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The contribution of naturally labelled ^{13}C fructose to glucose appearance in humans

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Summary. Among monosaccharides, fructose has a small hyperglycaemic effect. In order to better explain the mechanisms which cause this metabolic property, we used tracers labelled with stable isotopes (deuterated glucose and naturally ^{13}C labelled fructose) to quantify the overall glucose appearance, the rate of appearance in plasma of the ^{13}C glucose synthesized from fructose, and the fructose oxidation in vivo in man during a 6-h period following ingestion of 0.5 and 1 g · kg^{-1} fructose. Fructose had a very small effect on overall glucose appearance (NS). During the 6 h of the study, it was found that the overall glucose appearance was 0.87 ± 0.06 and 0.89 ± 0.06 g · kg^{-1} (NS). The amount of glucose synthesized from fructose was 0.27 ± 0.04 and 0.51 ± 0.03 g · kg^{-1} ($p < 0.01$) representing 31 % and 57 % of overall glucose appearance ($p < 0.01$); the non-fructose glucose production was 0.60 ± 0.02 and 0.38 ± 0.03 g · kg^{-1} ($p < 0.05$) after the 0.5 and 1 g · kg^{-1} load, respectively. Fructose oxidation was 0.28 ± 0.03 and 0.59 ± 0.07 g · kg^{-1} after the 0.5 and 1 g · kg^{-1} load respectively ($p < 0.01$) representing 56 % and 59 % of the fructose loads (NS). These data show that the low hyperglycaemic effect of fructose is explained by its very small effect on overall glucose appearance and that fructose has a sparing effect on glucose metabolism.

Key words: Fructose, glucose, stable isotopes, [^{13}C], mass spectrometry, nutrition, human.

Fructose is a naturally occurring keto sugar found in its free form in honey, fruit and vegetables and in its combined form as one-half of the disaccharide, sucrose. Although intake of the natural sources of fructose has remained reasonably stable, fructose consumption has increased over the last 20 years as many manufacturers now use high-fructose corn syrups in place of sucrose and glucose to sweeten processed foods and beverages. As reported by the United States Department of Agriculture, fructose consumption from corn sweeteners, honey and as a component of refined sugars amounts to about 70 g per capita per day in the United States [1]. Fructose is metabolized mainly in the liver and kidney in man through a specific metabolic pathway [2]. End-products of fructose metabolism in hepatocytes are glucose, glycogen, lactate, triglycerides and CO_2. Despite the fact that glucose is the main end-product of fructose metabolism in the liver, the glycaemic index following acute ingestion of an oral fructose load is low compared to glucose and saccharose [3, 4]. The mechanisms to explain such a low hyperglycaemic effect have not yet been studied in detail. It has been shown that the low hyperglycaemic effect observed after intravenous fructose administration could be related to its very small effect on total hepatic production [5]. This very small effect is in contrast with the known important metabolic conversion of fructose into glucose in the liver as demonstrated in vitro [2]. It has previously been shown that the use of two tracers of glucose labelled with stable isotopes, one to trace the total glucose production in plasma, and the other to trace the appearance in plasma of glucose from exogenous origin [6], was a useful technique with which to explain the mechanisms sustaining the hyperglycaemic effect of an oral glucose load [7]. In the present work we used the same methodology to study the metabolic fate of oral fructose in vivo in man, fructose being naturally labelled with ^{13}C and overall glucose metabolism being traced with deuterated glucose.

Subjects and methods

Subjects

Six healthy human volunteers (three women, three men; 23.7 ± 4.7 years old) were studied. All had normal body weight (59.8 ± 4 kg; body mass index 20.8 ± 2.53 kg/m^2) (mean ± SD) and none had a personal or family history of diabetes or had taken any drug during the previous 15 days. They were asked to continue their

normal diet but to omit the intake of alcohol and food naturally enriched in ^{13}C as previously described [8]; fructose intake was limited to about 20 g a day for 7 days before the test. The last evening meal was standardized to contain at least 125 g of carbohydrates. Each subject gave written consent to the study after being informed about its nature, purpose and possible risks. The scientific protocol was reviewed and accepted by the Ethical Committee of "Hospices Civils de Lyon".

Materials

Naturally ^{13}C labelled fructose (pure maize fructose), was obtained from Roquette Frères (Lestrem, France). D-[6, 6-$^2H_2$] glucose (99 mol % excess) was obtained from Commissariat à l'Energie Atomique (Gif-sur-Yvette, France); chemical and isotopic purity was confirmed by gas chromatography selected ion monitoring mass spectrometry analysis. It was dissolved in sterile isotonic saline (0.9% NaCl) and passed through a 0.22 µm Millipore filter (Millipore Corp., Bedford, Mass., USA) before infusion. The preparation was pyrogen free. The actual concentration of deuterated glucose in the infusate was determined at the end of each test.

Protocol

All tests were performed between 07.00 and 08.00 hours in the post-absorptive state 12 h after the last evening meal, after at least 30 min bed rest. Intravenous catheters were inserted into deep forearm veins in both arms for tracer infusion on one side, and blood sampling on the other side. A primed-continuous infusion of D-[6, 6-$^2H_2$] glucose (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was started 180 min before the fructose ingestion and was maintained during the next 6 h in order to determine the total rate of glucose appearance (R_aT). The priming dose was 80 times the infusion rate over 1 min. In a random order 1 to 3 weeks apart each subject absorbed a load of either 0.5 $g \cdot kg^{-1}$ or 1 $g \cdot kg^{-1}$ naturally ^{13}C-enriched fructose from maize diluted in 150 and 300 ml water, respectively. The fructose load was ingested over 5 min (time 0). Blood samples were taken sequentially over the 6 h following ingestion and were used for determining glucose, fructose, lactate, triglycerides and insulin concentrations in plasma, and for measuring plasma glucose enrichment in deuterium and ^{13}C. Indirect calorimetry (Deltatrac Metabolic Monitor; Datex Corporation, Helsinki, Finland) was performed in the basal state, then continuously during the first 3 h and for 30 min every hour during the next 3 h in order to measure VCO_2. Samples of expired gas were collected into a 5 litre-rubber balloon, then immediately transferred into two 50 ml glass containers under vacuum.

Analytical procedures

Glucose was determined on plasma by using the glucose-oxidase method (Beckman Glucose Analyser 2; Beckman Instruments, Gagny, France). Fructose and lactate were determined on plasma collected on ice-cold perchloric acid (6% volume/volume, v/v) by using standard enzymatic methods [9]. Plasma non-esterified fatty acids (NEFA) were determined by enzymatic methods [10]. Plasma triglycerides were assayed using an automated enzymatic method (Dimension Analyser, Dupond de Nemours, Les Ulis, France). Plasma immunoreactive insulin was determined by RIA [11].

The ^{13}C isotopic enrichment of absorbed maize fructose was determined by using an isotope ratio mass spectrometer (SIRA 10; Vacuum Generator Isogas, Middlewich, UK) after its transformation in CO_2 by complete oxidation in a furnace at around 1000 °C in the presence of O_2. The ^{13}C enrichment of the fructose ingested was –11.25 ∂‰ ^{13}C (1.09887 atom % ^{13}C).

In order to measure the isotopic enrichment of plasma glucose, the samples were deproteinized with ice-cold perchloric acid (6% v/v) before neutralization with K_2CO_3, and partially purified by sequential anion-cation exchange chromatography. The isotopic enrichment in deuterium was measured on the acetyl-bis-butane-boronyl derivative of glucose by using the method of Bier et al. [12] as previously described [13]. Briefly, the gas chromatography was performed by using a gas chromatograph oven (Fractovap 4160; Carlo Erba, Massy, France) coupled with a mass spectrometer (Quadrupole R 10–10 NERMAG, Rueil-Malmaison, France) as previously described [7]. Isotopic enrichment in ^{13}C was measured on the pentacetate derivative of glucose by using the recently developed gas chromatograph isotope ratio mass spectrometer Isochrom I (Vacuum Generator Isogas) as previously described [7]. The procedure of the chromatography which was used allowed a clear separation of fructose from glucose (data not shown). Owing to the low concentration of fructose in most of the samples, the analysis of ^{13}C isotopic enrichment in plasma fructose was unsuccessful. Breath CO_2 was purified by cryogenic distillation as described by Schoeller et al. [14], and $^{13}C/^{12}C$ ratio was measured with an isotope ratio mass spectrometer (SIRA 12; Vacuum Generator Isogas) and expressed as ∂ ^{13}C‰ as previously described [8].

Calculations

Calculation of the rate of total glucose appearance. The total rate of appearance of glucose in plasma (R_aT) was calculated from the enrichment of blood glucose in deuterium, by using the non-steady-state equation of Steele [15] as modified by DeBodo [16].

$$R_aT = \frac{F - pV\,[(C_1 + C_2)/2] \times [EI_2 - EI_1)/(t_2 - t_1)]}{(EI_1 + EI_2)/2} - F$$

where R_aT ($mg \cdot kg^{-1} \cdot min^{-1}$) is the rate of total glucose appearance
F ($mg \cdot kg^{-1} \cdot min^{-1}$) is the deuterated glucose infusion rate
p is the pool fraction = 0.75
V ($l \cdot kg^{-1}$) is the glucose distribution volume = 0.2
C_1 and C_2 (mg/l) are the glycaemia at time 1 and time 2
EI_1 and EI_2 (mol % excess) are the isotopic enrichments of plasma glucose at time 1 and time 2
t_1 and t_2 (min): time 1 and time 2

Calculation of the rate of appearance of glucose synthesized from fructose. It has been shown that the rate of exogenous glucose appearance following an oral glucose load could be calculated by using the transposition of Steele's equation proposed by Proietto et al. [7, 17]. This "classic" approach is valid when ^{13}C enriched glucose is ingested because the isotopic enrichment in ^{13}C of plasma glucose is known. In the present experimental design, the calculation of the rate of appearance of glucose synthesized from fructose (R_aE) was potentially arguable because the determination of the isotopic enrichment in ^{13}C of plasma fructose was unsuccessful (see analytical procedures). Therefore two approaches were used to calculate R_aE. The first method consisted in using the same method as used for an oral glucose load, knowing that the isotopic enrichment in carbons of glucose synthesized from fructose could have been slightly diluted in the liver by the carbons generated by gluconeogenesis from sources other than fructose. Thus, the rate of appearance of glucose synthesized from fructose could have been slightly overestimated with this method of calculation. Owing to the uncertainty about the isotopic enrichment of plasma fructose, we used a second method to calculate R_aE. The principle of this second method of calculation is to consider the D-[6, 6-$^2H_2$] glucose as the tracer of ^{13}C glucose appearance in the same way as it is usually used to calculate the rate of ^{12}C glucose appearance. This method was previously proposed by Abumrad et al. [18] for the calculation of the rate of appearance of an oral glucose load labelled with radioactive tracers. The two methods of calculation of the rate of appearance of glucose synthesized from fructose gave similar values for the total amount of glucose synthesized from fructose over the 6 h of the study. The results expressed in this paper are those obtained with the second method of calculation. The equations used are described below:

Table 1. Metabolites and hormone levels after ingestion of an oral fructose load

Load 0.5 g·kg^{-1}						
Time (min)	Glucose (mmol/l)	Fructose (mmol/l)	Lactate (mmol/l)	NEFA (μmol/l)	Triglycerides (mmol/l)	Insulin (mU/l)
0	4.6 ± 0.19	0.04 ± 0.03	1 ± 0.20	305 ± 44	0.88 ± 0.14	9.3 ± 0.45
30	4.9 ± 0.20[a]	0.32 ± 0.11[a]	1.59 ± 0.12[a]	179 ± 23[a]	0.90 ± 0.13	15.7 ± 1.57[a]
60	4.8 ± 0.14[a]	0.29 ± 0.03[b,c]	1.54 ± 0.19[a]	98 ± 14[b]	0.86 ± 0.15	13.3 ± 1.02[a]
90	4.5 ± 0.14	0.11 ± 0.03[a,d]	1.05 ± 0.14	149 ± 54[b]	0.87 ± 0.15	10.3 ± 0.87
120	4.4 ± 0.12	0.06 ± 0.04[d]	0.84 ± 0.11	153 ± 32[a]	0.92 ± 0.16	9 ± 0.83
150	4.5 ± 0.09	0.05 ± 0.03[d]	0.64 ± 0.05	262 ± 61	0.97 ± 0.17	8.8 ± 0.98
180	4.4 ± 0.10	0.02 ± 0.02[c]	0.62 ± 0.08	326 ± 61	1.02 ± 0.18[a]	7.8 ± 0.79
240	4.5 ± 0.09	0.02 ± 0.02	0.51 ± 0.05	438 ± 32	1.14 ± 0.20[a]	8.4 ± 0.83
300	4.4 ± 0.11	0.02 ± 0.02	0.50 ± 0.06	581 ± 26[b]	1.25 ± 0.21[a]	8 ± 0.87
360	4.6 ± 0.14	0.02 ± 0.02	0.52 ± 0.09	638 ± 27[b]	1.28 ± 0.23[a]	8.8 ± 0.91
Load 1 g·kg^{-1}						
Time (min)	Glucose (mmol/l)	Fructose (mmol/l)	Lactate (mmol/l)	NEFA (μmol/l)	Triglycerides (mmol/l)	Insulin (mU/l)
0	4.6 ± 0.14	0	0.63 ± 0.08	416 ± 43	0.86 ± 0.14	6.5 ± 1.97
30	5 ± 0.13[a]	0.41 ± 0.07[b]	1.48 ± 0.29[a]	240 ± 23[a]	0.90 ± 0.14	16 ± 1.51[b]
60	5 ± 0.24[a]	0.44 ± 0.07[b]	1.90 ± 0.31[b]	119 ± 28[b]	0.90 ± 0.14	14 ± 2[b]
90	4.7 ± 0.19	0.25 ± 0.07[b]	1.06 ± 0.22[b]	74 ± 12[b]	0.86 ± 0.13	13 ± 1.81[a]
120	4.5 ± 0.19	0.22 ± 0.07[b]	0.94 ± 0.14[b]	101 ± 17[b]	0.92 ± 0.14	10 ± 2.04
150	4.6 ± 0.18	0.20 ± 0.06[b]	0.73 ± 0.12[a]	148 ± 29[a]	0.92 ± 0.15	9.4 ± 1.81
180	4.6 ± 0.11	0.12 ± 0.02[a]	0.66 ± 0.10	257 ± 50[a]	1.04 ± 0.12	8.4 ± 1.78
240	4.5 ± 0.06	0.10 ± 0.04	0.55 ± 0.09	426 ± 48	1.18 ± 0.12	6.6 ± 1.36
300	4.5 ± 0.11	0.05 ± 0.02	0.50 ± 0.08	525 ± 67	1.19 ± 0.14[a]	6.8 ± 1.44
360	4.6 ± 0.08	0.07 ± 0.03	0.52 ± 0.09	641 ± 69	1.27 ± 0.16[b]	6.7 ± 1.93

[a] $p < 0.05$, [b] $p < 0.01$ significant difference vs values at time 0 min;
[c] $p < 0.05$, [d] $p < 0.01$ significant difference 0.5 vs 1 g·kg^{-1}
Values are given as mean ± SEM, $n = 6$

First method

$$R_aE = \frac{[R_aT \times (APE_2 + APE_1)/2] + [pV\,(C1 + C2)/2 \times (APE_2 - APE_1)/(t_2 - t_1)]}{APE\ Fruct_{ing}}$$

R_aT (mg·kg^{-1}·min^{-1}) is the rate of total glucose appearance
APE_2 and APE_1 (atom % excess) correspond to the ^{13}C APE of plasma glucose at time 2 and time 1. APE = $AP_S - AP_B$ where AP_S is the AP of the sample and AP_B is the AP of the plasma glucose in the basal state (before fructose ingestion).

$$AP = \frac{100\,R \times (0.001 \times \partial^{13}C_S + 1)}{1 + (R \times 0.001 \times \partial^{13}C_S + 1)}$$

where R is the $^{13}C/^{12}C$ of international Pee Dee Belemnite Limestone standard (R = 0.0112372) and $\partial^{13}C_S$ the $\partial^{13}C‰$ value of the sample. AP was determined on the pentacetate derivative of glucose.
$APE\ Fruct_{ing}$ corresponds to the ^{13}C APE of the ingested fructose

Second method
The first step **(equation 1)** of the calculation of R_aE using this method was to calculate the rate of appearance in plasma of ^{13}C uniformly enriched glucose coming from fructose (designated X). The Steele equation [15] modified by De Bodo [16] was used considering that deuterated glucose was the tracer and ^{13}C glucose coming from fructose was the tracee, in the same way that deuterated glucose is usually used to calculate the rate of appearance of ^{12}C glucose. The equation was adapted as following: the glucose pool was calculated from the sum of deuterated glucose molecules and ^{13}C glucose molecules.

X (μmol·kg^{-1}·min^{-1}): rate of appearance in plasma of ^{13}C enriched glucose (considering this glucose to be uniformly labelled with ^{13}C coming from fructose, without correction for isotopic enrichment of ingested fructose.
F (μmol·kg^{-1}·min^{-1}): deuterated glucose infusion rate
p (pool fraction) = 0.75
V: glucose distribution volume = 0.2 l·kg^{-1}
C_1 and C_2 (mg/l): plasma glucose concentration at time 1 and time 2
IE $^{13}C_1$ and IE $^{13}C_2$ (mol % excess): isotopic enrichment in ^{13}C of plasma glucose at time 1 and time 2 over isotopic enrichment at time 0; IE at times 0, 1 and 2 were calculated from $\partial\,^{13}C‰$ converted to mol % excess using a standard curve of pure beet glucose enriched with various amounts of U-^{13}C glucose (99.1 % enriched, Tracer Technology, Somerville, Mass., USA)
IE D_1 and IE D_2 (mol % excess): isotopic enrichment in deuterium of plasma glucose at time 1 and time 2
IE $D_2/^{13}C_2$ and IE $D_1/^{13}C_1$ (mol % excess): isotopic enrichment of deuterated glucose relative to ^{13}C glucose calculated as MR/(1 + MR) with MR equal to the molar ratio of deuterated glucose to ^{13}C glucose.
t_1 and t_2 (min): time 1 and time 2
The second step **(equation 2),** calculates the rate of appearance of ^{13}C in the glucose molecule which comes from ingested fructose. It is obtained from the ratio of the rate of appearance of ^{13}C – obtained by multiplying X by 6 (6 carbon atoms in the glucose molecule) and then dividing by the APE of ingested fructose.

$$\text{Rate of appearance of } ^{13}C \text{ from fructose} = \frac{X*6}{APE\ Fruct_{ing}} \qquad \textbf{(eq 2)}$$

$APE\ Fruct_{ing}$ = ^{13}C APE of ingested fructose.

$$X = \frac{F - pV \times \dfrac{[C_1 \times (IE^{13}C_1 + IE\ D_1) + C_2 \times (IE^{13}C_2 + IED_2)]}{2} \times \dfrac{(IE\ D_2/^{13}C_2 - IE\ D_1/^{13}C_1)}{t_2 - t_1}}{\dfrac{(IE\ D_2/^{13}C_2 + IE\ D_1/^{13}C_1)}{2}} - F \qquad \text{(eq 1)}$$

APE was calculated as $APFruct_{ing} - APCO_2$ basal where $APFruct_{ing}$ is the AP of ingested fructose calculated from the ∂‰ $^{13}CO_2$ obtained after complete oxidation and $APCO_2$ basal is the AP of expired CO_2 before fructose ingestion.

The third step **(equation 3)**, calculates the rate of appearance of exogenous glucose by dividing the rate of appearance of ^{13}C coming from fructose by 6 (6 carbons in glucose molecule).

$$R_aE = \frac{\text{Rate of appearance of } ^{13}C \text{ coming from fructose}}{6}$$

R_aE ($\mu mol \cdot kg^{-1} \cdot min^{-1}$): rate of apperance of exogenous glucose **(eq 3)**

The two methods of calculation gave similar results for the total amount of glucose synthesized from fructose over the 6 h: 0.23 ± 0.09 vs 0.50 ± 0.06 g · kg^{-1} (0.5 and 1 g · kg^{-1}), respectively obtained with the first method, and 0.27 ± 0.04 vs 0.51 ± 0.06 g · kg^{-1} (0.5 and 1 g · kg^{-1}), respectively obtained with the second method. The similarity of the results obtained with the two methods of calculation might suggest that the intra-hepatic dilution of the carbons coming from fructose, and going into glucose has been in fact almost negligible.

Calculation of the non-fructose glucose production (NFGP). NFGP, i.e. the glucose produced from sources other than fructose conversion, was calculated as R_aT minus R_aE.

Calculation of the fructose oxidation. The amount of ingested fructose oxidized was calculated by using ^{13}C enrichment in expired CO_2 and VCO_2 by using the following equation [19]:

$$\text{Exogenous fructose oxidized (mg} \cdot \text{min)} = \frac{\partial‰\ CO_2\ (t) - \partial‰\ CO_2\ (t_0)}{\partial‰\ \text{Fruct} - \partial‰\ CO_2\ (t_0)} \times \frac{VCO_2\ (t) \times 180}{22.29 \times 6 \times c}$$

where ∂‰ CO_2 (t) is the ∂ value of the expired CO_2 at time t (the end of each 30- or 60-min period), ∂‰ CO_2 (t_0) is the ∂‰ value of the expired CO_2 during the 30 min basal adaptation period, ∂‰ Fruct is the ∂ value of ingested fructose after complete combustion, VCO_2 (t), expressed in l/min, is the average volume of expired CO_2 during the 30 or 60 min which framed the sample of expired CO_2 at time t. The molecular weight of fructose is 180, 1/22.29 is for the conversion in moles of CO_2, and 1/6 is for the conversion in moles of fructose; c is the correction factor for CO_2 recovery and was chosen to be 0.8, the most usual value obtained in studies which evaluated the recovery [20].

For the estimation of the proportions of the fructose loads converted to glucose and to CO_2 we assumed that the entire loads were absorbed by the gut during the 6 h of the study.

Statistical analysis

The results are expressed as mean ± SEM ($n = 6$). ANOVA was used to compare the results obtained with the two fructose loads. When a significant difference was observed the paired Student's *t*-test was used. Differences were regarded as significant if p was less than 0.05.

Results

Metabolites and hormone responses (Table 1)

The increase in fructosaemia was different between the two loads ($p < 0.01$). The increase was rapid with a peak value of 0.29 ± 0.03 and 0.44 ± 0.07 mmol/l (0.5 and 1 g · kg^{-1}, respectively; $p < 0,01$) at time 60 min. Glycaemia was similar for the two loads and increased weakly (less than 9 %; $p < 0.05$) and transiently. Plasma lactate was not different between the two loads over the 6 h of the study, but the maximal increase was much lower with the 0.5 g · kg^{-1} load than the 1 g · kg^{-1} load: 59 % compared to time 0 for 0.5 g · kg^{-1} ($p < 0.05$) and 200 % for 1 g · kg^{-1} ($p < 0.01$). Plasma NEFA suppression was not different for the two loads; maximal suppression compared to time 0 was 68 % for 0.5 g · kg^{-1}; ($p < 0.001$) and 82 % for 1 g · kg^{-1}; ($p < 0.01$). Insulinaemia was not different between the two loads and increased weakly; the maximal increase was 69 % compared to time 0 for 0.5 g · kg^{-1}; ($p < 0.05$) and 146 % for 1 g · kg^{-1}; ($p < 0.05$). Triglycerides were not different between the two loads but increased linearly during the last 3 h, reaching a maximal value at 6 h (about 45 % increase from basal for the two loads; $p < 0.05$).

Evolution of ^{13}C in plasma glucose and expired CO_2

The evolution of ^{13}C enrichment in derivatized plasma glucose and in expired CO_2 is shown in Figure 1. The ∂‰ ^{13}C of derivatized plasma glucose before ingestion of fructose was remarkably stable (-37.71 ± 0.08) for the entire group with a coefficient of variation of 0.89 %. Basal values of ^{13}C enrichment in basal CO_2 were also very stable (-24.73 ± 0.17 ∂‰ ^{13}C) with a coefficient of variation of 2.6 %. In the basal state, the ∂‰ ^{13}C of CO_2 was higher than the ∂‰ ^{13}C of plasma glucose. The low ∂‰ ^{13}C of derivatized plasma glucose was related to a dilution phenomenon involved in the derivatization procedure with acetic anhydride as previously discussed in detail [7]. After fructose ingestion, the ∂ ^{13}C‰ of plasma glucose was significantly higher with the load of 1 g · kg^{-1} during the last 4 h ($p < 0.01$). The ∂ ^{13}C‰ increased rapidly with both loads and almost linearly during the first 90 min. Its increase was significant from time 10 min compared to the basal value ($p < 0.05$). It then followed a bell-shape curve before slightly declining, but never returning to the baseline values. The isotopic enrichment in ^{13}C of CO_2 was higher with 1 g · kg^{-1} than with 0.5 g · kg^{-1} during the last 4 h ($p < 0.01$). The increase in ∂‰ ^{13}C in CO_2 following fructose ingestion described the same profile as ∂‰ ^{13}C plasma glucose, but the curve was slightly shifted to the right of the curve of the ∂ ^{13}C‰ of plasma glucose (Fig. 1).

Glucose turnover and glucose production from fructose

The basal total glucose appearance rate (R_aT) averaged 2.40 ± 0.14 mg · kg^{-1} · min^{-1} (average of the basal values of the two loads). After the ingestion of fructose, R_aT increased weakly and transiently and was similar for the two loads (Fig. 2). The overall glucose appearance during the 6 h of experimental period was similar for the two loads: 0.87 g · kg^{-1} and 0.89 g · kg^{-1} for 0.5 and 1 g · kg^{-1}, respectively.

The rate of appearance of glucose synthesized from fructose (R_aE) was different between the two loads (ANOVA; $p < 0.01$) (Fig. 2). R_aE increased steadily and

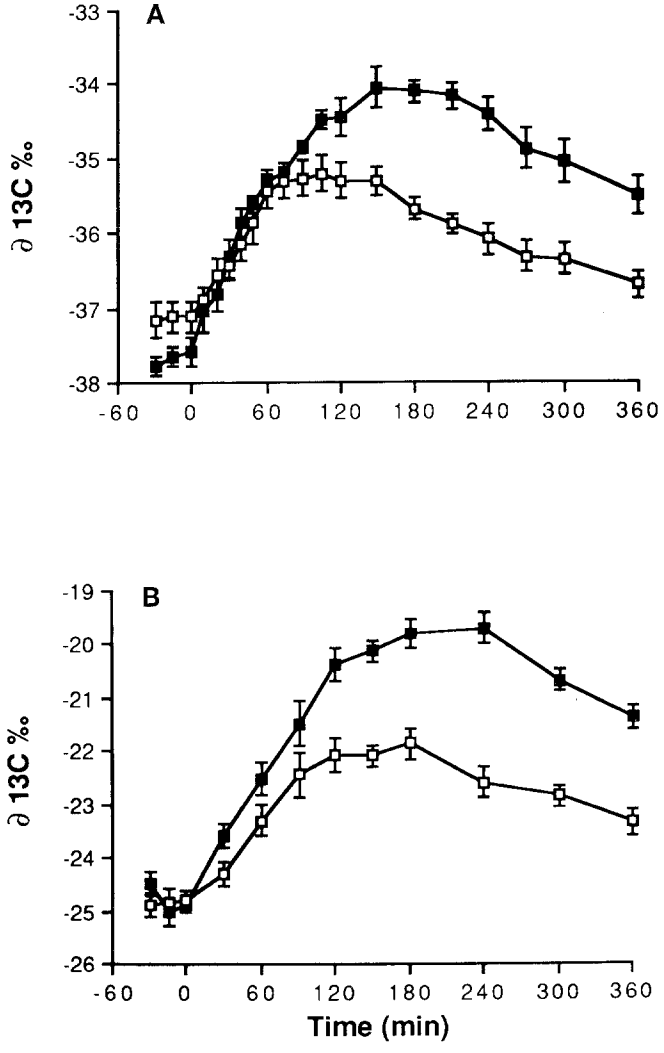

Fig. 1. A,B. Evolution of ^{13}C enrichment in derivatized plasma glucose (**A**) and in expired CO_2 (**B**) after the absorption of 1 (■) and 0.5 $g \cdot kg^{-1}$ (□) ^{13}C-naturally enriched fructose; (mean ± SEM) ($n = 6$)

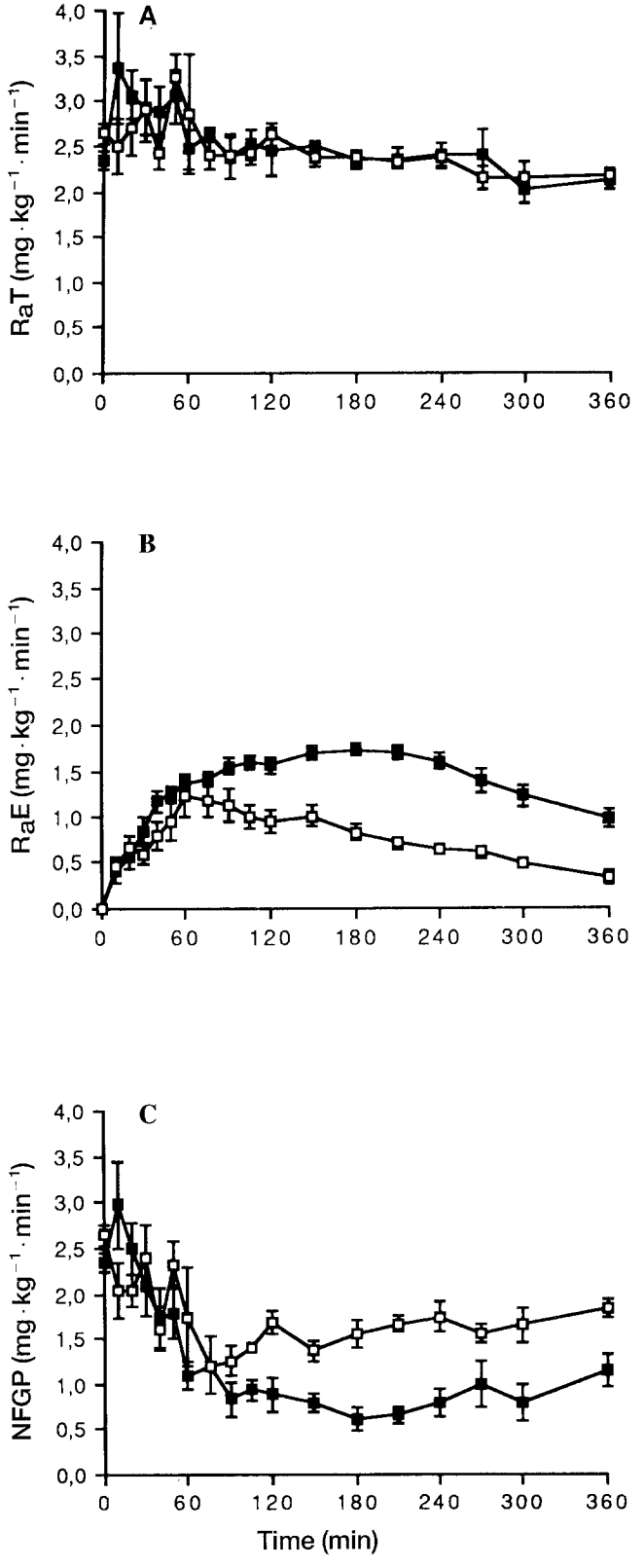

Fig. 2. A–C. Evolution of overall glucose appearance in plasma (R_aT) (**A**); rate of appearance of glucose-synthesized from fructose in plasma (R_aE) (**B**); rate of non-fructose glucose production (NFGP) (**C**), after the absorption of 1 (■) and 0.5 $g \cdot kg^{-1}$ (□) fructose; (mean ± SEM) ($n = 6$)

similarly for both loads for 60 min, after which the kinetics were clearly different. Following the 1 $g \cdot kg^{-1}$ load, R_aE plateaued between 1.55 ± 0.09 and 1.70 ± 0.08 mg · $kg^{-1} \cdot min^{-1}$ from the time 90 to 210 min, then declined progressively and did not return to 0 at the end of 6 h (0.98 ± 0.10 mg · $kg^{-1} \cdot min^{-1}$; $p < 0.05$). Following the 0.5 $g \cdot kg^{-1}$, R_aE reached a peak value of 1.22 ± 0.23 mg · $kg^{-1} \cdot min^{-1}$ at the time 60 min, then remained relatively constant until the time 150 min before progressively declining until time 360 min without returning to 0 (0.33 ± 0.08 mg · $kg^{-1} \cdot min^{-1}$; $p < 0.05$). However, its level at time 360 min was lower than with the 1 $g \cdot kg^{-1}$ load ($p < 0.01$). The overall appearance of glucose synthesized from fructose during the 6-h experiment was 0.27 ± 0.04 and 0.51 ± 0.03 $g \cdot kg^{-1}$ (0.5 vs 1 $g \cdot kg^{-1}$; $p < 0.01$), and amounted to 31 % and 57 % respectively (0.5 vs 1 $g \cdot kg^{-1}$; $p < 0.01$) of overall glucose appearance during this period. The appearance of glucose synthesized from fructose over the 6 h of the study accounted for 54 % and 50.7 % of the oral fructose load (0.5 vs 1 $g \cdot kg^{-1}$; NS).

As a result of the different R_aE, non-fructose glucose production (NFGP) was different between the two loads (ANOVA; $p < 0.05$) (Fig. 2). Maximal NFGP suppression was 52% (0.5 $g \cdot kg^{-1}$; $p < 0.01$) and 74% (1 $g \cdot kg^{-1}$; $p < 0.01$) and NFGP suppression could still be observed at the end of 6 h: 31% (0.5 $g \cdot kg^{-1}$; $p < 0.01$) and 51% (1 $g \cdot kg^{-1}$; $p < 0.01$). Overall NFGP was 0.60 ± 0.02 and 0.38 ± 0.03 $g \cdot kg^{-1}$ (0.5 vs 1 $g \cdot kg^{-1}$; $p < 0.05$) over the 6-h experimental period, and amounted to 69% and 43% (0.5 vs 1 $g \cdot kg^{-1}$; $p < 0.05$) of overall glucose appearance.

Fructose oxidation

The total amount of fructose oxidized during the 6-h experimental period was 0.28 ± 0.03 and 0.59 ± 0.07 $g \cdot kg^{-1}$ (0.5 vs 1 $g \cdot kg^{-1}$; $p < 0.01$) and amounted to 56% and 59% of the 0.5 and 1 $g \cdot kg^{-1}$ fructose loads, respectively (NS). There was a strong correlation between accumulated fructose oxidation and accumulated glucose synthesis from fructose over the 6 h of the study ($r = 0.99$; $p = 0.0001$ for both 0.5 and 1 $g \cdot kg^{-1}$).

Discussion

The use of the very sensitive technique of gas chromatography isotope ratio mass spectrometry enabled us to detect isotopic enrichment in ^{13}C of plasma glucose following ingestion of naturally ^{13}C labelled fructose. Thus, it was possible to study the precursor – product relationship between fructose and glucose in vivo in man. The quantification in the same study of the rate of appearance of glucose synthesized from fructose, of the contribution of this glucose to overall glucose production and of the fructose oxidation brings new insight in the field of fructose metabolism.

The glycaemic response following the ingestion of 0.5 (≈ 30 g) and 1 $g \cdot kg^{-1}$ (≈ 60 g) fructose by our subjects was small compared to the glycaemic response observed with comparable glucose loads [7]. Our data contribute to the understanding of the different well-known effects on the glycaemic response of fructose and glucose. It has been shown with the same methodology [7] that glucose ingestion induced a marked rise in the total rate of glucose appearance and that this increase was mainly determined by the appearance of the glucose of exogenous origin. A completely different pattern was observed with the fructose loads. In contrast to what was observed with a glucose load, fructose had a transient and very small effect on the total rate of glucose appearance. This result agrees with the observation by Björkman et al. [5], of the lack of a significant effect of a 45 g intravenous fructose load on the hepatic glucose production in normal man. The small effect of ingested fructose on the total rate of glucose appearance is in contrast to the importance of the rate of appearance of glucose synthesized from fructose. This represents 60–75% of the total rate of glucose appearance between time 90 and 240 min with the 1 $g \cdot kg^{-1}$ load, and 40–50% between time 60 to 90 min with the 0.5 $g \cdot kg^{-1}$ load. Thus, the glucose synthesized from fructose after the fructose ingestion is not an additive to the endogenous source of glucose present in the basal state, but rather replaces an important part of it.

The kinetics of the appearance of glucose synthesized from fructose deserves comment. The significant increase in ^{13}C enrichment in plasma glucose observed from time 10 min following fructose ingestion agrees with the well-known high capacity of the liver to metabolize fructose [2]. Such a rapid labelling of plasma glucose was observed after fructose infusion [21, 22]. The linear and fairly similar increase in the rate of exogenous glucose appearance during the first 60 min following the two fructose loads could suggest the existence of a rate-limiting step in glucose formation from fructose. This prevents, during the first hour, a greater increase in glucose synthesized from 1 $g \cdot kg^{-1}$ fructose than from 0.5 $g \cdot kg^{-1}$. The rate-limiting step could be the uptake of fructose by the liver. Fructose uptake by the liver can be limited by the activity of fructokinase. Indeed, owing to the low K_m of this enzyme (0.5 mmol/l) and to the reported fructose concentration in the portal vein [2] after an oral load (about 2 mmol/l), the enzyme is probably working at its maximal activity at least in the early phase of the experiment. The highly significant difference in fructosaemia observed with the two loads strongly supports this hypothesis. Secondly, whatever the importance of the limitation of fructose uptake by the liver, we may also assume that the similar increase in the rate of appearance of glucose synthesized from fructose observed with the two loads was due to a preferential diversion of glucose 6-phosphate towards glycogen synthesis with the larger fructose load. Analysis of the ^{13}C incorporation in glucose after the two loads, during the last 5 h strongly supports this hypothesis. This strongly suggests that fructose has been involved in glycogen formation by the liver; this storage being higher with the 1 $g \cdot kg^{-1}$ load than with the 0.5 $g \cdot kg^{-1}$ load [23].

The very small effect of the two fructose loads on overall glucose appearance, which contrasts with the importance of exogenous glucose appearance, implies an important, dose-dependent suppression of the NFGP: 57% and 31% following the 1 $g \cdot kg^{-1}$ and the 0.5 $g \cdot kg^{-1}$ load, respectively. The inhibition of the NFGP can result from the inhibition of glycogenolysis or gluconeogenesis from sources other than fructose, or both. The increase in insulinaemia observed with the two fructose loads probably contributes to the inhibition of NFGP. Moreover, increase in insulinaemia induced a sustained reduction in plasma NEFA concentrations. The reduced release of NEFA from the liver might have contributed to limiting the rate of gluconeogenesis. There is evidence in vitro in rats [24] and in vivo in man in euglycaemic and hypoglycaemic conditions that NEFA are important determinants of hepatic glucose output [25] and gluconeogenesis [26]. However, as insulinaemia and plasma NEFA suppression were similar for both loads, they cannot explain the dose-dependent inhibition of NFGP observed from time 90 to time 360 min. Therefore, the difference in NFGP suppression is probably more related to an effect of fructose on glycogenolysis, gluconeogenesis or both. A dose-dependent depletion in hepatic ATP has been shown to occur in vitro [2]

and in vivo [27] and could have interferred with the efficiency of gluconeogenesis.

The amount of fructose oxidized was about 57 % of the fructose loads and is in agreement with studies measuring fructose oxidation during fructose infusion or during an oral load [22, 28]. The study of kinetics of ^{13}C labelling in CO_2 does not reveal the relative amounts of ^{13}C coming from direct oxidation of fructose, glucose oxidation and lactate oxidation. Wolfe et al. [29] found that 15 % of the fructose taken up by the splanchnic bed was directly oxidized. These data favour at least part of direct fructose oxidation occurring rapidly in the liver. So it is quite possible that a significant amount of fructose could have been oxidized in liver even before becoming glucose. However, the strong correlation between the amounts of fructose oxidized and glucose synthesized from fructose also suggests that most of the expired $^{13}CO_2$ is in fact the product of glucose oxidation.

In this study we quantified fructose conversion into glucose and fructose oxidation. We found that over the 6 h of the study about 50 % of the two fructose loads were converted into glucose and about 60 % were oxidized. This conversion of 50 % of fructose into glucose after oral administration of fructose is superior to the 10 to 25 % which has been reported after intravenous fructose administration [5, 29] in studies using the splanchnic catheter technique. The difference may be related to the route of fructose administration. Indeed, it has been shown that after intravenous administration, about 20 % of the fructose was taken up by the kidney and that about 50 % of this fructose was converted into glucose [5]. Thus, when glucose appearance is only measured in the hepatic vein, glucose synthesized from fructose by the kidney is not considered, so that the interconversion of fructose into glucose is underestimated. In fact, the percentage of fructose taken up by liver and converted to glucose is 50 % [30]. This result agrees with our data in as much as a high percentage of the fructose ingested was probably directly absorbed and converted into glucose by the liver after its transport through the portal vein.

The conversion of only 50 % of the fructose load into glucose leaves the remaining 50 % unaccounted for. Some of this amount may be explained by a slight underestimation of fructose conversion into glucose as the rate of appearance of glucose synthesized from fructose did not completely return to 0 at the end of the study. Some fructose could have been diverted to triglyceride synthesis as suggested by the 45 % increase in triglyceridaemia at the end of the study, or to lactate formation, direct fructose oxidation in the liver and perhaps a possible direct uptake of fructose by adipose tissue [31]. Lastly, we cannot exclude that a small amount of fructose remained unabsorbed by the intestine [32]. If uncomplete absorption of fructose occurred, the amount of fructose converted to glucose and to CO_2 could have been underestimated, particularly during the 1 $g \cdot kg^{-1}$ load.

In conclusion, we have shown in this study that the low hyperglycaemic effect of fructose was explained by its small effect on the overall rate of glucose appearance. Despite this small effect on overall glucose production, glucose synthesized from fructose represented 30 to 60 % of the overall glucose production. Thus fructose has a sparing effect on endogenous glucose metabolism. Ingestion of either 30 or 60 g fructose led to a similar proportion being converted to glucose ($\approx$ 50 %). The remaining 50 % might have been used in the formation of the other end products of fructose metabolism.

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