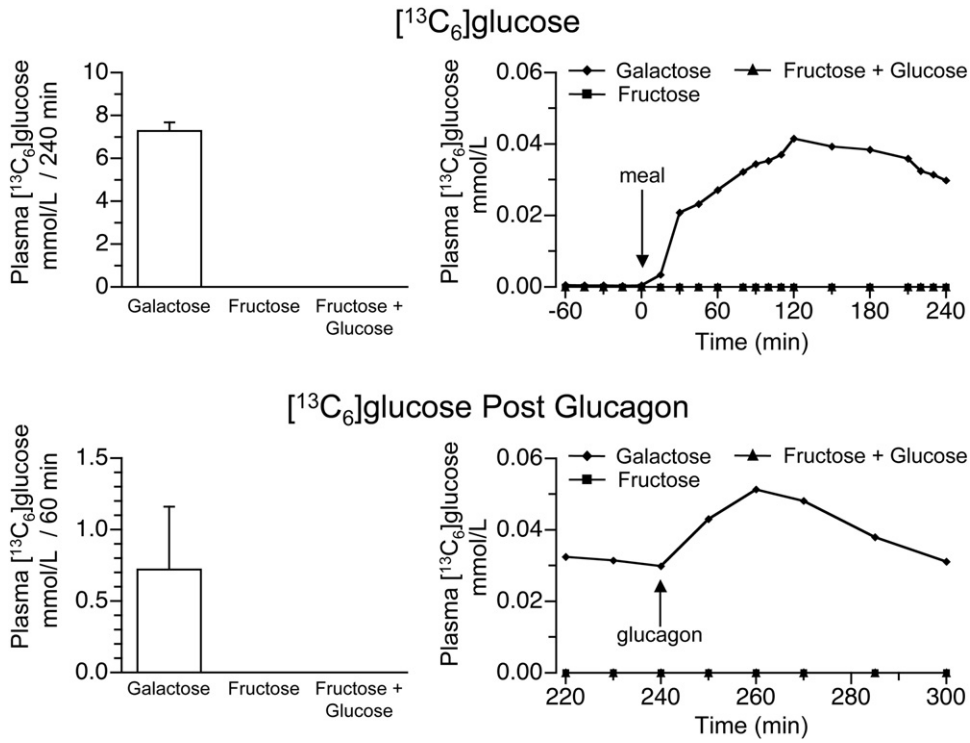

Fig. 4. [$^{13}C_6$]glucose. Left upper panel, [$^{13}C_6$]glucose (mmol/L)/240 minutes (0-240 minutes). Left bottom panel, ΔAUC for plasma [$^{13}C_6$]glucose (mmol/L)/60 minutes after the glucagon infusion (240-300 minutes after subtraction of preglucagon 240-minute value). Right upper panel, Average values of [$^{13}C_6$]glucose (mmol/L); 0 minute represents the time of the meal ingestion. Right bottom panel, Average values of [$^{13}C_6$]glucose (mmol/L) after the glucagon infusion (240-300 minutes); 240 minutes represents the time of the glucagon administration. Values represent mean ± SE for n = 3.

fructose-1-phosphate aldolase activity accounted for approximately 50% and approximately 30% of the total amount of hepatic fructose conversion to glucose in the control infants and children with hereditary fructose intolerance, respectively. These authors assumed that the probability of D-[U-^{13}C]glucose forming as a result of recombination of 2 molecules of [U-^{13}C]triose phosphates was negligible [13]. These results imply a direct pathway converting fructose-1-phosphate to fructose-1,6-bisphosphate. The enzymatic activity in the liver needed to accomplish that conversion has not been reported. Finally, these authors did not provide precision or accuracy data regarding their NMR method.

Table 3
AUC values for plasma enrichments of [$^{13}C_6$]glucose and [$^{13}C_3$]glucose

[$^{13}C_6$]glucose		
Protocol	Δ0-240 min	Δ240-300 min
A	1.48 ± 0.05	0.007 ± 0.02
B	0	0
C	0	0
[$^{13}C_3$]glucose		
Protocol	Δ0-240 min	Δ240-300 min
A	0.92 ± 0.16	−0.044 ± 0.003
B	4.52 ± 0.11*	−0.17 ± 0.05*
C	1.45 ± 0.04†	0.08 ± 0.03†

Mean ± SE. Protocols: A: galactose bolus; B: fructose bolus; C: fructose + glucose bolus.

* $P < .05$ vs galactose and fructose bolus.

† $P < .05$ vs galactose bolus.

Chandramouli et al [11] reexamined the pathway of fructose conversion to glucose in healthy adults under similar experimental conditions as Gopher et al [13]. Six healthy adult subjects received [1-^{14}C]lactate intravenously and unlabeled fructose with [6-^{14}C]sorbitol or [6-^{14}C]fructose either intravenously or intragastrically after an overnight fast. The authors measured the distribution of ^{14}C in blood glucose and calculated the ratio of ^{14}C in C1 (carbon 1 of glucose) to C6 and in C3 to C4. The results showed that, on average, 94.9% of the fructose converted to glucose underwent cleavage of the carbon skeleton of the fructose with only minimal direct conversion to glucose. Thus, both these indirect results and our direct results would dispute those of Gopher et al [13], thus supporting the main pathway of fructose to glucose conversion by an initial phosphorylation of fructose to fructose-1-phosphate, which enters the triose phosphates before ending up in glucose-6-phosphate and finally glucose.

Ingested fructose is known to be converted to glucose-6-phosphate and result in glycogen storage in the liver [21,22]. Several studies have investigated the effect of fructose ingestion on hepatic glucose metabolism in healthy human subjects [15,16,23]. Nuttall et al [15] demonstrated that, in normal male volunteers receiving intravenous infusion of [3-^{3}H]glucose plus a meal consisting of 50 g fructose, only

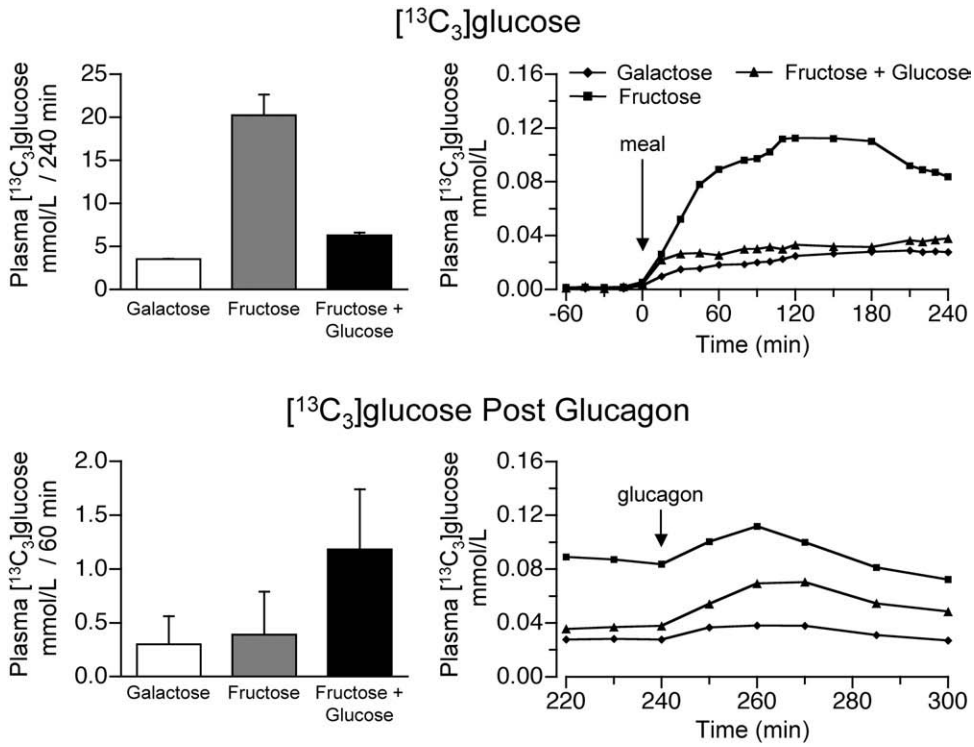

Fig. 5. [$^{13}C_3$]glucose. Left upper panel, [$^{13}C_3$]glucose (mmol/L)/240 minutes (0-240 minutes). ¶*P* less than .05, fructose alone vs galactose or fructose + glucose by ANOVA. Left bottom panel, ΔAUC for plasma [$^{13}C_3$]glucose (mmol/L)/60 minutes after the glucagon (240-300 minutes after subtraction of the preglucagon 240-minute value). Right upper panel, Average values of [$^{13}C_3$]glucose (mmol/L); 0 minute represents the time of the meal ingestion. Right bottom panel, Average values of [$^{13}C_3$]glucose (mmol/L) after the glucagon injection (240-300 minutes); 240 minutes represents the time of the glucagon administration. Values represent mean ± SE for n = 3.

20% of the ingested fructose could be accounted for as glucose appearing in the circulation. Presumably, the remaining fructose was stored as glycogen. Tounian et al [16] reported in healthy subjects infused with [1-^{13}C]fructose for 3 hours under a pancreatic clamp that approximately 67% of the fructose infused underwent nonoxidative disposal (fructose infusion rate minus fructose oxidation rate) and concluded that this value should represent the actual amount of glycogen synthesized from fructose.

Using the change in [$^{13}C_3$]glucose and [$^{13}C_6$]glucose plasma concentrations after the glucagon-stimulated release of hepatic glycogen as an indicator of uptake of labeled fructose ± glucose, our results demonstrate that a greater fraction of fructose was converted to glycogen when fructose was coingested with glucose as compared with fructose ingested alone. This indicates that, during the ingestion of fructose plus glucose, fructose carbons labeled with ^{13}C were deposited as glycogen in the liver and then released after the stimulation with glucagon. We believe this to be due to the glucose-mediated increase in plasma insulin and insulin effect on increasing hepatic glycogen content. In contrast, ingestion of fructose or galactose boluses without coingestion of glucose yielded negative values of the AUC of the plasma enrichment of [$^{13}C_3$]glucose. These results reflect release of a higher number of unlabeled glucose carbons in proportion to labeled carbons derived from labeled fructose or galactose, respectively, indicating that a smaller amount of labeled ^{13}C carbons was coming from liver glycogen.

We are aware that the small sample size limits the power of the analysis. Nonetheless, we believe that our data allow us to answer the questions outlined in the study aims. Secondly, given that values of several variables measured have not returned to baseline conditions, we believe that the interpretation of the data is still valid.

The present data demonstrate that, in healthy human subjects, (1) the majority of ingested galactose is converted to glucose, approximately 65% of which is derived via the direct pathway and approximately 35% via the indirect pathway; (2) when fructose or fructose plus glucose are given as an oral bolus, there is no direct conversion of fructose to glucose; and (3) a greater fraction of fructose carbon is converted to glycogen when fructose is coingested with glucose as compared with fructose or galactose alone.

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