Clinical Nutrition ESPEN 29 (2019) 125–132

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

The extra-splanchnic fructose escape after ingestion of a fructose–glucose drink: An exploratory study in healthy humans using a dual fructose isotope method

Célia Francey, Jérémy Cros, Robin Rosset, Camille Crézé, Valentine Rey, Nathalie Stefanoni, Philippe Schneiter, Luc Tappy, Kevin Seyssel*

Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, 7A Rue du Bugnon, Lausanne 1005, Switzerland

ARTICLE INFO

Article history:
Received 13 August 2018
Accepted 9 November 2018

Keywords:
Fructose metabolism
Human physiology
Isotope tracers

SUMMARY

Background & aims: The presence of specific fructose transporters and fructose metabolizing enzymes has now been demonstrated in the skeletal muscle, brain, heart, adipose tissue and many other tissues. This suggests that fructose may be directly metabolized and play physiological or pathophysiological roles in extra-splanchnic tissues. Yet, the proportion of ingested fructose reaching the systemic circulation is generally not measured. This study aimed to assess the amount of oral fructose escaping first-pass splanchnic extraction after ingestion of a fructose-glucose drink using a dual oral-intravenous fructose isotope method.

Methods: Nine healthy volunteers were studied over 2 h before and 4 h after ingestion of a drink containing 30.4 ± 1.0 g of glucose (mean ± SEM) and 30.4 ± 1.0 g of fructose labelled with 1% [U-$^{13}C_6$]-fructose. A 75%-unlabeled fructose and 25%-[6,6-$^{2}H_2$]-fructose solution was continuously infused (100 μg kg^{-1} min^{-1}) over the 6 h period. Total systemic, oral and endogenous fructose fluxes were calculated from plasma fructose concentrations and isotopic enrichments. The fraction of fructose escaping first-pass splanchnic extraction was calculated assuming a complete intestinal absorption of the fructose drink.

Results: Fasting plasma fructose concentration before tracer infusion was 17.9 ± 0.6 μmol.L^{-1}. Fasting endogenous fructose production detected by tracer dilution analysis was 55.3 ± 3.8 μg kg^{-1}min^{-1}. Over the 4 h post drink ingestion, 4.4 ± 0.2 g of ingested fructose (*i.e.* 14.5 ± 0.8%) escaped first-pass splanchnic extraction and reached the systemic circulation. Endogenous fructose production significantly increased to a maximum of 165.4 ± 10.7 μg kg^{-1}·min^{-1} 60 min after drink ingestion ($p < 0.001$).

Conclusions: These data indicate that a non-negligible fraction of fructose is able to escape splanchnic extraction and circulate in the periphery. The metabolic effects of direct fructose metabolism in extra-splanchnic tissues, and their relationship with metabolic diseases, remain to be evaluated. Our results also open new research perspectives regarding the physiological role of endogenous fructose production.

1. Introduction

Fructose intake has drastically increased over the past decades due to profound changes in global food habits [1–3]. There is growing suspicion that large amounts of fructose, mainly from added sugars, may play a role in the pathogenesis of obesity, diabetes, non-alcoholic fatty liver and cardiovascular diseases [4–7].

Until recently, the dogma was that fructose acted only on the liver and did not have direct effects on extra-splanchnic organs due to a nearly complete extraction by the intestine and the liver, as revealed

Abbreviations: $F_{infusion}$, fructose infusion; FRa_{tot}, total rate of fructose appearance; FRa_{oral}, rate of oral fructose appearance; EFP, endogenous fructose production; AUC, area under the curve; NOFD, non-oxidative fructose disposal; MPE, mol percent excess; APE, atom percent excess.

* Corresponding author.
E-mail address: kevin.seyssel@gmail.com (K. Seyssel).

https://doi.org/10.1016/j.clnesp.2018.11.008
2405-4577/

by post-prandial systemic fructose concentrations never exceeding the micromolar ranges [8,9]. Most research efforts have therefore focused on fructose-induced hepatic *de novo* lipogenesis and fructose effects on hepatic glucose and lipoprotein production to account for potential adverse effects of fructose [10]. However, some robust observations challenge the concept of a near-exclusive hepatic fructose metabolism. The presence of specific fructose transporters (GLUT5) and fructose metabolizing enzymes (fructokinase-A or C and aldolase A or C) has now been demonstrated in many tissues which were not presumed to metabolize fructose, such as white adipose tissue, skeletal muscle, heart, pancreas and brain [4,11,12]. This suggests that fructose may be directly metabolized in extra-hepatic tissues and may contribute to physiological processes. In addition, fructose metabolism has been linked to potentially pathogenic effects in the kidney [13], heart [14], adipose tissue [15] and brain [16], thereby suggesting that systemic fructose metabolism may be directly relevant for a vast array of organ dysfunctions. Therefore, the objective of our study was to quantify systemic fructose fluxes in healthy humans using a continuous infusion of [6,6-$^{2}H_2$]-fructose, and to calculate the proportion of fructose escaping splanchnic extraction after ingestion of fructose. Since fructose in our every-day diet is almost invariably consumed together with nearly isomolar amounts of glucose [17], these measurements were made after ingestion a glucose + fructose drink (including 1% [U-$^{13}C_6$]-fructose).

2. Materials and methods

2.1. Subjects

Nine volunteers (5 males and 4 females) participated in the study. All subjects were in good health based on a brief medical history and standard physical examination, had no family history of diabetes, were non-smoker and were moderately active (*i.e.* performing less than 4 h of exercise per week). The experimental protocol was approved by the Human Research Ethics Committee of Canton de Vaud and the trial was registered on the international clinical trial registry (clinicaltrials.gov, #NCT03195062). All participants provided a written informed consent.

Participants were recruited using posters displayed in various locations on the University of Lausanne campus. The recruitment flowchart is shown in Fig. 1A. Following first contacts, volunteers were pre-selected by email and if eligible, invited to a screening visit. Ten healthy subjects were included in the study. One subject had to be excluded from analyses due to omission of the [U-$^{13}C_6$]-fructose tracer during test drink preparation.

2.2. Study design

Each subject was studied after a 3-day run-in period of controlled weight-maintenance diet (*i.e.* energy requirements calculated with the Harris–Benedict equation and physical activity factor of 1.5) containing 55% carbohydrate (35% complex carbohydrate and 20% sugar), 15% protein and 30% fat. All meals and snacks were prepared by our staff and given to volunteers in containers labelled with information relative to consumption timing. During this run-in period, subjects were instructed to eat the entire pre-packed food provided, to consume nothing else except water (no alcohol, caffeine or sugar/sweetener-containing beverages) and to maintain their normal physical activity with no structured exercise. On the fourth day, subjects reported to the Clinical Research Center of the University of Lausanne at 6:30 am in a fasting state for a metabolic test.

The metabolic test included a 2 h-fasting and a 4 h post-prandial measurement periods, as depicted in Fig. 1B. Upon arrival, subjects were weighed (Seca 708, Seca GmbH, Hamburg, Germany) and transferred to a bed, where they remained quiet, but awake during the following 6 h. Their body composition was assessed by bioelectrical impedance (Biacorpus RX 400, Medi Cal HealthCare GmbH, Germany). Two catheters were then inserted, one in a vein of each forearm. The first one served to continuously infuse a 7.5 g L^{-1}unlabeled fructose and 2.5 g L^{-1} [6,6-$^{2}H_2$]-fructose solution (Cambridge Isotope Laboratories, Andover, Massachusetts, USA). This solution was prepared and tested for absence of pyrogenicity by the central pharmacy of Lausanne University Hospital. The infusion rate provided 100 μg kg^{-1} min^{-1}. The second catheter was used for repeated blood sampling at t = −120, −30, 0, 30, 60, 90, 120, 150, 180, 210 and 240 min. Breath samples were also collected at identical timings. Respiratory gas exchange was monitored throughout the test by indirect calorimetry (Quark RMR, Cosmed, Rome, Italy).

The fructose-glucose test drink provided individuals' energy requirements for a 240 min-period. This caloric equivalent was calculated for each subject using his/her resting metabolic rate data recorded with indirect calorimetry over the baseline period [18]. The drink consisted of equal amount of glucose (30.4 ± 1.0 g) and fructose (30.4 ± 1.0 g), including 1% of [U-$^{13}C_6$]-fructose (304 ± 10 mg) (Cambridge Isotope Laboratories, Andover, Massachusetts, USA), dissolved into water (243 ± 8 mL).

2.3. Analytic procedures

Plasma was separated from blood cells immediately after collection by centrifugation during 10 min at 3500 rpm at 4 °C. Plasma samples were stored at −20 °C until analyses. Plasma glucose and lactate concentrations were measured using enzymatic methods (RX Monza, Randox Laboratories Ltd., Crumlin, UK). Plasma insulin concentrations were measured with the use of radioimmunoassay kits (Merck Millipore, Billerica, MA, USA).

Plasma fructose concentrations, [6,6-$^{2}H_2$]- and [$^{13}C_6$]-fructose isotopic enrichments were measured by gas chromatography-mass spectrometry (GC–MS; Agilent Technologies, Santa Clara, CA, USA). For these analyses, 60 μL of 1 mM-mannitol was added to 200 μL of plasma as an internal standard. Plasma protein content was then precipitated with barium hydroxide and zinc sulfate, and the supernatant was partially purified by sequential anion/cation exchange chromatography using resins (AG 50 W-X8 and AG 1-X8; Bio-Rad, Richmond, CA, USA). The solute fraction thus obtained was vacuum-dried, acetylated with acetic anhydride and pyridine overnight, evaporated, re-suspended in 200 μL ethyl acetate and finally injected into the GC–MS using a HP-5MS 5% phenyl-methyl siloxane column. Analyses were performed in electron impact mode with selective monitoring of *m/z* 275 for unlabeled fructose, 277 for [6,6-$^{2}H_2$]-fructose, 280 for [$^{13}C_6$]-fructose and 361 for mannitol, respectively. Plasma fructose concentrations were determined from *m/z* 275/361 ratio by means of a standard curve ranging from 0 to 400 μmol L^{-1} fructose concentrations [19]. Plasma [6,6-$^{2}H_2$]- and [$^{13}C_6$]-fructose isotopic enrichments were determined from *m/z* 277/275 and 280/275 ratios. Breath $^{13}CO_2$ isotopic enrichments were determined by isotope-ratio mass spectrometry (IRMS) (SerCon Ltd., Crewe, UK) as previously described [20]. Baseline breath samples (t = −120 min) were used to measure individuals' $^{13}CO_2$ natural enrichment in breath.

2.4. Calculations

Total rate of fructose appearance (FRa_{tot}) over the baseline period (*i.e.* t = −30 and 0 min) was calculated using Steele's steady state equations. The FRa_{tot} during this period corresponds to the sum of endogenous fructose production (EFP) and the infusion of [6,6-$^{2}H_2$]-labelled/unlabeled fructose ($F_{infusion}$ = 100 μg kg^{-1} min^{-1}).

During the post-prandial period, the FRa_{tot} was calculated using non-steady state Steele's equations, as adapted by De Bodo et al.

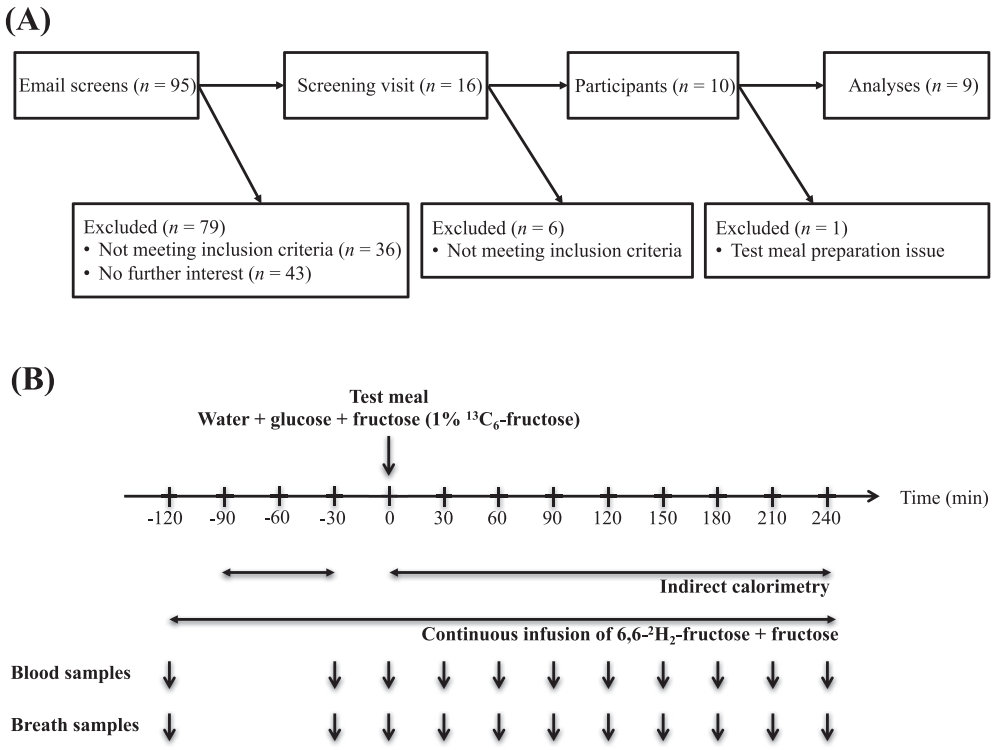

Fig. 1. Study flowchart (A) and metabolic test design (B).

[21] (supplementary material 1A). Calculations were done using a distribution volume for fructose of 0.2 L kg^{-1} and a pool fraction of 0.9, *i.e.* similar to that of glucose. The rate of oral fructose appearance (FRa_{oral}) was calculated from plasma [$^{13}C_6$]-fructose isotopic enrichments, as initially established in rats [22] and adapted for stable isotopes in humans [23] (supplementary data 1B). Endogenous fructose production (EFP) was calculated as the total rate of fructose appearance (FRa_{tot}) minus the rate of oral fructose appearance (FRa_{oral}) and fructose infusion ($F_{infusion}$). Oral fructose oxidation was calculated from breath $^{13}CO_2$ isotopic enrichments and dietary [$^{13}C_6$]-fructose isotopic enrichments as previously described by Theytaz et al. [8].

Total area under the curve (*i.e.* obtained by the trapezoidal rule) for FRa_{oral} and fructose oxidation were used to calculate the amounts of exogenous fructose escaping first pass splanchnic extraction and being oxidized (directly or indirectly). First-pass splanchnic fructose extraction was then calculated as the total amount of fructose contained in the test drink minus the amount of exogenous fructose escaping first pass splanchnic extraction. Non-oxidative fructose disposal (NOFD) was calculated as the total amount of fructose contained in the test drink minus the fructose oxidation. These calculations assumed that fructose was completely absorbed during this 240 min period. Although fructose alone is known to be poorly absorbed in some individuals [24], it is markedly facilitated by glucose co-ingestion, and Rumessen et al. have indeed documented a complete absorption of fructose within 4 h after ingestion of 50 g of fructose and 50 g of glucose [25].

2.5. Statistical analysis

All data are presented as mean ± SEM (standard error of mean). Normal distributions for each parameter were checked using the Shapiro–Wilk test. A linear model was used to investigate the effect of time on each parameter. Dependent variables were plasma glucose, insulin, fructose and lactate concentrations, [6,6-$^2H_2$]- and [$^{13}C_6$]-fructose isotopic enrichments, FRa_{tot}, FRa_{oral}, EFP and breath $^{13}CO_2$ isotopic enrichments. Time was considered as a fixed effect and subjects as a random effect. Whenever a main effect of Time was found, post-hoc Student's paired *t*-tests (two-sided) were conducted between each post-prandial time point (t = 30, 60, 90, 120, 150, 180, 210, 240 min) and the baseline (t = 0 min), in order to investigate the impact of drink ingestion. In addition, and to check the effect of fructose infusion on plasma glucose, insulin, lactate and fructose concentrations, we assessed differences in concentrations between time point t = −120 min versus t = −30 and 0 min using Student's paired *t*-tests (two-sided). All statistical analyses were performed with JMP 14 software (SAS Institute Inc., NC, USA) and *p*-values < 0.05 were considered as significant.

3. Results

Subject characteristics are presented in Table 1.

3.1. Blood hormones and metabolites concentrations

A main effect of Time was observed for plasma glucose, insulin, fructose and lactate concentrations (all $p < 0.001$). During the initial

Table 1
Study participants' characteristics at baseline (n = 9).

	Mean ± SEM
Age (years)	24.9 ± 1.9
Weight (kg)	62.9 ± 1.6
BMI (kg m^{-2})	21.4 ± 0.5
Fat mass (%)	23.8 ± 1.8
Systolic blood pressure (mmHg)	119.3 ± 2.4
Diastolic blood pressure (mmHg)	70.5 ± 2.2
Heart rate (beats min^{-1})	69.3 ± 4.2

2-h fasting period, the continuous fructose infusion did not alter glucose, insulin and lactate concentrations (Fig. 2) (all p = *NS*). However, fructose infusion induced a significant increase of plasma fructose concentrations (from 17.9 ± 0.6 μmol L^{-1} at t = −120 min, to 35.4 ± 1.3 μmol L^{-1} at t = −30 min, and 37.3 ± 1.4 μmol L^{-1} at t = 0 min (all $p < 0.001$)). Plasma glucose, insulin, fructose and lactate concentrations were all significantly increased, at 30 min after test drink ingestion, and remained elevated until 120 min for glucose and insulin, 150 min for lactate, and 210 min for fructose (Fig. 2).

3.2. Isotopic enrichments

A main effect of Time was observed for [6,6-$^2H_2$]-fructose, [$^{13}C_6$]-fructose and $^{13}CO_2$ isotopic enrichments (all $p < 0.001$). During the initial 2-h fasting period, the [6,6-$^2H_2$]-fructose isotopic enrichments were stable (*i.e.* no significant difference was detected between t = −30 and 0 min), as for baseline plasma fructose concentrations, indicating that steady state was reached (Fig. 3). The ingestion of the test drink induced a significant decrease in [6,6-$^2H_2$]-fructose isotopic enrichment associated with a significant

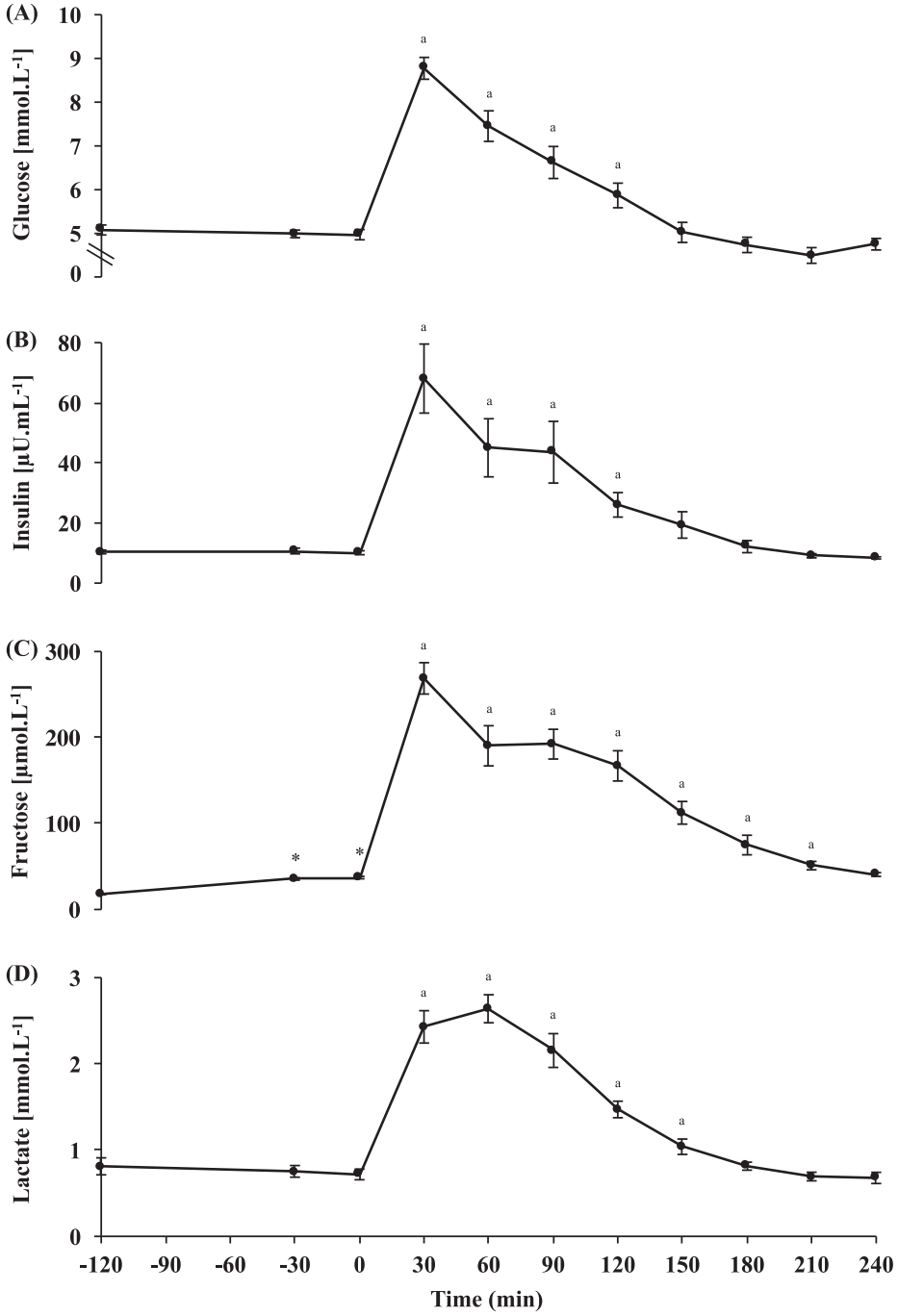

Fig. 2. Plasma concentrations of glucose (A), insulin (B), fructose (C) and lactate (D) in response to test drink ingestion at t = 0 min (n = 9). a: $p < 0.05$ for post-hoc Student's paired *t*-tests comparing respective post-prandial time points with t = 0 min. *: $p < 0.001$ for Student's paired *t*-tests comparing respective pre-prandial time points with t = −120 min. Data are presented as mean ± SEM for n = 9 subjects.

increase in [$^{13}C_6$]-fructose isotopic enrichment, respectively with minimal and maximal values at t = 30 min. Compared to baseline, [6,6-$^{2}H_2$]-fructose isotopic enrichment remained significantly decreased during the 4 h post-prandial period, whereas [$^{13}C_6$]-fructose isotopic enrichment remained significantly elevated after ingestion of the drink. Breath $^{13}CO_2$ isotopic enrichments were also significantly increased after drink ingestion reaching a maximal value of 0.164 ± 0.005 APE at t = 150 min. Then breath $^{13}CO_2$ isotopic enrichments decreased until 240 min and remained significantly elevated when compared to baseline (0.138 ± 0.004 APE, $p < 0.05$).

3.3. Calculated fructose fluxes

Calculated fructose fluxes are presented in Fig. 4. A main effect of Time was observed for FRa_{tot}, FRa_{oral} and EFP. Mean fasting EFP was 55.3 ± 3.8 μg kg^{-1} min^{-1}. After ingestion of the test drink at t = 0 min, FRa_{tot}, FRa_{oral} and EFP significantly increased, reaching maximal values at t = 60 min and decreased thereafter but remained significantly elevated compared to baseline until the end of the post-prandial period.

Overall, 4.4 ± 0.2 g of fructose escaped first-pass splanchnic extraction, as estimated from calculating the FRa_{oral} over the

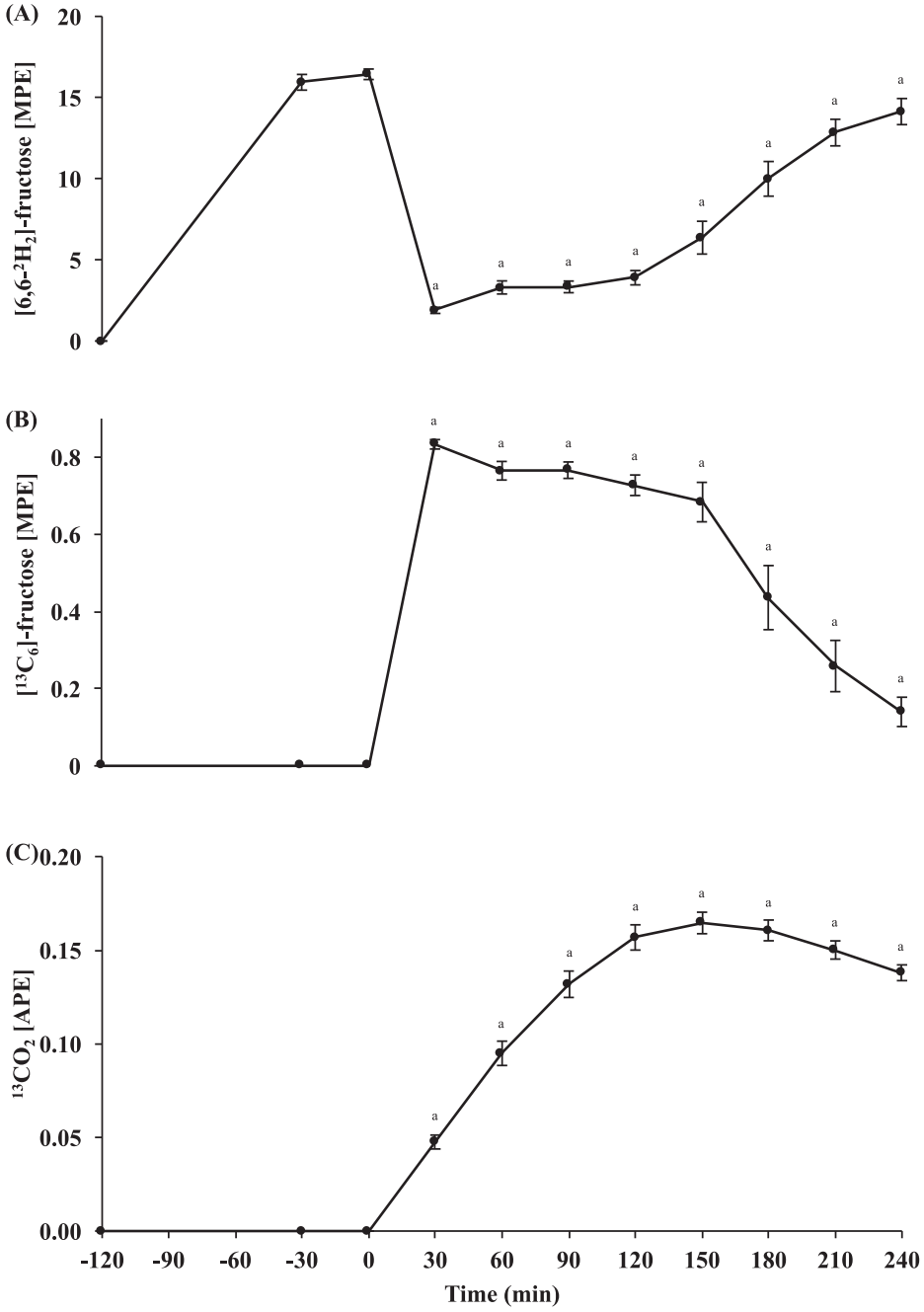

Fig. 3. Plasma [6,6-$^{2}H_2$]-fructose (A), [$^{13}C_6$]-fructose (B) and breath $^{13}CO_2$ (C) isotopic enrichments in response to test drink ingestion at t = 0 min. a: $p < 0.05$ for post-hoc Student's paired *t*-tests comparing respective post-prandial time points with t = 0 min. Data are presented as mean ± SEM for n = 9 subjects. MPE: mol % excess, APE: atom % excess.

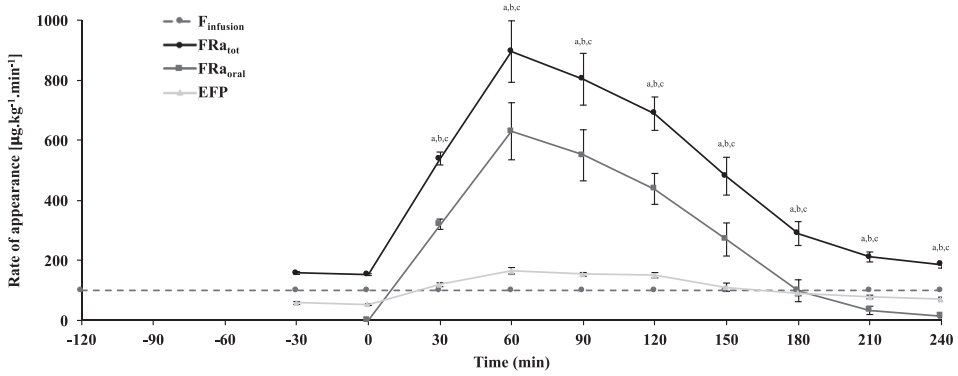

Fig. 4. Plasma fructose fluxes showing $F_{infusion}$ (fructose infusion), FRa_{tot} (total rate of fructose appearance), FRa_{oral} (rate of oral fructose appearance) and EFP (endogenous fructose production) in response to test drink ingestion at t = 0 min (n = 9). a,b,c: $p < 0.05$ for post-hoc Student's paired *t*-tests comparing respective post-prandial time points with t = 0 min for FRa_{tot}, FRa_{oral} and EFP respectively. Data are presented as mean ± SEM for n = 9 subjects.

240 min post-prandial period (4.2 ± 0.4 g for males and 4.6 ± 0.3 g for females; $p = NS$). This represented 14.5 ± 0.8% of the ingested fructose. First-pass splanchnic fructose extraction, was therefore of 85.5 ± 0.8%. Cumulated fructose oxidation over the 4 h post-prandial period was 9.2 ± 0.4 g. This represented 30.6 ± 1.5% of the ingested fructose. The NOFD was 21.2 ± 1.0 g for the whole 240 min post-prandial period.

4. Discussion

The aim of this exploratory study was to measure systemic endogenous and oral fructose fluxes in healthy normal-weight subjects, using a dual fructose isotope method. Here, we reproduced the same method used for glucose studies using deuterated- and ^{13}C-labelled glucose [26]. This dual isotope glucose approach has been well characterized and validated against catheterization studies [27].

Due to major differences between fructose and glucose metabolism in humans, the classical dual glucose isotope protocol required some specific adaptations. First, fructose concentrations in human blood are most of the time quite low, thereby potentially precluding GC–MS analyses. For this reason, we decided to infuse a mixture of labelled-unlabeled fructose at a low rate of 100 µg kg^{-1} min^{-1}, to ensure that plasma fructose concentration would be sufficient for fructose isotopic analysis with GC–MS. Even though this does not strictly correspond to a "tracer approach", we assumed that the amount of fructose infused was small and did not significantly alter endogenous or oral fructose fluxes. Second, since fasting blood fructose concentrations are particularly low, administration of a priming dose of tracer was obviously not warranted Third, fructose and glucose penta-acetate derivates co-elute simultaneously with GC, which represented a technical issue for the specific analysis of fructose enrichment. We therefore took advantage of the fact that, fructose, but not glucose, yields a 275 fragment when performing analyses in electron impact mode, thus allowing us to accurately measure fructose isotopic enrichments while avoiding glucose interference.

According to our results, 14.5 ± 0.8% of the 30.4 g of fructose ingested escaped first-pass splanchnic extraction, representing 4.4 ± 0.2 g of fructose. In other words, we found a first-pass splanchnic fructose extraction of 85.5 ± 0.8%. To our knowledge, this is the first measurement of splanchnic fructose extraction after ingestion of a test drink containing fructose and glucose in humans. In dogs, Shiota et al. [28] elegantly showed a fructose rate appearance of 1.62 µmol kg^{-1} min^{-1} in the portal vein (*i.e.* gut fructose uptake of 0.6 µmol kg^{-1} min^{-1}) and a net hepatic fructose uptake of 1.36 µmol kg^{-1} min^{-1} (*i.e.* an escape of 0.26 µmol kg^{-1} min^{-1} in the systemic blood stream) during an intraduodenal fructose infusion of 2.22 µmol kg^{-1} min^{-1} associated with glucose infusion. This corresponded to a 26% gut extraction and a 62% hepatic extraction, adding up to a 88% splanchnic fructose uptake. Even though we are not able to discriminate the relative gut's and liver's contributions with our protocol, our estimate of total splanchnic uptake is consistent with their data.

Until now, the only available data on splanchnic fructose uptake in humans had been estimated using intravenous fructose infusion. Björkman and Felig demonstrated that splanchnic uptake accounted for 38% of the administrated fructose load with a fructose infusion of 5.43 mg kg^{-1} min^{-1}, *i.e.* a splanchnic uptake capacity of 1.85 mg kg^{-1} min^{-1} [29]. Wolfe et al. also showed that about 46% of intravenous fructose infusion at 6.1 mg kg^{-1} min^{-1} was extracted by splanchnic tissues [30]. The fructose infusion rate used in both of these experiments was 2–12 times higher than the maximal fructose rate of appearance observed at t = 60 min in our study, however. When using arterio-venous differences in fructose concentrations in animals, some authors found a fractional hepatic fructose uptake of 54.9% and 71.5% in fed and fasting rats respectively [31], 77% in fasted dogs [32], and 32.6% in fed sheeps [33]. Altogether, except for sheeps, these data are consistent with ours. In addition, it has been recently observed in mice that hepatic fructose metabolism was dependant on fructose, since low fructose loads were almost entirely metabolized in the gut, while only high-doses of fructose outpassed intestinal fructose metabolic capacity and led to a fructose spillover reaching the liver [34].

Unexpectedly, infusion of labelled–unlabeled fructose mixture resulted in plasma fructose isotopic enrichments significantly lower than in the infusate, which indicated the presence of a small, but significant endogenous fructose production in fasting condition. This is consistent with the measurement of fasting plasma fructose concentrations above zero (17.9 ± 0.6 µmol L^{-1}) before tracer infusion, which can only be explained by an endogenous fructose production. Baseline fructosemia found in our study are in the same range as those reported in other studies which found between 8 and 35 µmol L^{-1} of plasma fructose in fasting state [8,35,36]. Brundin and Wahren indeed showed a splanchnic release of 43 ± 20 µmol min^{-1} in humans, using arterio-venous differences in fructose concentrations [35].

The existence of an active endogenous fructose production has been recently documented in several organs and tissues, both in animal and human studies [37,38]. Park et al. demonstrated the physiological role of endogenous fructose production in naked mole rats to survive against anoxia as revealed with a marked

increase in fructose concentration in the blood, liver and kidney [39]. Furthermore, Lanaspa et al. nicely demonstrated the existence of an hepatic endogenous fructose production in mice, and its contribution to the development of metabolic syndrome [37,40]. In humans, Hwang et al. showed that blood and intracerebral fructose levels significantly increased during a 4-h hyperglycaemic clamp at 12 mmol L^{-1} [38].

The origin of systemic endogenous fructose remains to be further investigated, regarding both the organ(s) and the metabolic pathway(s) involved. At least one pathway for fructose synthesis has been identified, *i.e.* the polyol pathway, which is a two-step metabolic pathway in which glucose is reduced to sorbitol and then converted to fructose [41]. It has been shown in mice that fructose production occurred in kidney through the activation of the polyol pathway and contributed to the pathogenesis of renal diseases [13,42,43]. The regulation of endogenous fructose production also remains unknown. However, hyperglycemia is known to activate the polyol pathway, and hence may possibly account for an increased endogenous fructose production after meal or drink ingestion, as in our study and also as demonstrated by others with glucose ingestion and increased fructose concentrations in human brain [38].

Our study is essentially exploratory and aimed at assessing the feasibility of measuring systemic fructose fluxes and extra-splanchnic fructose metabolism in humans using a dual fructose isotope method. Results reported here are for normal healthy subjects only. They prove consistent with animal studies and intravenous human studies, and hence may be of use to explore possible alterations of fructose kinetics in pathological conditions such as obesity, type 2 diabetes, hypoxia or cancer, where studies have documented various alterations of fructose metabolism [11,12,14,44].

This study has several limitations which need to be mentioned. First, the intravenous infusion of fructose was much higher than endogenous fructose fluxes, and significantly impacted plasma fructose concentration. Calculations of fasting endogenous fructose production were performed assuming no effect of fructose concentration, but the effects of fructosemia on this parameter remains to be assessed. In contrast, tracer fructose infusion was small in regard of oral fructose fluxes, and was therefore unlikely to impact on fructose fluxes after drink ingestion. Indeed, the evolution of post-prandial fructosemia is similar to that observed in other studies [8,38]. Second, the analysis of fructose isotope dilution in plasma only measure the portion of endogenous fructose production ending up in the blood stream, and we do not know what proportion of endogenously produced fructose is actually directly metabolized in fructose-forming organs; this limitation however does not apply to oral fructose fluxes, which transit in the blood stream before being metabolized. Third, we assumed that the parameters used for non-steady state calculations of fructose fluxes where similar to those used for glucose. This appears a reasonable assumption, however, given the very similar physico-chemical properties of these two hexoses. Fourth, there is evidence that some metabolic effects of fructose differ according to gender [45]. Although the results obtained in males and females were in the same range, our study was underpowered to accurately assess gender differences in splanchnic fructose extraction. And fifth, since breath $^{13}CO_2$ isotopic enrichment had not returned to basal levels at the end of our experiments, it is likely that total fructose oxidation was somewhat underestimated, and hence NOFD was overestimated. This however does not impact on our major conclusions regarding first-pass splanchnic fructose extraction.

In summary, we provide evidence that splanchnic fructose uptake and systemic fructose delivery can be non-invasively measured in humans using a dual fructose tracer method. Such studies challenge the dogma of a near-total splanchnic fructose extraction and open novel research perspectives regarding systemic fructose physiological and pathophysiological effects.

Funding sources

This project was supported by the Swiss National Science Foundation, Bern, Switzerland (grant n°32002B_156167).

Author's contribution

L.T. and K.S and conceived and designed the study; C.F. and K.S. enrolled participants; C.F., J.C., R.R., C.C., and K.S. performed the metabolic tests; C.F., V.R., N.S., P.S. and K.S. analyzed the data; C.F. and K.S. wrote the manuscript. J.C., R.R., C.C. and L.T. were involved with critical revision. All of the authors approved the final version of the manuscript.

Conflict of interest

L.T. has received research grants from Soremartec Italy Inc, and speaker's honoraria from Nestlé AG, Switzerland, the Gatorade Sport Science Institute, USA, and the Rippe Lifestyle Institute, USA. The other authors have no conflict of interest to disclose.

Acknowledgements

The authors would like to warmly thank all the participants and Françoise Secretan, Christiane Pellet and Laura Pezzi from the Clinical Research Center of the Lausanne University Hospital for their contribution to this study, Dr. Evrim Jaccard and Dr. Christel Tran for their availabilities on medical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2018.11.008.

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