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Fructose Metabolism and Exercise: Physiological Applications and Limitations

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Faculté de biologie et de médecine

Département de Physiologie

Fructose Metabolism and Exercise: Physiological Applications and Limitations

Thèse de doctorat ès sciences de la vie (PhD)

Présentée à la faculté de Biologie et de Médecine de l'Université de Lausanne par

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Titulaire d'une Maîtrise Universitaire en Biologie Médicale de l'Université de Lausanne

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Fructose Metabolism and Excercise :
Physiological Applications and Limitations

Lausanne, le 13 juillet 2017

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Michel Duchosal

Contents

I. Summary

Fructose has gained renewed interest for its suspected role in cardiometabolic diseases and as a performance enhancer in sports nutrition. Both are related to fructose metabolism in the liver resulting in no plasma glucose or insulin peaks, but causing postprandial hyperlactatemia and hypertriglyceridemia. The general aim of this work was to further investigate fructose metabolism and to find potential new applications of fructose that may improve performance.

In a first experimental study, we evaluated how fructose metabolism is modulated by an exercise session performed before or after fructose ingestion. Although confirming that fructose is extensively oxidized during exercise, our results also showed that fructose metabolism was largely unaltered when ingested during recovery. In a second study, we used fructose in mixed-meals provided for 24 h postexercise to specifically favor muscle energy storage and subsequent exercise performance. Compared to an isocaloric control, fructose did however not further improve muscle recovery, and fructose impaired whole-body glycogen storage and subsequent exercise performance. In a third study, we investigated the effects of glucose-fructose ingestion during training sessions on lactate metabolism, at rest and during exercise, pre-training and post-training. Interestingly, training with glucose-fructose increased lactate production, consumption and oxidation at rest but not during exercise, and an important part of lactate was directed to non-oxidative fates in all conditions.

Altogether, the present results indicate an ambivalent role of fructose, efficient to fuel muscle work, but inefficient during postexercise recovery. This may be largely due to fructose conversion into lactate, furnishing an alternative fuel during exercise that however needs to be stored for an extra energy cost in resting times. In turn, this “reverse Cori cycle” may represent an important mechanism by which fructose effects are matched to physical activity.

Résumé

Le fructose est suspecté de jouer un rôle dans les maladies cardio-métaboliques, et est largement utilisé en nutrition du sport. Ceci est lié à son métabolisme hépatique, entraînant l'absence de pics glycémiques et insulinémiques, mais causant une hyperlactatémie et hypertriglycéridémie postprandiale. Le but général de ce travail était de mieux comprendre le métabolisme du fructose et de l'utiliser afin d'améliorer la performance d'exercice physique.

Dans une première étude, nous avons évalué comment le métabolisme du fructose peut être modulé par un exercice effectué avant ou après ingestion. Tout en confirmant que le fructose est bien oxydé à l'exercice, nos résultats ont aussi montré que son métabolisme n'est pas modifié en récupération. La deuxième étude visait à utiliser des repas mixtes contenant fructose, graisse et protéines et ingérés pendant 24 h, afin d'améliorer la récupération des stocks énergétiques et la future performance. En comparaison avec un contrôle, le fructose n'a pas amélioré la récupération musculaire, a limité le stockage glycogénique total et a diminué la performance. Dans une troisième étude, nous avons étudié l'effet de l'ingestion de glucose-fructose durant l'entraînement sur le métabolisme du lactate, au repos et à l'exercice, avant et après entraînement. S'entraîner avec du glucose-fructose a augmenté la production, la consommation et l'oxydation de lactate plasmatique au repos mais pas à l'exercice, tandis qu'une part importante de ce lactate était dirigée vers des devenirs non-oxydatifs.

En résumé, nos résultats indiquent un rôle ambivalent du fructose, efficace pour nourrir le muscle à l'exercice, mais inefficace en récupération. Ceci pourrait être largement dû à la circulation du fructose en lactate, donnant un carburant alternatif à l'exercice qui cependant coûte de l'énergie lors de son restockage au repos. Ce « cycle de Cori inversé » pourrait être un important mécanisme par lequel les effets du fructose sont reliés à l'activité physique.

II. Remerciements

Ces années au Département de Physiologie ont été faites de hauts et de bas, et c'est grâce à un certain nombre de personnes que j'ai pu réaliser ce travail.

Ainsi, je voudrais remercier les membres de mon jury pour leur bienveillance, et tout particulièrement Luc, à qui j'exprime ma profonde gratitude pour m'avoir permis de me former dans un domaine qui me passionne. Merci pour ta patience, ta confiance et la liberté laissée. J'ai beaucoup appris à tes côtés durant ces années, qui m'ont apporté une expérience sans égale. Un vibrant hommage aussi à Philippe, pour tes qualités humaines et la rigueur que tu inspires, ainsi que pour m'avoir ouvert les portes du Département de Physiologie.

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Une immense reconnaissance à France, qui m'a accompagné depuis le début par son amour et m'a toujours soutenu, au point d'être devenue une experte du fructose. ☺

Enfin, cette thèse est dédiée à Michel à qui je dois toute ma passion pour les sciences, et à Marie-Claude qui serait fière de me voir finir cette aventure quand elle en vit de plus belles.

III. Abbreviations

Acetyl-CoA and Acyl-CoA: Acetyl-coenzyme A and acyl-coenzyme A

ATP, ADP and AMP: Adenosine tri-, di- and monophosphate

AMPK: AMP-kinase

ChREBP: Carbohydrate response-element binding protein

CO_2: Carbon dioxide

FAT/CD36/FABPpm: Fatty acid transporter cluster of differentiation 36/Fatty acid binding proteins

FBP: Fructose-bisphosphatase

FFA: Free fatty acids

GK: Glucokinase

GLUTs: Glucose transporters

HK: Hexokinase

IMCLs: Intramyocellular lipids

LDH: Lactate dehydrogenase

LPL: Lipoprotein lipase

MCTs: Monocarboxylate transporters

NAD^+ and NADH: nicotinamide dinucleotide oxidized and reduced

PFK: Phosphofructokinase

PHOS: Glycogen phosphorylase

P: Phosphate

PK: Pyruvate kinase

SGLT-1: Sodium-glucose co-transporter 1

SREBP1c: Sterol Regulatory-Element Binding Protein 1c

TG: Triglycerides

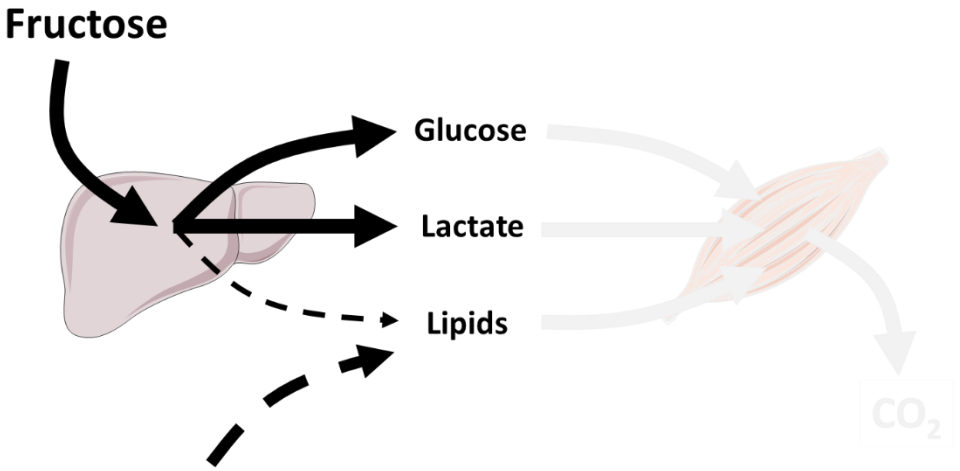
VLDL-TG: Very-low density lipoproteins bound triglycerides

VO_{2max}: Maximal oxygen consumption

$\Delta G°'$: Gibbs free energy change

IV. Fructose Metabolism

Fructose Metabolism: A Natural Sugar with Specific Effects



a. A general perspective

Carbohydrates have played a pivotal role in the co-evolution between plants and animals. This is visible with short-chain carbohydrates (i.e. 1-2 monomers, defined as sugars) in fruits and nectars, being specifically produced by plants for offering gifts to partner pollinators and/or seed dispersers animal species (Gonzalez-Teuber and Heil 2009). Natural sugars essentially consist in glucose and fructose, either separate or bound altogether as sucrose (i.e. sugar).

The ability to detect, digest and metabolize plants' carbohydrates also shaped animal physiology. Accordingly, the most frequent carbohydrate on Earth, glucose serves as an essential energy source for animals, in which it can be ingested, digested, absorbed and stored in glycogen granules (Campos and Tappy 2016). In mammal species, glucose can also be sensed, and it may have allowed for the development of the human brain (Hardy et al. 2015). Carbohydrates such as glucose have thus led to multiple adaptations in all living animals.

In contrast to glucose, fructose ingestion is more specific to frugivorous and omnivorous animal species. In these groups, the ingestion of fruits (but also of derived products like honey) can result in fructose intake being considerable, but limited in time to fructification periods. Thus, carbohydrate intake of our hunter-gatherers' ancestors typically consisted in a sugar-rich and thus fructose-rich diet during summer, and a high-protein/moderate-fat diet during winter (Tappy and Le 2010). The domestication of cooked cereals in the Neolithic then resulted in year-long availability of starch and its hundreds to thousands glucose molecules (Carmody et al. 2016), and humans adapted to the consumption of a carbohydrate-rich diet based on glucose, but in which fructose was present in low amounts (Tappy and Le 2010).

It is only since a few centuries that the development of trade, followed by extraction and refining inventions started to increase sugar (and thus fructose) availability. Yet, sugar

remained a costly product until the 19 and 20th centuries, when industrialization allowed to largely increase its availability and lower its cost, leading to the production of an infinite number of mixtures. Consequently, availability no longer limited sugars consumption, which has increased by one to two orders of magnitude in few generations (Tappy and Le 2010).

The main consequence of the increase in sugars consumption was to increase dietary fructose. From less than 5 g·d^{-1} at the beginning of the 18th century, fructose intake was estimated to have increased up to 49 g·d^{-1} at the beginning of the 21th century, with only 8 g·d^{-1} still deriving from natural products (Marriott et al. 2009). In turn, since fructose is nearly always consumed together with similar amounts of glucose, total sugars intake may be closer to ≈100 g·d^{-1}, representing 400 kcal (or 20% of a 2000 kcal·d^{-1} diet) (Welsh et al. 2011).

b. Overview of fructose metabolism

Fructose, or levulose, is mostly found as its D-fructose enantiomer equilibrated in solution between α-D-fructopyranose and α-D-fructofuranose. Of similar chemical formula ($C_6H_{12}O_6$; M_w: 180.16 g·mol^{-1}) as glucose or galactose, fructose however distinguishes by a ketone group that favors glucose and fructose association as sucrose (Hanover and White 1993). This ketone group may favor amino acids glycosylation by the Maillard reaction (Krajcovicova-Kudlackova et al. 2002), although its significance *in vivo* remains debated (Semchyshyn 2013).

Having been previously described in detail (Mayes 1993, Tappy and Le 2010), fructose metabolism will only be shortly depicted here. In the mouth, sugars fermentation by oral bacteria can increase dental plaque and carries risk (Frostell 1973), leading the World Health Organization to recommend to limit dietary sugars below 10% of total energy intake[1]. The

[1] http://www.who.int/mediacentre/news/releases/2015/sugar-guideline/en/

mouth is also acting as a first sensory interface between dietary carbohydrate and the organism that is then pursued along the digestive tract.

Fructose is sensed as sweeter than glucose (Nelson et al. 2001), leading to the suspicion that it may specifically stimulate appetite, may impair satiety and/or act as an addictive substance (Bray 2007). Remaining controverted (Ochoa et al. 2015), these aspects will not be further discussed here. Oral receptors may however play additional roles in metabolic regulation and exercise performance, as was illustrated in mouth-rinsing studies. Glucose presence in the mouth was shown to activate brain regions associated with reward and improved exercise performance (Chambers et al. 2009). Whether this may also be observed with fructose remains unknown, yet, since both sugars induce different neural responses (Luo et al. 2015).

The mouth is also the initiator of carbohydrates digestion. Sucrase, the enzyme responsible for sucrose hydrolysis into glucose and fructose, is present in saliva in which its content can increase upon frequent sugar consumption (Karjalainen et al. 1989). Gastric emptying seems sugar-specific and can also be increased upon frequent fructose consumption (Yau et al. 2014). These were considered adaptations toward a faster presentation of sugars to small intestine disaccharidases, which perform most of sugar digestion (Elias et al. 1968). Sucrose was suspected to induce distinct effects than free fructose and free glucose (provided in a ≈1:1 ratio in high-fructose corn syrups or in fruits). However, recent data suggest that sucrose digestion should be considered non-limiting, both at rest or during exercise (Wallis and Wittekind 2013). Sugar ingestion then results in a rapid substrates delivery to enterocytes.

Unlike glucose, which is actively absorbed through a sodium-mediated co-transport system (SGLT1), fructose intestinal absorption occurs passively through a low affinity transporter GLUT5 (K_M = 6-10 mmol·L^{-1}). One consequence is that fructose absorption is limited, with even

low doses causing fructose malabsorption (Douard and Ferraris 2013). However, gastrointestinal symptoms can be much diminished upon habituation (Douard and Ferraris 2008), or when fructose is co-ingested with glucose (Truswell et al. 1988). Interestingly, how glucose can favor fructose absorption is unknown, but the effect is maximal at an isomolar ratio (Latulippe and Skoog 2011). During exercise, glucose and fructose co-ingestion was shown to increase carbohydrate and fluid absorptions (Shi et al. 1995), and this was proposed a consequence of both hexoses using distinct main transport systems (Jeukendrup 2014). Yet, sugars absorption may be more complex with other transport systems, ion channels and intestinal metabolism also involved (Chen et al. 2016).

Fructose can be metabolized by the gut, which possesses all the required enzymes (Haidari et al. 2002). Yet, the importance and significance of intestinal fructose metabolism both remain uncertain (Iizuka 2017). Intestinal carbohydrate metabolism may affect food intake and hepatic metabolism through portal sensors (Mithieux and Gautier-Stein 2014). These topics, as well as the gluco-incretins responses elicited by fructose and glucose (Luo et al. 2015), will require further research. After absorption, sugars monomers are released in the portal blood.

The liver is the prime site of fructose metabolism. This is due to hepatic positioning in direct prolongation of the gut, forcing ingested carbohydrates and amino acids to pass through the liver before appearing in the systemic blood. Invasive animal experiments indicated that gradients in fructose concentrations between prehepatic and posthepatic blood can be large (Topping and Mayes 1971). In humans, a dose of 69 g sucrose elevated systemic fructose concentration, from virtually zero to only ≈0.3 $mmol \cdot L^{-1}$ (Le et al. 2012). In comparison, the increase in plasma glucose was much larger, and it is considered that >90% of fructose, as compared to only 15-30% glucose, is extracted by the liver at first-pass (Tappy and Le 2010).

Splanchnic fructose extraction is also explained by the kinetics of fructose metabolism (i.e. fructolysis). After intracellular entry, fructose phosphorylation can occur through a specific enzyme, fructokinase, of which the liver isoform has an exceptionally high affinity for fructose (Diggle et al. 2009). This isoform is also expressed in the gut and the kidneys, but not in skeletal muscle, adipose tissue or the brain (Diggle et al. 2009). Accordingly, fructose metabolism in splanchnic organs was found to be extensive, even with intravenous fructose infusions (Bjorkman et al. 1989, Ahlborg and Bjorkman 1990), and the extent of fructose metabolism in other organs is considered minimal. Hence, fructose hepatic metabolism is explained by both anatomy and fructokinase activity to rapidly convert fructose into fructose-1-P.

The second specialized enzyme of fructolysis is aldolase B, cleaving fructose-1-P into glyceraldehyde and dihydroxyacetone-P. The lower activity of aldolase B than that of fructokinase (Mayes 1993), explains that active fructolysis is associated with transient increases in fructose-1-P. In hereditary fructose intolerant subjects, loss of function aldolase B mutations can cause hepatomegaly, hepatic and renal failure caused by fructose-1-P accumulation (Cox 1994). In normal subjects, however, aldolase B expression is increased upon fructose consumption (Liu et al. 2011) to result in fructose metabolism be inducible upon frequent consumption, similar to its gastrointestinal disposal.

After a last phosphorylation of glyceraldehyde into glyceraldehyde-3-P, fructose carbons then join intracellular glucose metabolism at the level of trioses-P (dihydroxyacetone-P and glyceraldehyde-3-P). Interestingly, while the overall cost of fructolysis (2 ATP) is similar to that of glycolysis up to trioses-P, none of fructolysis enzymes are regulated by cellular energy status or hormones (Tappy et al. 2013). Bypassing the main regulatory steps of glycolysis, then led to consider fructose metabolism as unregulated (Bray 2007, Moore et al. 2014).

The main outcome of hepatic fructolysis is a rapid generation of trioses-P, which can then be directed to several pathways (**Figure 1**). A knowledge of the most important fates can be obtained using stable isotopes (i.e. labelling a small fructose amount, then following labelled products appearance). Recently reviewed (Sun and Empie 2012), these studies indicated that fructose carbons mainly recirculate as glucose (gluconeogenesis, ≥50%) and lactate (lacticogenesis, ≈25%), with much smaller amounts also converted into fatty acids (*de novo* lipogenesis) and glycerol (glyceroneogenesis) assembled as triglycerides (TG). In turn, non-circulating fructose carbons were assumed to be stored in the liver, either as glycogen (glyconeogenesis, ≈15%) or as TG that could later be secreted in the circulation. Total fructose oxidation, a surrogate for fructose direct oxidation and oxidation of fructose products (inseparable by current isotopic techniques), can cover ≈45% of fructose carbons over 6h. Importantly, these values should be viewed indicative of fructose disposal in overnight-fasted, resting subjects receiving a pure fructose load and can largely vary (Sun and Empie 2012).

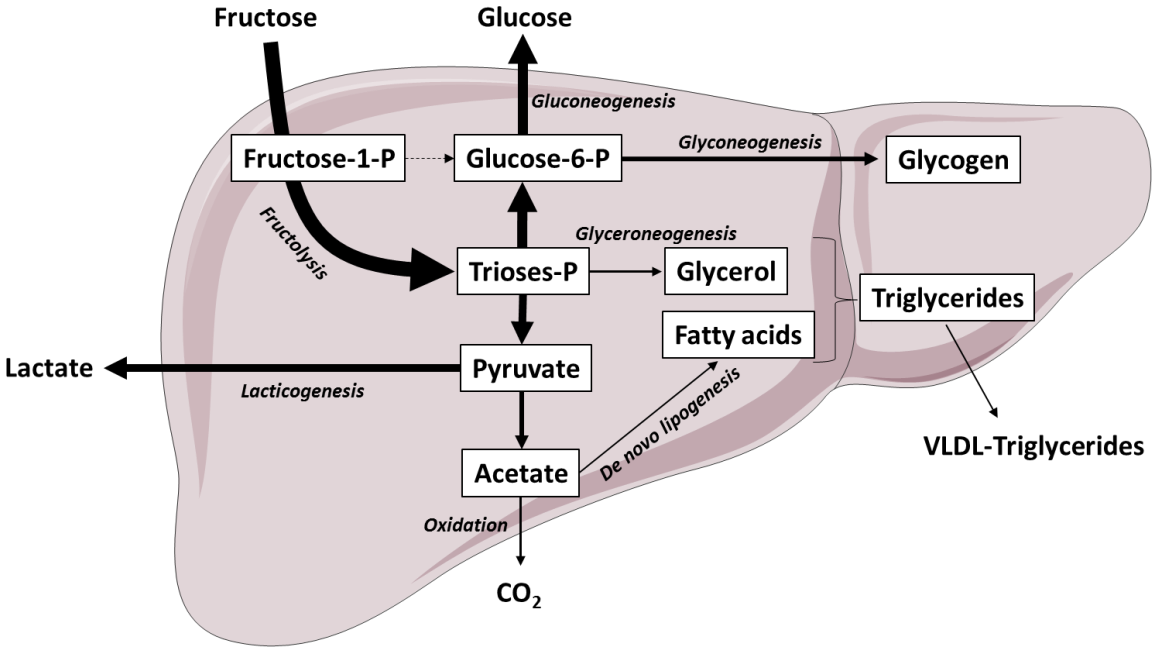


FIGURE 1: HEPATIC FRUCTOSE METABOLISM IN RESTING SUBJECTS

Fructose disposal is explained by liver function (Wasserman and Cherrington 1991, Moore et al. 2012). Briefly, the liver acts as a master metabolic organ by simultaneously consuming and producing substrates, both for its own energy needs and for those of other organs. The liver plays a main role in blood glucose control, with two complementary pathways (gluconeogenesis and glycogenolysis) be activated to increase hepatic glucose production and with two opposite pathways (glycolysis and glycogenesis) allowing glucose preferential and liver glycogen repletion. Hepatic glucose balance is known to be complexly regulated, principally by glucose, insulin and glucagon (Unger 1976), to result in glucose consumption during excess blood glucose availability and glucose production in case of blood glucose need.

Liver glucose metabolism is also interconnected with other pathways, and the balance of fluxes between glycolysis and gluconeogenesis can direct trioses-P deriving from fructolysis toward several endproducts. The liver is also central in fat metabolism through esterification of blood free fatty acids into intrahepatic TG either stored or released as VLDL-TG. Besides insulin and glucagon, hepatic metabolism is also under control of other hormones (f.eg. catecholamines), nervous signals or metabolic precursors' concentrations, with all signals susceptible to alter fructose disposal (Wasserman and Cherrington 1991, Moore et al. 2012).

Fructose can also catalyze liver metabolism. The fructolytic intermediate fructose-1-P was indeed shown to allosterically act on key enzymes to alter hepatic glucose balance (Mayes 1993). The fact that this resulted in blood glucose entry and storage as liver glycogen, made fructose be viewed as a "door-opener" for glucose metabolism (McGuinness and Cherrington 2003). Interestingly, a reverse effect was also reported with glucose also favoring fructose direction into liver glycogen by unclear mechanisms. Such synergies between glucose and fructose altogether (and thus of sucrose) can considerably increase hepatic glycogen synthesis

(Parniak and Kalant 1988, Coss-Bu et al. 2009), and fructose was also found to increase glucose production, resulting in a pseudo hepatic insulin-resistance state (Dirlewanger et al. 2000), that may be viewed as a means to distribute carbohydrates to the periphery (**Figure 2**).

Another effect of co-ingested glucose may be to activate fructose conversion into lactate. Indeed, the mass action of glucose molecules entering the liver, together with a high insulin:glucagon ratio, may both be suspected to channel trioses-P into pyruvate then lactate (Wasserman et al. 1991). Interestingly, while a study in rats (Underwood and Newsholme 1965) indeed corroborated such theoretical assumptions, no enhancement in lacticogenesis from fructose was detected in healthy, resting volunteers ingesting fructose-comprising mixed-meals supplemented with glucose (Theytaz et al. 2014). One possibility could be interference by other constituents of the mixed-meals in that study.

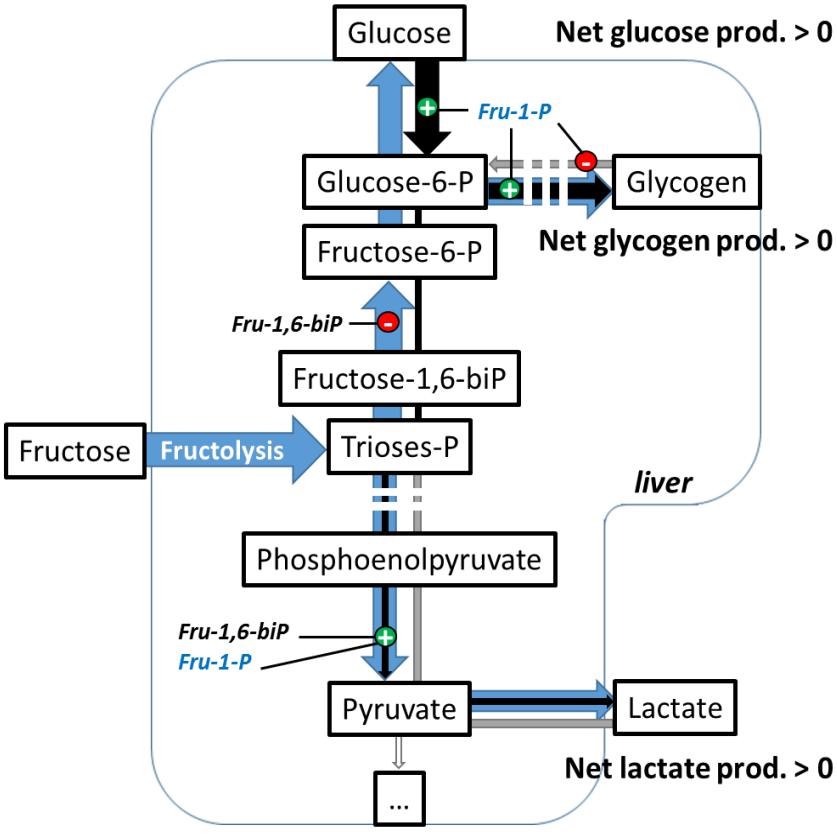


FIGURE 2: EFFECTS OF FRUCTOSE ON HEPATIC GLUCOSE METABOLISM IN RESTING SUBJECTS

Glycolytic intermediate themselves were also pointed to modulate glycolysis and gluconeogenesis fluxes. The repletion of fructose-1,6-biP by fructose carbons was considered to block further glycolysis at the phosphofructokinase step. Both fructose-1-P and fructose-1,6-biP were also shown to increase the activity of L-pyruvate kinase, thus channeling part of fructose carbons to pyruvate and lactate (Eggleston and Woods 1970). Initially reported in animal models, these allosteric mechanisms have further been observed in humans (Tounian et al. 1994, Paquot et al. 1996, Dirlewanger et al. 2000), confirming that fructose induce a large reorganization of hepatic glucose metabolism independently of the effects of hormones.

Fructose exerts profound effects on fat metabolism. This is considered partly a consequence of the interconnection of liver metabolic pathways (Bizeau and Pagliassotti 2005), with fructose-induced pyruvate winning out competition with fatty acids for mitochondrial oxidation (see below), and with unoxidized fatty acids being then esterified as intrahepatic TG. Besides a redirection of existing fatty acids, fructose conversion into fat is also considered to specifically affect fat metabolism. This was shown with a fructose-comprising mixed-meal inducing more *de novo* lipogenesis and glyceroneogenesis than an isocaloric glucose-containing meal, leading to increased postprandial VLDL-TG concentrations (Chong et al. 2007). Similarly, fructose metabolism in the gut can also lead to chylomicrons-TG secretion, and it remains unclear how both processes are regulated (Steenson et al. 2017).

Chronic effects of fructose may result from the induction of key transcription factors such as Sterol Regulatory-Element Binding Protein 1c (SREBP1c) and Carbohydrate Response-Element Binding Protein (ChREBP). Compared to glucose, fructose indeed increases more SREBP1c and ChREBP expression (Koo et al. 2009), and this is known to induce the genetic expression of GLUTs, fructokinase, aldolase B, triokinase, L-pyruvate kinase, glucose-6-phosphatase, acetyl-

CoA carboxylase and fatty acid synthase, that are all key proteins of liver metabolism. This may again collectively be viewed as an adaptation to favor fructose distribution into glucose, lactate and fatty acids products (Iizuka 2017), consistent with a 4-week high-fructose diet indeed increasing fasting glucose, lactate and TG concentrations (Le et al. 2006). Yet, other changes in liver function were also reported (Schaefer et al. 2009), and the long-term outcome of fructose exposure remains unclear. High-fructose diets can lead to intrahepatic TG accumulation (Ngo Sock et al. 2010), eventually leading to hepatic steatosis, and it is unclear how fructose influences hepatic TG metabolism between storage and secretion as VLDL-TG.

c. Overview of the metabolic effects of fructose

As a direct consequence of its hepatic metabolism, fructose ingestion has been long-known to induce no postprandial raise in blood glucose or insulin concentrations (Young 1957). In turn, it remains unclear if the low plasma insulin may also participate in increasing postprandial TG concentrations, another long-known effect of fructose, by limiting adipose tissue TG clearance (Chong et al. 2007). Similarly, fructose has been reported for decades to increase postprandial lactate and uric acid, but to lower plasma free fatty acids (FFA) (Froesch et al. 1965), resulting in a typical distinct profile of circulating substrates than glucose ingested in a meal.

This pattern of circulating substrates made fructose be viewed as a metabolic switch (Mayes 1993). Interestingly, fructose was shown to induce a high thermic effect (Schwarz et al. 1989) together with increased postprandial carbohydrate oxidation, but lowered fat oxidation compared to the rates observed after glucose ingestion (Tappy and Jequier 1993). Possibly related to fructose-induced hyperlactatemia (Brundin and Wahren 1993), these effects would however not be predicted by postprandial insulin concentrations. The low insulin coupled to high plasma TG concentrations was also suspected to induce redirection of circulating fat away

from the adipose tissue, promoting ectopic fat accumulation (Bray et al. 2004). In turn, others noticed in rodents that, while high-fructose diets increased fructolytic enzymes, they also downregulated those of glycolysis in the liver (Koo et al. 2008) and in muscle (Mayes 1993).

These effects may only reflect metabolic adaptations to a nutrient, especially since they generally remain within the physiological range (i.e. beyond pathological thresholds). Alternatively, since some of the effects of fructose (i.e. loss in hepatic and peripheral insulin sensitivity, raised fasting and postprandial TG, and raised ectopic fat (Le et al. 2009) have all been separately classified as non-communicable diseases risk-markers (Kolderup and Svihus 2015), it is unknown if fructose metabolic effects can turn detrimental to some point.

A decade ago, a seminal publication suggested an association between high-fructose corn syrup consumption and obesity prevalence (Bray et al. 2004). A link between fructose and noncommunicable diseases was made, in which fructose was suspected to be particularly detrimental to health and responsible for the “pure, white and deadly” reputation of sugar (Yudkin 1967). In addition to fructose metabolism, doubts were casted by animal data showing massive fructose doses inducing various deleterious effects (Hwang et al. 1987, Bizeau and Pagliassotti 2005). Epidemiological associations between sugar/fructose intake and various pathologies (Choi et al. 2010, Malik et al. 2010, Fuchs et al. 2014) and all-cause mortality (Barrington and White 2016), suggested an effect either secondary to weight gain (Mozaffarian et al. 2011), or through independent disease risk factors (Pollock et al. 2012). Yet, other epidemiological studies also reported that sugar from fruits was rather associated with a better health (Siegel et al. 2012) and the debate is continuing, focusing strong attention.

One consequence has been a better knowledge of the many factors influencing fructose metabolism. Unlike very large fructose overfeeding that was reported to induce specific

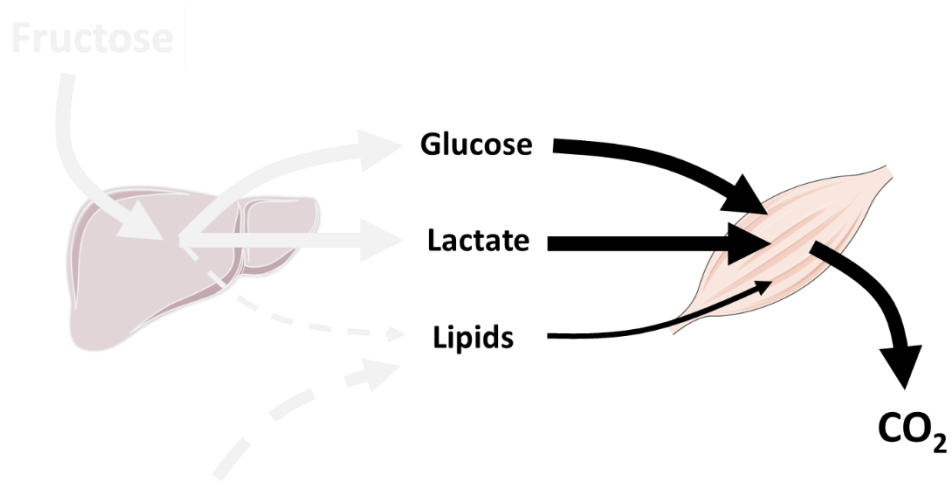
alterations (Seyssel et al. 2016), isocaloric substitution trials did generally not notice any pathological effect (Choo and Sievenpiper 2015), indicating a clear role of energy balance. Others found that, besides co-ingested glucose (see above), metabolic effects of fructose were modulated by amino acids (Bortolotti et al. 2012, Theytaz et al. 2012), fish oil (Faeh et al. 2005), polyphenols (Hokayem et al. 2013) or other constituents of coffee (Lecoultre et al. 2014). Women were shown to be somehow protected against the effects fructose overfeeding compared to men (Couchepin et al. 2008) and, in some cases, fructose could even be used as a tool to improve glucose control in diabetics, to increase alcohol metabolism or to improve mineral balance (Tygstrup et al. 1965, Holbrook et al. 1989). Other potent beneficial effects include slightly elevated uric acid concentrations acting as an antioxidant, or alleviated neuro-endocrine stress responses, that will require future studies (Tappy and Le 2015). Interestingly, these interactions were studied at rest, in which fructose is merely conserved within the body.

Another area in which fructose has found some interest is the sports field. It is indeed well-known that athletes intuitively choose (Burke et al. 2001) and are recommended (Cermak and van Loon 2013) to consume large amounts of energy-dense, ready-to-use food items typically rich in sugars. Sugars and fructose intake can then be large, leading to the ingestion of several hundred-grams by Tour de France cyclists (Saris et al. 1989) as a performance enhancer. Yet, if fructose was “toxic” (Lustig et al. 2012) should these habits be stopped or, alternatively, should be understood to determine when fructose effects turn from pathological to beneficial.

At the end of this overview, fructose appears as a natural nutrient that can cause specific effects through a complex metabolism. The following will consider fructose as a nutrient inducing physiological, non-pathological metabolic effects in a context of exercise. To be understood, these effects first require an overview of muscle metabolism.

V. **Exercise Physiology**

Exercise Physiology: Integrated Muscle Energetics



a. Overview of skeletal muscle energetics

From a metabolic standpoint, skeletal muscle is a remarkably plastic organ, able to increase its energy needs up to 1000-fold during exercise compared to resting conditions. This extremely high metabolic plasticity implies that muscle can be considered as the main driver of whole-body metabolism not only during exercise, but also during recovery. In turn, muscle metabolism requires an exquisite regulation both at the local and systemic levels, and peripheral fatigue has been associated with an overall failure to match energy requirements (Meeusen and Roelands 2017). This chapter will consider a few aspects of muscle energetics (that can also apply to non-muscle cells), before delineating some of its limitations.

Energy required by muscle contraction is obtained by the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate (P_i). Interestingly, body ATP stores are very small (≈8 $mmol \cdot kg^{-1}_{WW}$) despite of very high ATP turnovers at rest and even more during exercise, with many mechanisms supporting ATP resynthesis. One example is the phosphagen system that can buffer temporary decreased ATP concentrations by successive transfers of high energy phosphates. Yet, phosphagen stores are also small (≈26 $mmol \cdot kg^{-1}_{WW}$ at rest) and would still limit muscle energetics if intermediates of this system could not allosterically activate key cytosolic enzymes of a second system (Baker et al. 2010).

The second ATP production system through cytosolic glycolysis can be activated within seconds. Starting with glucose (or glycosyl-units) consumption, glycolysis ends with two pyruvate molecules generated through the sequential action of several enzymes. The net result is that glucose and NAD^+ is consumed to produce 2 moles pyruvate, 2 moles NADH and 2 moles ATP that can furnish ATP stores. Unlike the liver, muscle does not express the glucagon receptor (Maharaj et al. 2012), and glycolytic flux is principally locally regulated.

In man, muscle glycolysis seems to be rate-limited by phosphofructokinases (PFK) activity, being allosterically stimulated by AMP and ADP but inhibited by ATP (Newsholme 1971). The presence of exact opposite influences acting on the reverse enzyme, fructose-2,6-bisphosphatase (FBP), imply that the whole pathway depends on local energy status. Control of the PFK step can thus allow muscle to adapt its ATP needs, with these enzymes being also regulated by training (Jensen and Richter 2012).

A second major cytosolic enzyme is glycogen phosphorylase (PHOS), which controls the rate-limiting step of glycogenolysis. PHOS varies in its phosphorylation state between phosphorylase b (less active, favored by ATP) and phosphorylase a (more active, favored by AMP, contraction). PHOS can also be activated by sympathetic activation that simultaneously inhibit glycogen synthase, the key enzyme of glycogenesis also balancing between synthase a (active) and synthase b (less active) (Chasiotis et al. 1982). Since insulin can contrariwise stimulate glycogen synthase activity and diminish PHOS activity, this results in glycogen breakdown during stress situations, and glycogen synthesis after meals (Cross et al. 1995).

Interestingly, muscle glycogen is known to be simultaneously degraded (delivering 1 ATP) and synthesized in many situations, with a potent role in muscle energetics (Shulman and Rothman 2001). How this glycogen cycle is regulated during carbohydrate-fed exercise remains unclear, yet. Another regulatory factor is glycogen content itself, with a high content promoting glycogenolysis and a low content limiting glycogen breakdown during exercise (Hargreaves et al. 1995). In turn, this system can rapidly provide glucose-6-P to glycolysis, thereby raising ATP production when local energy status is insufficient or in situations of fight or flight.

Local energy status can also be sensed by other mechanisms. An example is AMP-kinase (AMPK) that, when activated by a high AMP/ATP ratio, can also activate PFK and thus glycolytic

flux. Interestingly, since activated AMPK also turns off biosynthetic pathways, it can be viewed as a molecular switch coupling anabolic and catabolic reactions to local energy status (Hardie et al. 2012). Other metabolic signals such as the NAD^+/NADH ratios may possibly be sensed (Madsen et al. 2016). A well-established effect of glycolysis is to reduce NAD^+ into NADH, with the recycling of NADH into NAD^+ either transferred to the mitochondria, or coupled to pyruvate reduction into lactate by lactate dehydrogenase (LDH).

Lactate production was viewed as a consequence of increased glycolytic flux, arising from the necessity to maintain redox status, buffer hydrogen ions (H^+) and process glycolytic pyruvate. From a stoichiometric point of view, LDH reduction of pyruvate into L-lactate is perfectly matched to these aims by consuming NADH and H^+ while regenerating NAD^+. Past research showed that muscle LDH can efficiently process pyruvate into lactate and, with a lower affinity, lactate into pyruvate (Li 1989). Once thought to be important in metabolic regulation, the role of interorgan differences in LDH isozymes was more recently minimized, considering that most LDH isozymes perform both lactate-pyruvate interconversions (van Hall 2010).

The first observations of lactate in amphibian muscle (Fletcher 1907), followed by correlations between plasma and muscle lactate accumulations and fatigue appearance (Gollnick et al. 1986) led to suspicions of lactate causing fatigue (Hermansen 1981). Lactate was then widely-popularized to cause burns or cramps synonyms of lactic acidosis during exercise. Yet, the last decades (Gladden 2004, Brooks 2016) showed that lactate is not a dead-end waste product and that lactate accumulations during high-intensity exercise are explained by pathways activities. Since maximal activities of glycolytic and glycogenolytic enzymes are higher than mitochondrial pyruvate disposal through pyruvate dehydrogenase, maximal stimulation of this system may result in lactate generation (Spriet et al. 2000). Lactate can also be implicated

in redox and pH homeostasis (Morris 2012) together with other mechanisms (Tanaka et al. 2016). Thus, the roles and regulation of muscle lactate production remain misunderstood.

In addition to cytosolic reduction into lactate, pyruvate can also be disposed in the mitochondria. This pathway depends on mitochondrial density and activity and is therefore directly related to fiber type (Peters et al. 2001). In turn, the pyruvate dehydrogenase (PDH) complex is also critically regulated by factors such as AMP, ADP and ATP concentrations to direct more pyruvate into mitochondrial conversion into acetyl-CoA in situations of energy needs. Activity of the PDH complex will not be comprehensively depicted here, neither will the subsequent mitochondrial processing of acetyl-CoA. Of importance, acetyl-CoA can then be fully oxidized to CO_2 by means of oxygen consumption as a third system generating ATP.

From the three systems, mitochondrial substrate oxidation is by far the most efficient by providing up to 15 ATP per mole pyruvate (and thus over 30 ATP per initial mole of glucose). However, this system requires oxygen and its activity was once thought to be limited by oxygen availability. Research from the last decades yet indicated that muscle oxygen tension remains generally sufficient during exercise performed at normobaric pressure, partly by compensatory vasodilation (Casey and Joyner 2011), and that mitochondrial oxidation is likely limited by other factors. Interestingly, moderate hypoxia can still dictate muscle phenotypic adaptations (Desplanches et al. 2014), with the links between oxygen, substrate oxidation and energy generation still implying that exercise intensity can be measured as a function of local and systemic VO_{2max}. Exercise intensities higher than that of VO_{2max} typically require an unsustainable ATP output and cannot be maintained longer than a few minutes. Consequently, they lead to systemic lactate accumulation (**Figure 3**), with recent evidence pointing that lactate production may account for most of glycolysis flux (Rogatzki et al. 2015).

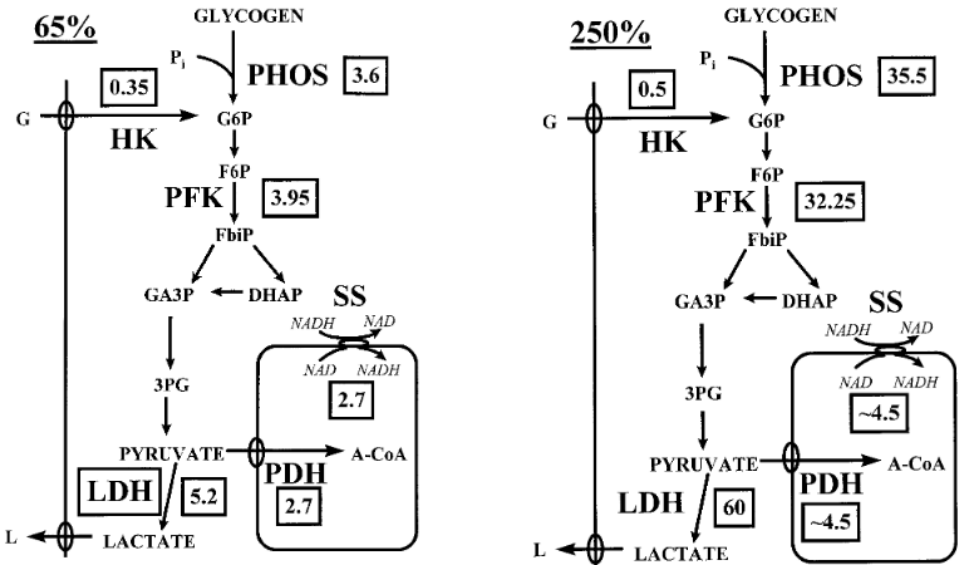


FIGURE 3: LACTATE PRODUCTION AT 65% AND 250% VO_{2MAX}. LOWER PDH MAXIMAL ACTIVITY THAN PHOS AND PFK CAN CAUSE PYRUVATE DIVERSION INTO LACTATE. SYMBOLS: G, GLUCOSE; L, LACTATE; A-COA, ACETYL-COA; SS, MALATE-ASPARTATE SHUTTLE SYSTEM; G6P AND F6P, GLUCOSE AND FRUCTOSE 6-PHOSPHATE; FBIP, FRUCTOSE 1,6-BISPHOSPHATE; DHAP, DIHYDROXYACETONE-PHOSPHATE; GA3P, GLYCERALDEHYDE 3-PHOSPHATE; 3PG, 3-PHSOPHOGLYCERATE; AND AAT, ALANINE AMINOTRANSFERASE (REPRODUCED FROM SPRIET AND HEIGENHAUSER 2002)

An aspect that has been so far here ignored is the possibility for muscle to derive energy from the blood. Expression of membrane receptors indeed allows muscle to import circulating substrates during both exercise and recovery. One example is GLUT4, of which the translocation to the plasma membrane is considered responsible for most of blood-borne glucose entry into myocytes. Interestingly, GLUT4 translocation can be induced by both insulin and contraction through separate pathways, whereas GLUT4 expression is also induced by exercise training (Richter and Hargreaves 2013). Working muscle can then obtain substantial energy from blood glucose that, by furnishing glucose-6-P pool, can inhibit muscle glycogenolysis. Accordingly, increased plasma glucose concentrations after glucose ingestion or intravenous glucose infusion could both increase plasma glucose oxidation during exercise while at the same time limiting glycogen use (i.e. glycogen sparing) (Hawley et al. 1994). This glycogen sparing effect however seems to be limited by maximal flux through hexokinase (HK), and can vary with exercise mode or duration (Tsintzas and Williams 1998).

The oxidation of fat can also provide an extensive energy source to working muscle. Like glucose, fat delivery first requires increased blood flow toward muscle beds (via vasodilation and capillary recruitment), together with sufficient plasma precursors concentrations. Fat essentially circulates as albumin-bound free fatty acids (FFA) and lipoprotein-bound triglycerides (TG), the latter requiring initial hydrolysis into FFAs and glycerol by lipoprotein lipase (LPL). The extent of circulating TG hydrolysis remains unclear during exercise due to LPL kinetics, and most of fat delivery is typically assumed as circulating FFAs deriving from adipose tissue lipolysis. Fat delivery is generally considered non-limiting during exercise compared to intracellular muscle fat metabolism (van Hall 2015).

Intramuscular fatty acids entry was long considered to occur by diffusion, until recent indications that fat can also be transported through specific FAT/CD36/FABPpm proteins. Interestingly, some of fat transporters seem to be translocated to the sarcolemma by insulin and contraction in a similar way to GLUT4, although this remains contested (Schwenk et al. 2010). Once into myocytes, fatty acids are converted into acyl-CoA either furnishing an intramyocellular lipid (IMCL) pool of triglycerides, or transported into the mitochondria. Control of muscle fat metabolism between fatty acids entry, acyl-CoA synthesis and esterification as IMCL, together with IMCL hydrolysis and acyl-CoA mitochondrial entry remains largely unclear, especially since all steps seem to be altered in diverse fashions depending on exercise modes, precursors availabilities and hormones (van Hall 2015).

Accumulating evidence supports that substrate partitioning (i.e. fuel selection) between carbohydrate and fat is exquisitely regulated at the level of the mitochondria (Romijn et al. 1993, Brooks and Mercier 1994). Fatty acid oxidation has been long-known to inhibit carbohydrate oxidation in resting conditions (Randle et al. 1963). Cytosolic fatty acyl-CoA can

indeed also be transported into the mitochondria, to increase mitochondrial concentrations of acyl-CoA. Through beta-oxidation, acyl-CoAs are then converted into acetyl-CoA, a part of which can then furnish mitochondrial and cytosolic citrate. In turn, cytosolic citrate concentrations were shown to inhibit key steps of glucose metabolism, namely transport through GLUT4, and HK, PHOS, PFK and PDH activities. The extent of this inhibition is generally considered most severe at the level of PDH, with alternative works also pointing that glucose transport may in fact be the main limiting site in human muscle (Roden et al. 1996). These mechanisms, possibly coupled to other mitochondrial events, explain that fat is the preferred substrate in any body cells under basal, unstressed conditions (Hue and Taegtmeyer 2009).

Fatty acid inhibition of glucose metabolism can be overruled in certain circumstances. In the glucose-fed resting state, elevated plasma glucose and insulin increase GLUT4 glucose transport, glycolytic flux and pyruvate oxidation. In turn, this elevates a cytosolic intermediate, malonyl-CoA, which is a strong inhibitor of fatty acid mitochondrial entry. Since insulin also favors fatty acid intracellular transport and cytosolic esterification, this results in diverting circulating fatty acids into IMCLs. This, and the effects of insulin to favor glucose entry and glycogen synthesis over glycogenolysis, result in local energy storage as both glycogen and IMCLs. Such conditions can arise during postexercise recovery, when low muscle glycogen content together with still translocated glucose transporters (among other local conditions) can further sensitize muscle to insulin action (Nagasawa et al. 1991).

During exercise, muscle contraction and systemic stress can also further modulate the balance between carbohydrate and fat oxidations. Once activated, key sensors such as AMPK strongly drive glycogenolytic and glycolytic fluxes. By favoring PDH activity, this results in increased pyruvate oxidation leading to malonyl-CoA synthesis. Unlike the fed resting state, however,

AMPK also favors malonyl-CoA degradation, resulting in restored fatty acid oxidation together with pyruvate oxidation. This system of multiple substrate switches allows to describe the dynamics of muscle fuel selection during submaximal exercise with the oxidation of fat being privileged at low-to-moderate intensity, then being lowered as carbohydrate oxidation increases with exercise intensity (Romijn et al. 1993). Furthermore, high rates of muscle glycolysis and glycogenolysis at the beginning of exercise, coupled with the time delay required to transport circulating substrates from their original organs, explain that local stores are typically used at the beginning of exercise (Romijn et al. 1993). Finally, muscle adaptations in enzymatic processes as well as decreased stress response explain that fat oxidation is increased when exercising at a given workload after training compared to pre-training.

b. Muscle as part of energy systems

Muscle metabolism should however not be seen in isolation, but from a systemic perspective. The maintenance of euglycaemia needs that glucose consumed by working muscle is replaced by equal glucose production. In unfed conditions, this is essentially performed by the liver which can mobilize its glycogen stores and synthesize glucose from gluconeogenic precursors. The result is an intensity-dependent glucose transfer with maximal reported values at ≈1.0 $g \cdot min^{-1}$ (Romijn et al. 1993, van Loon et al. 2001). This limit seems to result from endogenous glucose production, not glucose consumption (Hawley et al. 1994, Tounian et al. 1996).

In turn, the fact that lactate is also a precursor of hepatic gluconeogenesis led to the concept of an interorgan Cori cycle, in which muscle lactate production is consumed by the liver during exercise, sustaining hepatic glucose production for muscle energy needs (Simoni et al. 2002). Further work indicated that the Cori cycle is coupled to a similar cycle in which muscle alanine can also serve as a gluconeogenic precursor (Connolly et al. 1993) (**Figure 4**), to represent up

to 92% of endogenous glucose production in 40h-fasted humans (Katz and Tayek 1998). In turn, the Cori cycle was also shown to be lowered by glucose ingestion (Kreisberg et al. 1970).

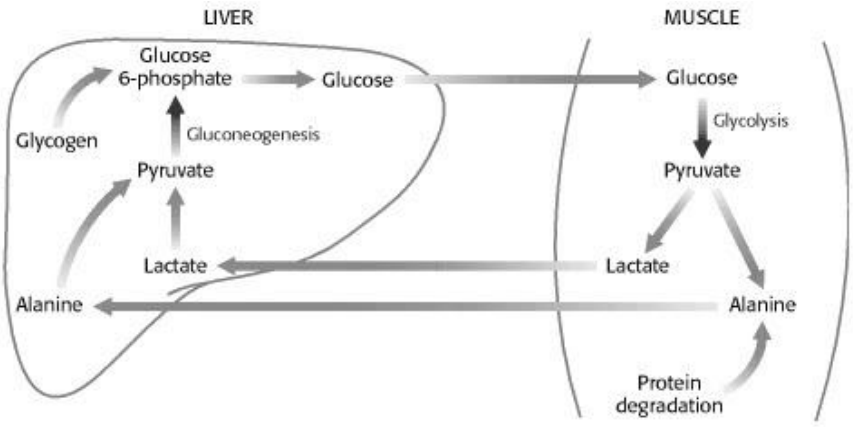


FIGURE 4: GLUCOSE-LACTATE (I.E. CORI CYCLE) AND GLUCOSE-ALANINE CYCLES BETWEEN THE LIVER AND MUSCLE DURING EXERCISE (REPRODUCED FROM CHHABRA 2014)

Research from the last decades however indicated that the Cori cycle may be a simplification, and that lactate should be viewed as an energy substrate that can be "shuttled" between cells and organs (and also possibly within cells) (Brooks 1986, Gladden 2004). Lactate shuttles were shown between cells in skeletal muscle, testes and brain tissue (Pellerin et al. 1998), between predominantly glycolytic (glycolysis > pyruvate oxidation) and oxidative (glycolysis < pyruvate oxidation) cells. Others broadened the concept to a vast system, in which lactate acts as a useful means to distribute energy between organs and in which muscle plays a central, pivotal role by turning from net lactate production into net consumption (van Hall 2010).

Lactate metabolism may also depend on specific transporters called monocarboxylate transporters (MCTs). Two members of the family, MCT1 and MCT4, were found to be specifically expressed in oxidative and glycolytic muscle fibers (Pilegaard et al. 1999). While both MCT1 and MCT4 are in fact co-transporters (lactate and a proton), they distinguish by very different affinity and saturation kinetics. Accordingly, their expression was considered to drive unidirectional lactate transport (MCT1 for lactate import and MCT4 for lactate export)

to explain muscle lactate transfers together with other MCTs, isoforms of LDH (Bonen et al. 1998) and lactate gradients. Increased muscle MCTs after exercise training (Dubouchaud et al. 2000) may partly explain that lactate clearance ability was related to VO_{2max} and sub-maximal exercise performance (Shephard 1992, Bentley et al. 2009). Others reported that MCT1 and MCT4 regulation is complex after both acute and chronic exercise, further depending on a variety of stimuli such as hypoxia, nutrition and metabolic perturbations (Thomas et al. 2012). Lactate itself was shown to stimulate MCTs and LDH expressions through binding to a specific membrane receptor (Philp et al. 2005, Brooks 2009), resulting in a feed-forward mechanism that may partly explain the adaptations observed in the training phenotype. Thus, lactate exchanges seem modulated by both systemic energy fluxes and transport-related mechanisms in a complex inter-organ system also susceptible of adaptations, to improve performance.

Fat, the most important energy source, can also be distributed between organs. Fat metabolism is however largely complicated by the circulation of both FFAs and lipoprotein-bound TG, and will only be shortly depicted here. Exercise is well-known to increase adipose tissue lipolysis, resulting in higher FFAs delivery to working muscle (van Hall 2015). The metabolism of lipoprotein-bound TG (either of intestinal or hepatic origin) remains much less understood. Circulating TG indeed require initial hydrolysis into glycerol and fatty acids then transported into cells. The enzyme performing this step, lipoprotein lipase (LPL), is known to be differently regulated close to tissues. One example is the difference between adipose tissue and muscle LPL isoforms, which are respectively activated and inhibited by insulin. In mice, this was shown to have large consequences by directing circulating FFAs and TGs to different organs when fasted or when fed (Teusink et al. 2003). Yet, since muscle LPL can also be activated by actual or past contraction, how all signals and hemodynamics coordinate to dictate fat distribution during exercise and recovery is largely unclear (Goldberg et al. 2009).

c. Muscle energy stores

Compared to adipose fat (≈100’000kcal in a typical 75kg-man with 15% fat mass), carbohydrate stores are generally assumed to be limited to ≈3000kcal, mainly as muscle and liver glycogen (Flatt 1987). In turn, since glycogen can be an important substrate source during muscle contraction, it is logically decreased by exercise and one adaptation to exercise training is precisely to increase muscle glycogen storage capacity in some fibers (Gonzalez et al. 2016).

After early indications that hypoglycemia can limit endurance performance, direct relationships between low muscle glycogen and fatigue appearance were first reported in the 1960s (Bergstrom et al. 1967). These works and many others allowed to delineate glycogen dynamics during exercise. Briefly, muscle glycogen was viewed as a store that can be lowered to a critical limit (i.e. “depletion”) below which peripheral fatigue occurs, sending nervous information to the brain. Others pointed that absolute glycogen depletion is never achieved and that the critical limit is highly variable and, in presence of between- and within-fiber heterogeneities, this model is likely an oversimplification (Gonzalez et al. 2016).

Nevertheless, the glycogen depletion model allows to describe how fatigue can also arise from liver glycogen depletion. Indeed, since myocellular glucose-6-P can result not only from muscle glycogen breakdown, but also from systemic glucose consumption, raising the contribution of plasma glucose can spare muscle glycogen. In fasted conditions, systemic glucose is essentially deriving from liver glycogen, and depletion of glycogen stores either in muscle or in the liver (causing an inability to match glucose production to glucose use) was proposed to increase relay to the other store, rapidly leading to fatigue (Gonzalez et al. 2016). Between sessions, the recovery of muscle glycogen occurs mainly from plasma glucose uptake, requiring insulin- or prior contraction-mediated GLUT4 translocation.

In comparison to glycogen, how IMCL stores affect muscle energetics and exercise performance remains largely misunderstood (Loher et al. 2016). IMCLs present some similarities to glycogen, including limited concentrations (mean 5 $g \cdot kg^{-1}_{ww}$ for a total of ≈3000kcal), a degradation during exercise and a competition with their circulating precursors as a fuel source (van Hall 2015). IMCL stores (Goodpaster et al. 2001) and IMCL turnover (Schrauwen-Hinderling et al. 2003) are both increased by training, as part of an adaptation to efficiently mobilize fat that may participate to improve endurance (Loher et al. 2016).

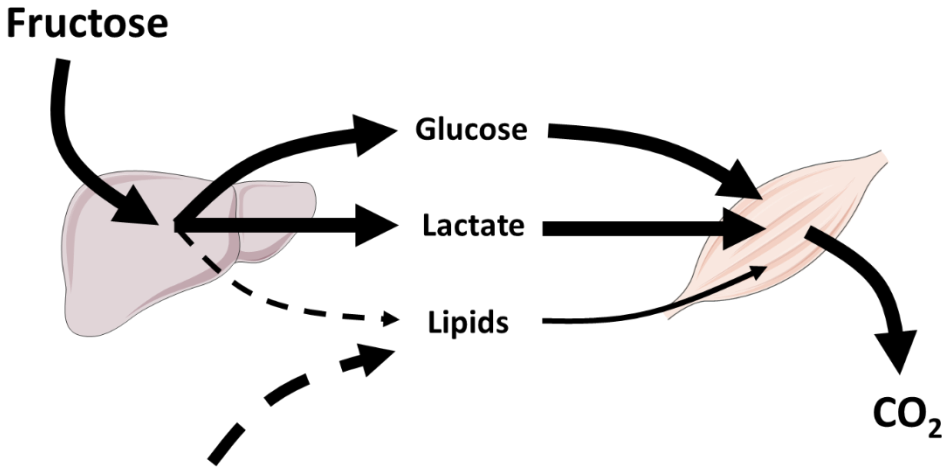
Studies in the 2000s found that IMCLs are normally replete within a few days fasting, but indicated that a faster repletion can be induced by diet (Boesch et al. 1999, Decombaz et al. 2000). Similar to high-carbohydrate diets that favor muscle glycogen repletion (Bergstrom et al. 1967), a 2006 study found that a high-carbohydrate diet supplemented with fat could restore not only glycogen, but also IMCLs stores (Zehnder et al. 2006). Interestingly, raised IMCLs were then used in proportion to content during subsequent exercise, suggesting that recovery diets increasing both circulating carbohydrates and fat precursors (dietary fat circulates as lipoprotein-bound TG) would favor optimal recovery. Yet, others found that low IMCLs did not cause premature fatigue (Larson-Meyer et al. 2008) and IMCL regulation, by involving all the steps of muscle fat metabolism (van Hall 2015), remains largely unclear.

At the end of this chapter, skeletal muscle appears to play a central metabolic role during both exercise and recovery. During endurance-type exercise, muscle needs are largely met by the oxidation of substrates from local glycogen and IMCL stores together with circulating glucose, FFA, lactate and TG. During recovery, the same circulating precursors can also favor muscle energy stores re-synthesis. The next chapter will return to fructose metabolism, discussing the situations in which fructose may be used as an exercise fuel.

VI. Sports Nutrition

Sports Nutrition: Is there a Role for Fructose?

Fructose metabolism: Is there a Role for Exercise?



a. Overview of fructose uses in sports nutrition

As mentioned in the first chapter, sugars are indeed recommended and used by many athletes especially in endurance sports (Burke et al. 2001). In turn, the last chapter noticed some limits in muscle energy furniture (low maximal uptake of plasma glucose, limited glycogen stores), that can impair exercise performance. The aim of this part is to summarize fructose uses as a tool in sports nutrition, then describe known metabolic effects of fructose during exercise, and finally evaluate how exercise may counteract side effects of fructose.

It is typically recommended that the last meal should be consumed 1-4 h before exercise, and should contain 1-4 $g \cdot kg^{-1}$ carbohydrates to ensure that glycogen stores are maximally filled (Burke et al. 2011). In such conditions, fructose improved subsequent performance compared to a non-caloric placebo (Okano et al. 1988), and it was proposed that hepatic fructose metabolism may specifically favor repletion of liver glycogen that had been lowered after an overnight fast. Fructose's low glycemic index was also supposed to protect against rebound hypoglycemia. Whether this occurs during exercise remains contested (Marmy-Conus et al. 1996), yet, and fructose and glucose elicited similar glucose control during subsequent exercise (Hargreaves et al. 1987). Interestingly, some authors suggested that sucrose may lead to different effects as a preexercise meal than separate hexoses (Wallis and Wittekind 2013).

Postexercise, most guidelines recommend ingesting ample amounts of carbohydrates in a degressive scheme (Burke et al. 2011). Fructose, glucose and sucrose have been compared only in a few studies (**Table 1**), that seemed to indicate that fructose is a poor precursor for muscle glycogen resynthesis, but can efficiently replete liver glycogen. This is consistent with fructose being primarily metabolized in the liver and will not be more commented here, only to mention that sucrose effects were grossly similar as glucose (Gonzalez et al. 2016).

TABLE 1: SUMMARY OF STUDIES THAT COMPARED FRUCTOSE, GLUCOSE AND/OR SUCROSE EFFECTS ON POSTEXERCISE LIVER AND MUSCLE GLYCOGEN RESYNTHESIS

						Liver glycogen			Muscle glycogen		
	Species	**Technique**	**Mode**	**Dose**	**Time**	**Glucose**	**Fructose**	**Sucrose**	**Glucose**	**Fructose**	**Sucrose**
Nilsson and Hultman 1974	Human	Biopsies	Infusion	21-26 mmol/kg/4h	4 h	+76 mmol glycos/kg_{ww}	+275 mmol glycos/kg_{ww}	--	+24 mmol glycos/kg_{ww}	+23 mmol glycos/kg_{ww}	--
Blom et al. 1987	Human	Biopsies	Oral	0.7 g/kg/4h	4 h	--	--	--	5.7 mmol/kg/h	3.2 mmol/kg/h	6.2 mmol/kg/h
Conlee et al. 1987	Rats	--	Oral	*Ad libitum*	2 h	+28.7 µmol/mg/h	+22.7 µmol/mg/h	--	+4.3 umol/mg/h	-0.8 umol/mg/h	--
	Rats	--	Oral	*Ad libitum*	4 h	+28.2 µmol/mg/h	+39.5 µmol/mg/h	--	+3.4 umol/mg/h	+1.3 umol/mg/h	--
Youn et al. 1987	Rats	Isotopes	Infusion	1-10 mM	1 h	--	72% label restraint	--	--	--	--
Bjorkman et al. 1989 and Ahlborg and Bjorkman 1990	Human	Catheters	Infusion	Arterial fructose: 5 mmol/L	1 h	--	+0.8 mmol/min A-V diff	--	--	+2.3 mmol/min A-V diff	--
Burke et al. 1993	Human	Biopsies	Oral	10 g/kg/24h	24 h	--	--	--	+106 mmol/kg_{ww}	+72 mmol/kg_{ww}	--
Van Den Bergh et al. 1996	Human	MRS	Oral	80 g	8 h	--	--	--	4.2 %/h	2.2 %/h	--
Decombaz et al. 2011	Human	MRS	Oral	450 g including MD	2 h	+32 mmol/L	+53 mmol/L	--	--	--	--
	Human	MRS	Oral		4.5 h	+45 mmol/L	+57 mmol/L	--	--	--	--
	Human	MRS	Oral		6.5 h	+10 mmol/L	+50 mmol/L	--	--	--	--
Trommelen et al. 2016	Human	MRS	Oral	1.5 g/kg/h	5 h	--	--	--	+54 mmol/kg_{ww}	+70 mmol/kg_{ww}	+79 mmol/kg_{ww}
Fuchs et al. 2016	Human	MRS	Oral	1.5 g/kg/h	2 h	+24 mmol/L	--	+36 mmol/L	+30 mmol/L	--	+34 mmol/L
	Human	MRS	Oral	1.5 g/kg/h	5 h	+67 mmol/L	--	+97 mmol/L	+50 mmol/L	--	+55 mmol/L

MD: MALTODEXTRIN, A SHORT-CHAIN GLUCOSE POLYMER; MRS: MAGNETIC RESONANCE SPECTROSCOPY; A-V DIFF: ARTERIOVENOUS DIFFERENCE

During exercise, current guidelines typically recommend ingesting between 1-1.5 $g \cdot min^{-1}$ carbohydrate (Jeukendrup 2014). Such rates are considered a compromise between maximum glycogen sparing (Rauch et al. 1995, Jeukendrup et al. 1999), and avoiding gastrointestinal symptoms. Accordingly, glucose ingestion was found to dose-dependently increase total glucose production, muscle glucose consumption and carbohydrate oxidation (Jeukendrup et al. 1999), causing a glycogen sparing both in the liver and in muscle. Yet, an important finding was that supra-physiological glucose infusions could elevate plasma glucose disposal to ≈2.5 $g \cdot min^{-1}$ during exercise (Hawley et al. 1994) and ≈0.7 $g \cdot min^{-1}$ at rest (Tounian et al. 1996). Since this is much higher than values observed in physiological conditions, this suggests that muscle could consume more glucose if more was entering the circulation.

Compared to glucose, fructose intestinal absorption was found to be markedly lowered by exercise (Fujisawa et al. 1993). This, and the effect of fructose to induce no postprandial glycemic peak, led to consider pure fructose of poor interest during exercise (Bjorkman et al. 1984). Accordingly, studies using isotopic labelling found that fructose was poorly oxidized during exercise (Wagenmakers et al. 1993), but interestingly found that its oxidation increased when exercising in previously fasted conditions than in the fed state (Massicotte et al. 1990).

Starting with a seminal study in 1994 (Adopo et al. 1994), another line of research noticed that fructose and glucose co-ingestion can increase total exogenous carbohydrate oxidation during exercise. Using ^{13}C-labelled sugars, these authors showed that, during exercise at 60% VO_{2max}, ingestion of 50 g or 100 g fructose led to lower $^{13}CO_2$ production than isocaloric glucose, but that a mixture of 50 g fructose and 50 g glucose increased $^{13}CO_2$ compared to 100 g glucose (Adopo et al. 1994). This effect was then considered to depend upon many factors such as carbohydrate ingestion rate, glucose:fructose ratio, exercise type and duration, training status

or gender, and was largely attributed to an improved gut absorption (Rowlands et al. 2015). Higher exogenous carbohydrates oxidation (Jentjens et al. 2004), time-trial gains (Currell and Jeukendrup 2008) and improved gastrointestinal comfort (Wilson and Ingraham 2015) then supported that glucose-fructose improve performance compared to glucose ingestion.

Ergogenic effects of glucose-fructose may also be metabolic. Besides gastrointestinal effects, a 2010 study aimed to measure plasma glucose and lactate fluxes in exercising subjects ingesting glucose-fructose or glucose-based drinks (Lecoultre et al. 2010). Interestingly, results indicated that glucose-fructose increased both glucose and lactate fluxes by respectively +10% and +30% compared to glucose ingestion (**Figure 5**). Furthermore, that study also showed that ^{13}C-fructose participated to ≈20% and ≈30% of glucose and lactate fluxes, and that the fate of fructose carbons was entirely oxidative (Lecoultre et al. 2010). These results indicate that, during exercise, an important part of fructose circulate as glucose and lactate that can fuel muscle work. One gap in that study was the involvement of well-trained endurance athletes, in which direct lactate oxidation is increased (Emhoff et al. 2013), and it was unknown if a similar lactate shuttle would be observed in exercising or non-exercising untrained subjects.

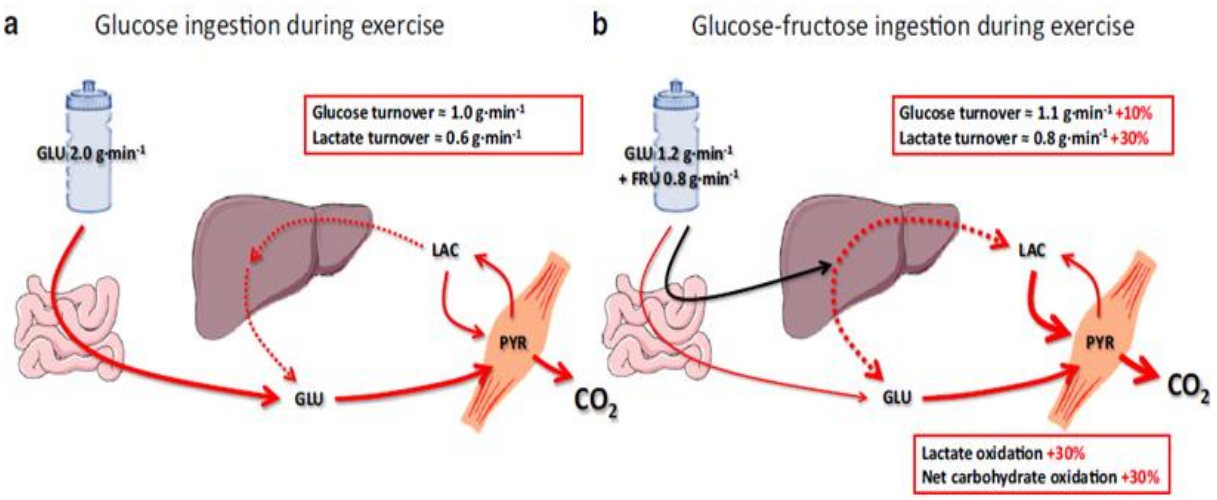


FIGURE 5: EFFECTS OF A) GLUCOSE AND B) GLUCOSE-FRUCTOSE INGESION ON SYSTEMIC GLUCOSE AND LACTATE FLUXES DURING EXERCISE AT ≈60% VO_{2MAX} (RETRIEVED FROM LECOULTRE ET AL. 2010)

Lactate may be preferred over other fuels (Brooks 1986). During exercise, lactate is indeed known to be extensively oxidized (Azevedo et al. 2007) and, like glucose, can inhibit fat oxidation. Compared to glucose, it however offers a more immediate conversion into pyruvate and is transported through separate transport systems. A few exercise experiments showed that infused lactate increased its oxidation, but also caused a ≈40% decrease in glucose oxidation (Miller et al. 2002, Miller et al. 2002). Similar studies at rest indicated that the decrease in glucose oxidation was only ≈20% in hyperinsulinemia (Paquot et al. 1995), to be related with recent findings that lactate can inhibit both HK and PFK, only in high glycolytic flux (Leite et al. 2011). Whether lactate can also inhibit glycogenolysis through attenuated catecholaminergic response (Fattor et al. 2005) is unknown, since no effect was observed *in vitro* (Lloyd et al. 2003). Hence, lactate may play a large role in fuel selection during exercise.

The effects of lactate on fuel selection may however differ in the trained state or at rest. Indeed, since training induces many adaptations in glycolytic enzymes (Baldwin 1985), how an inhibition of glucose oxidation would be affected in athletes is unclear. Moreover, some studies that infused lactate at rest noticed, unlike during exercise, that this was associated with a strong thermogenic effect (Ferrannini et al. 1993). It is therefore possible that providing muscle with a dual substrate source as both glucose and lactate results in different outcomes at rest and during exercise. In turn, if lactate derived from fructose, this could also affect energy efficiency, both in fructose-metabolizing and peripheral cells (Tappy et al. 2013). Such interactions between glucose, lactate and glycogen have so far been largely ignored and remain to be studied both at rest and during exercise, possibly by using fructose.

Lactate fates can also be non-oxidative. This was first shown in works with isolated myotubes that clearly indicated that lactate can serve as a glycogen precursor (Donovan and Pagliassotti

2000). Interestingly, such experiments also pointed that this may depend on muscle fibers type, and that glycogen formed from glucose or lactate was stored in different intramyocellular pools (Ryan and Radziuk 1995). Muscle glyconeogenesis from lactate was also implicated in post-exercise glycogen resynthesis in absence of food intake (Fournier et al. 2004), but was however estimated minor both in the fed state (when it is prevented by increased glycolytic flux) and also in the fasted state due to Cori cycle activity (Ferrannini et al. 1993). Recent evidence however indicate that lactate may contribute to muscle glyconeogenesis and glyceroneogenesis through a reversal of the pyruvate kinase reaction that would be thermodynamically more favorable than previously considered (Jin et al. 2015).

Hence, past findings suggest that glucose-fructose induce an interorgan lactate shuttle from the liver to muscle up to lactate oxidation during exercise. Since lactate plays a role in metabolic regulation (Sola-Penna 2008), and since chronic fructose can increase lactate fluxes (Abdel-Sayed et al. 2008), one could suspect lactate to be a major mediator of fructose effects.

b. Exercise protection against fructose's metabolic effects

Little is known regarding the modulation of other fructose effects by exercise. Several of fructose outcomes, including insulin resistance, ectopic fat accumulation, fasting and postprandial hypertriglyceridemia and hyperuricemia, were all classified as cardiometabolic risk factors (Kolderup and Svihus 2015). Alternatively, athletic populations that are consuming fructose in large amounts are generally metabolically healthy. This may indeed be related to exercise, that was shown to increase insulin sensitivity for 12-48 h after each session in direct relation to exercise energy expenditure (Nagasawa et al. 1991, Wojtaszewski et al. 1997).

Some authors then showed that exercise exerts hypolipemic effects that can largely lower postprandial TG concentrations after a fat meal (Magkos et al. 2008). The responsible organs

remain unknown, and it is unclear if this results from a lowered or delayed intestinal TG secretion and/or an improved blood TG clearance (Plaisance and Fisher 2014). Candidate organs in which circulating TG can be stored include the adipose tissue (i.e. the normal fat storage site) and ectopic tissues such as the liver or skeletal muscle and their IMCLs.

Fructose-induced hypertriglyceridemia (i.e. VLDL-TG), is somewhat different than dietary fat from intestine origin (i.e. chylomicrons-TG). However, both effects can combine with a fructose-and-fat mixed-meal inducing sustained high plasma TG, likely because of impaired adipose tissue TG clearance (Chong et al. 2007). A few recent studies indicated that these indirect effects of fructose could however be prevented by both endurance (Egli et al. 2013, Bidwell et al. 2014) and resistance (Wilburn et al. 2015) exercise. This may indeed be indicative of a synergy between fructose diverting dietary fat to ectopic tissues such as muscle (the low insulin favoring muscle LPL over adipose LPL activity), and previous exercise protecting against lipotoxicity and storing fat into IMCLs energy stores for the next session. These protective effects of exercise are likely dependent on energy balance and other factors, and how acute and chronic exercise could counterbalance other effects of fructose is largely unknown.

At the end of this literature overview, fructose appears as a very specific nutrient that can through its hepatic metabolism induce wide effects, some of them possibly detrimental during fructose overfeeding. Exercise is characterized by a large metabolic rate elevation due to muscle work, that profoundly alters liver function and thereby fructose metabolism. When co-ingested with glucose, fructose can efficiently fuel muscle work through a specific lactate shuttle, and may also specifically favor liver glycogen. Fructose-induced hypertriglyceridemia can be lowered by exercise. A part of this thesis was spent writing two review articles, manuscripts 1 and 2, which are mentioned next before continuing with the experimental part.

VII. **Manuscript 1:** Curr Hypertens Rep (2016) 18:44; DOI 10.1007/s11906-016-0652-7

Pathogenesis of Cardiovascular and Metabolic Diseases: Are Fructose-Containing Sugars More Involved Than Other Dietary Calories?

Robin Rosset, Anna Surowska and Luc Tappy

Contribution: Literature research, drafting and writing a part of the manuscript and figures.

Abstract: Cardiovascular diseases have become the major cause of mortality and morbidity worldwide, and were related to environmental factors such as obesity caused by specific nutrients. Of these, fructose was considered to play a large role by virtue of its hepatocentric metabolism. By activating *de novo* lipogenesis, it could increase both visceral, intrahepatic and circulating triglycerides, as well as being specifically atherogenic by acting on fatty acid saturation. In turn, it may induce hypertension by activating the sympathetic nervous system, raising plasma uric acid concentrations and act on kidneys function. Current evidence poorly supports such assertions, however, but merely suggests that most of the effects of fructose can be prevented by a healthy lifestyle. Potential future research areas include a better understanding of the functional significance of *de novo* lipid synthesis, how stress can modulate fructose metabolic effects, and how fructose or its intermediates act on the kidneys.

VIII. **Manuscript 2:** Sports Med (2017) Mar 22; DOI 10.1007/s40729-017-0692-4

Fructose Metabolism from a Functional Perspective: Implications for Athletes

Luc Tappy and Robin Rosset

Contribution: Literature research, drafting and writing of the manuscript. Figures preparation.

Abstract: Substantial amounts of fructose are present in our diet. Unlike glucose, this hexose cannot be metabolized by most cells and has first to be converted into glucose, lactate and fatty acids in enterocytes, hepatocytes and kidney proximal tubule cells, which all possess specific fructose-metabolizing enzymes. This particular metabolism may then be detrimental in resting, sedentary subjects. However, this may also present some advantages for athletes. First, since glucose and fructose are absorbed through distinct, saturable gut transporters, co-ingestion of glucose and fructose may increase total carbohydrate absorption and oxidation. Second, fructose is largely metabolized into glucose and lactate, resulting in a net local lactate release from splanchnic organs (mostly the liver). This “reverse Cori cycle” may be advantageous by providing lactate as an additional energy substrate to the working muscle. Following exercise, co-ingestion of glucose and fructose mutually enhance their own absorption and storage.

X. Working Hypothesis

Working Hypothesis: Delineating the Fructose-Exercise Relationship

a. Targeting fructose-induced hyperlactatemia and hypertriglyceridemia

The introduction provided an overview of fructose metabolism and muscle function, and the outcomes of fructose ingestion in an exercise context. The aim of the experimental part was first to better understand the mechanisms by which exercise can protect from fructose side effects, and second if new applications could derive from some of fructose effects in an exercise context. In accordance with the introduction, the experimental part largely focused on two main effects of fructose: postprandial hyperlactatemia and hypertriglyceridemia.

- Manuscript 3 (Egli et al. 2016) specifically aimed to understand the mechanisms by which exercise affects fructose metabolism. Suspecting that fructose would be efficiently used as a fuel during exercise and would be stored during recovery, we studied in a randomized order the fate of a ^{13}C-labelled oral fructose load provided either before, after, or in a no-exercise control condition.
- Manuscript 4 (Rosset et al. 2017) compared, between two exercise sessions, the effects of mixed-meals comprising fructose or glucose together with ^{13}C-labelled fat and protein on the recovery of muscle energy stores. Our hypothesis was that fructose-induced hypertriglyceridemia would specifically favor circulating ^{13}C-fat storage in IMCLs, with fructose carbons also replenishing muscle glycogen, and that this would result in an improved endurance performance during the second exercise session.

- Manuscript 5 (Rosset et al. 2017) evaluated how chronic ingestion of glucose-fructose during training sessions could cause specific adaptations in lactate metabolism. We suspected that repeated exposure to high lactate concentrations during sessions would raise glucose and lactate fluxes (assessed with $^{2}H_2$-glucose and ^{13}C-lactate), and that increased muscle lactate metabolism could further improve exercise performance.

XI. **Manuscript 3:** Am J Clin Nutr (2016) 103:348-55; DOI 10.3945/ajcn.115.116988

Exercise Performed Immediately after Fructose Ingestion Enhances Fructose Oxidation and Suppresses Fructose Storage

Léonie Egli, Virgile Lecoultre, Jérémy Cros, Robin Rosset, Anne-Sophie Marques, Philippe Schneiter, Leanne Hodson, Laure Gabert, Martine Laville and Luc Tappy

Contribution: Participation to metabolic tests, data analysis and writing of the manuscript.

This experiment involved three conditions, in which we investigated mechanisms by which exercise may prevent adverse effects of fructose. After a run-in period, a fructose load (0.75 $g \cdot kg^{-1}$) was provided to overnight-fasted men, and the response to that load was investigated: with an exercise session prior to fructose (ExFru), with no exercise (Fru) or with an exercise session after fructose ingestion (FruEx). ExFru increased energy expenditure and carbohydrate oxidation before fructose ingestion ($P<0.01$), but this prior depletion did not significantly alter postprandial fructose disposal compared to Fru (all P=N.S.). In contrast, exercise in FruEx largely altered fructose disposal (**Figure 6**), and close to 80% fructose was oxidized over 7h postprandial ($P<0.01$ vs. Fru). Fructose conversions into glucose and VLDL-palmitate, and plasma lactate concentrations were not different between conditions. In ExFru and FruEx, exercise increased energy expenditure and carbohydrate oxidation when the sessions were performed (i.e. before, after fructose) compared to Fru (all. $P<0.01$).

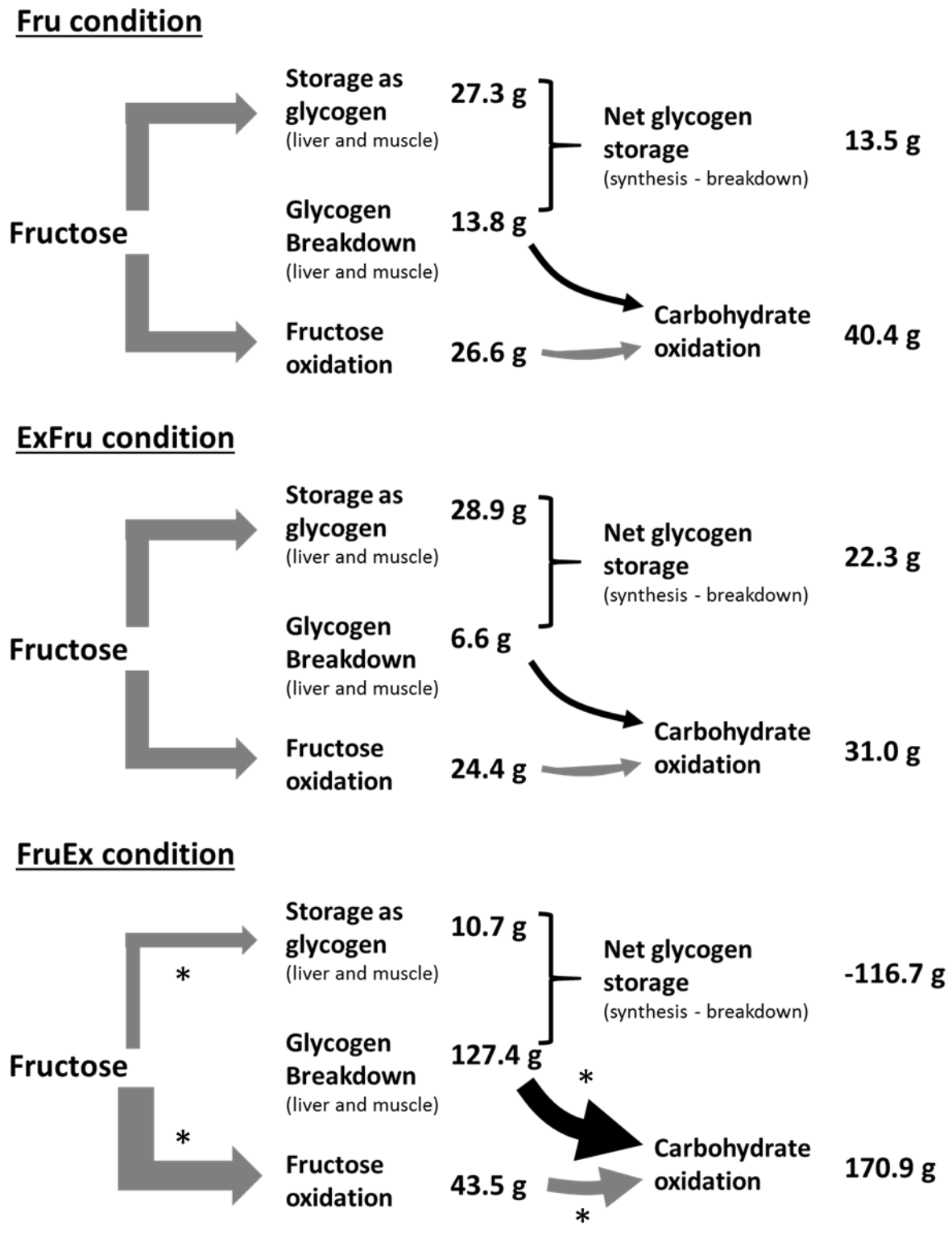


FIGURE 6: POSTRANDIAL, RESTING CARBOHYDRATE METABOLISM FOR THE NEXT 7H AFTER INGESTION OF A FRUCTOSE LOAD (0.75 G·KG^{-1}) PRECEDED OR FOLLOWED BY AN EXERCISE SESSION. *: FRUEX DIFFERENT FROM FRU (REDRAWN FROM EGLI ET AL. 2016)

XII. **Manuscript 4:** Am J Clin Nutr (2017) 105:609-17; DOI 10.3945/ajcn.116.138214

Postexercise Muscle Energy Storage Following Mixed-Meals Containing Fructose or Glucose

Robin Rosset, Virgile Lecoultre, Léonie Egli, Jérémy Cros, Ayse Sila Dokumaci, Karin Zwygart, Chris Boesch, Roland Kreis, Philippe Schneiter and Luc Tappy

Contribution: Study design, subject recruitment and metabolic tests, data analysis and drafting and writing of the manuscript.

This randomized crossover trial evaluated in trained athletes the effects of experimental mixed-meals (2.5 $g \cdot kg^{-1}$ fat, 1.9 $g \cdot kg^{-1}$ protein and 5.6 $g \cdot kg^{-1}$ carbohydrate) provided over 24h, on postexercise recovery of muscle energy stores. Replacing glucose (in GLU condition) by fructose (in FRU) was supposed to favor IMCLs repletion from dietary fat (traced using ^{13}C-palmitate), while also furnishing precursors for muscle glycogen. Yet, FRU and GLU induced similar restoration (**Figure 7**) of IMCLs (+2.4±0.4 vs. +2.0±0.6 $mmol \cdot kg^{-1}_{ww} \cdot 24h^{-1}$; P=0.45) and muscle glycogen (+10.9±0.9 vs. +12.3±1.9 $mmol \cdot kg^{-1}_{ww} \cdot 24h^{-1}$; P=0.45). Dietary fat oxidation and whole-body fat storage were also similar between conditions (unpublished results).

Interestingly, FRU increased postprandial lactate concentrations (P<0.01), that were linearly associated with glycogen recovery (r=0.75; P=0.03). FRU however also increased net carbohydrate oxidation followed over 6h postprandial (P<0.01) and decreased whole-body carbohydrate storage compared with GLU (+117±9 vs. +135±9 $g \cdot 6h^{-1}$; P<0.01). During the second exercise session performed the day after by overnight-fasted subjects, FRU lowered

plasma glucose concentrations and impaired endurance performance (P<0.01), presumably because of lower liver glycogen than in GLU.

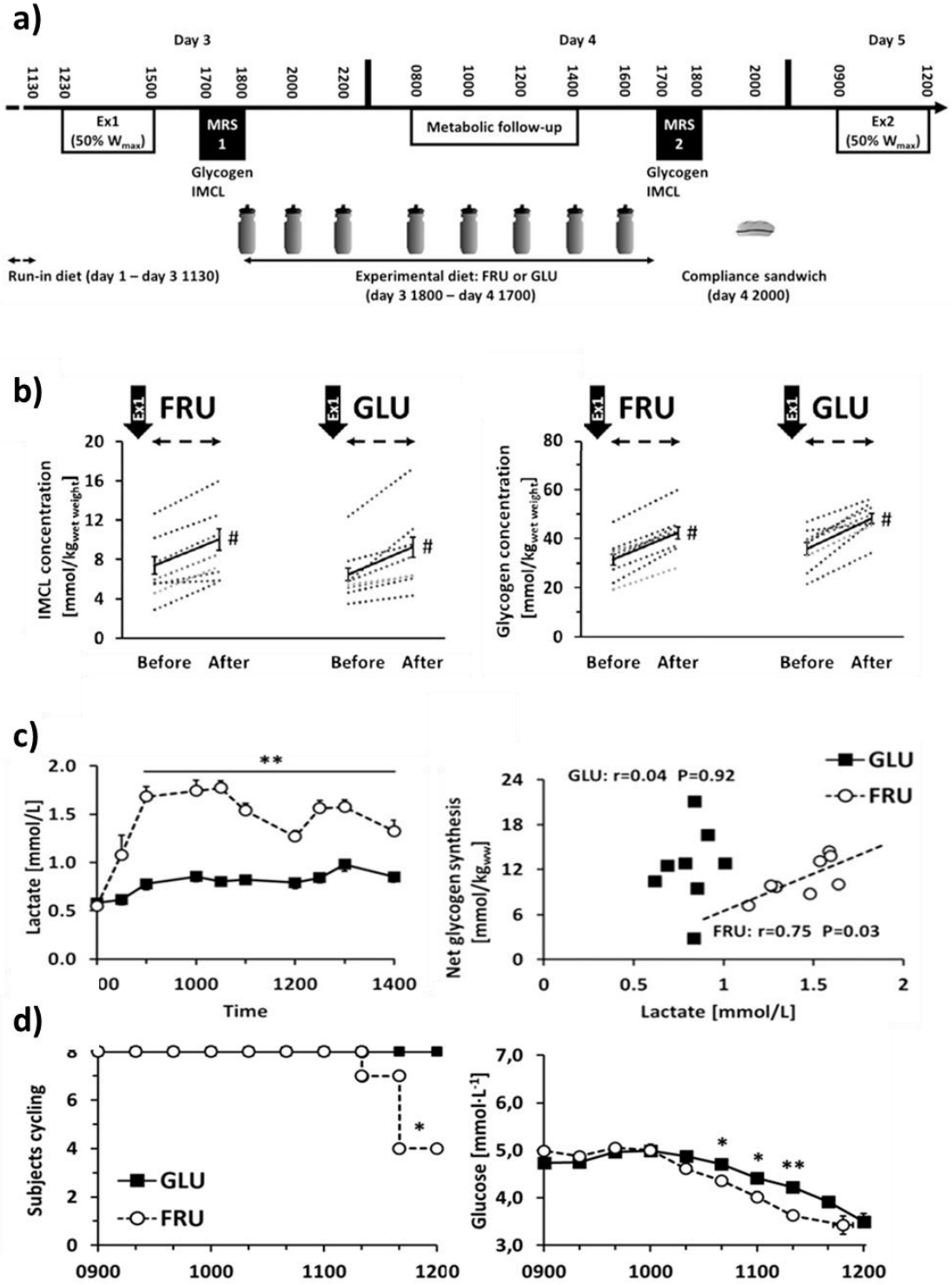


FIGURE 7: A) STUDY DESIGN; B) VASTUS IMCL AND GLYCOGEN CONCENTRATIONS INCREASED POSTEXERCISE COMPARED TO PREEXERCISE, SIMILARLY IN BOTH CONDITIONS; C) POSTPRANDIAL PLASMA LACTATE AFTER FRUCTOSE-BASED MIXED-MEALS WERE ASSOCIATED WITH 24H-POSTEXERCISE GLYCOGEN RECOVERY; D) THE DAY AFTER, FRU DECREASED PERFORMANCE AND PLASMA GLUCOSE COMPARED TO GLU. *: FRU DIFFERENT FROM GLU; #: BEFORE DIFFERENT FROM AFTER (ADAPTED FROM ROSSET ET AL. 2017)

XIII. **Manuscript 5:** Nutrients (2017) 9(4), 411; DOI 10.3390/nu9040411

Endurance Training with or without Glucose-Fructose Ingestion: Effects on Lactate Metabolism assessed in a Randomized Clinical Trial on Sedentary Men

Robin Rosset, Virgile Lecoultre, Léonie Egli, Jérémy Cros, Valentine Rey, Nathalie Stefanoni, Valérie Sauvinet, Martine Laville, Philippe Schneiter and Luc Tappy

Contribution: Study design, subject recruitment and metabolic tests, data analysis and drafting and writing of the manuscript.

This work aimed to evaluate how glucose-fructose drinks can affect lactate metabolism at rest and during exercise, pre- and post-training. After an initial metabolic evaluation with glucose-fructose drinks, two groups of sedentary men were endurance-trained while either ingesting glucose-fructose (GF intervention) or water (C intervention) drinks. By elevating lactate concentrations during training sessions, GF was expected to induce key adaptations in lactate metabolism and to improve post-training exercise performance compared to C (assessed in a second metabolic evaluation with glucose-fructose provided in both groups).

Pre-training, lactate production was increased by exercise compared to rest ($P<0.01$). Lactate disposal was mainly non-oxidative at rest, and mainly oxidative during exercise ($P<0.01$) in both GF and C. As compared to C, training with GF then increased lactate production and oxidation at rest (both $P<0.05$), but not during exercise (both P=N.S.). Both interventions had similar effects to increase ($P<0.01$) lactate clearance during exercise (+15.5 ± 9.2 and +10.1 ±

5.9 mL·kg^{-1}·min^{-1}; P=0.97), suggesting that muscle lactate transport systems were similarly improved (**Figure 8**). Both interventions also induced similar performance improvements.

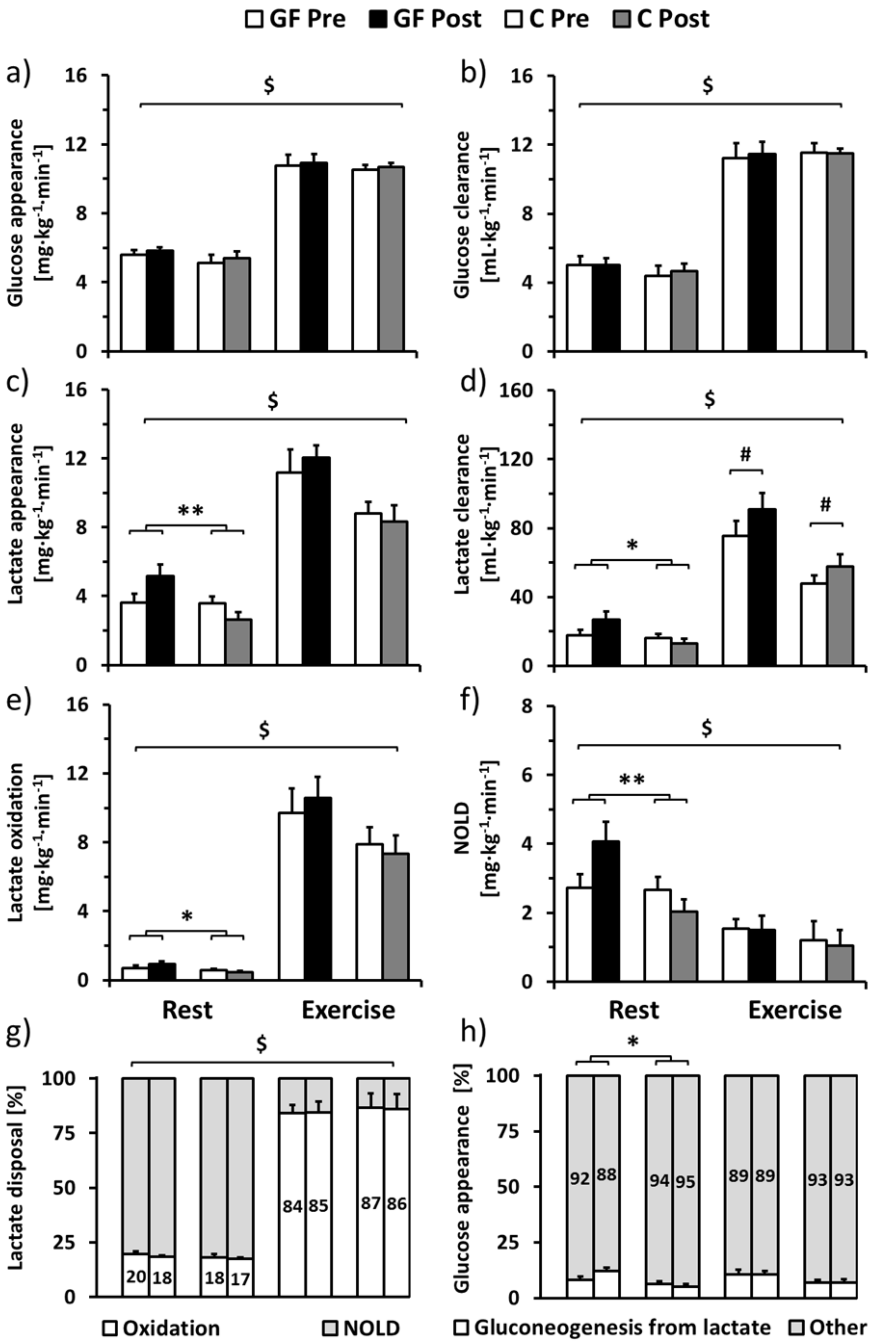


FIGURE 8: GLUCOSE AND LACTATE FLUXES AT REST AND DURING EXERCISE WITH GLUCOSE-FRUCTOSE INGESTION. A) AND B), GLUCOSE METABOLISM WAS INCREASED BY EXERCISE COMPARED TO REST, BUT WAS NOT AFFECTED BY TRAINING WITH/WITHOUT GF DRINKS. C) TO F), LACTATE FLUXES WERE INCREASED BY TRAINING WITH GF AT REST BUT NOT DURING EXERCISE, AND ALL LACTATE FATES FOLLOWED THESE CHANGES. G) AND H), INCREASED LACTATE FLUXES TRANSLATED AT REST INTO A HIGHER GLUCONEOGENESIS FROM LACTATE, WHILE A STABLE PROPORTION OF LACTATE WAS OXIDIZED DURING EXERCISE, AND DIRECTED TOWARD ENERGY CONSERVATION AT REST. $: REST DIFFERENT FROM EXERCISE; #: PRE-TRAINING DIFFERENT FROM POST-TRAINING; *: GF AND TRAINING INTERACTION (REDRAWN FROM ROSSET ET AL. 2017)

General Discussion: Lessons for Fructose Metabolism in an Exercise Context

a. An integrative model of fructose metabolism

The purpose of this thesis was to investigate how fructose metabolism can be modulated by exercise, and evaluate if some of fructose metabolic effects can improve performance. Our results suggest that fructose effects on fat metabolism were not altered by exercise, and could also not further replenish IMCL stores, questioning if there was a limitation in blood flow dynamics, in muscle fat uptake, or at any intracellular regulating point of muscle fat metabolism. The experimental protocols may also have not allowed to capture small or delayed changes in fat or IMCL metabolisms. Our results were more conclusive regarding fructose-induced hyperlactatemia and carbohydrate metabolism, indicating very different outcomes when fructose was ingested at rest or during exercise. This led to propose a simple, provisional model of fructose metabolism (**Figure 9**).

Our model is based on two fundamental rules: First, entropy or the measure of a system's disorder, is a natural law that needs to be fight by all living organisms. Second, mitochondria may derive from a former internalization of a prokaryotic into a eukaryotic cell (Martin et al. 2015), itself later organized as a pluricellular organism (giving a strict compartmentation: mitochondria<cells<organism). Each compartment may ensure its own energy homeostasis before sharing substrates with the next. Entropy would rapidly deplete all energy if uncontrolled, and varied strategies were developed to save as much energy as possible and only allowing for a limited, useful entropy to occur at a given time (Stettner and Segre 2013).

According with the previous postulate, fructose entry in fructose-metabolizing cells would result in a massive influx of energy, that would be first naturally directed downward of the energy gradient (fructose: $\Delta G^{\circ\prime} \approx 220$ kgcal in aqueous solution (Krebs et al. 1957)) to full oxidation as CO_2 (providing 31.5 $ATP \cdot mol^{-1}$). At some point, ATP generation could however find

a maximum, after which it would be controlled by the mitochondria to ensure a proper functioning. Fructose was indeed shown to alter liver ATP equilibrium (Gaussin et al. 1997, Abdelmalek et al. 2012), yet being unable to specifically determine mitochondrial ATP.

Fructose oxidation occurs as pyruvate in the mitochondria, replenishing mitochondrial intermediates, blocking fat oxidation and directing fat toward esterification (Hue and Taegtmeyer 2009). The result may be an indirect link between fructose oxidation and fat esterification (Softic et al. 2016), that may explain why high-fructose diets can cause intrahepatic fat accumulation (Bortolotti et al. 2012). In turn, raised mitochondrial intermediates would lead to energy crisis if there was no compensation. Interestingly, high ATP and high mitochondrial intermediates is perfectly adapted to *de novo* lipogenesis which, by consuming both precursors, may serve as a local buffer. Manuscript 3 indeed indicated a constant, low-grade *de novo* lipogenesis independent on metabolic rate (Egli et al. 2016), and future studies could focus on a relation between fructose oxidation and *de novo* lipogenesis.

A consequence of the coupling between fructose oxidation and *de novo* lipogenesis could be a sort of balance, after which fructose carbons may be directed to the most favorable fates according to energy gradients. Glycolysis is well-known to present a free energy gradient with three difficultly-reversible reactions. Our model then speculates that this would direct trioses-P from fructose to pyruvate and lactate. Interestingly, recent results indicate that fructose can be directed to glutamate (Varma et al. 2015), questioning if fructose may alter splanchnic amino acids fluxes *in vivo*. Fructose direction to pyruvate and lactate could then be constitutively activated when local energy homeostasis is met.

The fate of lactate produced from fructose is then to be shared in a vast system (van Hall 2010) in which it can serve as a fuel or can be disposed non-oxidatively as glucose, glycogen and

possibly lipids. Our model will not cover the complexity of lactate exchanges, but will consider thermodynamics of the whole system. Interestingly, lactate action to limit upstream glycolytic activity at the PFK step (Leite et al. 2011), may be viewed as a possible energy conservation mechanism limiting use of carbohydrates of higher free energy. Lactate seems then to be preferred over other fuel sources (Brooks 1986), and manuscript 5 indicated that ≈20% and ≈80% of lactate disposal was oxidized at rest and during exercise, respectively (Rosset et al. 2017). While this confirms that lactate oxidation depends on metabolic rate, our data also indicate that an important part of lactate is directed to non-oxidative, anabolic fates.

Accordingly, manuscript 4 indicated that postprandial lactate concentrations were associated with muscle glycogen storage, but also that whole-body carbohydrate storage was impaired by fructose ingestion (Rosset et al. 2017). Manuscript 3 confirmed that fructose was extensively oxidized when followed by exercise but, interestingly, indicated that fructose was not further stored when preceded by exercise (Egli et al. 2016). This may support that lactate from fructose is shared in a vast system of organs in which its ideal entropic fate would be oxidation, but in which each cell first considers its metabolic needs and prefers to store currently unnecessary substrates at the prize of an energy cost when escalating glycolysis. Lactate, but also acetate and beta-hydroxybutyrate infusions, have all been shown to induce thermic effects at rest when the metabolic rate was insufficient (Chiolero et al. 1993), but to our knowledge not during exercise when entire oxidation is possible.

A consequence of this model is that, if fructose conversion to lactate is actually rate-limited, then the liver should massively store fructose as glycogen and/or intrahepatocellular fat. This seems indeed to be the case, but to a limited extent (Tappy and Le 2010), yet, possibly due to mechanisms trying to keep energy stores constant and preventing too much local energy

storage. Such effects have been largely documented for muscle glycogen (Hargreaves et al. 1995), and whether this also exists for liver glycogen (Gonzalez et al. 2016) and/or intrahepatic and intramuscular fat remains unclear. Interestingly, fructose can affect hepatic glucose and glycogen cycling by varied mechanisms (Tounian et al. 1994, Dirlewanger et al. 2000), possibly indicating an adaptation against excessive storage.

Our results support that mechanisms trying to stabilize glycogen may also echo in a systemic manner. In manuscript 4, we observed that previously exercised muscle was efficiently replete by fructose, likely at the expense of liver glycogen (Rosset et al. 2017). This is at odds with fructose being generally considered efficient for liver but not muscle glycogen storage (Conlee et al. 1987), and our work is also one of the single (Burke et al. 1993) having compared fructose and glucose for a long recovery period (>8h). It is therefore also possible that exercised muscle progressively recovered its glycogen from the liver over time, and that this effect was revealed by suboptimal carbohydrate amounts in our study (Gonzalez et al. 2017). Muscle glycogen storage from lactate was previously shown in extreme conditions (Fournier et al. 2004), and whether fructose causes an inefficient transfer through lactate will require future evaluation.

These examples illustrate that fructose is unique in furnishing carbohydrate energy at the trioses-P level, while other sources (f.eg. galactose) can also target the liver, their entry at a higher free energy level results in similar effects than glucose regarding energy conservation. In turn, the absence of regulation of fructolysis forces fructose carbons to enter the lower part of the glycolysis energy gradient, from which an anabolic disposal (gluconeogenesis, glyconeogenesis) may explain its higher thermic effect than glucose (Tappy et al. 2013).

Exercise is particular in that it causes working muscle literally “pump” circulating substrates to meet increased energy needs. This indeed causes lactate use as a fuel as supposed in the

model, but the extremely high metabolic rate of working myocytes also requires glucose uptake (and muscle glycogen consumption) that will translate in more constraint put on liver glucose production. A result is that fructose can be directed to glucose during exercise and, more generally, at any time when plasma glucose control requires counter-regulation. This may also depend on the larger mass of muscle to that of the liver, implying that plasma glucose comes as a last possibility for fructose carbons, but that can be put first (or at least second) in case of impaired glucose homeostasis sensed by hypothalamic neurons. Fructose fates as CO_2, *de novo* lipogenesis, lactate, glycogen then glucose would be primarily locally mediated, and interactions between systemic metabolic rate, plasma lactate and glucose would come as an overriding factor under central command. Yet, each situation in which an intermediate is moved to an upper free energy level (potential energy) would be associated with a cost for the cell first, for the living organism second, and thus would be avoided if possible.

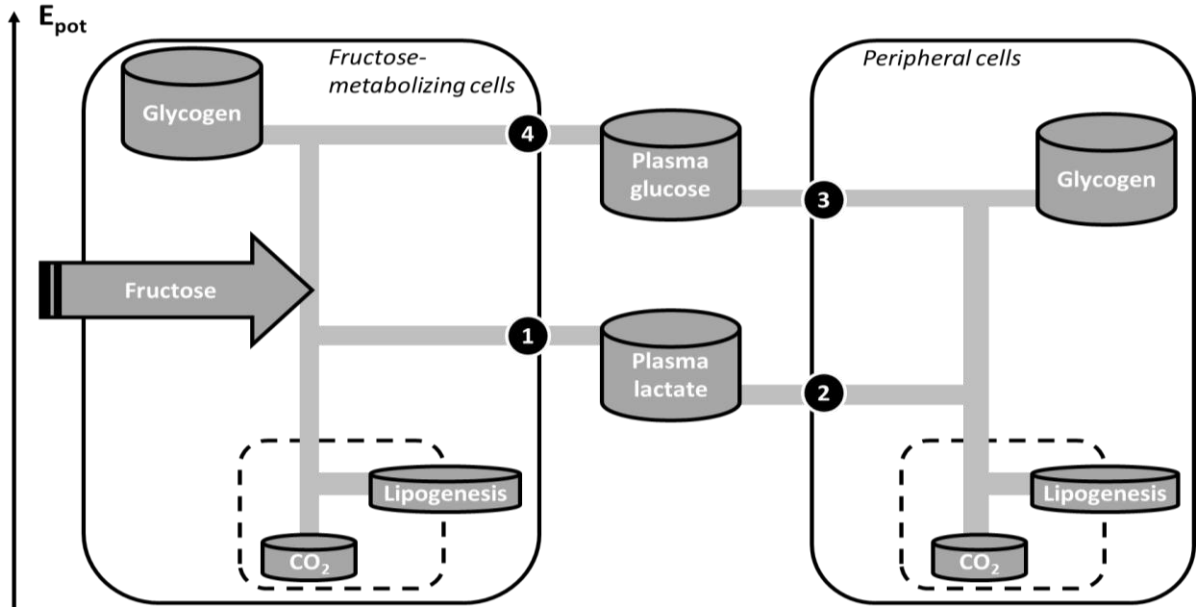


FIGURE 9: PROPOSED DISTRIBUTION OF FRUCTOSE CARBONS MAY FOLLOW ENERGY GRADIENTS IN A FIRST-LOCAL, THEN-SYSTEMIC MANNER. 1. AFTER SATURATION OF CO2 AND DE NOVO LIPOGENESIS, FRUCTOSE CONSTITUTIVE EFFLUX AS LACTATE IS NUTRITIONNALY TRAINABLE. 2. LACTATE INFLUX IS INCREASED BY EXERCISE, NOT FRUCTOSE TRAINING AND METABOLIC RATE (OXIDATIVE CAPACITY) THEN DICTATES THE PART OF LACTATE OXIDIZED (FRUCTOSE FREE ENERGY OPTIMALLY USED) OR DIRECTED TOWARD ANABOLIC FATES (ENERGY COST). 3. DEPENDING UPON CONDITIONS, THE PERIPHERY CAN PUMP PLASMA GLUCOSE. 4. COUNTER-REGULATION THEN CAUSES FRUCTOSE REROUTING TOWARD GLUCONEOGENESIS (ENERGY COST)

Several limitations may arise from this model. First, it overemphasizes the role of energy, without considering alternative regulation of the many enzymes involved. Second, it does not address whether fluxes derive from transport limitations, enzymes kinetics or precursors concentrations. It also little considers fat and protein metabolisms, hormones or blood flow control. Far from denying these aspects, they may however come as supplementary sources of regulation that alter fructose metabolism on top of the basal tendency to fight entropy (f.eg. lactate transport limitations may delay compartment equilibrations (Omlin et al. 2014)).

Finally, this model does not consider transfers going in the opposite direction such as the Cori and glucose-alanine cycles. These cycles may be driven by peripheral metabolism (MacRae et al. 1992), while the "reverse Cori cycle" induced by fructose ingestion results from fructose-lactate conversion and/or lactate efflux from fructose-metabolizing cells. A compilation of studies suggests that glucose and lactate fluxes are raised by carbohydrate ingestion during rest and exercise, and indicates that lactate production may depend on fructose dose (**Figure 10**). How fructose contribution, that was only once measured during exercise as ≈30% of lactate fluxes (Lecoultre et al. 2010), adapts to exercise modalities is unknown, yet. A similar reverse cycle may also apply to glycerol, also converted to a triose-P (Trimmer et al. 2001).

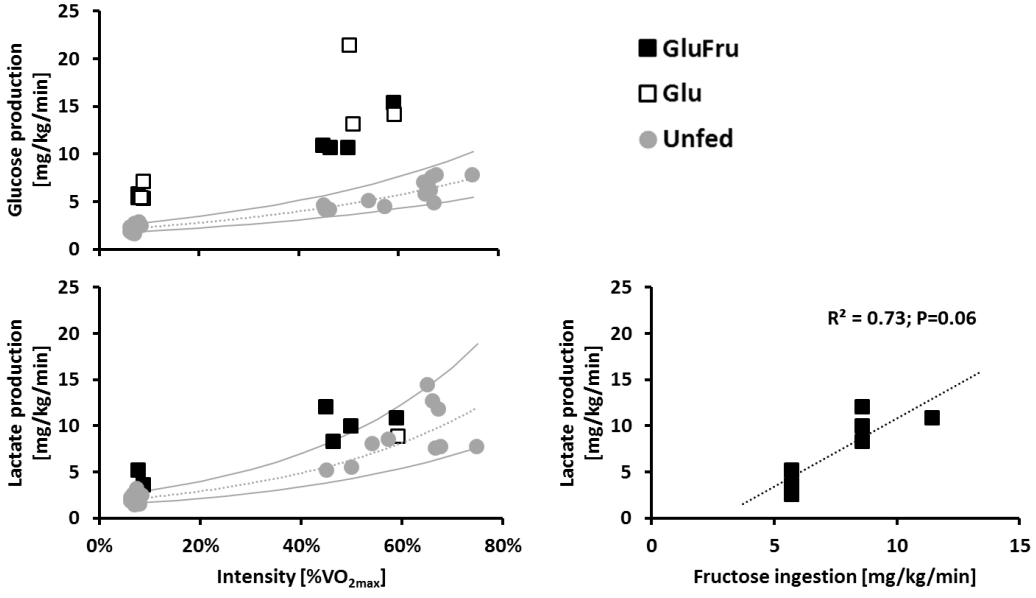


FIGURE 10: EFFECT OF METABOLIC RATE ON GLUCOSE AND LACTATE FLUXES IN CARBOHYDRATE-FED AND UNFED SUBJECTS. REVERSE CORI CYCLE ACTIVITY MAY BE PROPORTIONAL TO CARBOHYDRATE INGESTION RATE WHILE TOTAL LACTATE PRODUCTION DEPENDS ON METABOLIC RATE (RETRIEVED FROM FERRANNINI ET AL. 1993, HAESLER ET AL. 1995, PAQUOT ET AL. 1995, TAPPY ET AL. 1995, HUIE ET AL. 1996, FRIEDLANDER ET AL. 1997, FRIEDLANDER ET AL. 1998, BERGMAN ET AL. 1999, BERGMAN ET AL. 1999, JEUKENDRUP ET AL. 1999, MILLER ET AL. 2002, MILLER ET AL. 2002, TRIMMER ET AL. 2002, ABDEL-SAYED ET AL. 2008, LECOULTRE ET AL. 2010, EMHOFF ET AL. 2013, EMHOFF ET AL. 2013, ROSSET ET AL. 2017)

b. Practical implications

A first consequence of this work is that fructose may be optimally used as a fuel for athletes when the reverse Cori cycle activity is maximal. Unpublished results from this thesis suggest that glucose-fructose can differently alter lactate concentrations at varied exercise intensities, questioning how the reverse Cori cycle interacts with muscle metabolism. One possibility would be an increased muscle carbohydrate uptake and oxidation during exercise. How such effects can be optimized by glucose-fructose co-ingestion to improve performance compared to glucose-fed exercise was discussed in a last publication, manuscript 6.

In other situations than exercise, when systemic metabolic rate may not match with available lactate free energy, fructose appears to be associated with diminished energy conservation. Consequently, fructose may not be recommended for the recovering athlete aiming for future performance, or should be ingested in higher amounts than pure glucose to obtain the same glycogen repletion. Not restricted to muscle (Balon et al. 1992), this energy loss may also protect against fructose overfeeding by providing an accessible way to dispend energy.

Indirect effects of fructose on plasma TG were also investigated in manuscript 4 but, as mentioned, were inconclusive regarding IMCL metabolism (Rosset et al. 2017). Interestingly, this study provided very high fructose doses (308±13 $g \cdot 24h^{-1}$) being, to our knowledge, the largest used in humans. Even at these doses, fructose induced no noticeable effect on insulin

resistance or cholesterol profiles in fasted subjects (unpublished results). This supports that the variable of interest regarding fructose pathophysiological effects is energy balance, and suggests that there may be no reason to target fructose more than another nutrient for the current epidemic of metabolic diseases. Of note, the entry of fructose as trioses-P may even be associated with an obligatory energy cost when at rest.

Having ingested glucose-fructose during training sessions did not further increase lactate fluxes during exercise. This suggests that splanchnic adaptations to fructose were minor comparing to the effects of training on muscle lactate metabolism (MacRae et al. 1992) and/or that a limitation in the gut or the liver prevented splanchnic adaptations to fructose and muscle adaptations to training to act in synergy. How this limitation was related to glucose co-ingestion is unknown. If confirmed, our results may suggest that habituation toward carbohydrates ingestion during exercise do generally not further increase carbohydrate fluxes after training due to hepatic limitations. Alternatively, suspicions that chronic fructose ingestion may induce peripheral insulin resistance were not observed.

c. Conclusions

The results from this thesis have allowed for a better understanding of fructose metabolism and the consequences of fructose ingestion in an exercise context. Experimental results indicate that fructose ingested as an exercise fuel, as a precursor for energy storage or aiming to reinforce adaptations all induced very different outcomes. We stipulate that this was largely due to fructose conversion into lactate, referring to previous suspicions that conversion into lactate may protect against fructose overfeeding (Brundin and Wahren 1993). However, our work allowed to reconcile this concept with results showing increased lactate fluxes after glucose-fructose co-ingestion during exercise (Lecoultre et al. 2010), to further understand

the fructose-exercise relationship. Fructose disposal as lactate may then be viewed as an important mechanism of distribution of fructose energy to the periphery to maintain proper liver function. In turn, the peripheral fate of lactate between oxidative and non-oxidative disposal can be viewed as largely dependent on metabolic rate. As a whole, this system would then imply that fructose, as a subsidiary substrate, causes a systematic energy cost when metabolized, and that the only means to completely use fructose energy is through complete oxidation. Comparison of data collected at rest and during exercise indicate a large dependency on metabolic rate, in line with lactate being used as a preferred fuel whenever metabolic rate is sufficient, and be otherwise stored for an extra cost.

Future studies could further investigate fructose lactate conversion that may be key to fructose metabolic effects. How lactate from fructose may furnish extrahepatic glycogenesis, lipogenesis and gluconeogenesis remains unclear, similarly as how it may affect substrates hierarchy. Besides its roles as an energy substrate, lactate is also known to have diverse effects on metabolic regulation, such as hormone-like actions in the brain (Proia et al. 2016), and how fructose itself (Moulin et al. 2017) or through its metabolite lactate may induce non-metabolic signaling will require further studies. To which extent these conclusions similarly apply to fructose ingested with other nutrients such as glucose (f.eg. in sucrose) is also unknown.

From an evolutionary perspective, fructose may interestingly have served as a metabolic driver. During fructification periods, its ingestion by animal species may have signaled a period of energy storage necessary to maintain body mass in prevision of winter times. In turn, its conversion into secondary metabolites that can be oxidized during exercise may also be seen as a means to escape from predators. This thesis then only partly unveiled a history that has been lasting since the apparition of physically active animals ingesting natural carbohydrates.

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Glucose-Fructose Ingestion and Exercise Performance: The Gastrointestinal Tract and Beyond

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Contribution: Literature research, drafting and writing of the manuscript. Figures preparation.

Abstract: Carbohydrate ingestion can improve endurance exercise performance. In the past two decades, research has repeatedly reported the performance benefits of formulations comprising both glucose and fructose (GLUFRU) over those based on glucose (GLU). This has been usually related to additive effects of these two monosaccharides on the gastrointestinal tract whereby intestinal carbohydrate absorption is enhanced and discomfort limited. This is only a partial explanation, since glucose and fructose are also metabolized through different pathways after being absorbed from the gut. In contrast to glucose that is readily used by every body cell type, fructose is specifically targeted to the liver where it is mainly converted into glucose and lactate. The ingestion of GLUFRU may thereby profoundly alter hepatic function ultimately raising both glucose and lactate fluxes. During exercise, this particular profile of circulating carbohydrate may induce a spectrum of effects on muscle metabolism possibly resulting in an improved performance. Compared to GLU alone, GLUFRU ingestion could also induce several non-metabolic effects which are so far largely unexplored. Through its metabolite lactate, fructose may act on central fatigue and/or alter metabolic regulation. Future research could further define the effects of GLUFRU over other exercise modalities and different athletic populations, using several of the hypotheses discussed in this review.

XVI. References

- **Abdel-Sayed, A., C. Binnert, K. A. Le, M. Bortolotti, P. Schneiter and L. Tappy**. A high-fructose diet impairs basal and stress-mediated lipid metabolism in healthy male subjects. *Br J Nutr* **2008**, *100*, 393-399.
- **Abdelmalek, M. F., M. Lazo, A. Horska, S. Bonekamp, E. W. Lipkin, A. Balasubramanyam, J. P. Bantle, R. J. Johnson, A. M. Diehl, J. M. Clark and A. R. G. Fatty Liver Subgroup of Look**. Higher dietary fructose is associated with impaired hepatic adenosine triphosphate homeostasis in obese individuals with type 2 diabetes. *Hepatology* **2012**, *56*, 952-960.
- **Adopo, E., F. Peronnet, D. Massicotte, G. R. Brisson and C. Hillaire-Marcel**. Respective oxidation of exogenous glucose and fructose given in the same drink during exercise. *J Appl Physiol (1985)* **1994**, *76*, 1014-1019.
- **Ahlborg, G. and O. Bjorkman**. Splanchnic and muscle fructose metabolism during and after exercise. *J Appl Physiol (1985)* **1990**, *69*, 1244-1251.
- **Azevedo, J. L., E. Tietz, T. Two-Feathers, J. Paull and K. Chapman**. Lactate, fructose and glucose oxidation profiles in sports drinks and the effect on exercise performance. *PLoS One* **2007**, *2*, e927.
- **Baker, J. S., M. C. McCormick and R. A. Robergs**. Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. *J Nutr Metab* **2010**, *2010*, 905612.
- **Baldwin, K. M.** Effects of chronic exercise on biochemical and functional properties of the heart. *Med Sci Sports Exerc* **1985**, *17*, 522-528.
- **Balon, T. W., J. L. Treadway, J. B. Hughes, J. C. Young and N. B. Ruderman**. Effect of prior exercise and insulin on potential thermogenic systems in rat skeletal muscle. *J Appl Physiol (1985)* **1992**, *72*, 2203-2209.
- **Barrington, W. E. and E. White**. Mortality outcomes associated with intake of fast-food items and sugar-sweetened drinks among older adults in the Vitamins and Lifestyle (VITAL) study. *Public Health Nutr* **2016**, *19*, 3319-3326.
- **Bentley, D. J., B. Roels, C. Thomas, R. Ives, J. Mercier, G. Millet and D. Cameron-Smith**. The relationship between monocarboxylate transporters 1 and 4 expression in skeletal muscle and endurance performance in athletes. *Eur J Appl Physiol* **2009**, *106*, 465-471.
- **Bergman, B. C., G. E. Butterfield, E. E. Wolfel, G. D. Lopaschuk, G. A. Casazza, M. A. Horning and G. A. Brooks**. Muscle net glucose uptake and glucose kinetics after endurance training in men. *Am J Physiol* **1999**, *277*, E81-92.
- **Bergman, B. C., E. E. Wolfel, G. E. Butterfield, G. D. Lopaschuk, G. A. Casazza, M. A. Horning and G. A. Brooks**. Active muscle and whole body lactate kinetics after endurance training in men. *J Appl Physiol (1985)* **1999**, *87*, 1684-1696.
- **Bergstrom, J., L. Hermansen, E. Hultman and B. Saltin**. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* **1967**, *71*, 140-150.
- **Bidwell, A. J., T. J. Fairchild, J. Redmond, L. Wang, S. Keslacy and J. A. Kanaley**. Physical activity offsets the negative effects of a high-fructose diet. *Med Sci Sports Exerc* **2014**, *46*, 2091-2098.
- **Bizeau, M. E. and M. J. Pagliassotti**. Hepatic adaptations to sucrose and fructose. *Metabolism* **2005**, *54*, 1189-1201.
- **Bjorkman, O., R. Gunnarsson, E. Hagstrom, P. Felig and J. Wahren**. Splanchnic and renal exchange of infused fructose in insulin-deficient type 1 diabetic patients and healthy controls. *J Clin Invest* **1989**, *83*, 52-59.
- **Bjorkman, O., K. Sahlin, L. Hagenfeldt and J. Wahren**. Influence of glucose and fructose ingestion on the capacity for long-term exercise in well-trained men. *Clin Physiol* **1984**, *4*, 483-494.
- **Blom, P. C., A. T. Hostmark, O. Vaage, K. R. Kardel and S. Maehlum**. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med Sci Sports Exerc* **1987**, *19*, 491-496.
- **Boesch, C., J. Decombaz, J. Slotboom and R. Kreis**. Observation of intramyocellular lipids by means of 1H magnetic resonance spectroscopy. *Proc Nutr Soc* **1999**, *58*, 841-850.
- **Bonen, A., K. J. McCullagh, C. T. Putman, E. Hultman, N. L. Jones and G. J. Heigenhauser**. Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. *Am J Physiol* **1998**, *274*, E102-107.
- **Bortolotti, M., J. Dubuis, P. Schneiter and L. Tappy**. Effects of dietary protein on lipid metabolism in high fructose fed humans. *Clin Nutr* **2012**, *31*, 238-245.
- **Bray, G. A.** How bad is fructose? *Am J Clin Nutr* **2007**, *86*, 895-896.
- **Bray, G. A., S. J. Nielsen and B. M. Popkin**. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr* **2004**, *79*, 537-543.
- **Brooks, G. A.** The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc* **1986**, *18*, 360-368.
- **Brooks, G. A.** Cell-cell and intracellular lactate shuttles. *J Physiol* **2009**, *587*, 5591-5600.

- **Brooks, G. A.** Energy Flux, Lactate Shuttling, Mitochondrial Dynamics, and Hypoxia. *Adv Exp Med Biol* **2016**, *903*, 439-455.
- **Brooks, G. A. and J. Mercier**. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J Appl Physiol (1985)* **1994**, *76*, 2253-2261.
- **Brundin, T. and J. Wahren**. Whole body and splanchnic oxygen consumption and blood flow after oral ingestion of fructose or glucose. *Am J Physiol* **1993**, *264*, E504-513.
- **Burke, L. M., G. R. Collier and M. Hargreaves**. Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. *J Appl Physiol (1985)* **1993**, *75*, 1019-1023.
- **Burke, L. M., G. R. Cox, N. K. Culmmings and B. Desbrow**. Guidelines for daily carbohydrate intake: do athletes achieve them? *Sports Med* **2001**, *31*, 267-299.
- **Burke, L. M., J. A. Hawley, S. H. Wong and A. E. Jeukendrup**. Carbohydrates for training and competition. *J Sports Sci* **2011**, *29 Suppl 1*, S17-27.
- **Campos, V. C. and L. Tappy**. Physiological handling of dietary fructose-containing sugars: implications for health. *Int J Obes (Lond)* **2016**, *40 Suppl 1*, S6-11.
- **Carmody, R. N., M. Dannemann, A. W. Briggs, B. Nickel, E. E. Groopman, R. W. Wrangham and J. Kelso**. Genetic Evidence of Human Adaptation to a Cooked Diet. *Genome Biol Evol* **2016**, *8*, 1091-1103.
- **Casey, D. P. and M. J. Joyner**. Local control of skeletal muscle blood flow during exercise: influence of available oxygen. *J Appl Physiol (1985)* **2011**, *111*, 1527-1538.
- **Cermak, N. M. and L. J. van Loon**. The use of carbohydrates during exercise as an ergogenic aid. *Sports Med* **2013**, *43*, 1139-1155.
- **Chambers, E. S., M. W. Bridge and D. A. Jones**. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol* **2009**, *587*, 1779-1794.
- **Chasiotis, D., K. Sahlin and E. Hultman**. Regulation of glycogenolysis in human muscle at rest and during exercise. *J Appl Physiol Respir Environ Exerc Physiol* **1982**, *53*, 708-715.
- **Chen, L., B. Tuo and H. Dong**. Regulation of Intestinal Glucose Absorption by Ion Channels and Transporters. *Nutrients* **2016**, *8*.
- **Chiolero, R., P. Mavrocordatos, P. Burnier, M. C. Cayeux, C. Schindler, E. Jequier and L. Tappy**. Effects of infused sodium acetate, sodium lactate, and sodium beta-hydroxybutyrate on energy expenditure and substrate oxidation rates in lean humans. *Am J Clin Nutr* **1993**, *58*, 608-613.
- **Choi, H. K., W. Willett and G. Curhan**. Fructose-rich beverages and risk of gout in women. *JAMA* **2010**, *304*, 2270-2278.
- **Chong, M. F., B. A. Fielding and K. N. Frayn**. Mechanisms for the acute effect of fructose on postprandial lipemia. *Am J Clin Nutr* **2007**, *85*, 1511-1520.
- **Choo, V. L. and J. L. Sievenpiper**. The ecologic validity of fructose feeding trials: supraphysiological feeding of fructose in human trials requires careful consideration when drawing conclusions on cardiometabolic risk. *Front Nutr* **2015**, *2*, 12.
- **Conlee, R. K., R. M. Lawler and P. E. Ross**. Effects of glucose or fructose feeding on glycogen repletion in muscle and liver after exercise or fasting. *Ann Nutr Metab* **1987**, *31*, 126-132.
- **Connolly, C. C., R. W. Stevenson, D. W. Neal, D. H. Wasserman and A. D. Cherrington**. The effects of lactate loading on alanine and glucose metabolism in the conscious dog. *Metabolism* **1993**, *42*, 154-161.
- **Coss-Bu, J. A., A. L. Sunehag and M. W. Haymond**. Contribution of galactose and fructose to glucose homeostasis. *Metabolism* **2009**, *58*, 1050-1058.
- **Couchepin, C., K. A. Le, M. Bortolotti, J. A. da Encarnacao, J. B. Oboni, C. Tran, P. Schneiter and L. Tappy**. Markedly blunted metabolic effects of fructose in healthy young female subjects compared with male subjects. *Diabetes Care* **2008**, *31*, 1254-1256.
- **Cox, T. M.** Aldolase B and fructose intolerance. *FASEB J* **1994**, *8*, 62-71.
- **Cross, D. A., D. R. Alessi, P. Cohen, M. Andjelkovich and B. A. Hemmings**. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **1995**, *378*, 785-789.
- **Currell, K. and A. E. Jeukendrup**. Superior endurance performance with ingestion of multiple transportable carbohydrates. *Med Sci Sports Exerc* **2008**, *40*, 275-281.
- **Decombaz, J., M. Fleith, H. Hoppeler, R. Kreis and C. Boesch**. Effect of diet on the replenishment of intramyocellular lipids after exercise. *Eur J Nutr* **2000**, *39*, 244-247.
- **Decombaz, J., R. Jentjens, M. Ith, E. Scheurer, T. Buehler, A. Jeukendrup and C. Boesch**. Fructose and galactose enhance postexercise human liver glycogen synthesis. *Med Sci Sports Exerc* **2011**, *43*, 1964-1971.
- **Desplanches, D., M. Amami, S. Dupre-Aucouturier, P. Valdivieso, S. Schmutz, M. Mueller, H. Hoppeler, R. Kreis and M. Fluck**. Hypoxia refines plasticity of mitochondrial respiration to repeated muscle work. *Eur J Appl Physiol* **2014**, *114*, 405-417.

- **Diggle, C. P., M. Shires, D. Leitch, D. Brooke, I. M. Carr, A. F. Markham, B. E. Hayward, A. Asipu and D. T. Bonthron**. Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. *J Histochem Cytochem* **2009**, *57*, 763-774.
- **Dirlewanger, M., P. Schneiter, E. Jequier and L. Tappy**. Effects of fructose on hepatic glucose metabolism in humans. *Am J Physiol Endocrinol Metab* **2000**, *279*, E907-911.
- **Donovan, C. M. and M. J. Pagliassotti**. Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. *Med Sci Sports Exerc* **2000**, *32*, 772-777.
- **Douard, V. and R. P. Ferraris**. Regulation of the fructose transporter GLUT5 in health and disease. *Am J Physiol Endocrinol Metab* **2008**, *295*, E227-237.
- **Douard, V. and R. P. Ferraris**. The role of fructose transporters in diseases linked to excessive fructose intake. *J Physiol* **2013**, *591*, 401-414.
- **Dubouchaud, H., G. E. Butterfield, E. E. Wolfel, B. C. Bergman and G. A. Brooks**. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab* **2000**, *278*, E571-579.
- **Eggleston, L. V. and H. F. Woods**. Activation of liver pyruvate kinase by fructose-1-phosphate. *FEBS Lett* **1970**, *6*, 43-45.
- **Egli, L., V. Lecoultre, J. Cros, R. Rosset, A. S. Marques, P. Schneiter, L. Hodson, L. Gabert, M. Laville and L. Tappy**. Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage. *Am J Clin Nutr* **2016**, *103*, 348-355.
- **Egli, L., V. Lecoultre, F. Theytaz, V. Campos, L. Hodson, P. Schneiter, B. Mittendorfer, B. W. Patterson, B. A. Fielding, P. A. Gerber, V. Giusti, K. Berneis and L. Tappy**. Exercise prevents fructose-induced hypertriglyceridemia in healthy young subjects. *Diabetes* **2013**, *62*, 2259-2265.
- **Elias, E., G. J. Gibson, L. F. Greenwood, J. N. Hunt and J. H. Tripp**. The slowing of gastric emptying by monosaccharides and disaccharides in test meals. *J Physiol* **1968**, *194*, 317-326.
- **Emhoff, C. A., L. A. Messonnier, M. A. Horning, J. A. Fattor, T. J. Carlson and G. A. Brooks**. Direct and indirect lactate oxidation in trained and untrained men. *J Appl Physiol (1985)* **2013**, *115*, 829-838.
- **Emhoff, C. A., L. A. Messonnier, M. A. Horning, J. A. Fattor, T. J. Carlson and G. A. Brooks**. Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold. *J Appl Physiol (1985)* **2013**, *114*, 297-306.
- **Faeh, D., K. Minehira, J. M. Schwarz, R. Periasamy, S. Park and L. Tappy**. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. *Diabetes* **2005**, *54*, 1907-1913.
- **Fattor, J. A., B. F. Miller, K. A. Jacobs and G. A. Brooks**. Catecholamine response is attenuated during moderate-intensity exercise in response to the "lactate clamp". *Am J Physiol Endocrinol Metab* **2005**, *288*, E143-147.
- **Ferrannini, E., A. Natali, L. S. Brandi, R. Bonadonna, S. V. De Kreutzemberg, S. DelPrato and D. Santoro**. Metabolic and thermogenic effects of lactate infusion in humans. *Am J Physiol* **1993**, *265*, E504-512.
- **Flatt, J. P.** The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. *Ann N Y Acad Sci* **1987**, *499*, 104-123.
- **Fletcher, W. M.** Lactic acid in amphibian muscle. *J Physiol* **1907**, *35*, 247-309.
- **Fournier, P. A., T. J. Fairchild, L. D. Ferreira and L. Brau**. Post-exercise muscle glycogen repletion in the extreme: effect of food absence and active recovery. *J Sports Sci Med* **2004**, *3*, 139-146.
- **Friedlander, A. L., G. A. Casazza, M. A. Horning, M. J. Huie and G. A. Brooks**. Training-induced alterations of glucose flux in men. *J Appl Physiol (1985)* **1997**, *82*, 1360-1369.
- **Friedlander, A. L., G. A. Casazza, M. A. Horning, M. J. Huie, M. F. Piacentini, J. K. Trimmer and G. A. Brooks**. Training-induced alterations of carbohydrate metabolism in women: women respond differently from men. *J Appl Physiol (1985)* **1998**, *85*, 1175-1186.
- **Froesch, E. R., H. Burgi, P. Bally and A. Labhart**. Insulin inhibition of spontaneous adipose tissue lipolysis and effects upon fructose and glucose metabolism. *Mol Pharmacol* **1965**, *1*, 280-296.
- **Frostell, G.** Effects of mouth rinses with sucrose, glucose, fructose, lactose, sorbitol and Lycasin on the pH of dental plaque. *Odontol Revy* **1973**, *24*, 217-226.
- **Fuchs, C. J., J. T. Gonzalez, M. Beelen, N. M. Cermak, F. E. Smith, P. E. Thelwall, R. Taylor, M. I. Trenell, E. J. Stevenson and L. J. van Loon**. Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion compared with glucose ingestion in trained athletes. *J Appl Physiol (1985)* **2016**, *120*, 1328-1334.
- **Fuchs, M. A., K. Sato, D. Niedzwiecki, X. Ye, L. B. Saltz, R. J. Mayer, R. B. Mowat, R. Whittom, A. Hantel, A. Benson, D. Atienza, M. Messino, H. Kindler, A. Venook, S. Ogino, K. Wu, W. C. Willett, E. L. Giovannucci and J. A. Meyerhardt**. Sugar-sweetened beverage intake and cancer recurrence and survival in CALGB 89803 (Alliance). *PLoS One* **2014**, *9*, e99816.

- **Fujisawa, T., K. Mulligan, L. Wada, L. Schumacher, J. Riby and N. Kretchmer**. The effect of exercise on fructose absorption. *Am J Clin Nutr* **1993**, *58*, 75-79.
- **Gaussin, V., P. Gailly, J. M. Gillis and L. Hue**. Fructose-induced increase in intracellular free Mg2+ ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism. *Biochem J* **1997**, *326 (Pt 3)*, 823-827.
- **Gladden, L. B.** Lactate metabolism: a new paradigm for the third millennium. *J Physiol* **2004**, *558*, 5-30.
- **Goldberg, I. J., R. H. Eckel and N. A. Abumrad**. Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways. *J Lipid Res* **2009**, *50 Suppl*, S86-90.
- **Gollnick, P. D., W. M. Bayly and D. R. Hodgson**. Exercise intensity, training, diet, and lactate concentration in muscle and blood. *Med Sci Sports Exerc* **1986**, *18*, 334-340.
- **Gonzalez-Teuber, M. and M. Heil**. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. *Plant Signal Behav* **2009**, *4*, 809-813.
- **Gonzalez, J. T., C. J. Fuchs, J. A. Betts and L. J. van Loon**. Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am J Physiol Endocrinol Metab* **2016**, *311*, E543-553.
- **Gonzalez, J. T., C. J. Fuchs, J. A. Betts and L. J. van Loon**. Glucose Plus Fructose Ingestion for Post-Exercise Recovery-Greater than the Sum of Its Parts? *Nutrients* **2017**, *9*.
- **Goodpaster, B. H., J. He, S. Watkins and D. E. Kelley**. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* **2001**, *86*, 5755-5761.
- **Haesler, E., P. Schneiter, E. Temler, E. Jequier and L. Tappy**. Effects of lactate infusion on hepatic gluconeogenesis and glycogenolysis. *Clin Physiol* **1995**, *15*, 581-595.
- **Haidari, M., N. Leung, F. Mahbub, K. D. Uffelman, R. Kohen-Avramoglu, G. F. Lewis and K. Adeli**. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. *J Biol Chem* **2002**, *277*, 31646-31655.
- **Hanover, L. M. and J. S. White**. Manufacturing, composition, and applications of fructose. *Am J Clin Nutr* **1993**, *58*, 724S-732S.
- **Hardie, D. G., F. A. Ross and S. A. Hawley**. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* **2012**, *13*, 251-262.
- **Hardy, K., J. Brand-Miller, K. D. Brown, M. G. Thomas and L. Copeland**. The Importance of Dietary Carbohydrate in Human Evolution. *Q Rev Biol* **2015**, *90*, 251-268.
- **Hargreaves, M., D. L. Costill, W. J. Fink, D. S. King and R. A. Fielding**. Effect of pre-exercise carbohydrate feedings on endurance cycling performance. *Med Sci Sports Exerc* **1987**, *19*, 33-36.
- **Hargreaves, M., G. McConell and J. Proietto**. Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *J Appl Physiol (1985)* **1995**, *78*, 288-292.
- **Hawley, J. A., A. N. Bosch, S. M. Weltan, S. C. Dennis and T. D. Noakes**. Effects of glucose ingestion or glucose infusion on fuel substrate kinetics during prolonged exercise. *Eur J Appl Physiol Occup Physiol* **1994**, *68*, 381-389.
- **Hawley, J. A., A. N. Bosch, S. M. Weltan, S. C. Dennis and T. D. Noakes**. Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects. *Pflugers Arch* **1994**, *426*, 378-386.
- **Hermansen, L.** Effect of metabolic changes on force generation in skeletal muscle during maximal exercise. *Ciba Found Symp* **1981**, *82*, 75-88.
- **Hokayem, M., E. Blond, H. Vidal, K. Lambert, E. Meugnier, C. Feillet-Coudray, C. Coudray, S. Pesenti, C. Luyton, S. Lambert-Porcheron, V. Sauvinet, C. Fedou, J. F. Brun, J. Rieusset, C. Bisbal, A. Sultan, J. Mercier, J. Goudable, A. M. Dupuy, J. P. Cristol, M. Laville and A. Avignon**. Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients. *Diabetes Care* **2013**, *36*, 1454-1461.
- **Holbrook, J. T., J. C. Smith, Jr. and S. Reiser**. Dietary fructose or starch: effects on copper, zinc, iron, manganese, calcium, and magnesium balances in humans. *Am J Clin Nutr* **1989**, *49*, 1290-1294.
- **Hue, L. and H. Taegtmeyer**. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab* **2009**, *297*, E578-591.
- **Huie, M. J., G. A. Casazza, M. A. Horning and G. A. Brooks**. Smoking increases conversion of lactate to glucose during submaximal exercise. *J Appl Physiol (1985)* **1996**, *80*, 1554-1559.
- **Hwang, I. S., H. Ho, B. B. Hoffman and G. M. Reaven**. Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **1987**, *10*, 512-516.
- **Iizuka, K.** The Role of Carbohydrate Response Element Binding Protein in Intestinal and Hepatic Fructose Metabolism. *Nutrients* **2017**, *9*.
- **Jensen, T. E. and E. A. Richter**. Regulation of glucose and glycogen metabolism during and after exercise. *J Physiol* **2012**, *590*, 1069-1076.

- **Jentjens, R. L., J. Achten and A. E. Jeukendrup**. High oxidation rates from combined carbohydrates ingested during exercise. *Med Sci Sports Exerc* **2004**, *36*, 1551-1558.
- **Jeukendrup, A.** A step towards personalized sports nutrition: carbohydrate intake during exercise. *Sports Med* **2014**, *44 Suppl 1*, S25-33.
- **Jeukendrup, A. E., A. Raben, A. Gijsen, J. H. Stegen, F. Brouns, W. H. Saris and A. J. Wagenmakers**. Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *J Physiol* **1999**, *515 (Pt 2)*, 579-589.
- **Jin, E. S., A. D. Sherry and C. R. Malloy**. Lactate Contributes to Glyceroneogenesis and Glyconeogenesis in Skeletal Muscle by Reversal of Pyruvate Kinase. *J Biol Chem* **2015**, *290*, 30486-30497.
- **Karjalainen, S., P. Hannula, E. Soderling, M. Hamalainen, K. Makinen and A. Scheinin**. Sucrose consumption and salivary sucrase activity in a 2-year longitudinal study. *Scand J Dent Res* **1989**, *97*, 401-404.
- **Katz, J. and J. A. Tayek**. Gluconeogenesis and the Cori cycle in 12-, 20-, and 40-h-fasted humans. *Am J Physiol* **1998**, *275*, E537-542.
- **Kolderup, A. and B. Svihus**. Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. *J Nutr Metab* **2015**, *2015*, 823081.
- **Koo, H. Y., M. Miyashita, B. H. Cho and M. T. Nakamura**. Replacing dietary glucose with fructose increases ChREBP activity and SREBP-1 protein in rat liver nucleus. *Biochem Biophys Res Commun* **2009**, *390*, 285-289.
- **Koo, H. Y., M. A. Wallig, B. H. Chung, T. Y. Nara, B. H. Cho and M. T. Nakamura**. Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. *Biochim Biophys Acta* **2008**, *1782*, 341-348.
- **Krajcovicova-Kudlackova, M., K. Sebekova, R. Schinzel and J. Klvanova**. Advanced glycation end products and nutrition. *Physiol Res* **2002**, *51*, 313-316.
- **Krebs, H. A., H. L. Kornberg and K. Burton**. A survey of the energy transformations in living matter. *Ergeb Physiol* **1957**, *49*, 212-298.
- **Kreisberg, R. A., L. F. Pennington and B. R. Boshell**. Lactate turnover and gluconeogenesis in normal and obese humans. Effect of starvation. *Diabetes* **1970**, *19*, 53-63.
- **Larson-Meyer, D. E., O. N. Borkhsenious, J. C. Gullett, R. R. Russell, M. C. Devries, S. R. Smith and E. Ravussin**. Effect of dietary fat on serum and intramyocellular lipids and running performance. *Med Sci Sports Exerc* **2008**, *40*, 892-902.
- **Latulippe, M. E. and S. M. Skoog**. Fructose malabsorption and intolerance: effects of fructose with and without simultaneous glucose ingestion. *Crit Rev Food Sci Nutr* **2011**, *51*, 583-592.
- **Le, K. A., D. Faeh, R. Stettler, M. Ith, R. Kreis, P. Vermathen, C. Boesch, E. Ravussin and L. Tappy**. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. *Am J Clin Nutr* **2006**, *84*, 1374-1379.
- **Le, K. A., M. Ith, R. Kreis, D. Faeh, M. Bortolotti, C. Tran, C. Boesch and L. Tappy**. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. *Am J Clin Nutr* **2009**, *89*, 1760-1765.
- **Le, M. T., R. F. Frye, C. J. Rivard, J. Cheng, K. K. McFann, M. S. Segal, R. J. Johnson and J. A. Johnson**. Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects. *Metabolism* **2012**, *61*, 641-651.
- **Lecoultre, V., R. Benoit, G. Carrel, Y. Schutz, G. P. Millet, L. Tappy and P. Schneiter**. Fructose and glucose co-ingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. *Am J Clin Nutr* **2010**, *92*, 1071-1079.
- **Lecoultre, V., G. Carrel, L. Egli, C. Binnert, A. Boss, E. L. MacMillan, R. Kreis, C. Boesch, C. Darimont and L. Tappy**. Coffee consumption attenuates short-term fructose-induced liver insulin resistance in healthy men. *Am J Clin Nutr* **2014**, *99*, 268-275.
- **Leite, T. C., R. G. Coelho, D. Da Silva, W. S. Coelho, M. M. Marinho-Carvalho and M. Sola-Penna**. Lactate downregulates the glycolytic enzymes hexokinase and phosphofructokinase in diverse tissues from mice. *FEBS Lett* **2011**, *585*, 92-98.
- **Li, S. S.** Lactate dehydrogenase isoenzymes A (muscle), B (heart) and C (testis) of mammals and the genes coding for these enzymes. *Biochem Soc Trans* **1989**, *17*, 304-307.
- **Liu, J., R. Wang, K. Desai and L. Wu**. Upregulation of aldolase B and overproduction of methylglyoxal in vascular tissues from rats with metabolic syndrome. *Cardiovasc Res* **2011**, *92*, 494-503.
- **Lloyd, S., C. Brocks and J. C. Chatham**. Differential modulation of glucose, lactate, and pyruvate oxidation by insulin and dichloroacetate in the rat heart. *Am J Physiol Heart Circ Physiol* **2003**, *285*, H163-172.
- **Loher, H., R. Kreis, C. Boesch and E. Christ**. The Flexibility of Ectopic Lipids. *Int J Mol Sci* **2016**, *17*.

- **Luo, S., J. R. Monterosso, K. Sarpelleh and K. A. Page**. Differential effects of fructose versus glucose on brain and appetitive responses to food cues and decisions for food rewards. *Proc Natl Acad Sci U S A* **2015**, *112*, 6509-6514.
- **Lustig, R. H., L. A. Schmidt and C. D. Brindis**. Public health: The toxic truth about sugar. *Nature* **2012**, *482*, 27-29.
- **MacRae, H. S., S. C. Dennis, A. N. Bosch and T. D. Noakes**. Effects of training on lactate production and removal during progressive exercise in humans. *J Appl Physiol (1985)* **1992**, *72*, 1649-1656.
- **Madsen, A. S., C. Andersen, M. Daoud, K. A. Anderson, J. S. Laursen, S. Chakladar, F. K. Huynh, A. R. Colaco, D. S. Backos, P. Fristrup, M. D. Hirschey and C. A. Olsen**. Investigating the Sensitivity of NAD+-dependent Sirtuin Deacylation Activities to NADH. *J Biol Chem* **2016**, *291*, 7128-7141.
- **Magkos, F., Y. E. Tsekouras, K. I. Prentzas, K. N. Basioukas, S. G. Matsama, A. E. Yanni, S. A. Kavouras and L. S. Sidossis**. Acute exercise-induced changes in basal VLDL-triglyceride kinetics leading to hypotriglyceridemia manifest more readily after resistance than endurance exercise. *J Appl Physiol (1985)* **2008**, *105*, 1228-1236.
- **Maharaj, A., L. Zhu, F. Huang, H. Qiu, H. Li, C. Y. Zhang, T. Jin and Q. Wang**. Ectopic expression of glucagon receptor in skeletal muscles improves glucose homeostasis in a mouse model of diabetes. *Diabetologia* **2012**, *55*, 1458-1468.
- **Malik, V. S., B. M. Popkin, G. A. Bray, J. P. Despres, W. C. Willett and F. B. Hu**. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* **2010**, *33*, 2477-2483.
- **Marmy-Conus, N., S. Fabris, J. Proietto and M. Hargreaves**. Preexercise glucose ingestion and glucose kinetics during exercise. *J Appl Physiol (1985)* **1996**, *81*, 853-857.
- **Marriott, B. P., N. Cole and E. Lee**. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *J Nutr* **2009**, *139*, 1228S-1235S.
- **Martin, W. F., S. Garg and V. Zimorski**. Endosymbiotic theories for eukaryote origin. *Philos Trans R Soc Lond B Biol Sci* **2015**, *370*, 20140330.
- **Massicotte, D., F. Peronnet, G. Brisson, L. Boivin and C. Hillaire-Marcel**. Oxidation of exogenous carbohydrate during prolonged exercise in fed and fasted conditions. *Int J Sports Med* **1990**, *11*, 253-258.
- **Mayes, P. A.** Intermediary metabolism of fructose. *Am J Clin Nutr* **1993**, *58*, 754S-765S.
- **McGuinness, O. P. and A. D. Cherrington**. Effects of fructose on hepatic glucose metabolism. *Curr Opin Clin Nutr Metab Care* **2003**, *6*, 441-448.
- **Meeusen, R. and B. Roelands**. Fatigue: Is it all neurochemistry? *Eur J Sport Sci* **2017**, 1-10.
- **Miller, B. F., J. A. Fattor, K. A. Jacobs, M. A. Horning, F. Navazio, M. I. Lindinger and G. A. Brooks**. Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion. *J Physiol* **2002**, *544*, 963-975.
- **Miller, B. F., J. A. Fattor, K. A. Jacobs, M. A. Horning, S. H. Suh, F. Navazio and G. A. Brooks**. Metabolic and cardiorespiratory responses to "the lactate clamp". *Am J Physiol Endocrinol Metab* **2002**, *283*, E889-898.
- **Mithieux, G. and A. Gautier-Stein**. Intestinal glucose metabolism revisited. *Diabetes Res Clin Pract* **2014**, *105*, 295-301.
- **Moore, J. B., P. J. Gunn and B. A. Fielding**. The role of dietary sugars and de novo lipogenesis in non-alcoholic fatty liver disease. *Nutrients* **2014**, *6*, 5679-5703.
- **Moore, M. C., K. C. Coate, J. J. Winnick, Z. An and A. D. Cherrington**. Regulation of hepatic glucose uptake and storage in vivo. *Adv Nutr* **2012**, *3*, 286-294.
- **Morris, D.** Effects of oral lactate consumption on metabolism and exercise performance. *Curr Sports Med Rep* **2012**, *11*, 185-188.
- **Moulin, S., G. Seematter and K. Seyssel**. Fructose use in clinical nutrition: metabolic effects and potential consequences. *Curr Opin Clin Nutr Metab Care* **2017**.
- **Mozaffarian, D., T. Hao, E. B. Rimm, W. C. Willett and F. B. Hu**. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med* **2011**, *364*, 2392-2404.
- **Nagasawa, J., Y. Sato and T. Ishiko**. Time course of in vivo insulin sensitivity after a single bout of exercise in rats. *Int J Sports Med* **1991**, *12*, 399-402.
- **Nelson, G., M. A. Hoon, J. Chandrashekar, Y. Zhang, N. J. Ryba and C. S. Zuker**. Mammalian sweet taste receptors. *Cell* **2001**, *106*, 381-390.
- **Newsholme, E. A.** The regulation of phosphofructokinase in muscle. *Cardiology* **1971**, *56*, 22-34.
- **Ngo Sock, E. T., K. A. Le, M. Ith, R. Kreis, C. Boesch and L. Tappy**. Effects of a short-term overfeeding with fructose or glucose in healthy young males. *Br J Nutr* **2010**, *103*, 939-943.
- **Nilsson, L. H. and E. Hultman**. Liver and muscle glycogen in man after glucose and fructose infusion. *Scand J Clin Lab Invest* **1974**, *33*, 5-10.

- **Ochoa, M., J. P. Lalles, C. H. Malbert and D. Val-Laillet**. Dietary sugars: their detection by the gut-brain axis and their peripheral and central effects in health and diseases. *Eur J Nutr* **2015**, *54*, 1-24.
- **Okano, G., H. Takeda, I. Morita, M. Katoh, Z. Mu and S. Miyake**. Effect of pre-exercise fructose ingestion on endurance performance in fed men. *Med Sci Sports Exerc* **1988**, *20*, 105-109.
- **Omlin, T., K. Langevin and J. M. Weber**. Exogenous lactate supply affects lactate kinetics of rainbow trout, not swimming performance. *Am J Physiol Regul Integr Comp Physiol* **2014**, *307*, R1018-1024.
- **Paquot, N., P. Schneiter, M. C. Cayeux, R. Chiolero, E. Temler, E. Jequier and L. Tappy**. Effects of infused sodium lactate on glucose and energy metabolism in healthy humans. *Diabete Metab* **1995**, *21*, 345-352.
- **Paquot, N., P. Schneiter, E. Jequier, R. Gaillard, P. J. Lefebvre, A. Scheen and L. Tappy**. Effects of ingested fructose and infused glucagon on endogenous glucose production in obese NIDDM patients, obese non-diabetic subjects, and healthy subjects. *Diabetologia* **1996**, *39*, 580-586.
- **Parniak, M. A. and N. Kalant**. Enhancement of glycogen concentrations in primary cultures of rat hepatocytes exposed to glucose and fructose. *Biochem J* **1988**, *251*, 795-802.
- **Pellerin, L., G. Pellegri, J. L. Martin and P. J. Magistretti**. Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. *Proc Natl Acad Sci U S A* **1998**, *95*, 3990-3995.
- **Peters, S. J., R. A. Harris, G. J. Heigenhauser and L. L. Spriet**. Muscle fiber type comparison of PDH kinase activity and isoform expression in fed and fasted rats. *Am J Physiol Regul Integr Comp Physiol* **2001**, *280*, R661-668.
- **Philp, A., A. L. Macdonald and P. W. Watt**. Lactate--a signal coordinating cell and systemic function. *J Exp Biol* **2005**, *208*, 4561-4575.
- **Pilegaard, H., G. Terzis, A. Halestrap and C. Juel**. Distribution of the lactate/H+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am J Physiol* **1999**, *276*, E843-848.
- **Plaisance, E. P. and G. Fisher**. Exercise and dietary-mediated reductions in postprandial lipemia. *J Nutr Metab* **2014**, *2014*, 902065.
- **Pollock, N. K., V. Bundy, W. Kanto, C. L. Davis, P. J. Bernard, H. Zhu, B. Gutin and Y. Dong**. Greater fructose consumption is associated with cardiometabolic risk markers and visceral adiposity in adolescents. *J Nutr* **2012**, *142*, 251-257.
- **Proia, P., C. M. Di Liegro, G. Schiera, A. Fricano and I. Di Liegro**. Lactate as a Metabolite and a Regulator in the Central Nervous System. *Int J Mol Sci* **2016**, *17*.
- **Randle, P. J., P. B. Garland, C. N. Hales and E. A. Newsholme**. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1963**, *1*, 785-789.
- **Rauch, L. H., A. N. Bosch, T. D. Noakes, S. C. Dennis and J. A. Hawley**. Fuel utilisation during prolonged low-to-moderate intensity exercise when ingesting water or carbohydrate. *Pflugers Arch* **1995**, *430*, 971-977.
- **Richter, E. A. and M. Hargreaves**. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev* **2013**, *93*, 993-1017.
- **Roden, M., T. B. Price, G. Perseghin, K. F. Petersen, D. L. Rothman, G. W. Cline and G. I. Shulman**. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* **1996**, *97*, 2859-2865.
- **Rogatzki, M. J., B. S. Ferguson, M. L. Goodwin and L. B. Gladden**. Lactate is always the end product of glycolysis. *Front Neurosci* **2015**, *9*, 22.
- **Romijn, J. A., E. F. Coyle, L. S. Sidossis, A. Gastaldelli, J. F. Horowitz, E. Endert and R. R. Wolfe**. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol* **1993**, *265*, E380-391.
- **Rosset, R., V. Lecoultre, L. Egli, J. Cros, A. S. Dokumaci, K. Zwygart, C. Boesch, R. Kreis, P. Schneiter and L. Tappy**. Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals. *Am J Clin Nutr* **2017**, *105*, 609-617.
- **Rosset, R., V. Lecoultre, L. Egli, J. Cros, V. Rey, N. Stefanoni, V. Sauvinet, M. Laville, P. Schneiter and L. Tappy**. Endurance Training with or without Glucose-Fructose Ingestion: Effects on Lactate Metabolism Assessed in a Randomized Clinical Trial on Sedentary Men. *Nutrients* **2017**, *9*.
- **Rowlands, D. S., S. Houltham, K. Musa-Veloso, F. Brown, L. Paulionis and D. Bailey**. Fructose-Glucose Composite Carbohydrates and Endurance Performance: Critical Review and Future Perspectives. *Sports Med* **2015**, *45*, 1561-1576.
- **Ryan, C. and J. Radziuk**. Distinguishable substrate pools for muscle glyconeogenesis in lactate-supplemented recovery from exercise. *Am J Physiol* **1995**, *269*, E538-550.
- **Saris, W. H., M. A. van Erp-Baart, F. Brouns, K. R. Westerterp and F. ten Hoor**. Study on food intake and energy expenditure during extreme sustained exercise: the Tour de France. *Int J Sports Med* **1989**, *10 Suppl 1*, S26-31.

- **Schaefer, E. J., J. A. Gleason and M. L. Dansinger**. Dietary fructose and glucose differentially affect lipid and glucose homeostasis. *J Nutr* **2009**, *139*, 1257S-1262S.
- **Schrauwen-Hinderling, V. B., P. Schrauwen, M. K. Hesselink, J. M. van Engelshoven, K. Nicolay, W. H. Saris, A. G. Kessels and M. E. Kooi**. The increase in intramyocellular lipid content is a very early response to training. *J Clin Endocrinol Metab* **2003**, *88*, 1610-1616.
- **Schwarz, J. M., Y. Schutz, F. Froidevaux, K. J. Acheson, N. Jeanpretre, H. Schneider, J. P. Felber and E. Jequier**. Thermogenesis in men and women induced by fructose vs glucose added to a meal. *Am J Clin Nutr* **1989**, *49*, 667-674.
- **Schwenk, R. W., G. P. Holloway, J. J. Luiken, A. Bonen and J. F. Glatz**. Fatty acid transport across the cell membrane: regulation by fatty acid transporters. *Prostaglandins Leukot Essent Fatty Acids* **2010**, *82*, 149-154.
- **Semchyshyn, H. M.** Fructation in vivo: detrimental and protective effects of fructose. *Biomed Res Int* **2013**, *2013*, 343914.
- **Seyssel, K., E. Meugnier, K. A. Le, C. Durand, E. Disse, E. Blond, L. Pays, S. Nataf, J. Brozek, H. Vidal, L. Tappy and M. Laville**. Fructose overfeeding in first-degree relatives of type 2 diabetic patients impacts energy metabolism and mitochondrial functions in skeletal muscle. *Mol Nutr Food Res* **2016**, *60*, 2691-2699.
- **Shephard, R. J.** Blood lactate and the Canadian Home Fitness Test. *Can J Sport Sci* **1992**, *17*, 72-73.
- **Shi, X., R. W. Summers, H. P. Schedl, S. W. Flanagan, R. Chang and C. V. Gisolfi**. Effects of carbohydrate type and concentration and solution osmolality on water absorption. *Med Sci Sports Exerc* **1995**, *27*, 1607-1615.
- **Shulman, R. G. and D. L. Rothman**. The "glycogen shunt" in exercising muscle: A role for glycogen in muscle energetics and fatigue. *Proc Natl Acad Sci U S A* **2001**, *98*, 457-461.
- **Siegel, K. R., J. B. Echouffo-Tcheugui, M. K. Ali, N. K. Mehta, K. M. Narayan and V. Chetty**. Societal correlates of diabetes prevalence: An analysis across 94 countries. *Diabetes Res Clin Pract* **2012**, *96*, 76-83.
- **Simoni, R. D., R. L. Hill and M. Vaughan**. Carbohydrate Metabolism: Glycogen Phosphorylase and the Work of Carl F. and Gerty T.Cori. 1928-1943. *J Biol Chem* **2002**, *277*, 18e.
- **Softic, S., D. E. Cohen and C. R. Kahn**. Role of Dietary Fructose and Hepatic De Novo Lipogenesis in Fatty Liver Disease. *Dig Dis Sci* **2016**, *61*, 1282-1293.
- **Sola-Penna, M.** Metabolic regulation by lactate. *IUBMB Life* **2008**, *60*, 605-608.
- **Spriet, L. L. and G. J. Heigenhauser**. Regulation of pyruvate dehydrogenase (PDH) activity in human skeletal muscle during exercise. *Exerc Sport Sci Rev* **2002**, *30*, 91-95.
- **Spriet, L. L., R. A. Howlett and G. J. Heigenhauser**. An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med Sci Sports Exerc* **2000**, *32*, 756-763.
- **Steenson, S., A. M. Umpleby, J. A. Lovegrove, K. G. Jackson and B. A. Fielding**. Role of the Enterocyte in Fructose-Induced Hypertriglyceridaemia. *Nutrients* **2017**, *9*.
- **Stettner, A. I. and D. Segre**. The cost of efficiency in energy metabolism. *Proc Natl Acad Sci U S A* **2013**, *110*, 9629-9630.
- **Sun, S. Z. and M. W. Empie**. Fructose metabolism in humans - what isotopic tracer studies tell us. *Nutr Metab (Lond)* **2012**, *9*, 89.
- **Tanaka, H., K. Nakamura, M. Mizuno, K. Ishikawa, K. Takeda, H. Kajiyama, F. Utsumi, F. Kikkawa and M. Hori**. Non-thermal atmospheric pressure plasma activates lactate in Ringer's solution for anti-tumor effects. *Sci Rep* **2016**, *6*, 36282.
- **Tappy, L., M. C. Cayeux, P. Schneiter, C. Schindler, E. Temler, E. Jequier and R. Chiolero**. Effects of lactate on glucose metabolism in healthy subjects and in severely injured hyperglycemic patients. *Am J Physiol* **1995**, *268*, E630-635.
- **Tappy, L., L. Egli, V. Lecoultre and P. Schneider**. Effects of fructose-containing caloric sweeteners on resting energy expenditure and energy efficiency: a review of human trials. *Nutr Metab (Lond)* **2013**, *10*, 54.
- **Tappy, L. and E. Jequier**. Fructose and dietary thermogenesis. *Am J Clin Nutr* **1993**, *58*, 766S-770S.
- **Tappy, L. and K. A. Le**. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev* **2010**, *90*, 23-46.
- **Tappy, L. and K. A. Le**. Health effects of fructose and fructose-containing caloric sweeteners: where do we stand 10 years after the initial whistle blowings? *Curr Diab Rep* **2015**, *15*, 54.
- **Teusink, B., P. J. Voshol, V. E. Dahlmans, P. C. Rensen, H. Pijl, J. A. Romijn and L. M. Havekes**. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes* **2003**, *52*, 614-620.
- **Theytaz, F., S. de Giorgi, L. Hodson, N. Stefanoni, V. Rey, P. Schneiter, V. Giusti and L. Tappy**. Metabolic fate of fructose ingested with and without glucose in a mixed meal. *Nutrients* **2014**, *6*, 2632-2649.

- **Theytaz, F., Y. Noguchi, L. Egli, V. Campos, T. Buehler, L. Hodson, B. W. Patterson, N. Nishikata, R. Kreis, B. Mittendorfer, B. Fielding, C. Boesch and L. Tappy**. Effects of supplementation with essential amino acids on intrahepatic lipid concentrations during fructose overfeeding in humans. *Am J Clin Nutr* **2012**, *96*, 1008-1016.
- **Thomas, C., D. J. Bishop, K. Lambert, J. Mercier and G. A. Brooks**. Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. *Am J Physiol Regul Integr Comp Physiol* **2012**, *302*, R1-14.
- **Topping, D. L. and P. A. Mayes**. The concentration of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. *Nutr Metab* **1971**, *13*, 331-338.
- **Tounian, P., P. Schneiter, S. Henry, E. Jequier and L. Tappy**. Effects of infused fructose on endogenous glucose production, gluconeogenesis, and glycogen metabolism. *Am J Physiol* **1994**, *267*, E710-717.
- **Tounian, P., P. Schneiter, S. Henry and L. Tappy**. Effects of infused glucose on glycogen metabolism in healthy humans. *Clin Physiol* **1996**, *16*, 403-416.
- **Trimmer, J. K., G. A. Casazza, M. A. Horning and G. A. Brooks**. Autoregulation of glucose production in men with a glycerol load during rest and exercise. *Am J Physiol Endocrinol Metab* **2001**, *280*, E657-668.
- **Trimmer, J. K., J. M. Schwarz, G. A. Casazza, M. A. Horning, N. Rodriguez and G. A. Brooks**. Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis. *J Appl Physiol (1985)* **2002**, *93*, 233-241.
- **Trommelen, J., M. Beelen, P. J. Pinckaers, J. M. Senden, N. M. Cermak and L. J. Van Loon**. Fructose Coingestion Does Not Accelerate Postexercise Muscle Glycogen Repletion. *Med Sci Sports Exerc* **2016**, *48*, 907-912.
- **Truswell, A. S., J. M. Seach and A. W. Thorburn**. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. *Am J Clin Nutr* **1988**, *48*, 1424-1430.
- **Tsintzas, K. and C. Williams**. Human muscle glycogen metabolism during exercise. Effect of carbohydrate supplementation. *Sports Med* **1998**, *25*, 7-23.
- **Tygstrup, N., K. Winkler and F. Lundquist**. The Mechanism of the Fructose Effect on the Ethanol Metabolism of the Human Liver. *J Clin Invest* **1965**, *44*, 817-830.
- **Underwood, A. H. and E. A. Newsholme**. Some Properties of Fructose 1,6-Diphosphatase of Rat Liver and Their Relation to the Control of Gluconeogenesis. *Biochem J* **1965**, *95*, 767-774.
- **Unger, R. H.** Glucagon and insulin: a bihormonal system. *Compr Ther* **1976**, *2*, 20-26.
- **Van Den Bergh, A. J., S. Houtman, A. Heerschap, N. J. Rehrer, H. J. Van Den Boogert, B. Oeseburg and M. T. Hopman**. Muscle glycogen recovery after exercise during glucose and fructose intake monitored by 13C-NMR. *J Appl Physiol (1985)* **1996**, *81*, 1495-1500.
- **van Hall, G.** Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol (Oxf)* **2010**, *199*, 499-508.
- **van Hall, G.** The Physiological Regulation of Skeletal Muscle Fatty Acid Supply and Oxidation During Moderate-Intensity Exercise. *Sports Med* **2015**, *45 Suppl 1*, S23-32.
- **van Loon, L. J., P. L. Greenhaff, D. Constantin-Teodosiu, W. H. Saris and A. J. Wagenmakers**. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol* **2001**, *536*, 295-304.
- **Varma, V., L. G. Boros, G. T. Nolen, C. W. Chang, M. Wabitsch, R. D. Beger and J. Kaput**. Metabolic fate of fructose in human adipocytes: a targeted 13C tracer fate association study. *Metabolomics* **2015**, *11*, 529-544.
- **Wagenmakers, A. J., F. Brouns, W. H. Saris and D. Halliday**. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. *J Appl Physiol (1985)* **1993**, *75*, 2774-2780.
- **Wallis, G. A. and A. Wittekind**. Is there a specific role for sucrose in sports and exercise performance? *Int J Sport Nutr Exerc Metab* **2013**, *23*, 571-583.
- **Wasserman, D. H. and A. D. Cherrington**. Hepatic fuel metabolism during muscular work: role and regulation. *Am J Physiol* **1991**, *260*, E811-824.
- **Wasserman, D. H., C. C. Connolly and M. J. Pagliassotti**. Regulation of hepatic lactate balance during exercise. *Med Sci Sports Exerc* **1991**, *23*, 912-919.
- **Welsh, J. A., A. J. Sharma, L. Grellinger and M. B. Vos**. Consumption of added sugars is decreasing in the United States. *Am J Clin Nutr* **2011**, *94*, 726-734.
- **Wilburn, J. R., J. Bourquin, A. Wysong and C. L. Melby**. Resistance Exercise Attenuates High-Fructose, High-Fat-Induced Postprandial Lipemia. *Nutr Metab Insights* **2015**, *8*, 29-35.
- **Wilson, P. B. and S. J. Ingraham**. Glucose-fructose likely improves gastrointestinal comfort and endurance running performance relative to glucose-only. *Scand J Med Sci Sports* **2015**, *25*, e613-620.
- **Wojtaszewski, J. F., B. F. Hansen, B. Kiens and E. A. Richter**. Insulin signaling in human skeletal muscle: time course and effect of exercise. *Diabetes* **1997**, *46*, 1775-1781.

- **Yau, A. M., J. McLaughlin, R. J. Maughan, W. Gilmore and G. H. Evans**. Short-term dietary supplementation with fructose accelerates gastric emptying of a fructose but not a glucose solution. *Nutrition* **2014**, *30*, 1344-1348.
- **Youn, J. H., H. R. Kaslow and R. N. Bergman**. Fructose effect to suppress hepatic glycogen degradation. *J Biol Chem* **1987**, *262*, 11470-11477.
- **Young, F. G.** Claude Bernard and the discovery of glycogen; a century of retrospect. *Br Med J* **1957**, *1*, 1431-1437.
- **Yudkin, J.** Why blame sugar? *Chem Ind* **1967**, *35*, 1464-1466.
- **Zehnder, M., E. R. Christ, M. Ith, K. J. Acheson, E. Pouteau, R. Kreis, R. Trepp, P. Diem, C. Boesch and J. Decombaz**. Intramyocellular lipid stores increase markedly in athletes after 1.5 days lipid supplementation and are utilized during exercise in proportion to their content. *Eur J Appl Physiol* **2006**, *98*, 341-354.

XVII. List of Publications

Published as part of this thesis

- Manuscript 1: **Rosset R.**, Surowska A. and Tappy L. (2016). *Pathogenesis of Cardiovascular and Metabolic Diseases: Are Fructose-Containing Sugars More Involved Than Other Dietary Calories?* Current Hypertension Reports 18(6). **RR performed literature research and wrote a part of the manuscript.** Article in annex.

- Manuscript 2: Tappy L. and **Rosset R.** (2017). *Fructose Metabolism from a Functional Perspective: Implications for Athletes.* Sports Medicine. **RR performed literature research and wrote a part of the manuscript.** Article in annex.

- Manuscript 3: Egli L., Lecoultre V., Cros J., **Rosset R.**, Marques A.S., Schneiter P., Hodson L., Gabert L., Laville M. and Tappy L. (2017). *Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage.* American Journal of Clinical Nutrition. **RR participated to data acquisition, data analysis and writing of the manuscript.** Article in annex.

- Manuscript 4: **Rosset R.**, Lecoultre V., Egli L., Cros J., Dokumaci A.S., Zwygart K., Boesch C., Kreis R., Schneiter P., Tappy L. (2017). *Postexercise Muscle Energy Storage Following Mixed-Meals Containing Fructose or Glucose.* American Journal of Clinical Nutrition. **RR designed the study, performed experiments, analyzed data and wrote the manuscript.** Article in annex.

- Manuscript 5: **Rosset R.**, Lecoultre V., Egli L., Cros J., Rey V., Stefanoni N., Sauvinet V., Laville M., Schneiter P., Tappy L. (2017). *Endurance Training with or without Glucose-Fructose Ingestion: Effects on Lactate Metabolism assessed in a Randomized Clinical Trial on Sedentary Men.* Nutrients. **RR designed the study, performed experiments, analyzed data and wrote the manuscript.** Article in annex.

- Manuscript 6: **Rosset R.**, Egli L. and Lecoultre V. (2017). *Glucose-Fructose Ingestion and Exercise Performance: The Gastrointestinal Tract and Beyond.* European Journal of Sport Science. **Invited review paper for which RR performed literature research and wrote the manuscript.** Article in annex.

Publications in collaboration

- Bally L., Zueger T., Buehler T., Dokumaci A.S., Speck C., Pasi N., Ciller C., Paganini D., Feller K., Loher H., **Rosset R.**, Wilhelm M., Tappy L., Boesch C., Stettler C. (2016). *Metabolic and hormonal response to intermittent high-intensity and continuous moderate intensity exercise in individuals with type 1 diabetes: a randomized crossover study.* Diabetologia. **RR participated to study design and data acquisition.**
- Bally L., Kempf P., Zueger T., Speck C., Pasi N., Ciller C., Feller K., Loher H., **Rosset R.**, Wilhelm M., Boesch C., Buehler T., Dokumaci A.S., Tappy L. and Stettler C. (2017). *Metabolic Effects of Glucose-Fructose Co-Ingestion Compared to Glucose Alone during Exercise in Type 1 Diabetes.* Nutrients. **RR participated to study design and data acquisition.**
- Von Tobel J.S., Antinori P., Zurich M.G., **Rosset R.**, Aschner M., Glück F., Scherl A. and Monnet-Tschudi F. (2014). *Repeated exposure to Ochratoxin A generates a neuroinflammatory response, characterized by neurodegenerative M1 microglial phenotype.* Neurotoxicology. **RR participated to data acquisition.**

Oral presentations

- *Comparison of a bolus approach to Steele's equation in modelling in vivo glucose kinetics: a randomized cross-over controlled trial* at European Society for Clinical Nutrition and Metabolism (ESPEN) expert course on tracer methodology in metabolism, Stockholm, Sweden, 2014. **RR presented a 15-min talk.**
- *Fructose-induced hypertriglyceridemia does not enhance post-exercise resynthesis of intramyocellular lipid stores* at European College of Sport Science (ECSS), Malmö, Sweden, 2015. **RR presented a 10-min talk.**
- *Glucose-fructose beverages do not alter the effects of training on lactate metabolism* at European College of Sport Science (ECSS), Vienna, Austria, 2016. **3th place at Youth Investigator Award. RR presented a 4-min talk.**

Posters

- *L'exercice aérobie module l'hypertriglycéridémie induite par le fructose en atténuant la lipogenèse hépatique et intestinale* at congrès de l'Association des Chercheurs en Activités Physiques et Sportives (ACAPS), Grenoble, France, 2013. **Presentation by RR.**

Manuscript 1

Pathogenesis of Cardiovascular and Metabolic Diseases: Are Fructose-Containing Sugars more Involved than other Dietary Calories?

Curr Hypertens Rep (2016) 18:44
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Pathogenesis of Cardiovascular and Metabolic Diseases: Are Fructose-Containing Sugars More Involved Than Other Dietary Calories?

Robin Rosset[1] & Anna Surowska[1] & Luc Tappy [1]

Abstract There is increasing concern that sugar consumption may be linked to the development of metabolic and cardiovascular diseases. There is indeed strong evidence that consumption of energy-dense sugary beverages and foods is associated with increased energy intake and body weight gain over time. It is further proposed that the fructose component of sugars may exert specific deleterious effects due to its propension to stimulate hepatic glucose production and de novo lipogenesis. Excess fructose and energy intake may be associated with visceral obesity, intrahepatic fat accumulation, and high fasting and postprandial blood triglyceride concentrations. Additional effects of fructose on blood uric acid and sympathetic nervous system activity have also been reported, but their link with metabolic and cardiovascular diseases remains hypothetical. There is growing evidence that fructose at physiologically consumed doses may exert important effects on kidney function. Whether this is related to the development of high blood pressure and cardiovascular diseases remains to be further assessed.

Keywords Fructose and cardiovascular disease · Sugars and cardiovascular disease · Metabolic disease and sugar · Visceral obesity · Uric acid · Sympathetic nervous system · Endothelial dysfunction

Abbreviations

ATP, ADP, AMP	Adenosine tri-, di- and monophosphate
ChREBP	Carbohydrate-responsive element-binding protein
SREBP-1c	Sterol-regulatory element-binding protein 1c
CoA	Coenzyme A
ACC	Acetyl-CoA carboxylase
FAS	Fatty acid synthase
SCD	Stearoyl-CoA desaturases
HDL	High-density lipoproteins
VLDL	Very low-density lipoproteins
SNS	Sympathetic nervous system
NO	Nitric oxide

Introduction

Obesity prevalence was low in Western societies and virtually absent in most South-American, African, and Asian countries at the beginning of the twentieth century. The number of individuals overweight or obese (i.e., body mass index ≥25 kg m^{-2}) has however increased dramatically since then, to presently affect close to 40 % of the population worldwide [1]. This augmentation closely paralleled rises in the prevalence of coronary heart diseases, hypertension, and stroke [2].

The development of obesity results from a long-lasting imbalance between energy intake and energy expenditure and may occur following the excess consumption of any energy-containing nutrient. There has however been a growing concern that the deposition of body fat may be particularly favored by dietary sugars, that would at first sight appear at odds with the recommendations to consume at least five daily servings of fruits and vegetables. Yet, these adverse health

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effects were specifically assigned to Bfree^ sugars, defined as Bmono- and disaccharides added to foods by the manufacturer, cook, or consumer, plus sugars naturally present in honey, syrups, and fruit juices^ [3]. Collectively, free sugars are estimated to represent ≈20 % of daily energy intake in westernized countries, mostly as mixtures of glucose and fructose [4].

Why May Fructose Specifically Contribute to Cardiometabolic Risk?

Suspicions on fructose were initially raised by observational studies showing associations between cardiometabolic diseases and consumption of fructose-containing sugars (i.e., sucrose, high-fructose corn-syrup, and fruit juices) but not with lactose (a glucose-galactose dimer). These considerations were also confirmed by several pre-clinical and clinical studies globally showing that dietary fructose can induce several metabolic alterations bearing close similarity with the metabolic syndrome [5, 6]. The existence of direct causal link between fructose intake and the development of metabolic and cardiovascular diseases, however, remains also disputed [7, 8].

Unlike glucose and fatty acids that are key energy substrates and are metabolizable by most cells, fructose cannot be used as such and requires to be pre-processed in splanchnic organs [9]. In the liver, fructose undergoes a three-step intermediate metabolism (i.e., Bfructolysis^) generating trioses-phosphates that join the glycolysis pathway. Since the main phosphorylating enzyme governing this sequence (fructokinase, converting fructose to fructose-1-phosphate) is unregulated, fructose intake has been proposed to lower hepatic ATP pools, thus stimulating hepatic nucleotide turnover and uric acid production [10]. Furthermore, fructose was considered to induce an overload of trioses phosphates that could either be converted to glucose, lactate, glycerol or fatty acids, or be directed to mitochondrial oxidation. The synthesized products, in turn, could be secreted in the circulation or furnish liver glycogen and triglycerides pools [11•]. As a remarkable result of this hepatocentric metabolism, fructose induces very little insulin secretion. These fructose-induced increases in hepatic glucose production and VLDL-TG secretion were proposed by several authors as being early markers of cardiometabolic diseases [5, 6]. In the next sections, we will discuss how fructose could specifically increase cardiovascular risk by promoting visceral and intrahepatic fat deposition, hypertriglyceridemia, and hypertension.

Effects of Fructose on Visceral and Intrahepatic Fat Deposition

Results from many large and medium-sized cohort studies clearly indicate that the consumption of sugar, and more specifically of sugar-sweetened beverages, is related with weight gain over time [12•]. This association becomes weaker when adjusted for total energy intake, however, indicating that it is confounded by obesity. Of interest, some small-sized short-term intervention studies noticed that dietary fructose may be more specifically associated with visceral fat than glucose. The mechanisms responsible remain to be understood, yet, particularly since this effect was observed only in males in one of these trials [13]. If these data were to be replicated in other well-controlled larger studies, such an effect of fructose would indeed be a major health concern, given the strong association between visceral adipose tissue and both insulin resistance and cardiovascular risk.

The relationship between cardiovascular and/or metabolic risk and visceral fat depots in general has been recently reconsidered in favor of a prevailing role of intrahepatic fat. It is indeed well established that the prevalence of non-alcoholic fatty liver disease is very high among subjects with abdominal obesity and insulin resistance. Furthermore, the study of subgroups of subjects with similar visceral fat mass but differing intrahepatic fat suggested that ectopic fat depots in the liver, rather than omental fat, may be directly linked with insulin resistance and cardiovascular risk [14]. Interestingly, it was observed that switching healthy volunteers from a low to a high-fructose diet significantly increased intrahepatic fat content within a few days [15]. This effect may be related with the conversion of a portion of fructose carbons into lipids through hepatic de novo lipogenesis. In this energetically costly anabolic pathway, mitochondrial citrate is first transported to the cytosol, then converted to acetyl-CoA and to malonyl-CoA. The latter, in turn, acts as a precursor for the iterative addition of two-carbon acetate chains to acetyl-CoA, resulting in the synthesis of palmitate (C16:0). Palmitate can be elongated to stearate (C18:0) and to fatty acids of longer carbon backbone by elongases, and both palmitate and stearate can be desaturated to palmitoleate (C16:1) and oleate (C18:1), respectively (Fig. 1).

The net effect of de novo lipogenesis on cardiovascular risk remains unclear and could largely depend on factors beyond the net mass of acetyl-CoA polymerized. The type of synthesized fatty acids may be particularly important, especially since high concentrations of palmitate have been well-described to promote atherogenesis. Alternatively, the activation of ChREBP and specifically of SREBP-1c could limit palmitate and stearate accumulation by promoting their desaturation into palmitoleate and oleate by SCD. Oleate, in turn, was found in vitro to protect against palmitate-induced lipotoxicity [16]. Consistent with a key role of saturated to unsaturated fatty acid ratio (SFA:UFA), SCD-deficient mice are highly insulin-sensitive and resistant to diet-induced obesity [17]. Similarly, the ratio of dietary fatty acids was also shown to critically affect carbohydrate-induced steatosis [18]. How these effects interplay in humans, and how they alter cardiovascular health could be investigated in

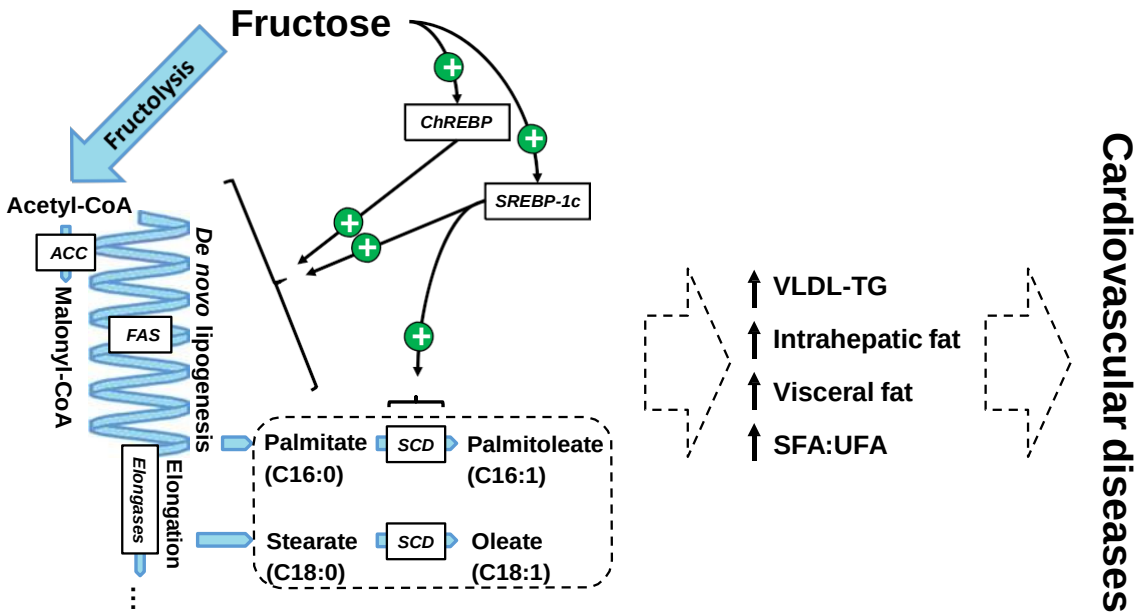

Fig. 1 Potential roles of fructose-derived de novo lipogenesis on cardiovascular health. De novo lipogenesis, i.e., synthesis of long-chain fatty acids from acetyl-coA precursors, can occur following the pre-respiratory degradation of any nutrients. This pathway is particularly active during fructose overfeeding experiments, however, and the high lipogenic potential of fructose is due to (1) an unregulated synthesis of pyruvate and acetyl-CoA and (2) a stimulation of lipogenic transcription factors CHREBP and SREBP-1c by intracellular fructose metabolites. In turn, this stimulation of de novo lipogenesis may be associated with increased VLDL-triglyceride secretion and intrahepatic fat accumulation. The outcome of this process may also depend on the ratio of saturated to unsaturated fatty acids (SFA:UFA) in blood lipids, affected by dietary fat as well as by the relative activities of the enzymes involved in synthesis (fatty acid synthase), elongation (elongases), and desaturation (stearoyl-CoA desaturase) of newly generated fatty acids. Abbreviations: ChREBP carbohydrate-responsive element-binding protein, SREBP-1c sterol regulatory element-binding protein 1c, CoA coenzyme A, ACC acetyl-CoA carboxylase, FAS fatty acid synthase, SCD stearoyl-CoA desaturases, VLDL-TG triglycerides in very low-density lipoprotein fraction, SFA:UFA saturated-to-unsaturated fatty acids ratio

Inuits, in which specific variants of desaturases and elongases were recently found [19].

Fructose has been amply documented to stimulate hepatic de novo lipogenesis more efficiently than glucose, and this was proposed to account for its role in non-alcoholic fatty liver disease. Recent short-term intervention studies, however, revealed that hypercaloric high-fructose and high-glucose diets both increased intrahepatic fat content to the same extent in healthy human volunteers [15, 20•]. The same was also observed for high-fat hypercaloric diet [21•]. In contrast, the consumption of weight-maintenance high-fructose diet led to a moderate stimulation of hepatic de novo lipogenesis [22••, 23•], but neither high-fructose nor high-glucose diets were associated with increased intrahepatic fat content [20•]. This suggests that, more than fructose per se, excess energy intake may be responsible for the development of hepatic steatosis.

Effects of Fructose on Fasting and Postprandial Triglycerides

Many epidemiological studies indicated a strong association between sugar intake and blood triglyceride concentrations [24]. Fructose, in particular, was found in numerous short-term intervention studies to further increase fasting and postprandial triglycerides [13, 25] and may also alter cholesterol metabolism [26].

The mechanisms underlying fructose-induced hypertriglyceridemia remain controversial. On one hand [27], the conversion of fructose carbons into fatty acids by de novo lipogenesis may be responsible for both increased hepatic triglyceride synthesis and VLDL-triglyceride secretion, concomitantly enhancing circulating triglycerides appearance [28]. On the other hand, fructose was suggested to impair plasma clearance of triglyceride-rich lipoproteins, since co-ingested fructose enhanced the triglyceride response to a mixed meal [25]. In turn, both processes may vary according to population, fructose dose, and co-ingested nutrients.

Since high fasting and postprandial blood triglyceride are recognized to be independent risk factors for cardiovascular diseases, their augmentation following fructose intake is consistent with a role of fructose in cardiovascular risk. Accordingly, when fructose replaced starch in weight-maintaining controlled diets, it significantly raised blood triglyceride concentrations. Interestingly, these increases were larger and mainly observed when fructose was administered as part of hypercaloric diets, suggesting a modulation of this effect by energy balance. In turn, other factors may be involved, since moderate exercise normalized fasting and

postprandial triglycerides concentrations, even in conditions of neutral energy balance [23•]. The available data, however, clearly indicate that fructose-induced hypertriglyceridemia generally remains within the normal range (i.e., under pathological thresholds), and whether this reflects a normal effect of fructose [29] or a true risk factor is still debated.

Effects of Fructose on Blood Pressure and Hemodynamics

Recent epidemiological data from large cohort studies show a strong positive relationship between sugars [24] or fructose [30] intake and hypertension. Both associations, interestingly, were markedly attenuated when adjusted for body weight, suggesting that obesity was an important confounder. Few short-term intervention studies reported the effects of altering dietary fructose content on blood pressure. Although most of them did not report any significant effect of on blood pressure [13, 31], one study documented a 6 % increase in ambulatory blood pressure in middle-aged men with the metabolic syndrome [32]. Several mechanisms may potentially account for hypertensive effects of fructose (Fig. 2) [33]:

– Sympathetic Nervous System (SNS) Activation

  Carbohydrate ingestion and intravenous infusion of glucose both increase sympathetic nervous system activity, the effects of which are highly complex and only partly elucidated. SNS activity was suspected to be protective against obesity in some individuals through allowing energy wasting. Consequently, it has been intensively investigated in studies measuring the concentrations and turnover of norepinephrine [34], as well as organ-specific microneurography [35]. These reports, however, resulted in little support of an anti-obesogenic effect, but demonstrated that SNS activation was much more complexly orchestrated than previously thought, and was differentially regulated in various organs.

  The functional significance of food-induced sympathetic activation remains largely hypothetical. Glucose and insulin infusion both increase markers of SNS activity, and at the same time, stimulate resting energy expenditure. Since this effect can be partially inhibited by administration of beta-adrenergic antagonists [36], SNS was considered to be involved in adaptive thermogenesis. Such SNS-mediated heat generation was initially proposed to mainly occur in brown adipocytes in rodents [34] and in skeletal muscle in humans [37]. This concept, however, may need to be revised following demonstrations of brown adipose tissue existence in healthy human adults [38]. These specialized cells, alike recently discovered Bbeige^ adipocytes [39], can generate heat by uncoupling mitochondrial respiration from ATP synthesis and may be active during stressful situations such as cold exposure or exercise [40]. Whether this is related to food-induced sympathetic activation and regulation of body temperature remains speculative, however.

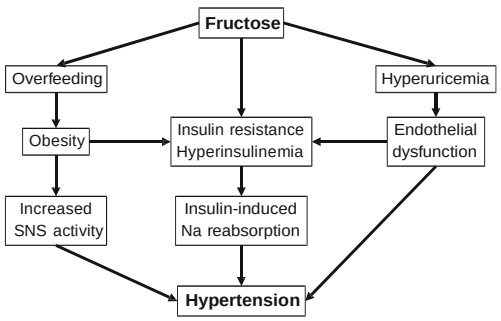

Fig. 2 Potential mechanisms linking dietary fructose and hypertension. See text for detailed descriptions

  SNS activation varies according to the type of carbohydrate consumed and to the organ considered. Glucose administration, either intravenous or oral, stimulates muscle sympathetic nerve activity [41]. This effect of glucose appears to be primarily mediated by insulin and is coupled with insulin-induced NO-ergic vasodilation of skeletal muscle arterioles [42, 43]. Interestingly, fructose, as glucose, increases postprandial energy expenditure, and this effect could be lowered by about 30 % using beta-adrenergic antagonists [44]. However, glucose activates muscle sympathetic nerve activity while fructose does not, suggesting that stimulation of adaptive thermogenesis by fructose is independent of insulin secretion and does not involve muscle sympathetic nerve activation.

  The effects of the sympathetic nervous system on cardiovascular and hemodynamic functions are highly complex and only partially elucidated. They involve the coordinated activation of several subsets of sympathetic nervous fibers, as was illustrated by studies on mental stress [45, 46]. Mental stress can be elicited in animals by forced immobilization and in humans by complex experimental paradigms reproducing what happens in a prey when encountering a predator. Activation of the sympathetic nervous system altogether with stimulation of glucocorticoids and catecholamines from the adrenal medulla then elicits a set of coordinated hemodynamic and metabolic responses to allow for Bfight or flight^ responses (i.e. aiming to immediately optimize muscle functions to outrun the predator or eventually to fight for life). These include increases in cardiac output and ventilation in order to raise systemic blood flow and oxygenation. Simultaneously, circulating substrates are raised following the stimulation of hepatic glucose output and adipose tissue lipolysis by glucocorticoids and sympathetic mediators. Finally, muscle sympathetic nerve activation occurs, inducing a local vasodilation that favors as a whole

an increased delivery of oxygen and energy substrates to skeletal muscle, ensuring optimal immediate response.

Food intake also elicits a set of integrated hemodynamic adaptations to favor blood perfusion in digestive organs at the expense of most other tissues excepting the brain. SNS activation, again, is instrumental in many of these effects, notably by increasing both heart rate and contraction force, thus raising cardiac output [47]. Even if digestive organs vasodilation is essentially produced by local autoregulatory mechanisms, episodes of severe postprandial systemic hypotension in individuals with autonomic neuropathy indicate that SNS activation is still required to maintain physiological blood pressure [48]. This is mainly attained by alpha-adrenergic vasoconstriction of arterioles in non-digestive vascular beds in the kidneys and the skin. Surprisingly, ingestion of a meal is associated with sympathetic nerve activation altogether with arteriolar vasodilation in skeletal muscle. This effect appears mediated by insulin-induced release of nitric oxide from muscle endothelium, possibly triggered by activation of sympathetic NO-ergic fibers. This unexpected vasodilation in skeletal muscle induced by meal ingestion has also now been recognized to contribute to insulin sensitivity by facilitating the delivery of both glucose and insulin to skeletal muscle.

Acute administration of a fructose drink, but not of glucose, produces a small and transient increase in systolic and diastolic blood pressure in healthy humans [49]. This may indicate that fructose activates vasoconstrictive sympathetic fibers in some non-digestive organs to a larger extent than glucose and hence may favor the development of hypertension is some individuals. This effect was however observed with administration of pure fructose only, but disappeared when glucose and fructose were co-ingested due to glucose-induced, insulin-dependent muscle vasodilation [50]. This therefore suggests that ingestion of fructose-containing sweeteners such as sucrose, honey, and high-fructose corn syrup is unlikely to produce large acute increases in blood pressure.

– Hyperuricemia

Intravenous infusion of large fructose loads has been recognized since several decades to result in several metabolic perturbations including hyperlactatemia, hypoglycemia, and hyperuricemia [10]. The same effects can be observed when subjects with hereditary fructose intolerance ingest even small amounts of fructose or sorbitol. This condition is due to an inherited deficiency of aldolase B, with the consequence that fructose is phosphorylated to fructose-1-P in fructokinase-expressing cells, a reaction which is associated with ATP consumption [51]. The absence of aldolase B thereafter impairs further metabolism of fructose-1-P and thus prevents ATP regeneration. This leads to ATP depletion and energy crisis in fructokinase-expressing cells such as hepatocytes, in which this impairs glucose production, thus leading to hypoglycemia. In the kidneys, it leads to proximal tubular dysfunction and acidosis. In addition, ATP depletion stimulates the degradation of ADP to AMP and inositol, thus increasing uric acid production [52]. It is likely that, in normal subjects, the i.v. administration of large amounts of fructose leads to a rapid phosphorylation of fructose to fructose-1-phosphate, which outpaces temporarily the ability of aldolase B to clear fructose-1-phosphate. It results that i.v. administration of fructose is associated with an acute increase in blood uric acid and can occasionally cause acute metabolic defects similar to those encountered in hereditary fructose intolerance.

Administration of oral physiological fructose loads is occasionally associated with moderate postprandial increases in blood uric acid concentration, the latter effect being however inconstant [53]. In contrast, an increase in dietary fructose intake is associated with moderate, yet highly significant elevations in fasting uric acid concentrations [53]. This effect is consistently observed in hypercaloric, high-fructose diets when fructose isoenergetically replaces starch or fat. This long-term moderate hyperuricemia is unlikely to be explained by ATP depletion, since it is not associated with the landmark effects of energy crisis in liver cells (i.e., hypoglycemia) or in renal tubules (i.e., proximal tubule dysfunction and renal acidosis). Instead, most subjects with hyperuricemia secondary to ingestion of a high-fructose diet present evidence of increased hepatic glucose production and de novo lipogenesis [54], two energy-requiring metabolic processes unlikely to be active in ATP-depleted cells. In turn, consumption of a high-fructose diet is almost invariably associated with an increase in blood lactate concentrations [46], but this merely reflects a physiological conversion of fructose into a more ubiquitously usable energy substrate, rather than a state of acidosis. Early isotope studies indicated that fructose may stimulate the endogenous synthesis of uric acid [55], but the exact mechanism and functional significance of this remains unknown. In turn, the study of kidneys transporter proteins has dramatically progressed over the past decades and provides additional putative mechanisms. Uric acid and lactate share the same transporter in proximal tubule cells, URAT1, and the high blood lactate concentrations observed after fructose intake may compete with uric acid filtration and decrease uric acid renal clearance (Fig. 3) [56].

Interestingly, it has recently been documented that uric acid may exert important regulatory effects in several physiological processes [52]. Hyperuricemia potently activates the NALP3 inflammasome (cryopyrin) [57] and may therefore trigger a low-grade chronic inflammation

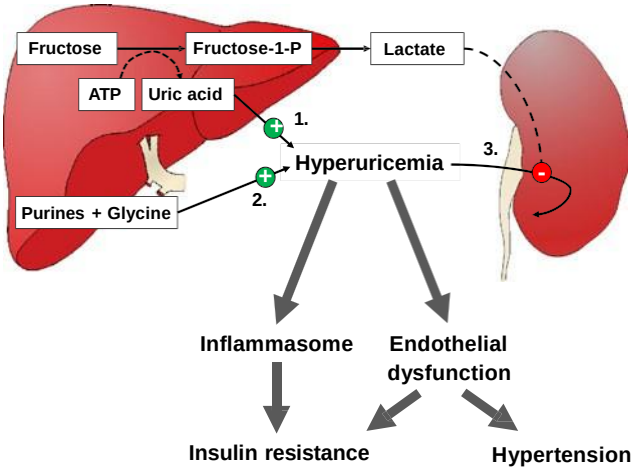

Fig. 3 Potential deleterious effects of fructose-induced hyperuricemia. Fructose may increase plasma uric (i.e., hyperuricemia) acid through (1) transient intrahepatocellular energy depletion (due to ATP consumption by fructokinase following large i.v. fructose loads), (2) stimulation of endogenous uric acid synthesis from purine and glycine precursors (by unknown mechanisms), and (3) decreased uric acid excretion (by competition with lactate for secretion into urinary tubules). In turn, hyperuricemia may contribute to the metabolic effects of fructose by triggering inflammation through the NALP3 inflammasome or by impairing endothelial function, in turn promoting insulin resistance and hypertension. Hyperuricemia may also activate lipogenic enzymes (not shown). ATP adenosine triphosphate, Fructose-1-P fructose-1-phosphate

secondarily responsible the development of insulin resistance [58]. An increase in uric acid in the liver may stimulate fructose metabolism [59•] and lipogenic enzymes [60•] and may thus contribute to further enhance de novo lipogenesis. High uric acid concentration may also impair normal endothelial function and may contribute to the development of hypertension or to that of insulin resistance by preventing insulin-induced vasodilation [61]. The latter mechanisms have been reported in rodent models of high-fructose diet, while human observations were more mitigated. One study reported that middle-aged, hypertensive, obese high-fructose consumers had significantly reduced blood pressure when their blood uric acid concentrations were lowered with administration of allopurinol [32]. However, this was not associated with any improvement of markers of insulin resistance such as glucose and insulin concentration nor of dyslipidemia.

– Kidneys Dysfunction

The effects of dietary fructose on blood pressure remain altogether highly controversial. Fructose is associated with the development of hypertension in some, but not all rat models fed a high-fructose diet. The explanation responsible between studies differences remains unknown, but other co-variables are likely to be involved. Some investigators have suggested that hypertension occurring in some high-fructose fed animals may be due to some experimental artifacts such as diet copper [62] or magnesium [63] deficiencies.

Of more importance, there is growing evidence that fructose enhances the effects of a high-salt diet on blood pressure [64••]. This may result from multiple effects of fructose on renal handling of sodium and other ions. Fructose has indeed been shown to increase the reabsorption of sodium in the proximal tubule through various mechanisms, including enhanced bicarbonate reabsorption and increased sodium-hydrogen exchangers [65].

Major regulatory steps of sodium and water metabolism linked to blood pressure control take place in the distal renal tubule. Of interest, filtered glucose is normally completely reabsorbed from the primary urine in the proximal tubule and does not reach the distal renal tubule apart in overt diabetes mellitus. Chronic hyperglycemia and glycosuria in diabetes mellitus are associated with increased kidneys size and the progressive development of renal and cardiovascular dysfunction. Compared to glucose, blood fructose concentrations remain most of the time very low and can only transiently reach 0.5–0.8 mmol L^{-1} following a fructose-containing meal. Under such conditions, however, fructose can be filtered by the kidneys and appears in primary urine. In contrast to glucose, which can be efficiently reabsorbed in the proximal tubule through an active sodium-glucose co-transport, fructose is only removed from the proximal tubule by passive diffusion. It seems thus conceivable that dietary fructose can reach the distal parts of the nephron, where its effects on renal function remain to be evaluated [66].

Epidemiological studies show an association between sugar [24] and fructose [30] intake and the development of hypertension or cardiovascular diseases, yet obesity is an important confounder in these analyses. There is strong evidence that a high sugar intake is frequently encountered in obese subjects, and part of these associations may therefore be related to excess body weight or insulin resistance. In support of this hypothesis, insulin-resistant subjects have chronically elevated hyperinsulinemia, which has been shown to increase renal sodium reabsorption [67]. There is also evidence that endothelial function is frequently impaired in insulin resistant subjects, possibly due to lipid-mediated endothelial toxicity. Such endothelial dysfunction have minimal effects on resting arterial pressure, but can be associated with excessive blood pressure rise in response to external stimuli such as stress [45, 46].

Conclusion

In a well-balanced analysis, Casazza and colleagues noticed that Bpassionate interests, the human tendency to seek explanations for observed phenomena, and everyday experience appear to contribute to strong convictions about obesity^ [68]. In the context of fructose research, these words of caution sound

particularly pertinent, and many mechanisms still need to be understood before reaching definitive conclusions. There is indeed a strong evidence that a high sugar intake is present in many obese patients, and that can be associated with the development of non-communicable diseases. In our opinion, this is mostly explained by the hedonic properties of sugary foods, which tend to favor the overconsumption of highly palatable energy-dense foods when available. Compared to other nutrients such as glucose or fat, fructose is first processed in splanchnic organs and then released as glucose, lactate, or VLDL-TG into the systemic circulation. This may contribute to the development of and hepatic insulin resistance during chronic excessive intake of both total energy and sugar. Additional effects of fructose on blood uric acid and sympathetic nervous system activity have also been reported, but their link with metabolic and cardiovascular diseases remains hypothetical. Interestingly, there is growing evidence that more fructose may escape first pass hepatic liver metabolism than previously thought, and that systemic low fructose concentrations may exert important effects on kidney function. Whether this is related to the development of high blood pressure and cardiovascular diseases remains to be further assessed.

Compliance with Ethical Standards

Conflict of Interest MSc. Rosset, Surowska and Dr. Tappy declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any original data from studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Ng M et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766–81.
2. DeMarco VG, Aroor AR, Sowers JR. The pathophysiology of hypertension in patients with obesity. Nat Rev Endocrinol. 2014;10(6):364–76.
3. World Health Organisation. Guideline: sugars intake for adults and children. Geneva: World Health Organization; 2015. 49.
4. Sun SZ et al. Fructose and non-fructose sugar intakes in the US population and their associations with indicators of metabolic syndrome. Food Chem Toxicol. 2011;49(11):2875–82.
5. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. Obes Rev. 2013;14(8):606–19.
6. Bray GA, Popkin BM. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes? Health be damned! Pour on the sugar. Diabetes Care. 2014;37(4):950–6.
7. Kaiser KA et al. Will reducing sugar-sweetened beverage consumption reduce obesity? Evidence supporting conjecture is strong, but evidence when testing effect is weak. Obes Rev. 2013;14(8):620–33.
8. Kahn R, Sievenpiper JL. Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes? We have, but the pox on sugar is overwrought and overworked. Diabetes Care. 2014;37(4):957–62.
9. Campos VC, Tappy L. Physiological handling of dietary fructose-containing sugars: implications for health. Int J Obes. 2016;40 Suppl 1:S6–11.
10. Van den Berghe G. Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog Biochem Pharmacol. 1986;21:1–32.
11.• Sun SZ, Empie MW. Fructose metabolism in humans—what isotopic tracer studies tell us. Nutr Metab (Lond). 2012;9(1):89. A very complete review on the state of knowledge of human fructose metabolism, as assessed by studies using isotopic tracers.
12.• Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ. 2013;346:e7492. The reference meta-analysis regarding the relationships between sugar intake and body weight.
13. Stanhope KL et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 2009;119(5):1322–34.
14. Fabbrini E et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci U S A. 2009;106(36):15430–5.
15. Ngo Sock ET et al. Effects of a short-term overfeeding with fructose or glucose in healthy young males. Br J Nutr. 2010;103(7):939–43.
16. Ricchi M et al. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. J Gastroenterol Hepatol. 2009;24(5):830–40.
17. Miyazaki M et al. Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. Cell Metab. 2007;6(6):484–96.
18. Siddiqui RA et al. Comparative study of the modulation of fructose/sucrose-induced hepatic steatosis by mixed lipid formulations varying in unsaturated fatty acid content. Nutr Metab (Lond). 2015;12:41.
19. Fumagalli M et al. Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science. 2015;349(6254):1343–7.
20.• Johnston RD et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. Gastroenterology. 2013;145(5):1016–1025 e2. One of the few intervention studies having compared fructose to another reference nutrient (glucose) at both weight maintenance energy intake hypercaloric energy intake.
21.• Lecoultre Vet al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. Obesity (Silver Spring). 2013;21(4):782–5. A compilation of studies comparing the effects of fructose, fat and glucose on hepatic fat content.
22.•• Schwarz JM et al. Effect of a high-fructose weight-maintaining diet on lipogenesis and liver fat. J Clin Endocrinol Metab. 2015;100(6):2434–42. One of the few intervention studies having assessed the effects of fructose ingested as part of a weight maintenance energy intake.

23.• Egli L et al. Exercise prevents fructose-induced hypertriglyceridemia in healthy young subjects. Diabetes. 2013;62(7):2259–65. One of the few intervention studies having assessed the effects of fructose ingested as part of a weight maintenance energy intake.
24. Te Morenga LA et al. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. Am J Clin Nutr. 2014;100(1):65–79.
25. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr. 2007;85(6):1511–20.
26. Zhang YH et al. Very high fructose intake increases serum LDL-cholesterol and total cholesterol: a meta-analysis of controlled feeding trials. J Nutr. 2013;143(9):1391–8.
27. Reaven GM, Ho H, Hoffman BB. Effects of a fructose-enriched diet on plasma insulin and triglyceride concentration in SHR and WKY rats. Horm Metab Res. 1990;22(7):363–5.
28. Taghibiglou C et al. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model. J Biol Chem. 2000;275(12):8416–25.
29. Laughlin MR. Normal roles for dietary fructose in carbohydrate metabolism. Nutrients. 2014;6(8):3117–29.
30. Jalal DI et al. Increased fructose associates with elevated blood pressure. J Am Soc Nephrol. 2010;21(9):1543–9.
31. Le KA et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. Am J Clin Nutr. 2006;84(6):1374–9.
32. Perez-Pozo SE et al. Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: role of uric acid in the hypertensive response. Int J Obes. 2010;34(3):454–61.
33. Klein AV, Kiat H. The mechanisms underlying fructose-induced hypertension: a review. J Hypertens. 2015;33(5):912–20.
34. Young JB, Weiss J, Boufath N. Effects of dietary monosaccharides on sympathetic nervous system activity in adipose tissues of male rats. Diabetes. 2004;53(5):1271–8.
35. Berne C, Fagius J, Niklasson F. Sympathetic response to oral carbohydrate administration. Evidence from microelectrode nerve recordings. J Clin Invest. 1989;84(5):1403–9.
36. Acheson K, Jequier E, Wahren J. Influence of beta-adrenergic blockade on glucose-induced thermogenesis in man. J Clin Invest. 1983;72(3):981–6.
37. Astrup A et al. Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. Am J Physiol. 1985;248(5 Pt 1):E507–15.
38. van Marken Lichtenbelt WD et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med. 2009;360(15):1500–8.
39. Wu J et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012;150(2):366–76.
40. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med. 2013;19(10):1252–63.
41. Vollenweider P et al. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest. 1993;92(1):147–54.
42. Scherrer U et al. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest. 1994;94(6):2511–5.
43. Steinberg HO et al. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest. 1994;94(3):1172–9.
44. Schwarz JM et al. Thermogenesis and fructose metabolism in humans. Am J Physiol. 1992;262(5 Pt 1):E591–8.
45. Seematter G et al. Effects of mental stress on insulin-mediated glucose metabolism and energy expenditure in lean and obese women. Am J Physiol Endocrinol Metab. 2000;279(4):E799–805.
46. Abdel-Sayed A et al. A high-fructose diet impairs basal and stress-mediated lipid metabolism in healthy male subjects. Br J Nutr. 2008;100(2):393–9.
47. Seematter G et al. Relationship between stress, inflammation and metabolism. Curr Opin Clin Nutr Metab Care. 2004;7(2):169–73.
48. Hoeldtke RD, Streeten DH. Treatment of orthostatic hypotension with erythropoietin. N Engl J Med. 1993;329(9):611–5.
49. Brown CM et al. Fructose ingestion acutely elevates blood pressure in healthy young humans. Am J Physiol Regul Integr Comp Physiol. 2008;294(3):R730–7.
50. Grasser EK, Dulloo A, Montani JP. Cardiovascular responses to the ingestion of sugary drinks using a randomised cross-over study design: does glucose attenuate the blood pressure-elevating effect of fructose? Br J Nutr. 2014;112(2):183–92.
51. Ali M, Rellos P, Cox TM. Hereditary fructose intolerance. J Med Genet. 1998;35(5):353–65.
52. Johnson RJ et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62(10):3307–15.
53. Wang DD et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. J Nutr. 2012;142(5):916–23.
54. Faeh D et al. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes. 2005;54(7):1907–13.
55. Raivio KO et al. Stimulation of human purine synthesis de novo by fructose infusion. Metabolism. 1975;24(7):861–9.
56. MacFarlane LA, Kim SC. Gout: a review of nonmodifiable and modifiable risk factors. Rheum Dis Clin North Am. 2014;40(4):581–604.
57. Martinon F et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440(7081):237–41.
58. de Rooij SR et al. Low-grade chronic inflammation in the relationship between insulin sensitivity and cardiovascular disease (RISC) population: associations with insulin resistance and cardiometabolic risk profile. Diabetes Care. 2009;32(7):1295–301.
59.• Lanaspa MA et al. Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. PLoS One. 2012;7(10):e47948. This experiment on hepatocytes describes new potentiel mechanism relating fructose intake and cardiovascular diseases.
60.• Lanaspa MA et al. Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. J Biol Chem. 2012;287(48):40732–44. One of the few intervention studies having assessed the effects of fructose ingested as part of a weight maintenance energy.
61. Kanbay M et al. The role of uric acid in the pathogenesis of human cardiovascular disease. Heart. 2013;99(11):759–66.
62. Fields M, Lewis CG. Dietary fructose but not starch is responsible for hyperlipidemia associated with copper deficiency in rats: effect of high-fat diet. J Am Coll Nutr. 1999;18(1):83–7.
63. Busserolles J et al. High fructose feeding of magnesium deficient rats is associated with increased plasma triglyceride concentration and increased oxidative stress. Magnes Res. 2003;16(1):7–12.
64.•• Ares GR, Ortiz PA. Direct renal effects of a fructose-enriched diet: interaction with high salt intake. Am J Physiol Regul Integr Comp Physiol. 2015;309(9):R1078–81. This minireview hints to specific effects of fructose on kidney function.
65. Song J et al. Effects of dietary fat, NaCl, and fructose on renal sodium and water transporter abundances and systemic blood pressure. Am J Physiol Renal Physiol. 2004;287(6):F1204–12.
66. Douard V, Ferraris RP. The role of fructose transporters in diseases linked to excessive fructose intake. J Physiol. 2013;591(2):401–14.
67. Quinones-Galvan A, Ferrannini E. Renal effects of insulin in man. J Nephrol. 1997;10(4):188–91.
68. Casazza K et al. Myths, presumptions, and facts about obesity. N Engl J Med. 2013;368(5):446–54.

Manuscript 2

Fructose Metabolism from a Functional Perspective:

Implications for Athletes

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REVIEW ARTICLE

Fructose Metabolism from a Functional Perspective: Implications for Athletes

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Abstract Substantial amounts of fructose are present in our diet. Unlike glucose, this hexose cannot be metabolized by most cells and has first to be converted into glucose, lactate or fatty acids by enterocytes, hepatocytes and kidney proximal tubule cells, which all express specific fructose-metabolizing enzymes. This particular metabolism may then be detrimental in resting, sedentary subjects; however, this may also present some advantages for athletes. First, since fructose and glucose are absorbed through distinct, saturable gut transporters, co-ingestion of glucose and fructose may increase total carbohydrate absorption and oxidation. Second, fructose is largely metabolized into glucose and lactate, resulting in a net local lactate release from splanchnic organs (mostly the liver). This 'reverse Cori cycle' may be advantageous by providing lactate as an additional energy substrate to the working muscle. Following exercise, co-ingestion of glucose and fructose mutually enhance their own absorption and storage.

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1 Introduction: Fructose from an Historical Perspective

Carbohydrates have been at the root of the co-evolution of plants and animals. On one hand, some plants developed a supraphysiological production of glucose, fructose and sucrose to attract pollinators and/or seed dispersers, while, on the other hand, mutualistic animal species adapted to detect and metabolize these compounds. Since these interrelations may explain speciation [1], the ancestors of many present animal species acquired the capacity to metabolize both glucose and fructose. Fructose, in particular, is naturally present in fruits, vegetables, nectars and honey. It is present either as a monosaccharide (i.e. 'free' fructose) or as part of sucrose (hereby referred to as sugar), a disaccharide formed from one molecule of glucose linked to one molecule of fructose. As such, fructose has been a nutrient long before the appearance of humans.

Fructose, sucrose and glucose, when present in aqueous solutions, activate sweet taste receptors present in the mouth, particularly on the tongue [2]. The resulting pleasant sensation has been shown to be associated with activation of dopaminergic reward pathways located in the mesolimbic nervous system [3]. This positive hedonic response to sugars is thought to have developed as a means of promoting the consumption of energy-dense, sugary foods. Interestingly, food protein and fat contents are also detected by umami taste receptors and fat transporting proteins (fatty acid transporter cluster of differentiation 36 [FAT/CD36]) expressed in taste buds, and could similarly reinforce the consumption of protein-rich and fat-rich energy dense foods, respectively [4]. The implications of fructose being sensed as sweeter than both sucrose and glucose remain misunderstood.

In pre-agricultural, hunter-gatherer humans, dietary patterns showed a high geographic variability, with vegetal-based products being principally consumed in most temperate areas. In such places, the proportion of energy derived from fruits was estimated to be as high as 42%, which is noteworthy and suggests that fructose consumption may have been substantial [5]. However, at that time, fructose intake was likely to show important fluctuations with geographical migrations and seasonal rhythmicity. In the Neolithic period, with the introduction of farming, cereals became the main food component until modern times (at least in European regions), and the most prevalent carbohydrate source was starch, a glucose polymer. With the appearance of trade, some sugar extracted from sugarcane was imported from the Middle East, but it remained a very expensive product that was mostly used as a spice. It was only during the colonial period that sugar became widely available at an affordable price in Europe and North America, and its consumption increased markedly in the 17th and 18th centuries. Interestingly, this was also associated with the consumption of novel plant extracts such as tea, coffee and chocolate [6].

More recently, sugar consumption further increased with the invention of sodas, ice creams, chocolate bars, and many other sugary products. At the turn of the second millennium, estimated sugar intake was 100–150 kg person^{-1} year^{-1} in Europe, North America and Australasia, but remained markedly lower (30–50 kg person^{-1} year^{-1}) in Asia and Africa [7]. In 1997, total individual fructose consumption, accounting for all fructose present in natural and artificial foods either as sucrose, high-fructose corn syrup (HFCS) or free fructose, was, on average, 60 g day^{-1} in the US. Of this, approximately 75% was from sugar-sweetened beverages and industrial foods (cereal products, confectionary, candies) to which sugar had been added, and only 25% from fresh fruits and vegetables [8, 9]. Thus, the major part of our dietary fructose presently comes from refined sugar (sucrose) and HFCS, a North-American industrial mixture of free glucose and fructose comprising 42–55% fructose. Since both contain approximately as much glucose as fructose, 60 g·day^{-1} fructose corresponds to 120 g·day^{-1} of sugar, containing approximately 500 kcal·day^{-1}, i.e. 20–25% of total energy requirements; however, these values can vary greatly among individuals depending on age, sex and country.

2 Fructose Metabolism in the Liver

Glucose represents the preferred substrate for eukaryotic cells and can be used as an energy source by all cells of the human organism. Due to the need for conserving energy between meals, and the fact that fat is more compact and lighter than carbohydrate as an energy storage form, most human cells (with the exception of the brain) have evolved to rely on glucose in the hours after meals, and on fatty acids otherwise (Fig. 1a). Accordingly, starch (which, after digestion, is absorbed as glucose) and fat provide the bulk of our daily energy intake.

In addition to these nutrients, we also consume foods containing protein (constituted of approximately 25 different amino acids), sugars or alcohol. Since the energy contained in these nutrients requires a large number of specific enzymes, it would not be efficient to express all of

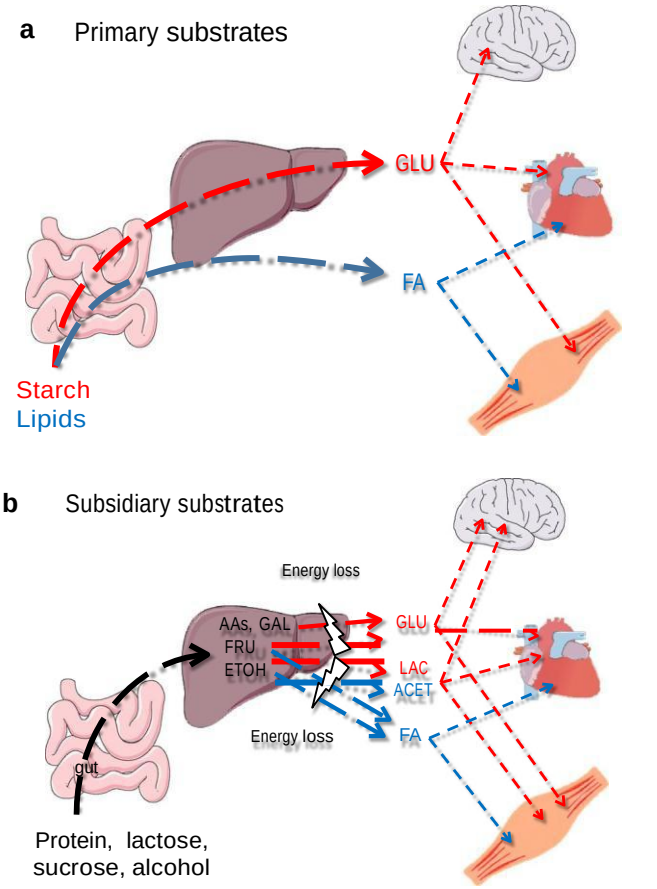

Fig. 1 Metabolism of 'primary' and 'subsidiary' substrates. Dietary GLU (pure or from starch) and lipids (containing FAs) are absorbed from the gut as GLU, triglycerides and other lipids. Both GLU and FA can then be directly used as energy substrates by most cells of the organism with minimal transformation (a). Other nutrients, including protein, lactose, sucrose and alcohol, also result in the absorption of metabolites such as AAs, GAL, FRU and ETOH, which cannot be directly used by most cells. These subsidiary substrates are dispensable and are inconstantly present in our diet. They need to be processed into GLU and FAs by specialized tissues (mainly the liver) before becoming a source of energy for other cells; this processing results in energy loss. They may also be processed into LAC and ACET, which, as metabolites of GLU and fat, can also be used by all cells as sources of energy (b). GLU glucose, FAs fatty acids, AAs amino acids, GAL galactose, FRU fructose, ETOH ethanol, LAC lactate, ACET acetate

them in every single cell, including highly specialized cells such as brain cells or skeletal muscle. Instead, these 'subsidiary' energy substrates are first metabolized in the liver, which is a specialized metabolic organ expressing all needed metabolic enzymes. There, they are converted into glucose, fatty acids, lactate (which, as the product of glucose degradation in glycolysis, can be used by any cell), or acetate (which, as a precursor of acetyl-coenzyme A [acetyl-CoA], can also be used by any cell) (Fig. 1b).

Fructose is typically a 'subsidiary substrate'. It is metabolized in the liver through a pathway known as 'fructolysis' by a set of three specific enzymes: (1) fructokinase, which catalyzes the synthesis of fructose-1-phosphate; (2) aldolase B, which further catalyzes the degradation into glyceraldehyde and dihydroxyacetone-phosphate (DHAP); and (3) triokinase, which finally converts glyceraldehyde into glyceraldehyde-3-phosphate (GAP) [10]. The end-products of fructolysis, the triose phosphates GAP and DHAP, are also intermediates of glycolysis and hence further metabolic steps share those of glucose metabolism. When glucose is used as an energy substrate in the liver or in any cell type of the organism, glycolysis is tightly regulated to match cellular energy demand. This is attained by an inhibition of phosphofructokinase (the enzyme converting fructose-6-phosphate into fructose 1,6-bisphosphate in the glycolytic pathway) by adenosine triphosphate (ATP) and citrate levels. In contrast, when fructose is metabolized in hepatocytes, there is no negative feedback on fructolysis enzymes, and fructose molecules are completely converted into triose phosphates, which are then further processed into acetyl-CoA, lactate, glucose, and eventually fatty acids and triglycerides [11].

The relative proportion of fructose metabolized to each of these end-products has been generally evaluated in isotope studies. Over 4–6 h postprandial, a pure fructose load was shown to be mostly directed to glucose-6-phosphate synthesis (gluconeogenesis), with 40–50% being slowly released as circulating glucose and 10–15% being stored as hepatic glycogen [12]. In comparison, the well-known effect of fructose to increase blood lactate concentration by inducing a net hepatic lactate release [13, 14] was relatively understudied, but estimated to occur with up to 25% of ingested fructose [10]. Dietary fructose oxidation was also shown to be significant, with 40–50% of fructose carbons being recovered in breath carbon dioxide (CO_2) over 4–6 h postprandial. Of note, this method cannot discriminate direct and indirect (issuing from newly synthesized substrates) fructose oxidation, and the relative proportions remain largely unknown [15]. In addition, some fructose carbons can be metabolized to acetyl-CoA, which is then converted into fatty acids later assembled into triglycerides in a metabolic pathway known as 'de novo lipogenesis'. The exact importance of this fate is not known accurately, but it is generally recognized as quantitatively small, based on estimates of fructose metabolism in other pathways [12]. Yet, de novo lipogenesis is nonetheless associated with increased secretion of very-low-density lipoproteins (VLDL) by the liver [16], as well as with some intrahepatocellular triglyceride storage [17]. While the factors governing fructose distribution between these fates are still incompletely understood, recent studies showed that fructose conversion into glucose and postprandial fructose oxidation, albeit somewhat less common, remain the major disposal pathways for fructose ingested in a mixed meal [18].

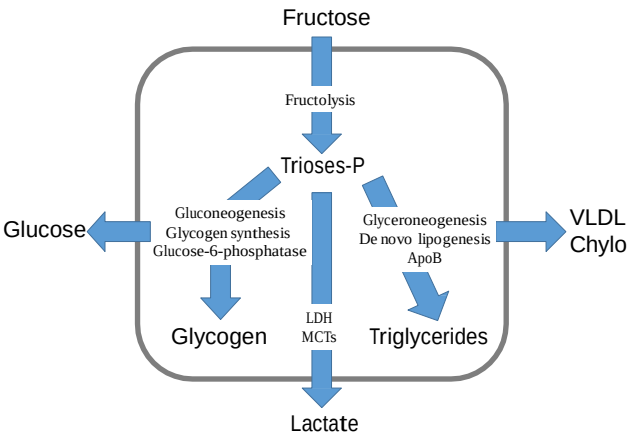

Fig. 2 Metabolism of fructose in liver, jejunum and kidney specialized cells. Fructose is thought to be essentially metabolized in cells expressing specific fructose-metabolizing enzymes, i.e. fructokinase, aldolase B, and triokinase. These enzymes are responsible for the degradation of fructose into Triose-P. This process, called fructolysis, is equivalent to glycolysis for glucose metabolism. In order to further process triose phosphates into glucose, lactate and circulating lipids, fructose-metabolizing cells also express gluconeogenic enzymes such as glucose-6-phosphatase and lipogenic enzymes, and can promote lactate efflux. They can also store fructose carbons as glycogen or intracellular triglycerides. VLDL very-low-density lipoproteins, Chylo chylomicrons, LDH lactate dehydrogenase, MCTs monocarboxylate transporters, ApoB apolipoprotein B, Triose-P triose phosphates

As a consequence of these multiple fates, fructose metabolism in hepatocytes, in addition to fructolytic enzymes, requires the presence of a whole set of metabolic enzymes and transporters not expressed in most other cells of the organism. In particular, hepatocytes express the proteins, allowing for the synthesis and systemic release of glucose and lactate, as well as fat synthesis, and lipoprotein secretion (Fig. 2).

3 Fructose Metabolism in Kidney Proximal Tubule Cells and Enterocytes

While it is generally assumed, for simplification, that fructose is almost entirely metabolized in the liver, it has long been known that renal proximal tubule cells also

express fructolytic enzymes. The functional significance and possible pathological dysfunctions of kidney fructose metabolism still remain largely unexplored. It may merely metabolize whatever fructose escaped first-pass hepatic metabolism. Circulating fructose concentrations generally do not exceed 0.6 mmol·L^{-1} after meals [19, 20], but can increase up to 1–3 mmol·L^{-1} as a result of intravenous fructose infusion. Under such conditions, the kidneys contribute up to 20% of total fructose metabolism [21, 22]. Like hepatocytes, kidney proximal tubule cells are among the few cell types that express the enzyme glucose-6-phosphatase, a glucose transporter (GLUT) able to carry fructose (i.e. GLUT2), and monocarboxylate transporters, and are hence able to release fructose carbons as glucose or lactate in the systemic circulation [23, 24]. In addition, kidney cells also express lipogenic enzymes [25].

Beside hepatocytes and kidney proximal tubule cells, small bowel enterocytes also express the complete enzymatic machinery required for fructose metabolism [26, 27]. Enterocytes thus contribute to overall gluconeogenesis from fructose and endogenous glucose production, as well as de novo lipogenesis and secretion of triglyceride-rich lipoprotein particles; however, the local function of these pathways in enterocytes, and the relative contribution of the gut to overall fructose metabolism, both remain speculative. One hypothesis is that intracellular fructose metabolism may be instrumental in promoting gut fructose absorption. Unlike glucose, which is mostly absorbed through a secondary active sodium-glucose cotransport, fructose absorption is relatively slow compared with glucose, and some degree of fructose malabsorption is commonly encountered when pure fructose is ingested; however, co-ingestion of glucose substantially facilitates gut fructose absorption [28]. An active fructose metabolism may therefore be a requisite for maintaining intracellular fructose concentrations lower than in the gut lumen, and passive fructose diffusion into the cells. In addition, enterocytes may well have yet unrecognized metabolic functions, and it can be speculated that intestinal fructose conversion into glucose may increase portal blood glucose concentration and may thus elicit regulatory signals through activation of portal glucose sensors [29]; however, these functional roles for intestinal fructose metabolism (Fig. 3) will require further dedicated research.

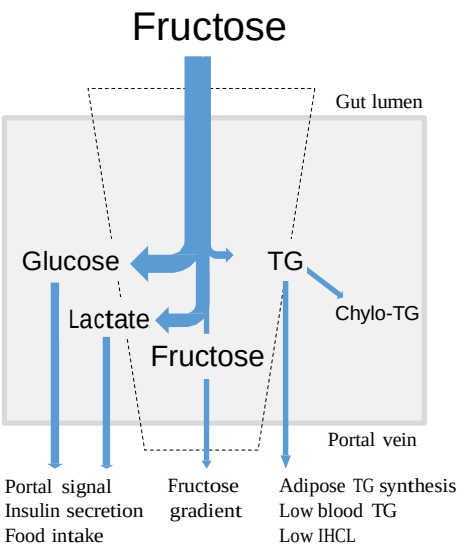

Fig. 3 Putative functional significance for fructose metabolism in enterocytes. Unlike glucose, which is absorbed through a secondary active transport, fructose is mainly absorbed from the gut by a facilitated diffusion operated by facilitated fructose transporters (mainly GLUT5). This requires fructose concentration to be lower inside the enterocytes than in the gut lumen. Fructose metabolism within the enterocyte may primarily facilitate fructose absorption by maintaining low intracellular fructose concentrations. In addition, it can be hypothesized that fructose conversion into glucose and lactate increases the concentration of these substrates in the portal vein, and may activate sensors responsible for the regulation of hepatic glucose metabolism, insulin secretion, or food intake. It can also be hypothesized that de novo lipogenesis in enterocytes may favor the storage of newly synthesized fat into adipocytes, thus limiting IHCL storage or very-low-density lipoprotein secretion. Chylo-TG chylomicrons containing triglycerides, IHCL intrahepatocellular lipid, GLUT5 glucose transporter 5

4 Effect of Fructose Overfeeding in Humans

In healthy subjects, fructose overfeeding is associated with increased endogenous glucose production, elevated fasting and postprandial plasma triglycerides and lactate concentrations, as well as increased intrahepatocellular lipid concentrations. These metabolic alterations are the direct consequence of fructose processing in fructokinase-expressing cells in the splanchnic area, and hence may be considered as normal adaptations to a fructose-rich diet; however, when associated with a high-energy intake and low physical activity, they may favor the development of diabetes and cardiovascular diseases [17, 30]. Nonetheless, a few recent reports also indicate that early markers of these alterations can be corrected when appropriate physical activity is performed [31, 32].

5 Fructose Metabolism During Exercise

During exercise, athletes face a very high energy need and hence a high requirement for metabolic substrates for their contracting muscles. This energy can be obtained either from carbohydrate (glucose) or fat oxidation, or, still (for a short period of time only), from glycolysis alone. The relative contributions of blood glucose, hepatic and muscle glycogen, blood fatty acids and intramyocellular lipids vary

according to the type and intensity of exercise performed, as well as sex, nutritional and training status of the exercising subject [33].

Carbohydrate oxidation during exercise is also dependent on exogenous carbohydrate intake. Indeed, glucose ingestion was found to increase total carbohydrate oxidation in a dose-dependent manner, partly replacing endogenous carbohydrate sources [34]. Importantly, this effect may be mediated by increased exogenous glucose oxidation, which is measurable by ^{13}C-labeling and was shown to be maximal at rates of glucose ingestion close to 1.1 g·min^{-1} [34]. The widely-accepted explanation for this plateau relates to the intestine, where glucose absorption is saturable during exercise. Many studies have also evaluated the potential benefits of fructose ingestion, and ^{13}C-labeled fructose was indeed shown to be oxidized during exercise [35]; however, pure fructose did not confer any advantage compared with glucose, but caused gastrointestinal distress secondary to incomplete gut absorption [36].

Nonetheless, fructose may have beneficial effects when administered together with glucose by increasing total absorption of gut hexoses. Indeed, it enters the enterocyte through a different apical transporter (GLUT5) than glucose or galactose, and several authors have shown that a larger maximal total and exogenous carbohydrate oxidation (up to 1.75 g·min^{-1}) was obtained with ingestion of fructose-glucose mixtures than with glucose alone [35, 36]. The increase in total carbohydrate oxidation with the addition of fructose to glucose drinks in exercising athletes may appear surprising given the absence of fructokinase in skeletal muscle, and the fact that muscle hexokinase has much lower affinity for fructose than glucose. When ^{13}C-labeled fructose is ingested immediately before or during exercise, breath $^{13}CO_2$ elimination nonetheless increases rapidly and may account for between 50 and 100% of fructose carbon disposal [35]. This most likely reflects oxidation of glucose synthesized from fructose in skeletal muscle.

5.1 Reverse Hepatic-Muscle Cori Cycle During Exercise

Although fructose administration has been repeatedly reported to elevate arterial lactate concentrations, the functional role of this effect has been largely ignored. Interestingly, there are rare inborn errors of fructose metabolism that help better understand the consequences of normal fructose metabolism. Inherited absence of fructokinase causes essential fructosuria, a benign condition associated with high plasma fructose concentration and urinary fructose excretion after ingestion of fructose-containing meals. In contrast, a deficiency of aldolase B causes hereditary fructose intolerance, a severe pathology associated with life-threatening hypoglycemia and renal tubular acidosis after ingestion of even small amounts of fructose or sorbitol [10]. In patients with hereditary fructose intolerance, lactic acidosis reflects the consequences of acute liver energy depletion on glucose and glycogen metabolism [37, 38]. Similarly, intravenous administration of large doses of pure fructose can cause acute hyperlactatemia and hyperuricemia, consistent with acute hepatic energy crisis [39]. This has led to consideration of fructose-induced hyperlactatemia as a possible toxic effect of fructose.

In contrast to the preceding, there is solid experimental evidence that fructose conversion into lactate in splanchnic tissue is one significant metabolic pathway for fructose disposal. Catheterization studies in dogs have documented that the liver maintains a net lactate uptake in fasting conditions and after a glucose meal, but switches to a net lactate production after fructose administration [40]. Other animal studies have postulated that up to 25% of fructose carbons may be disposed through this pathway in resting conditions [41]. In humans, the administration of ^{13}C-fructose was associated with an increase in plasma ^{13}C-lactate, indicating that fructose was indeed a precursor for lactate production [12].

There are very few human studies that have actually assessed lactate kinetics and fructose-lactate conversion in humans during exercise. Using stable isotopes, one trial documented the pathways used for fructose disposal in athletes fed glucose alone or glucose with fructose during prolonged exercise (Fig. 4) [42]. When glucose was administered orally as repeated bolus drinks at a rate of 2.0 g·min^{-1} (Fig. 4a), whole-body glucose production and utilization (corresponding to the sum of endogenous glucose production and glucose having been absorbed from the gut minus hepatic glucose uptake) was estimated to be approximately 1.0 g·min^{-1}, and was equal to whole-body carbohydrate oxidation. Lactate production and utilization were approximately 0.6 g·min^{-1}, and mainly reflected glucose-lactate shuttles between skeletal muscle fibers [43]. In contrast, when some of the ingested glucose was replaced by fructose 0.8 g·min^{-1} (Fig. 4b), total carbohydrate oxidation further increased by 30%. Further analyses showed that this was accounted for by increases in the oxidation of fructose released as blood glucose and lactate. In this condition, lactate was presumably produced from splanchnic organs, then extracted and used by peripheral tissues, including contracting skeletal muscle. This contrasts with the general concept that lactate is mainly produced by skeletal muscle to be used by the liver to synthesize glucose (Cori cycle) [44] and by other muscle fibers to be oxidized as an energy fuel (concept of cell-cell lactate shuttle) [43].

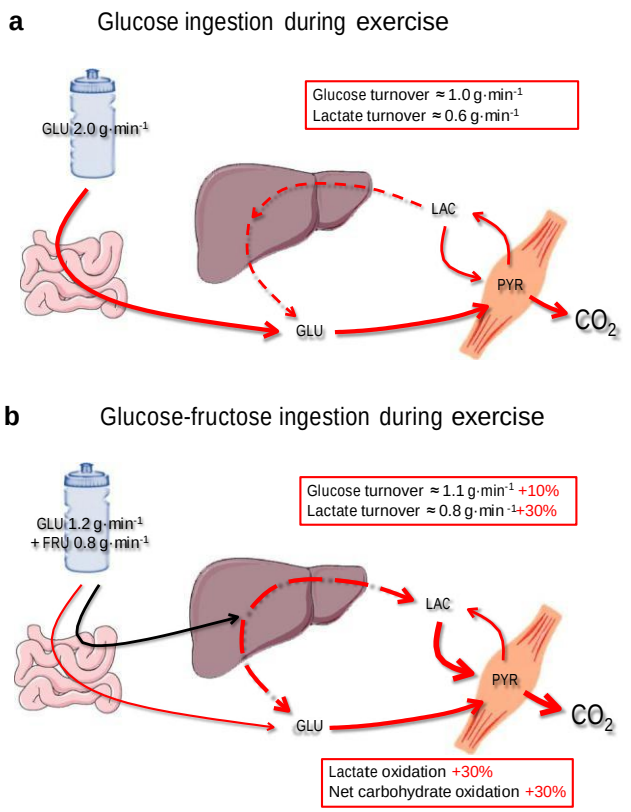

Fig. 4 GLU and LAC fluxes in exercising subjects consuming GLU or a GLU-FRU mixture. In these experiments, subjects performed a prolonged exercise session at 60% of maximal oxygen uptake while ingesting 2.0 g·min^{-1} of GLU (a) or a GLU-FLU mixture (b) while monitoring total GLU turnover by [6,6-$^{2}H_2$]glucose infusion. Oral FRU was ^{13}C-labeled on one occasion to monitor FRU conversion into GLU and net FRU oxidation, and intravenous ^{13}C-LAC was administered on another occasion to calculate LAC fluxes and oxidation. Compared with GLU alone, ingestion of GLU-FRU increased total GLU turnover by 10% and both total LAC turnover and oxidation by 30%. This indicates that approximately 50% of ingested FRU was released from splanchnic tissues as GLU, and the other 50% as LAC, before being oxidized to CO_2 in skeletal muscle. GLU glucose, FRU fructose, LAC lactate, CO_2 carbon dioxide, PYR pyruvate Adapted from Lecoultre et al. [42], with permission

5.2 Energetics of Fructose and Glucose During Exercise

The indirect oxidation of fructose as glucose or lactate in skeletal muscle has potential effects on energy efficiency that have remained largely unexplored (Fig. 5) [45]. The metabolism of one molecule of glucose, which remains the 'standard' energy source for skeletal muscle, uses 6 O_2 and 2 ATP and produces 6 CO_2 and 29.5 ATP, corresponding to 27.5 ATP gained in working muscle, i.e. 4.58 ATP per oxygen (Fig. 5a). In comparison, when fructose is converted into glucose in splanchnic organs (mostly the liver) to be secondarily oxidized in muscle, an additional 2 ATP are consumed, decreasing the net gain of ATP from 27.5 to 25.5 and corresponding to 4.25 ATP per oxygen (Fig. 5b). Interestingly, this pathway is associated with increased energy expenditure in liver cells, but energy yield in skeletal muscle is identical to that of glucose [45].

In a third pathway, splanchnic fructose-metabolizing cells convert fructose into lactate, which is subsequently oxidized in contracting muscle (Fig. 5c). Overall, this process is very similar to muscle glucose processing by using 6 O_2 and 2 ATP, and producing 6 CO_2 and 29.5 ATP; however, the consequences of splanchnic fructose-lactate conversion may be different at the organ level. In the liver and other fructose-metabolizing organs, fructolysis consumes 2 ATP, and conversion of DHAP and GAP into pyruvate produces 4 ATP, resulting in 2 ATP gained. In skeletal muscle, two lactates are transported into the cells through facilitated diffusion, i.e. without energy

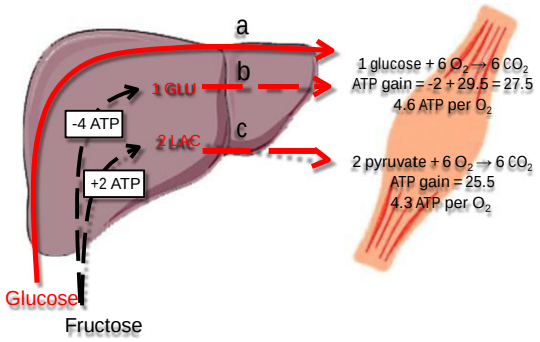

Fig. 5 GLU and FRU energy efficiency in skeletal muscle. The majority of GLU bypasses the liver to be metabolized in skeletal muscle (a), where it requires the initial hydrolysis of 2 ATP for the synthesis of FRU-1-6 diphosphate, and generates a total of 29.5 ATP when FRU-1-6 diphosphate is oxidized to CO_2 and water. This corresponds to a net gain of 27.5 ATP, or 4.6 ATP per O_2 consumed. FRU is metabolized as a first step in specialized cells or certain organs (e.g. the liver) and as a second step in muscle. When it is converted into GLU in FRU-metabolizing cells before being oxidized in muscle, 4 ATP are used in splanchnic organs; 2 for the synthesis of triose phosphates, and 2 for synthesizing GLU from triose phosphates. The oxidation of GLU in muscle then uses 2 ATP and produces 29.5 ATP. The whole process thus consumes 6 ATP and produces 29.5 ATP, corresponding to a net gain of 23.5 ATP. However, the additional energy is only expended in splanchnic FRU-metabolizing cells, and the yield of net ATP gained per O_2 in muscle is the same as for GLU (b). When FRU is first converted into LAC in specialized cells (c), this process generates a net gain of 2 ATP (4 ATP synthesized during conversion of two 1,3-diphosphoglycerates into two 2,3-phosphoglycerates and two phosphoenol pyruvates into two pyruvates, minus 2 ATP used for the synthesis of 2 triose phosphates). Oxidation of two molecules of LAC in skeletal muscle then proceeds without any ATP hydrolysis, but generates only 25.5 ATP (i.e. 29.5 ATP as for GLU minus 4 ATP generated in splanchnic organs by glycolytic metabolism of triose phosphates). The whole process thus consumes 2 ATP and produces 29.5 ATP, corresponding to a net gain of 27.5 ATP, as for GLU. However, 2 ATP are gained in FRU-metabolizing cells, and only 25.5 ATP are gained in muscle, corresponding to 4.3 ATP per O_2 consumed. GLU glucose, FRU fructose, ATP adenosine triphosphate, CO_2 carbon dioxide, O_2 oxygen, LAC lactate

cost, bypassing the glycolysis pathway. Then, complete mitochondrial oxidation requires 6 O_2 and produces 25.5 ATP, corresponding to 4.25 ATP per oxygen. In summary, the energy efficiency for fructose oxidation in muscle is somewhat lower than for dietary glucose or starch oxidation. Oxidation of glucose synthesized from fructose is associated with an increased energy use in fructose-metabolizing splanchnic cells, while oxidation of lactate synthesized from glucose is associated with a lower energy gain in muscle.

Based on these theoretical considerations, one may consider that fructose may not confer any energetic advantage to the working muscle. Alternatively, in situations in which glycolytic flux would be rate-limiting muscle ATP synthesis, hepatic fructolysis into lactate may provide support to the contracting myocytes. Lactate is indeed well known to be a preferred energy substrate for working skeletal muscle, and the existence of muscle lactate shuttling, i.e. glycolytic glucose metabolism in some muscle fibers linked to oxidative metabolism of lactate by other muscle fibers, has been well documented [43]. In addition, similar cooperative intercellular shuttles have been described in the brain between astrocytes and neurons [46], or in testes between Sertoli cells and germ cells [47]. However, the common view remains that muscle lactate production during exercise exceeds muscle lactate utilization [48], resulting in net lactate and alanine efflux from the muscle to the liver, net hepatic lactate uptake and recycling of lactate carbons to glucose through gluconeogenesis (Cori and glucose-alanine cycles) [44, 49].

Whether muscle performance would actually be modified when fructose replaced part of the glucose in exercise drinks, and what the maximal hepatic lactate output and muscle lactate utilization during exercise would be, remain to be investigated.

6 Effects of Exogenous Fructose During Recovery

After exercise, athletes face a second metabolic challenge by having to restore their initial muscle and liver energy stores. This is a major issue when involved in repeated strenuous activity, such as multiday cycling or running races, as well as in general training. In these cases, the recovery of energy stores plays a fundamental role in performance and training adaptation.

From a theoretical point of view, hepatic glycogen synthesis from fructose requires that fructose is first converted into triose phosphates that enter gluconeogenesis and then glycogen synthesis. In comparison to glycogen directly synthesized from glucose, these additional metabolic steps require the use of ATP, and hence glycogen storage from fructose is certainly less efficient than from glucose [45]. The same considerations apply to muscle glycogen synthesis, whether from fructose-derived glucose or lactate (Fig. 5). The storage of fructose as intrahepatocellular lipids or intramyocellular lipids is even less energy efficient since de novo lipogenesis is associated with approximately 30% energy losses.

In contrast to these energetic predictions, it has been well documented that both hepatic and muscle glycogen synthesis can occur after ingestion of fructose [50]. Some studies even suggested that postprandial hepatic glycogen synthesis was larger after ingestion of fructose than glucose [51]. There is also limited evidence that a high fructose diet may also increase intramyocellular lipid concentrations [52]. A definitive conclusion regarding the effect of fructose is complicated by the fact that the recovery of these energy stores is affected not only by diet but also by the timeframe of ingestion. Whether it may be advantageous to ingest fructose to accelerate energy recovery has not been definitely assessed.

Interestingly, whether in fruits or honey, refined cane or beet sugar, or HFCS, fructose is always associated with approximately equivalent amounts of glucose. This constant association may be more than fortuitous since both hexoses participate in each other's metabolism in the gut and liver (Fig. 6). Indeed, the maximal rate of absorption of pure fructose is limited in many individuals, and partial malabsorption, with or without gastrointestinal symptoms, is frequently observed after ingestion of large fructose loads [53]. Interestingly, the co-ingestion of glucose together with fructose enhances gut fructose absorption and prevents malabsorption [53], and some data indicate fructose absorption to be maximal when equivalent amounts of glucose are provided. In the long-term, glucose has also been considered to increase fructose transporter activity and expression in enterocytes [54].

The synergies between fructose and glucose may be even more important in the liver. It has been documented, both in humans and dogs, that small amounts of fructose ingested together with glucose significantly enhance hepatic glucose disposal and glycogen synthesis [55]. Indeed, the fructolysis intermediate fructose-1-phosphate has been shown to interact with a glucokinase regulatory protein, releasing and activating glucokinase. With the activity of glucokinase being specifically to phosphorylate glucose to retain it in the liver, this process results in a several-fold increase in hepatic glucose disposal. Interestingly, the fructose-1-phosphate concentrations needed to achieve this effect are low, and hence this can be elicited by small 'catalytic' doses of fructose added to a glucose meal [56]. Thus, both fructokinase (producing fructose-1-phosphate from fructose in an unregulated manner) and glucokinase can act in synergy to increase the hepatic metabolizing capacity of glucose and fructose. This, as well as the

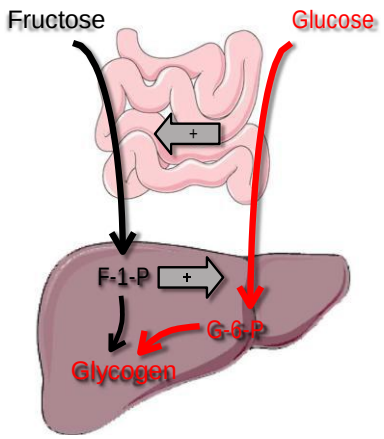

Fig. 6 Synergic effects of fructose and glucose for energy storage. Glucose increases the expression and activity of a specialized gut fructose transporter, and hence hastens fructose absorption and reduces the amount of unabsorbed fructose. F-1-P issued from fructose metabolism then catalyzes glucokinase activity to favor glucose phosphorylation to G-6-P. The overall effect of these synergies is to promote absorption and storage of hexoses as hepatic glycogen. F-1-P fructose-1-phosphate, G-6-P glucose-6-phosphate

unique ability to convert fructose into fat, may actually be important physiological mechanisms, allowing the rapid storage of energy from energy-dense fruits and honey, for instance in hibernating animals and migratory birds. Whether these intestinal and hepatic interactions could also have implications for athletes with a limited recovery timeframe remains to be properly assessed.

7 Conclusions and Perspectives

Fructose can constitute a substantial energy source in the human diet. It is obviously a dispensable nutrient, and no adverse effects of a fructose-deprived diet have been reported. However, specific fructose-metabolizing enzymes are expressed in most mammals, including humans, indicating that this source of energy most likely conferred some metabolic advantage at some point during evolution. While glucose and fatty acids remain the primary energy substrates for contracting skeletal muscle, fructose presents some potential advantages for individuals involved in strenuous physical exercise.

The current knowledge of fructose physiological effects in relation to exercise can be summarized as follows:

- There is no risk associated with ingesting reasonable amounts of fructose when performing physical activity, and exercise attenuates the possibly deleterious effects of fructose overfeeding.
- Immediately before and during exercise, fructose can be conveniently ingested in easily consumed, energy-rich, solid or liquid foods. Compared with glucose, it elicits minimal glycemic responses. It is metabolized in a two-step process, characterized by the initial conversion of fructose into glucose or lactate in splanchnic fructose-metabolizing cells. As a consequence, it is, in part, oxidized as lactate in skeletal muscle through a 'reverse Cori cycle'. This, and the fact that it is absorbed by different intestinal transporters as those for glucose absorption, may allow it to increase muscle energy provision when glucose utilization is maximally stimulated.
- In the recovery period, fructose administered together with glucose may be a convenient, well tolerated way of increasing total energy intake. Furthermore, fructose and glucose exert mutually synergic effects to enhance intestinal fructose absorption, hepatic energy uptake and glycogen synthesis.

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References

1. Eriksson O. Evolution of angiosperm seed disperser mutualisms: the timing of origins and their consequences for coevolutionary interactions between angiosperms and frugivores. Biol Rev Camb Philos Soc. 2016;91:168–86.
2. Treesukosol Y, Smith KR, Spector AC. The functional role of the T1R family of receptors in sweet taste and feeding. Physiol Behav. 2011;105:14–26.
3. Lenoir M, Serre F, Cantin L, et al. Intense sweetness surpasses cocaine reward. PloS One. 2007;2:e698.
4. Breslin PA. An evolutionary perspective on food and human taste. Curr Biol. 2013;23:R409–18.
5. Cordain L, Miller JB, Eaton SB, et al. Macronutrient estimations in hunter-gatherer diets. Am JClin Nutr. 2000;72:1589–92.
6. Standage T. A history of the world in 6 glasses. Palo Alto: Walker & Company; 2005.
7. Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev. 2010;90:23–46.
8. Marriott BP, Cole N, Lee E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. J Nutr. 2009;139:1228S–35S.
9. Marriott BP, Olsho L, Hadden L, et al. Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003–2006. Crit Rev Food Sci Nutr. 2010;50:228–58.

10. Van den Berghe G. Inborn errors of fructose metabolism. Ann Rev Nutr. 1994;14:41–58.
11. Mayes PA. Control of hepatic triacylglycerol metabolism. Biochem Soc Trans. 1976;4:575–80.
12. Sun SZ, Empie MW. Fructose metabolism in humans: what isotopic tracer studies tell us. Nutr Metab. 2012;9:89.
13. McGuinness OP, Cherrington AD. Effects of fructose on hepatic glucose metabolism. Curr Opin Clin Nutr Metab Care. 2003;6:441–8.
14. Bjorkman O, Crump M, Phillips RW. Intestinal metabolism of orally administered glucose and fructose in Yucatan miniature swine. J Nutr. 1984;114:1413–20.
15. Delarue J, Normand S, Pachiaudi C, et al. The contribution of naturally labelled ^{13}C fructose to glucose appearance in humans. Diabetologia. 1993;36:338–45.
16. Parks EJ, Hellerstein MK. Thematic review series: patient-oriented research. Recent advances in liver triacylglycerol and fatty acid metabolism using stable isotope labeling techniques. J Lipid Res. 2006;47:1651–60.
17. Lecoultre V, Egli L, Carrel G, et al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. Obesity. 2013;21:782–5.
18. Theytaz F, de Giorgi S, Hodson L, et al. Metabolic fate of fructose ingested with and without glucose in a mixed meal. Nutrients. 2014;6:2632–49.
19. Macdonald I, Keyser A, Pacy D. Some effects, in man, of varying the load of glucose, sucrose, fructose, or sorbitol on various metabolites in blood. Am J Clin Nutr. 1978;31:1305–11.
20. Simonson DC, Tappy L, Jequier E, et al. Normalization of carbohydrate-induced thermogenesis by fructose in insulin-resistant states. Am J Physiol. 1988;254:E201–7.
21. Björkman O, Gunnarsson R, Hagströ m E, et al. Splanchnic and renal exchange of infused fructose in insulin-deficient type 1 diabetic patients and healthy controls. J Clin Invest. 1989;83:52–9.
22. Bjorkman O, Felig P. Role of the kidney in the metabolism of fructose in 60-hour fasted humans. Diabetes. 1982;31:516–20.
23. Kranhold JF, Loh D, Morris RC Jr. Renal fructose-metabolizing enzymes: significance in hereditary fructose intolerance. Science. 1969;165:402–3.
24. Diggle CP, Shires M, Leitch D, et al. Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. J Histochem Cytochem. 2009;57:763–74.
25. Lanaspa MA, Sanchez-Lozada LG, Choi YJ, et al. Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. J Biol Chem. 2012;287:40732–44.
26. Haidari M, Leung N, Mahbub F, et al. Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing lipoprotein overproduction. J Biol Chem. 2002;277:31646–55.
27. Rajas F, Bruni N, Montano S, et al. The glucose-6 phosphatase gene is expressed in human and rat small intestine: regulation of expression in fasted and diabetic rats. Gastroenterology. 1999;117:132–9.
28. Douard V, Ferraris RP. The role of fructose transporters in diseases linked to excessive fructose intake. J Physiol. 2013;591:401–14.
29. Mithieux G, Misery P, Magnan C, et al. Portal sensing of intestinal gluconeogenesis is a mechanistic link in the diminution of food intake induced by diet protein. Cell Metab. 2005;2:321–9.
30. Johnston RD, Stephenson MC, Crossland H, et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. Gastroenterology. 2013;145(1016–25):e2.
31. Egli L, Lecoultre V, Theytaz F, et al. Exercise prevents fructose-induced hypertriglyceridemia in healthy young subjects. Diabetes. 2013;62:2259–65.
32. Wilburn JR, Bourquin J, Wysong A, et al. Resistance exercise attenuates high-fructose, high-fat-induced postprandial lipemia. Nutr Metab Insights. 2015;8:29–35.
33. Romijn JA, Coyle EF, Sidossis LS, et al. Substrate metabolism during different exercise intensities in endurance-trained women. J Appl Physiol. 2000;88:1707–14.
34. Hawley JA, Dennis SC, Noakes TD. Oxidation of carbohydrate ingested during prolonged endurance exercise. Sports Med. 1992;14:27–42.
35. Jandrain BJ, Pallikarakis N, Normand S, et al. Fructose utilization during exercise in men: rapid conversion of ingested fructose to circulating glucose. J Appl Physiol. 1993;74:2146–54.
36. Jeukendrup AE. Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. Curr Opin Clin Nutr Metab Care. 2010;13:452–7.
37. Perheentupa J, Raivio KO. Fructose-induced hyperuricemia. Lancet. 1967;2:528–31.
38. Bouteldja N, Timson DJ. The biochemical basis of hereditary fructose intolerance. J Inherit Metab Dis. 2010;33:105–12.
39. Bergstrom J, Hultman E, Roch-Norlund AE. Lactic acid accumulation in connection with fructose infusion. Acta Med Scand. 1968;184:359–64.
40. Donmoyer CM, Lacy DB, Zhang Y, et al. Impact of chronic fructose infusion on hepatic metabolism during TPN administration. Am J Physiol. 2002;283:E1151–8.
41. Topping DL, Mayes PA. The concentration of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. Nutr Metab. 1971;13:331–8.
42. Lecoultre V, Benoit R, Carrel G, et al. Fructose and glucose coingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. Am J Clin Nutr. 2010;92:1071–9.
43. Brooks GA. Intra- and extra-cellular lactate shuttles. Med Sci Sports Exerc. 2000;32:790–9.
44. Reichard GAJ, Moury NF, Hochella NJ, et al. Quantitative estimation of the Cori cycle in the human. J Biol Chem. 1963;238:495–501.
45. Tappy L, Egli L, Lecoultre V, et al. Effects of fructose-containing caloric sweeteners on resting energy expenditure and energy efficiency: a review of human trials. Nutr Metab. 2013;10:54.
46. Pellerin L. Brain energetics (thought needs food). Curr Opin Clin Nutr Metab Care. 2008;11:701–5.
47. Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. Trends Endocrinol Metab. 2004;15:345–50.
48. Stallknecht B, Vissing J, Galbo H. Lactate production and clearance in exercise. Effects of training. A mini-review. Scand J Med Sci Sports. 1998;8:127–31.
49. Felig P. The glucose-alanine cycle. Metabolism. 1973;22:179–207.
50. Nilsson LH, Hultman E. Liver and muscle glycogen in man after glucose and fructose infusion. Scand J Clin Lab Invest. 1974;33:5–10.
51. Decombaz J, Jentjens R, Ith M, et al. Fructose and galactose enhance postexercise human liver glycogen synthesis. Med Sci Sports Exerc. 2011;43:1964–71.
52. Le KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr. 2009;89:1760–5.
53. Latulippe ME, Skoog SM. Fructose malabsorption and intolerance: effects of fructose with and without simultaneous glucose ingestion. Crit Rev Food Sci Nutr. 2011;51:583–92.

54. Patel C, Douard V, Yu S, et al. Fructose-induced increases in expression of intestinal fructolytic and gluconeogenic genes are regulated by GLUT5 and KHK. Am J Physiol. 2015;309:R499–509.
55. Moore MC, Cherrington AD, Mann SL, et al. Acute fructose administration decreases the glycemic response to an oral glucose tolerance test in normal adults. J Clin Endocrin Metab. 2000;85:4515–9.
56. Moore MC, Coate KC, Winnick JJ, et al. Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr. 2012;3:286–94.

Manuscript 3

Exercise Performed immediately after Fructose Ingestion Enhances Fructose Oxidation and Suppresses Fructose Storage

See corresponding editorial on page 301.

Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage[1]

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ABSTRACT

Background: Exercise prevents the adverse effects of a high-fructose diet through mechanisms that remain unknown.

Objective: We assessed the hypothesis that exercise prevents fructose-induced increases in very-low-density lipoprotein (VLDL) triglycerides by decreasing the fructose conversion into glucose and VLDL-triglyceride and fructose carbon storage into hepatic glycogen and lipids.

Design: Eight healthy men were studied on 3 occasions after 4 d consuming a weight-maintenance, high-fructose diet. On the fifth day, the men ingested an oral ^{13}C-labeled fructose load (0.75 g/kg), and their total fructose oxidation ($^{13}CO_2$ production), fructose storage (fructose ingestion minus ^{13}C-fructose oxidation), fructose conversion into blood ^{13}C glucose (gluconeogenesis from fructose), blood VLDL-^{13}C palmitate (a marker of hepatic de novo lipogenesis), and lactate concentrations were monitored over 7 postprandial h. On one occasion, participants remained lying down throughout the experiment [fructose treatment alone with no exercise condition (NoEx)], and on the other 2 occasions, they performed a 60-min exercise either 75 min before fructose ingestion [exercise, then fructose condition (ExFru)] or 90 min after fructose ingestion [fructose, then exercise condition (FruEx)].

Results: Fructose oxidation was significantly ($P < 0.001$) higher in the FruEx (80% ± 3% of ingested fructose) than in the ExFru (46% ± 1%) and NoEx (49% ± 1%). Consequently, fructose storage was lower in the FruEx than in the other 2 conditions ($P < 0.001$). Fructose conversion into blood ^{13}C glucose, VLDL-^{13}C palmitate, and postprandial plasma lactate concentrations was not significantly different between conditions.

Conclusions: Compared with sedentary conditions, exercise performed immediately after fructose ingestion increases fructose oxidation and decreases fructose storage. In contrast, exercise performed before fructose ingestion does not significantly alter fructose oxidation and storage. In both conditions, exercise did not abolish fructose conversion into glucose or its incorporation into VLDL triglycerides. This trial was registered at clinicaltrials.gov as NCT01866215. *Am J Clin Nutr* 2016;103:348–55.

Keywords: de novo lipogenesis, energy output, fructose, gluconeogenesis, lactic acid

INTRODUCTION

It has been proposed that dietary fructose may play a causal role in the development of obesity, diabetes, and cardiovascular diseases. The consumption of a diet rich in fructose or fructose-containing caloric sweeteners causes hepatic insulin resistance (1, 2), stimulates hepatic de novo lipogenesis (3), increases blood triglyceride concentrations (4), and promotes liver-fat deposition (5) in humans. There is currently much concern that these alterations may contribute to the development of insulin resistance and nonalcoholic liver disease in the long term.

An increase in physical activity has been shown to efficiently improve metabolic homeostasis and reduce cardiovascular disease risk in patients with the metabolic syndrome as well as in healthy participants (6–8). Several studies have specifically reported that an increase in physical activity also prevented fructose-induced metabolic alterations (9, 10) possibly by altering the metabolic fate of fructose ingested before or while exercising.

The disposal of ingested fructose carbons is mainly partitioned into lactic acid production, fructose conversion into plasma glucose and hepatic glycogen, and lipid synthesis (11, 12). Because lactic acid production is more energy efficient than fructose conversion into glucose and glycogen is and much-more efficient than lipid synthesis is (13), we have proposed that fructose conversion into glucose and fat would occur mainly when lactic acid production is saturated (14). Therefore, in this study, we assessed the hypothesis that exercise may prevent a fructose-induced rise in VLDL triglycerides (VLDL-TGs)[6] by decreasing fructose conversion into glucose and VLDL-TGs and fructose carbon storage into hepatic glycogen and lipids.

To evaluate this hypothesis, we assessed how a single exercise session altered the metabolic fate of a pure fructose load in

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[6] Abbreviations used: ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; NEFA, nonesterified fatty acid; NoEx, fructose treatment alone with no exercise condition; VLDL-TG, VLDL triglyceride.

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a group of healthy nonobese subjects. Because exercise is expected to differentially affect endogenous glycogen and blood glucose use when performed in the fasting state or after ingestion of a meal (15, 16), we monitored the fate of an acute ^{13}C-fructose load ingested either immediately before or immediately after a standardized exercise (**Figure 1**). We specifically assessed the partitioning of fructose carbons into oxidation and storage by monitoring breath $^{13}CO_2$ production and the conversion of fructose carbon into blood glucose by monitoring total ^{13}C glucose appearance with intravenous deuterated glucose. We also assessed the effects of exercise on blood lactate and VLDL-^{13}C palmitate concentrations as potential clues to exercise-induced alterations of hepatic lactate production from fructose and de novo lipogenesis, respectively.

METHODS

Subjects

Eight sedentary male volunteers [mean ± SD age: 22.5 ± 2.2 y; mean ± SD BMI (in kg/m^2): 22.8 ± 0.8] participated in the study. The subjects were in good health, nonsmokers, and not presently taking any medications and had no history of diabetes in their first-degree relatives. The study protocol was approved by the Human Research Ethics Committee of Canton de Vaud, and the participants provided informed written consent. The procedures were conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. This trial was registered at clinicaltrials.gov as NCT01866215.

Study design

Participants were studied on 3 occasions with fructose ingested on one occasion in sedentary conditions (i.e., without exercise [fructose treatment alone with no exercise condition (NoEx)] or on the other occasions with an exercise bout performed immediately before fructose ingestion (exercise, then fructose condition (ExFru)] or after fructose ingestion [fructose, then exercise condition (FruEx)] in a randomized order. On each occasion, subjects received a controlled, high-fructose, weight-maintenance diet that contained 55% carbohydrate (30% fructose, 20% starch, and 5% nonfructose sugars), 30% lipids, and 15% proteins during the 4 d before each experiment. Dietary intake was calculated for each participant according to the Harris-Benedict equation (17) and a physical activity factor of 1.5. Diets were provided as prepacked food items and drinks. Fructose was provided as lemon-flavored drinks. Volunteers received instructions to consume all the foods and beverages that they received at specified times and to not consume any other foods or beverages except water. Subjects were also requested to restrain from physical activity during these 4 d.

On the fifth day, subjects underwent one of 3 experimental tests (**Figure 2**). For this purpose, they reported to the Clinical Research Center of Lausanne University Hospital at 0700 after a 12-h overnight fast. On arrival, subjects were weighed (Seca 708; Seca GmbH), and their body compositions were measured with the use of a bioelectrical impedance analysis (Imp DF50; ImpediMed). Participants were transferred to a bed, and a cannula was inserted into a vein of the right forearm for blood sampling.

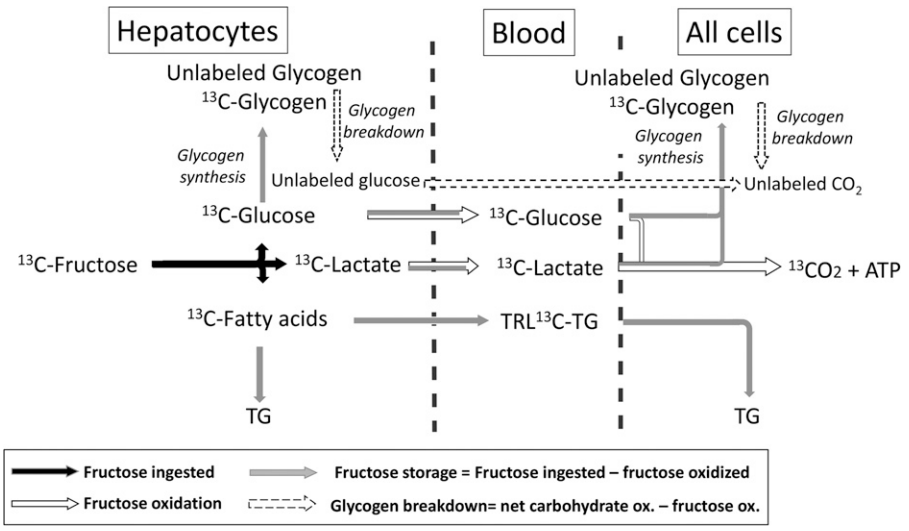

FIGURE 1 Overview of major pathways used for fructose metabolism. Fructose absorbed from the gut (solid black arrow) is mainly extracted by hepatic cells expressing fructokinase and aldolase B (i.e., hepatocytes, intestinal cells, and proximal renal tubule cells) where it is converted into lactate, glucose, and fatty acids. These metabolites are ubiquitous energy substrates that can be released into the bloodstream to be either oxidized or stored as glycogen or triglycerides in all cells of the organism. Because of the complexity of its metabolism, no single tracer method accurately assesses all pathways of fructose disposal. When a ^{13}C-labeled fructose load is administered, breath $^{13}CO_2$ production provides an estimate of total fructose oxidation (direct oxidation of trioses-phosphate formed from fructose in the liver (not shown) and extrahepatic oxidation of lactate, glucose, and fatty acids synthesized from fructose). With the assumption that all ^{13}C-labeled fructose is completely absorbed during the experiment, (fructose ingested) – (fructose oxidized) represents fructose storage as intracellular triglycerides or glycogen. The measurement of net carbohydrate oxidation with the use of indirect calorimetry does not differentiate the oxidation of glycogen, glucose, lactate, or fructose because all these substrates have the same respiratory quotient, and it provides an estimate of the sum of their net oxidations. Note that standard indirect calorimetry equations compute the oxidation of glucose synthesized from amino acids as being part of net protein oxidation and not of net carbohydrate oxidation. The immediate oxidation of VLDL-TG synthesized from fructose can be assumed to be small, and thus was neglected. When a pure fructose load is ingested, [net carbohydrate oxidation (indirect calorimetry)] – [fructose oxidation ($^{13}CO_2$)] corresponds to the oxidation of endogenous glycogen (i.e., glycogen breakdown), and the rate of ^{13}C-glucose appearance in blood (measured with intravenous tracer infusion of deuterated glucose) corresponds to the release of fructose carbons as glucose in the systemic circulation. ox., oxidized; TG, triglycerides; TRL, triglyceride-rich lipoproteins.

A second cannula was inserted into a vein of the left forearm for tracer infusion. A primed, continuous infusion of [6,6-$^2H_2$]-glucose (Cambridge Isotope Laboratories) (bolus: 3 mg/kg body weight; continuous infusion: 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was administered throughout the test. Blood samples were collected at baseline (T = 0) and after 60, 120, 180, 240, 300, 360, 420, 480, and 540 min for the measurement of plasma substrate concentrations and isotopic enrichments. Energy expenditure and substrate oxidation were measured with the use of indirect calorimetry (Quark RMR; Cosmed Srl). An oral fructose load (0.75 g/kg body weight; D-Fructose, Fluka Analytic; Sigma Aldrich) that was enriched with 0.1% [U-$^{13}C_6$]-fructose (Cambridge Isotope Laboratories) was given at time 120 min. Breath samples were collected every 60 min to measure breath $^{13}CO_2$ enrichment. A timed urine collection was performed throughout the test to determine urinary nitrogen excretion.

Each volunteer was studied during 3 experimental conditions according to a randomized order. The sequence of conditions was determined with the use of computer-generated random numbers (R software, version 3.0.2; CRAN). A 4-wk washout period separated the following 3 experimental conditions.

1) No exercise (NoEx): the participant remained installed in bed over the 9 h of the metabolic test and received a fructose load at time 120 min.
2) Exercise before fructose ingestion (ExFru): the subject cycled during 1 h at 100 W starting at time 45 min and received a fructose load at time 120 min while remaining in bed the rest of the time. The cycling exercise was performed on an ergometer (Ergoline GmbH). During exercise, oxygen uptake and carbon dioxide production were measured breath by breath (SensorMedicsVmax; Sensormedics Corp.) during 5 min every 20 min. Blood and breath samples were also collected 20, 40, and 60 min after the beginning of the exercise session.
3) Exercise after fructose ingestion (FruEx): the participant received a fructose load at time 120 min and performed 1 h of cycling exercise at 100 W starting at time 210 min. To account for the exercise-induced increase in glucose fluxes, the 6,6-$^2H_2$-glucose infusion rate was increased to 75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ at the beginning of exercise and was reduced to 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ at the end of exercise.

Analytic procedures

Plasma glucose, lactate, nonesterified fatty acids (NEFAs), triglycerides, and urinary urea were measured with the use of enzymatic methods (Randox Laboratories). Insulin and glucagon were measured with the use of a radioimmunoassay (Millipore). The VLDL subfraction was separated by ultracentrifugation as previously described (18). VLDL concentrations were measured with the use of an enzymatic method (Randox Laboratories).

For the plasma glucose isotopic analysis, plasma was deproteinized with the use of barium hydroxide, neutral compounds were isolated by passing the supernatant over anion- and cation-exchange resins, and glucose penta-acetyl derivatives were obtained by adding acetic anhydride and pyridine. Plasma 6,6-$^2H_2$ isotopic enrichment was measured with the use of gas chromatography–mass spectrometry (Agilent Technologies). Plasma ^{13}C glucose isotopic enrichment was measured with the use of gas chromatography–combustion–isotope ratio mass

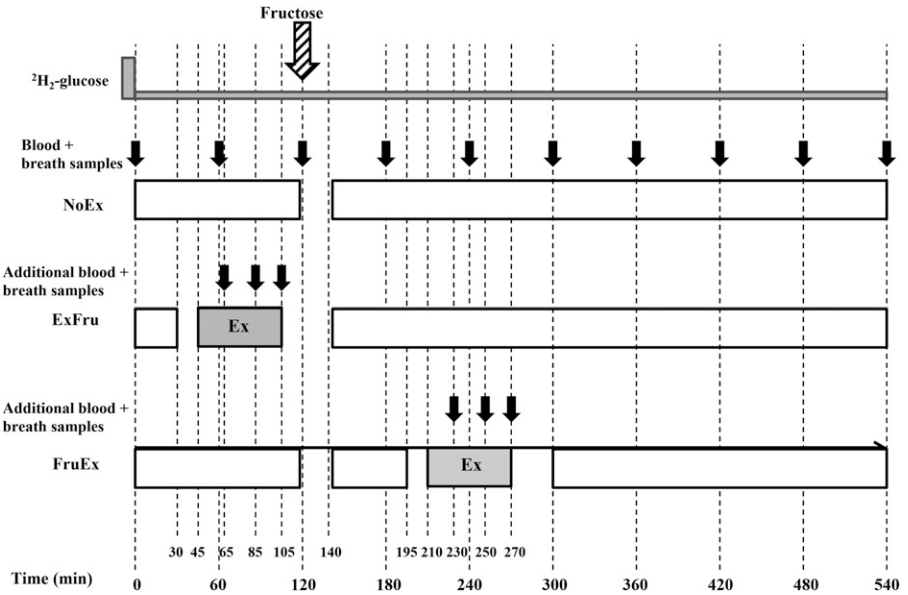

FIGURE 2 Experimental design of day 5 for each condition. In the NoEx, the participant remained in bed over the 9 h of the metabolic test and received a fructose load enriched with 0.1% [U-$^{13}C_6$]-fructose, which was given at time (T) = 120 min (striped arrow). Blood and breath samples (black arrows) were collected at baseline (T = 0) and after 60, 120, 180, 240, 300, 360, 420, 480, and 540 min for measurements of plasma substrate concentrations and isotopic enrichments as well as measurements of breath $^{13}CO_2$ enrichment. [6,6-$^2H_2$]-glucose (bolus: 3 mg/kg body weight; continuous infusion: 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused throughout the test. In the ExFru, the subject cycled during 1 h at 100 W starting at T = 45 min and received a fructose load at T = 120 min while remaining in bed the rest of the time. White bars represent the time when the volunteers were resting, and gray bars represent the exercise sessions. Blood and breath samples were also collected 20, 40, and 60 min after the beginning of the exercise session. The [6,6-$^2H_2$]-glucose infusion rate was increased to 75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during exercise. In the FruEx, 1 h of cycling exercise at 100 W was performed starting at T = 210 min. The 3 experimental conditions were followed in a randomized order. Ex, 60-min exercise; ExFru: exercise, then fructose condition; FruEx, fructose, then exercise condition; NoEx, fructose treatment alone with no exercise condition.

TABLE 1
Anthropometric variables and fasting metabolic substrates and hormones[1]

	NoEx	ExFru	FruEx
Body mass, kg	71.9 ± 5.3	71.1 ± 4.8	72.3 ± 4.9
Fat mass, kg	13.4 ± 3.5	13.1 ± 4.0	13.7 ± 3.6
Systolic blood pressure, mm Hg	118 ± 6	120 ± 8	119 ± 6
Diastolic blood pressure, mm Hg	69 ± 4	70 ± 4	70 ± 5
Glucose, mmol/L	4.89 ± 0.38	4.92 ± 0.37	4.89 ± 0.41
Lactate, mmol/L	0.84 ± 0.36	0.80 ± 0.17	0.84 ± 0.32
Insulin, mU/mL	7.50 ± 2.12	8.00 ± 3.42	7.89 ± 3.04
Glucagon, pg/mL	67.5 ± 32.2	69.7 ± 38.4	69.0 ± 24.9
Triglycerides, mmol/L	1.01 ± 0.42	0.87 ± 0.25	1.07 ± 0.5
VLDL-TGs, mmol/L	0.33 ± 0.12	0.29 ± 0.09	0.34 ± 0.13
NEFAs, mmol/L	0.40 ± 0.11	0.41 ± 0.09	0.46 ± 0.15

[1]All values are means ± SDs (n = 8 participants in each analysis; analyzed by original assigned groups). The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. Skewed distributions (lactate) were log transformed before the statistical analysis. Changes in anthropometric variables and fasting metabolic variables at $T = 0$ min of each condition were assessed by Student's paired t tests with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. Participants showed no significant difference in anthropometric variables and fasting metabolic substrates and hormones between the 3 tests performed. All P values were NS. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; NEFA, nonesterified fatty acid; NoEx, fructose treatment alone with no exercise condition; VLDL-TG, VLDL triglyceride.

spectrometry (Isoprime; Isoprime Ltd.) at the Centre for Research in Human Nutrition Rhône-Alpes.

Breath $^{13}CO_2$ enrichment was measured by isotope-ratio mass spectrometry (Sercon Ltd.). ^{13}C-palmitate enrichment and concentrations in VLDL were measured after total lipid extractions from plasma and the preparation of fatty acid methyl esters from triglyceride fractions. The palmitate ^{13}C:^{12}C ratio in fatty acid methyl ester derivatives was determined with the use of Delta Plus XP GC-combustion isotope ratio mass spectrometry (Thermo Electron Corporation, Bremen, Germany). VLDL-TG palmitate content (percentage of weight) was measured by GC-isotope-ratio mass spectrometry (Thermo Electron, Bremen, Germany) as reported previously (9).

Calculations

Plasma concentrations of VLDL ^{13}C palmitate (nmol/L) were calculated as

$$[\text{VLDL-TG-}^{13}\text{C-palmitate isotopic enrichment (atom percent excess)}] \times \text{weight \% palmitate} \times [\text{VLDL-TG (nmol/L)}] \quad (1)$$

Fructose conversion into plasma glucose was calculated as

$$\text{EGP} \times \{(^{13}\text{CG}_1 + {}^{13}\text{CG}_2) \div 2 + \text{pV} \times [(G_1 + G_2) \div 2 \times (^{13}G_2 - {}^{13}G_1) \div (t_2 - t_1)]\} \div {}^{13}\text{CF} \quad (2)$$

where EGP is the endogenous glucose production ($g \cdot kg^{-1} \cdot h^{-1}$) calculated with [6,6-$^2H_2$]-glucose and Steele's equations for non–steady state conditions (19), ^{13}CG is the isotopic enrichment of plasma glucose (atom percent excess), G is the glucose concentration (g/L), ^{13}CF is the isotopic enrichment of oral fructose, p is the pool fraction (set at 0.75), V is the glucose distribution space (set at 0.2 L/kg body weight), and t is the time (h).

Total fructose oxidation (g/min) was calculated as

$$\frac{\text{CO}_2\text{ IE}}{\text{Fructose IE} \times 0.8} \times \frac{\dot{V}\text{CO}_2}{0.134} \times \frac{180}{10^6} \quad (3)$$

where CO_2 IE is the isotopic enrichment of breath carbon dioxide, and fructose IE is the isotopic enrichment of the oral fructose load, both of which are expressed as the atom percent excess, 0.8 is the recovery of ^{13}C from fructose in breath carbon dioxide, $\dot{V}CO_2$ is the total respiratory carbon dioxide (mL/min), 0.134 is the volume of carbon dioxide (mL) produced by the oxidation of 1 μmol fructose, 180 is the molar weight of fructose, and 10^6 allows for the conversion from micrograms to grams.

When cumulated over a 7-h postprandial period, the absorption of ingested fructose can be assumed to be essentially complete, although a few grams may be malabsorbed (20). In such conditions, the amount of fructose ingested minus fructose oxidation represents the sum of fructose carbons retained in the body as glycogen and fat (i.e., fructose storage).

The net carbohydrate oxidation calculated with the use of indirect calorimetry (21) includes the conversion of fructose (or glucose) into fat as long as the fat synthesis does not exceed fat oxidation (i.e., nonnet fat synthesis) (22). With the ingestion of pure fructose (i.e., with no exogenous carbohydrate other than fructose), the difference between the amount of fructose ingested and the cumulated net carbohydrate oxidation represents exclusively the net storage of hepatic and muscle glycogen. The net glycogen storage between times 120 and 540 was calculated as

$$\text{Fructose ingested (g)} - \text{net carbohydrate oxidation (g)} \quad (4)$$

Glycogen breakdown between times 120 and 540 (g) was calculated as

$$(\text{Net carbohydrate oxidation}) - (^{13}\text{C fructose oxidation}) \quad (5)$$

Statistical analysis

Anthropometric variables are expressed as means ± SDs, whereas all other values are expressed as means ± SEMs. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. When necessary, skewed distributions (baseline lactate) were log transformed before statistical analyses.

Changes in anthropometric variables and fasting metabolic variables at $T = 0$ min of each condition were assessed with the use of Student's paired t tests with Bonferroni corrections for multiple testing. Metabolic effects of the conditions (NoEx, ExFru, and FruEx) were evaluated by the calculation of incremental AUCs either over 9 or 7 h after fructose consumption and compared with the use of Student's paired t tests with Bonferroni correction for multiple testing. All statistical calculations were performed with R software (version 3.0.2). $P < 0.05$ was considered statistically significant.

RESULTS

All participants were recruited and completed the 3 experimental conditions between July 2012 and June 2013. One

participant who was initially included had to stop the exercise session (because of faintness) and was removed from the analysis and replaced to reach the number of 8 completed participants that were needed on the basis of the sample size calculation. No other harms or unintended effects were reported by participants or observed by investigators during the metabolic tests.

Anthropometric variables and fasting metabolic variables

Participants showed no significant differences in body weight, body fat mass, or blood pressure between the 3 experimental conditions (**Table 1**). Their fasting plasma metabolite and hormone concentrations were also not significantly different (Table 1).

Metabolic effects of fructose without exercise (NoEx)

Fructose ingestion in subjects remaining awake and lying in their bed (NoEx) induced a slight increase in blood glucose and insulin, a marked increase in blood lactate, and a marked suppression of NEFA (**Figure 3**). Estimated pathways used for oral fructose disposal, total energy expenditure, and net substrate oxidation, cumulated over 2-h basal and over 7 h postprandial are shown in **Table 2**. Postprandial oxidation of exogenous fructose corresponded to 49.3 ± 0.9%, total fructose storage to 50.7 ± 0.9%, net glycogen storage to 25.3 ± 5.6%, and fructose conversion into plasma glucose to 15.5 ± 0.7% of the ingested load (Table 2). Blood ^{13}C-palmitate enrichment and ^{13}C palmitate concentrations in VLDL increased progressively from time 120 to reach a peak at time 300, and declined slowly thereafter (**Figure 4**).

Effects of exercise performed before fructose ingestion (ExFru)

In the ExFru, total energy expenditure and net carbohydrate oxidation ($P < 0.001$ compared with NoEx; Table 2) were significantly higher than in the NoEx during the preprandial 2-h period during which exercise was performed. Glycogen breakdown during this period amounted to 139.0 ± 6.0 g over 2 h (data not shown). Thereafter, the ingestion of the fructose load elicited plasma glucose and lactate responses that did not significantly differ from those in the NoEx (all P = NS compared with NoEx; Figure 3). In contrast, plasma insulin concentrations tended to be lower ($P = 0.06$ compared with NoEx; Figure 3), and plasma NEFA concentrations were significantly lower ($P < 0.01$ compared with NoEx; Figure 3). Fructose oxidation (45.7 ± 1.2%), fructose storage (54.3 ± 1.2%), net glycogen storage (41.3 ± 4.5% of the fructose load), and glycogen breakdown that were cumulated over 7 h postprandial, did not show any significant difference compared with in the NoEx (all P = NS; Table 2).

Postprandial total plasma triglyceride concentrations were not significantly altered (P = NS compared with NoEx), but VLDL-TG concentrations were lower ($P < 0.05$ compared with NoEx). AUCs for VLDL-^{13}C palmitate enrichment and concentrations were not significantly different from those in the NoEx (Figure 4).

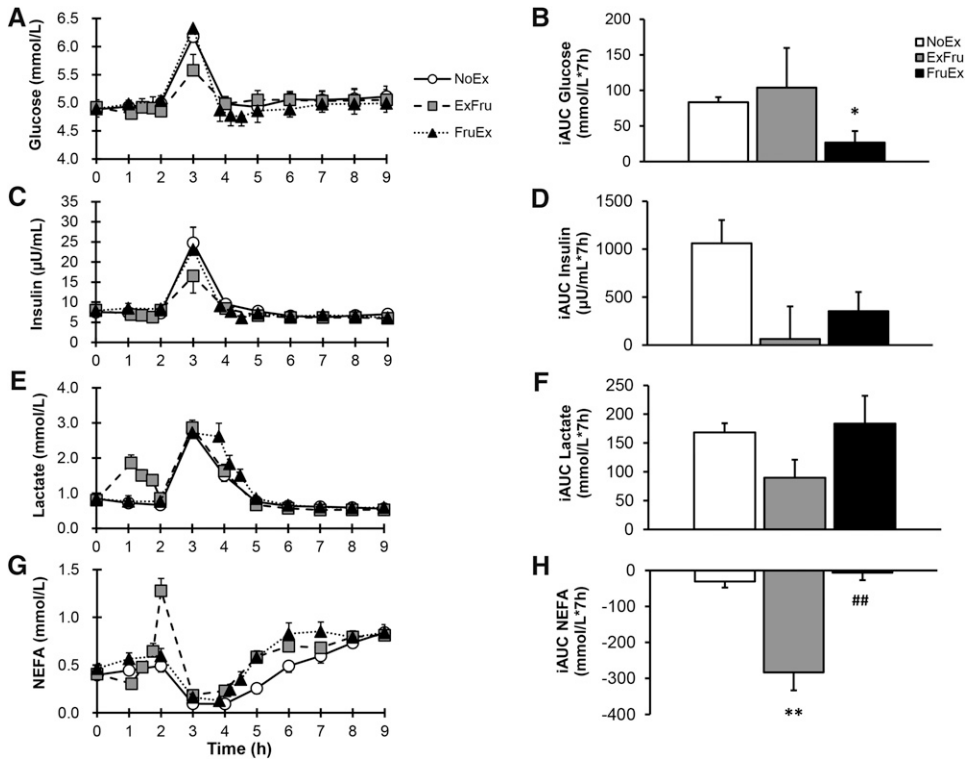

FIGURE 3 Mean ± SEM glucose, insulin, lactate, and NEFA concentrations and postprandial AUCs. n = 8 participants in each analysis; analyzed by original assigned groups. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of the iAUC over 7 h after fructose consumption and compared with the use of Student's paired t test, with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. In the ExFru, insulin tended to be lower ($P = 0.06$ compared with NoEx). $^{*}P < 0.05$ compared with NoEx; $^{**}P < 0.01$ compared with NoEx; $^{\#\#}P < 0.01$ compared with ExFru. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; iAUC, incremental AUC; NEFA, nonesterified fatty acids; NoEx, fructose treatment alone with no exercise condition.

TABLE 2
Estimated pathways used for oral fructose disposal, total energy expenditure, and net substrate oxidation[1]

					P		
	Period AUC, min	NoEx	ExFru	FruEx	NoEx vs. ExFru	NoEx vs. FruEx	ExFru vs. FruEx
Fructose oxidation, g over 7 h	120–540	26.6 ± 1.0	24.4 ± 1.0	43.5 ± 1.9	0.053	<0.001	<0.001
Fructose storage, g over 7 h	120–540	27.3 ± 0.6	28.9 ± 0.8	10.7 ± 1.4	0.25	<0.001	<0.001
Net glycogen storage, g over 7 h	120–540	13.5 ± 2.9	22.3 ± 2.7	−116.7 ± 5.8	0.24	<0.001	<0.001
Glycogen breakdown, g over 7 h	120–540	13.8 ± 3.0	6.6 ± 2.2	127.4 ± 5.1	0.32	<0.001	<0.001
Fructose conversion into plasma glucose, g over 7 h	120–540	8.4 ± 0.5	8.4 ± 0.4	8.6 ± 0.7	1.00	1.00	1.00
Energy expenditure, kcal	0–120	141 ± 5.2	635.3 ± 12.1	152.3 ± 6.9	<0.001	0.21	<0.001
over 2, 7, or 9 h	120–540	537.0 ± 16.2	528.6 ± 20.4	1034.8 ± 29.9	1.00	<0.001	<0.001
	0–540	678 ± 20.9	1163.9 ± 28.6	1187.1 ± 36.3	<0.001	<0.001	0.81
CHOX, g over 2, 7, or 9 h	0–120	9.3 ± 0.9	139 ± 6	7.7 ± 1.6	<0.001	0.81	<0.001
	120–540	40.4 ± 3.5	31 ± 1.9	170.9 ± 6.4	0.20	<0.001	<0.001
	0–540	49.8 ± 4.2	170 ± 6.0	178.6 ± 7.5	<0.001	<0.001	1.00

[1]All values are means ± SEMs ($n = 8$ participants in each analysis; analyzed by original assigned groups). The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of incremental AUCs over 120 min (prefructose load), 420 min (after fructose load), or 540 min (overall) and comparison with the use of Student's paired *t* tests with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. CHOX, carbohydrate oxidation; ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; NoEx, fructose treatment alone with no exercise condition.

Effects of exercise performed after fructose ingestion (FruEx)

In the FruEx, there were no significant differences in plasma insulin, lactate, and triglyceride concentrations compared with those in the NoEx and ExFru except for a lower plasma glucose concentration over the 7 h after fructose ingestion ($P = 0.038$ compared with NoEx' Figures 3 and 4). Exercise performed 90 min after fructose ingestion significantly increased the total energy expenditure and net carbohydrate oxidation (both $P < 0.001$ compared with NoEx; Table 2) to values similar to those observed in the ExFru, whereas lipid oxidation remained comparable to that in the NoEx and ExFru (data not shown). Postprandial fructose oxidation (80.2 ± 2.6% of the fructose load) was nearly doubled compared with the NoEx and ExFru (both $P < 0.001$), and glycogen breakdown was higher than in the NoEx and ExFru ($P < 0.001$). Fructose storage (19.8 ± 2.6% of the fructose load) and net glycogen storage were both lower than in the NoEx and ExFru (all $P < 0.001$; Table 2)

The AUC of VLDL-^{13}C palmitate enrichment was significantly reduced compared with in the NoEx ($P = 0.039$ compared with NoEx; Figure 4). In contrast, AUCs for VLDL-^{13}C-palmitate concentrations were not significantly different from those in the NoEx (Figure 4).

DISCUSSION

To our knowledge, this study allowed us to obtain novel original data regarding the effects of exercise on the fructose metabolic pathways used for its postprandial metabolism.

When fructose was administered in resting conditions, 49% of the exogenous load was oxidized over 7 h postprandial. The amount of newly synthesized, labeled glucose released in the systemic circulation during this time corresponded to 15% of the fructose load. Fifty-one percent of ingested fructose was stored, but the net glycogen storage corresponded only to 25% of ingested fructose. The remaining 26% may be explained by the loss of ^{13}C carbon labels that were due to *1*) whole-body glycogen turnover (i.e., storage of ^{13}C-labeled glycogen with the simultaneous breakdown and oxidation of unlabeled glycogen), *2*) storage of fructose as fat, or *3*) incomplete gut absorption of oral fructose. The appearance of ^{13}C carbons in VLDL palmitate unequivocally indicated that hepatic de novo lipogenesis from fructose occurred. However, a quantitative estimate of hepatic fat synthesis was not possible. Therefore, we conclude that hepatic de novo lipogenesis was active after fructose ingestion. However, the total amount of fat synthesis was likely to be small.

When exercise was performed before fructose ingestion, postprandial fructose oxidation tended to be lower, whereas fructose storage was not significantly different from that in sedentary conditions (i.e., without exercise). Cumulative energy expenditure was largely superior to the energy content of the fructose meal, resulting in an energy deficit of 950 kcal. As a consequence, glycogen utilization during exercise was not fully compensated by glycogen storage after fructose ingestion, which resulted in a global glycogen deficit of 146 g over the total 9 h. Labeled fructose carbons were nonetheless recovered as VLDL palmitate, which indicated that de novo lipogenesis remained active and, hence, that fructose carbons were not exclusively channeled toward glycogen storage.

In contrast, exercise performed immediately after fructose ingestion had substantial effects on postprandial fructose metabolism. It increased the total energy expenditure and net carbohydrate oxidation to the same extent as with the ExFru and resulted in the same total energy and glycogen deficits. However, it markedly enhanced fructose oxidation and decreased fructose and net glycogen storage. Despite this preferential fructose oxidation, the fructose conversion into blood glucose was unchanged, and fat synthesis was still active, as documented by the ongoing incorporation of labeled carbons in VLDL palmitate. Blood lactate, insulin, and triglyceride concentrations were hardly altered compared with in the other 2 conditions.

Our current data support our postulation that exercising after fructose ingestion increases fructose conversion into lactic acid followed by its oxidation in the working muscle. This process has previously been shown with the use of ^{13}C-labeled tracers in

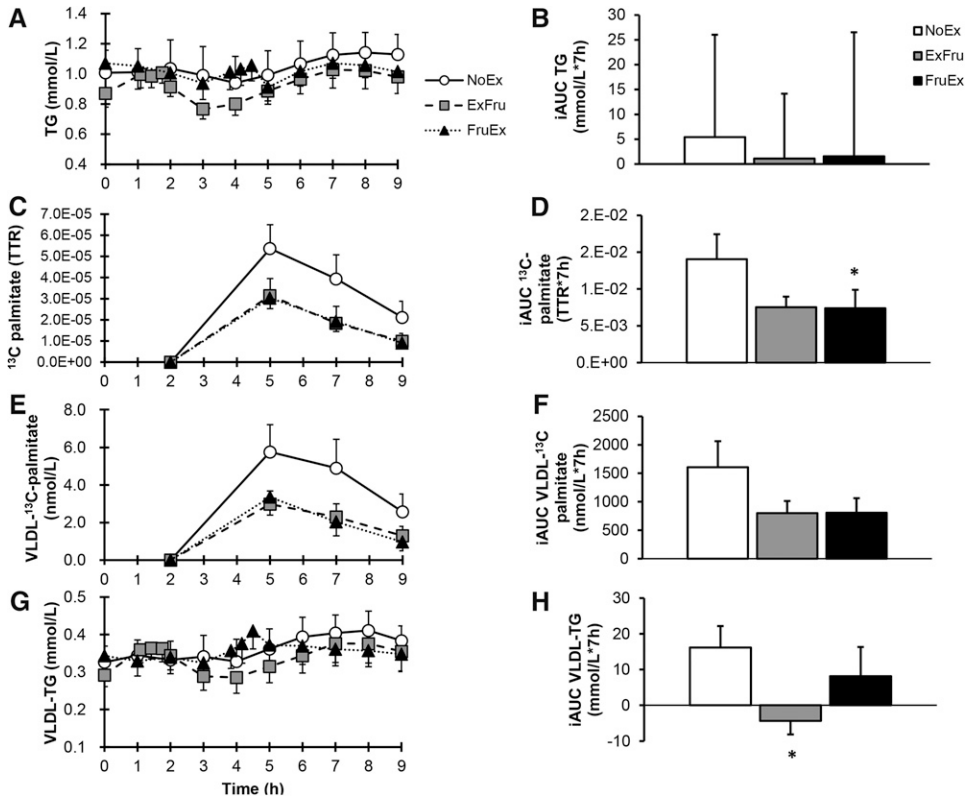

FIGURE 4 Mean ± SEM TG concentrations, ^{13}C-palmitate enrichment, ^{13}C palmitate concentrations in VLDL, VLDL-TG concentrations, and postprandial AUCs. $n = 8$ participants in each analysis; analyzed by original assigned groups. The normality of the distribution was inspected visually and checked with the use of Shapiro-Wilk tests. All data were normally distributed. Metabolic effects of the conditions (NoEx, FruEx, and ExFru) were evaluated by the calculation of the iAUC over 7 h after fructose consumption and compared with the use of Student's paired *t* tests with Bonferroni corrections for multiple testing. $P < 0.05$ was considered significant. *$P < 0.05$. ExFru, exercise, then fructose condition; FruEx, fructose, then exercise condition; iAUC, incremental AUC; NEFA, nonesterified fatty acids; NoEx, fructose treatment alone with no exercise condition; TG, triglyceride; TTR, tracer-to-tracee ratio.

exercising subjects who were fed glucose-fructose mixtures (23). In contrast, the data do not confirm our hypothesis that exercising before fructose ingestion would massively divert fructose carbons into glycogen storage. Furthermore, our observations that exercise did not decrease fructose conversion into plasma glucose and did not abolish de novo lipogenesis were in contradiction with our starting hypothesis that de novo lipogenesis would become active only when other pathways were saturated.

It has been reported that exercise can revert hypertriglyceridemia induced by high-fructose or high-carbohydrate weight-maintenance diets (9, 24), and this effect has been proposed as one major mechanism by which exercise may protect against the development of cardiovascular diseases. However, in our current experiments, exercise had no significant effects on plasma triglyceride concentrations. This finding was not unexpected because the ingestion of pure fructose elicits no or only minimal increases in plasma triglyceride concentrations. The addition of fructose to a mixed meal is known to enhance postprandial triglyceride excursions, possibly by impairing extrahepatic triglyceride clearance (25). Such an effect could not be observed in our experiment because no exogenous lipids were administered together with fructose. Our current data provide targeted information regarding the effects of exercise on fructose disposal but provide little information on how exercise specifically prevents the development of hypertriglyceridemia. Previously, we have documented that hepatic de novo lipogenesis activity increased over time in subjects who were fed a weight-maintenance high-fructose diet. In addition, we have also reported that exercise prevented both this upregulation of hepatic de novo lipogenesis and the development of fasting and postprandial hypertriglyceridemia (9). The mechanisms that are responsible for these effects of exercise on short-term adaptations to dietary fructose remain unknown. We can only speculate that exercise, when performed after fructose ingestion, may contribute to this effect by decreasing the need to store fructose carbons within the organism.

Our current study had important limitations. First, the monitoring of breath $^{13}CO_2$ release provided a reasonable estimate of total fructose oxidation but did not allow for the differentiation of $^{13}CO_2$ produced from lactate, glucose, and fatty acids. Second, we did not monitor intestinal fructose absorption in this study. Nonetheless, we are confident that fructose malabsorption, if present, was quantitatively small because participants did not get diarrhea or bloating, which are side effects typically elicited by malabsorption of as little as 5–10 g carbohydrate (20). However, it remains possible that fructose malabsorption <5g passed undetected in some of the participants and, hence, that fructose storage was somewhat overestimated (26). Third, we obtained only qualitative estimates for de novo lipogenesis from fructose because the need to simultaneously assess $^{13}CO_2$ production and the cost of ^{13}C fructose precluded the use of

highly enriched fructose and the calculation of intrahepatic acetyl-CoA enrichment by mass isotopomer analysis. Fourth, the amount of fructose disposed through systemic lactate was not measured by tracer methods because this would have required a duplication of all tests in each volunteer to separately monitor glucose and lactate kinetics. As a consequence, our data did not allow for a quantitative estimate of the amount of fructose ultimately stored as glycogen and fat or in which organs or tissues this action occurred. Nonetheless, the data provide reliable estimates for fructose oxidation, fructose conversion into plasma glucose, and net glycogen storage and do not support allegations that fructose is mainly converted into fat (27). Nonetheless, fat synthesis from fructose, even if quantitatively minor, may play an important role in the development of fructose-induced hypertriglyceridemia (5).

In conclusion, our results indicate that exercise performed after fructose ingestion markedly enhances fructose oxidation and decreases fructose storage, whereas a similar exercise performed before fructose ingestion does not alter postprandial fructose disposal in healthy individuals. Exercise does not inhibit fructose conversion into plasma glucose and does not abolish fructose conversion into fat. These results suggest that exercise improves sugar-induced hypertriglyceridemia by other mechanisms.

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The authors' responsibilities were as follows—LE, VL, JC, and RR: analyzed the data and performed the statistical analysis; LE, VL, JC, RR, and A-SM: recruited participants and performed tests; LE, VL, PS, and LT: designed the study; LE and LT: wrote the manuscript; LH: analyzed the isotopic enrichment of plasma lipids; LG and ML: analyzed the isotopic enrichment of plasma glucose; LT: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. LT has previously received research support from Nestlé SA and Ajinomoto Co. Inc. for research unrelated to this article. None of the other authors reported conflicts of interest related to the study.

REFERENCES

1. Wei Y, Pagliassotti MJ. Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance. Am J Physiol Endocrinol Metab 2004;287:E926–33.
2. Lecoultre V, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, Boss A, Zwygart K, Le KA, Bortolotti M, et al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. Obesity (Silver Spring) 2013;21:782–5.
3. Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 2005; 54:1907–13. Erratum in: Diabetes 2006;55:563.
4. Stanhope KL, Bremer AA, Medici V, Nakajima K, Ito Y, Nakano T, Chen G, Fong TH, Lee V, Menorca RI, et al. Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women. J Clin Endocrinol Metab 2011;96:E1596–605.
5. Lê KA, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P, Boesch C, Ravussin E, Tappy L. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. Am J Clin Nutr 2006;84:1374–9.
6. Magkos F, Wright DC, Patterson BW, Mohammed BS, Mittendorfer B. Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. Am J Physiol Endocrinol Metab 2006; 290:E355–62.
7. Venables MC, Jeukendrup AE. Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. Diabetes Metab Res Rev 2009;25(Suppl 1):S18–23.
8. Nseir W, Hellou E, Assy N. Role of diet and lifestyle changes in nonalcoholic fatty liver disease. World J Gastroenterol 2014;20:9338–44.
9. Egli L, Lecoultre V, Theytaz F, Campos V, Hodson L, Schneiter P, Mittendorfer B, Patterson BW, Fielding BA, Gerber PA, et al. Exercise prevents fructose-induced hypertriglyceridemia in healthy young subjects. Diabetes 2013;62:2259–65.
10. Bidwell AJ, Fairchild TJ, Redmond J, Wang L, Keslacy S, Kanaley JA. Physical activity offsets the negative effects of a high-fructose diet. Med Sci Sports Exerc 2014;46:2091–8.
11. Parks EJ, Skokan LE, Timlin MT, Dingfelder CS. Dietary sugars stimulate fatty acid synthesis in adults. J Nutr 2008;138:1039–46.
12. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr 2007;85:1511–20.
13. Tappy L, Egli L, Lecoultre V, Schneider P. Effects of fructose-containing caloric sweeteners on resting energy expenditure and energy efficiency: a review of human trials. Nutr Metab (Lond) 2013;10:54.
14. Tappy L, Le KA. Health effects of fructose and fructose-containing caloric sweeteners: where do we stand 10 years after the initial whistle blowings? Curr Diab Rep 2015;15:627.
15. Schneiter P, Di Vetta V, Jequier E, Tappy L. Effect of physical exercise on glycogen turnover and net substrate utilization according to the nutritional state. Am J Physiol 1995;269:E1031–6.
16. Schneiter P, Pasche O, Di Vetta V, Jequier E, Tappy L. Noninvasive assessment of in vivo glycogen kinetics in humans: effect of increased physical activity on glycogen breakdown and synthesis. Eur J Appl Physiol Occup Physiol 1994;69:557–63.
17. Harris JA, Benedict FG. A biometric study of human basal metabolism. Proc Natl Acad Sci USA 1918;4:370–3.
18. Theytaz F, Noguchi Y, Egli L, Campos V, Buehler T, Hodson L, Patterson BW, Nishikata N, Kreis R, Mittendorfer B, et al. Effects of supplementation with essential amino acids on intrahepatic lipid concentrations during fructose overfeeding in humans. Am J Clin Nutr 2012;96:1008–16.
19. Debodo RC, Steele R, Altszuler N, Dunn A, Bishop JSON. The hormonal regulation of carbohydrate metabolism; studies with C14 glucose. Recent Prog Horm Res 1963;19:445–88.
20. Rumessen JJ, Hamberg O, Gudmand-Hoyer E. Interval sampling of end-expiratory hydrogen (H2) concentrations to quantify carbohydrate malabsorption by means of lactulose standards. Gut 1990;31:37–42.
21. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am J Clin Nutr 1988;47:608–28.
22. Frayn KN. Indirect calorimetry. Metabolism 1988;37:1185.
23. Lecoultre V, Benoit R, Carrel G, Schutz Y, Millet GP, Tappy L, Schneiter P. Fructose and glucose co-ingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. Am J Clin Nutr 2010;92:1071–9.
24. Koutsari C, Karpe F, Humphreys SM, Frayn KN, Hardman AE. Exercise prevents the accumulation of triglyceride-rich lipoproteins and their remnants seen when changing to a high-carbohydrate diet. Arterioscler Thromb Vasc Biol 2001;21:1520–5.
25. Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 2010;90:23–46.
26. Fujisawa T, Mulligan K, Wada L, Schumacher L, Riby J, Kretchmer N. The effect of exercise on fructose absorption. Am J Clin Nutr 1993;58: 75–9.
27. Hudgins LC, Parker TS, Levine DM, Hellerstein MK. A dual sugar challenge test for lipogenic sensitivity to dietary fructose. J Clin Endocrinol Metab 2011;96:861–8.

Manuscript 4

Postexercise Repletion of Muscle Energy Stores with Fructose or Glucose in Mixed Meals

Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals[1,2]

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ABSTRACT

Background: Postexercise nutrition is paramount to the restoration of muscle energy stores by providing carbohydrate and fat as precursors of glycogen and intramyocellular lipid (IMCL) synthesis. Compared with glucose, fructose ingestion results in lower postprandial glucose and higher lactate and triglyceride concentrations. We hypothesized that these differences in substrate concentration would be associated with a different partition of energy stored as IMCLs or glycogen postexercise.

Objective: The purpose of this study was to compare the effect of isocaloric liquid mixed meals containing fat, protein, and either fructose or glucose on the repletion of muscle energy stores over 24 h after a strenuous exercise session.

Design: Eight male endurance athletes (mean ± SEM age: 29 ± 2 y; peak oxygen consumption: 66.8 ± 1.3 mL · kg^{-1} · min^{-1}) were studied twice. On each occasion, muscle energy stores were first lowered by a combination of a 3-d controlled diet and prolonged exercise. After assessment of glycogen and IMCL concentrations in vastus muscles, subjects rested for 24 h and ingested mixed meals providing fat and protein together with 4.4 g/kg fructose (the fructose condition; FRU) or glucose (the glucose condition; GLU). Postprandial metabolism was assessed over 6 h, and glycogen and IMCL concentrations were measured again after 24 h. Finally, energy metabolism was evaluated during a subsequent exercise session.

Results: FRU and GLU resulted in similar IMCL [+2.4 ± 0.4 compared with +2.0 ± 0.6 mmol · kg^{-1} wet weight · d^{-1}; time × condition (mixed-model analysis): $P = 0.45$] and muscle glycogen (+10.9 ± 0.9 compared with +12.3 ± 1.9 mmol · kg^{-1} wet weight · d^{-1}; time × condition: $P = 0.45$) repletion. Fructose consumption in FRU increased postprandial net carbohydrate oxidation and decreased net carbohydrate storage (estimating total, muscle, and liver glycogen synthesis) compared with GLU (+117 ± 9 compared with +135 ± 9 g/6 h, respectively; $P < 0.01$). Compared with GLU, FRU also resulted in lower plasma glucose concentrations and decreased exercise performance the next day.

Conclusions: Mixed meals containing fat, protein, and either fructose or glucose elicit similar repletion of IMCLs and muscle glycogen. Under such conditions, fructose lowers whole-body glycogen synthesis and impairs subsequent exercise performance, presumably because of lower hepatic glycogen stores. This trial was registered at clinicaltrials.gov as NCT01866215. *Am J Clin Nutr* 2017;105:609–17.

Keywords: athletes, intramyocellular lipids, muscle glycogen, recovery, hypertriglyceridemia, lactate shuttle

INTRODUCTION

Adequate recovery of endogenous energy stores is critical for exercise performance and is largely dependent on nutrition (1, 2). The maintenance of muscle glycogen, in particular, has been considered a major challenge for endurance athletes because of its limited content, importance as a substrate source, and association with the development of fatigue during exercise (3, 4). Consequently, most nutritional guidelines recommend the increase of postexercise dietary carbohydrate content to favor muscle glycogen repletion between sessions (2).

Consumption of beverages or foods containing glucose or maltodextrins is generally advocated to improve postexercise muscle glycogen repletion because of the increase in plasma glucose and insulin concentrations they elicit. In contrast, fructose is considered not to be a prime energy source for muscle, and most (5–7) but not all (1) studies have found lower muscle glycogen repletion after fructose ingestion than after glucose ingestion. However, these observations were made with pure glucose and maltodextrin rather than with mixed meals. In addition, muscle glycogen synthesis was monitored during the hours immediately after exercise (<8 h postexercise), which corresponds with a period in which muscle insulin sensitivity is enhanced. Unlike glucose, fructose is primarily metabolized in the liver, and pure fructose was shown to efficiently restore hepatic glycogen after exercise (8). Substantial amounts of fructose are also released as blood glucose and lactate from the liver (9, 10). In addition, fructose metabolism can be modulated by coingested nutrients such as glucose or amino acids (9, 10). For these reasons, the comparison of the presence of fructose and glucose in a mixed meal on muscle glycogen repletion still remains unclear. Fructose and glucose could also differentially affect muscle fat storage. Indeed, beside glycogen, intramyocellular

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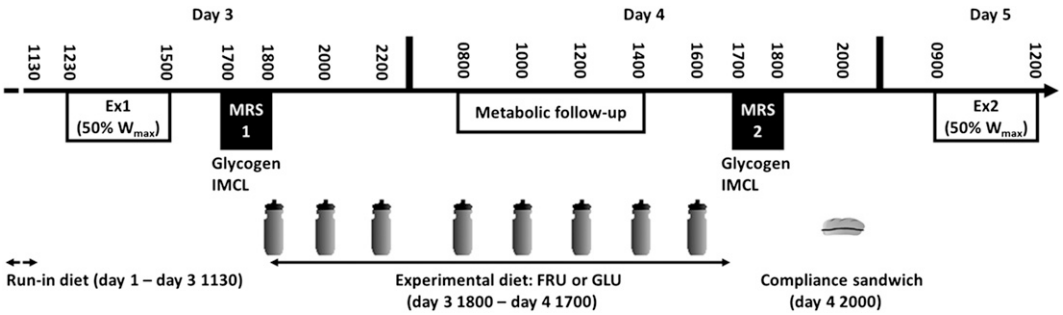

FIGURE 1 Setting of the 5-d experimental sequences. Subjects were studied on 2 occasions, each consisting of a 5-d sequence of dietary and physical activity control. Having first ingested a weight-maintenance controlled diet between day 1 and day 3, subjects were supervised while cycling for 2 h 30 min at 50 W_{max} (Ex1: 1230–1500 on day 3). Lowered IMCL and glycogen stores in vastus muscles were then measured by MRS (MRS1: 1700–1800 on day 3) before subjects started a 24-h period of dietary recovery during which they remained inactive and received the experimental diets. A total of 2.5 g fat/kg, 1.9 g protein/kg, and 5.6 g carbohydrate/kg, including 4.4 g/kg of either pure fructose or pure glucose, was administered in 8 liquid mixed meals provided at 1800, 2000, and 2200 (day 3), and 0800, 1000, 1200, 1400, and 1600 (day 4). The effect of 3 of these meals was assessed during a postprandial metabolic follow-up of 6 h (0800–1400 on day 4), and muscle energy stores were again measured after the end of the 24-h period of recovery (MRS2, day 4: 1700–1800). To improve compliance, subjects received a standardized small sandwich to ingest at the end of day 4. Finally, subjects, who fasted overnight, participated in a second endurance session to assess the effect of the previous diet on energy metabolism and subsequent exercise capacity (Ex2: 0900–1200 on day 5). Ex, exercise; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy; W_{max}, maximal workload.

lipids (IMCLs)[5] represent an alternative energy source to muscle glycogen, which can be mobilized during exercise (11). As for muscle glycogen, IMCL stores are repleted in the postexercise period. However, the relative contributions of plasma free fatty acids (FFAs) (12) and lipoprotein-bound triglycerides from dietary fat (13) to postexercise IMCL repletion remain controversial (14). High-fructose diets are consistently associated with increased fasting and postprandial plasma triglyceride concentrations (15). These effects are accounted for by both increased hepatic VLDL triglyceride secretion (16) and decreased adipose tissue triglyceride clearance (17). We therefore hypothesized that the consumption of fructose might stimulate IMCL repletion more efficiently than might glucose consumption.

The primary aim of this study (NCT01866215) was therefore to compare the effectiveness of isocaloric mixed meals containing fat and protein with either pure fructose or pure glucose on IMCL and muscle glycogen repletion over a 24-h period after a strenuous exercise session. We also wanted to assess whether these mixed meals containing fructose (the fructose condition; FRU) or glucose (the glucose condition; GLU) had different effects on whole-body metabolism during a subsequent standardized exercise session.

METHODS

All variables are presented as means ± SEMs. Eleven well-trained young men were recruited from local triathlon and cycling clubs. Two of them dropped out during the experiment because of gastrointestinal symptoms during the fructose condition. One subject completed the study but was excluded from analysis because of apparent failure to comply with dietary instructions (see Results section). The 8 remaining subjects [age: 29 ± 2 y; weight: 71.2 ± 1.9 kg; BMI (in kg/m^2): 22.6 ± 0.4; peak oxygen consumption: 66.8 ± 1.3 mL · kg^{-1} · min^{-1}; maximal workload (W_{max}): 368 ± 12 W] were nonsmokers and weight-stable, took no medication, had no family history of diabetes and had ≥3 y of cycling training and racing experience. In addition, all of them were fully informed about the nature of and risks involved in the testing procedure and provided written consent before beginning the study. Except for magnetic resonance spectroscopy (MRS) measurements, which were done at the Department of Clinical Research and Radiology, University of Bern, the experiments were performed at the Clinical Research Center, University of Lausanne, Switzerland. The experimental protocol was approved by the Ethics Committee of the Canton de Vaud, Lausanne, Switzerland, and all procedures were performed in accordance with the 1983 revision of the Declaration of Helsinki.

Study design

The subjects of this double-blind, crossover-controlled, randomized clinical trial were studied on 2 occasions separated by 2–4 wk. The experiments (**Figure 1**) started with a run-in period aimed at lowering the muscle energy stores by using a 2.5-d controlled diet (day 1 to 1130 on day 3) followed by a prolonged exercise session (exercise 1: 1230–1500 on day 3). IMCL and muscle glycogen concentrations were then measured (MRS1: 1700 on day 3). Thereafter, 2 experimental isocaloric mixed diets that contained either fructose or glucose were provided as 8 liquid meals ingested at 1800, 2000, and 2200 on day 3, and at 0800, 1000, 1200, 1400, and 1600 on day 4. To evaluate the effects of these diets, plasma metabolic markers and energy metabolism were monitored after an overnight fast (0800 on day 4) and over 6 h postprandial (0800–1400 on day 4). Thereafter, IMCLs and muscle glycogen were measured at 1700 (MRS2). The next day, all subjects reported to the Clinical Research Center after an overnight fast to have their metabolism monitored during 3 h of standardized exercise (exercise 2: 0900–1200 on day 5).

Preliminary visit

Between 1 and 2 wk before the first experimental condition, subjects performed an incremental test to exhaustion on an electrically braked leg cycle ergometer (Excalibur; Lode). Peak

[5] Abbreviations used: FFA, free fatty acid; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy; W_{max}, maximal workload; WW, wet weight.

oxygen consumption was determined by the continuous monitoring of respiratory gas exchange with the use of indirect calorimetry (Quark CPET; Cosmed) as previously described (18). Briefly, after a 5-min warm-up at 60 W, ergometer power was increased by 35 W every 3 min. W_{max} was calculated by linear regression of the time completed and was used for setting subsequent exercise intensities.

Experimental sequences: run-in period (day 1 to 1700 on day 3)

The purpose of this period was to lower muscle energy stores at the beginning of the 2 experimental periods. Subjects were provided an outpatient weight-maintenance diet (day 1 to 1130 on day 3) providing 1.5 times calculated basal energy expenditure (19) and containing 55% carbohydrate, 30% fat, and 15% protein. Food items were weighed (P10; Mettler Toledo) and prepacked by the experimenters. Subjects were instructed to ingest all the food supplied and nothing else except water, and were asked to return emptied containers. To further standardize muscle energy stores, subjects were instructed to perform, under both conditions, 1 h of endurance exercise on day 1, remain sedentary on day 2, and perform a supervised constant-load cycling exercise session (exercise 1: 2.5 h at 50% W_{max}) between 1230 and 1500 on day 3. After this session, subjects traveled by public transportation to the MRI scanning facility for an initial assessment of muscle IMCL and glycogen concentrations by MRS (MRS1: 1700–1800 on day 3). Travel time was ~90 min, with a walk of <5 min. Subjects were escorted by an investigator to ensure that physical activity remained minimal and that no food was consumed. Altogether, a comparable protocol was previously used to standardize baseline IMCL and muscle glycogen concentrations (13).

MRS measurements

Noninvasive measurements of IMCLs, followed by glycogen concentrations, in the vastus muscles were performed with the use of a 3-Tesla whole-body scanner (Verio; Siemens) before (MRS1: 1700 on day 3) and after (MRS2: 1700 on day 4) the dietary interventions. To ensure reproducible anatomical positioning, the subjects' right legs were fixed in a specially designed cast and the first session positioning served as an intraindividual reference. Care was taken to minimize MRI-visible extramyocellular lipids in the voxel, and measurements were performed at the same time of day, by the same operator. A double-tuned, linearly polarized proton-MRS/^{13}C-MRS surface coil (Lammers Medical Technology) was used, and constant magnetic field was assessed by a shimming package (Siemens), followed by post hoc calculation of the shim currents. To determine vastus intermedius IMCL contents by proton MRS, a $9 \times 9 \times 18$ mm^3 voxel was used, and nonwater-suppressed spectra (1 acquisition) and water-suppressed spectra (96 averages) were acquired with the use of a point-resolved spectroscopy localization sequence (retention time: 3 s; echo time: 30 ms). Glycogen measures by ^{13}C-MRS targeted a larger region in the vastus lateralis and medialis, and 3 consecutive spectra (retention time: 200 ms; 2048 averages each; 7 min/spectrum) were acquired and compared with a reference spectrum (retention time: 200 ms; 32 averages; chromium-doped acetone, keto-group singlet on center frequency). Relaxation effects were not corrected, and spectra were analyzed with the use of jMRUI version 3.1 (20). Water and total creatine signals were used as internal standards to determine IMCL and glycogen concentrations, respectively [water reference: 39.8 mol/kg wet weight (WW); total creatine reference: 33.8 mmol/kg WW] (13, 21).

Dietary intervention (1700 on day 3 to 1700 on day 4)

The experimental diets were provided during the whole experimental period, and were consumed partly under investigators' supervision. Random assignment and meal preparation were performed by an experimenter who was not otherwise involved in the experiments, leaving subjects and investigators blinded. Both FRU and GLU contained the same total energy and macronutrient composition (3508 ± 154 kcal as 5.6 g carbohydrate/kg, 2.5 g fat/kg, and 1.9 g protein/kg), and were consumed as 8 equal liquid meals (308 ± 13 mL each) ingested at 1800, 2000, and 2200 (day 3, unsupervised), and 0800, 1000, 1200, 1400, and 1600 (day 4, supervised). This timing was selected to ensure continuous energy provision while limiting the effect of prior exercise. The choice of a liquid meal was made because of the convenience of preparing accurate, tailor-made liquid diets according to the energy needs of each subject. The total energy administered during the experimental period was calculated to match total energy expenditure. This was done by summing each participant's daily metabolic requirements [basal energy expenditure (19) with a 1.4 physical activity level to account for the fact that subjects remained in bed most of the day] and the energy expended during exercise 1 (assuming a muscle energy efficiency of 25%). Fat was provided with the use of a mixture of fresh full cream, soy cream, and soy milk, and protein was provided with the use of soy milk protein and whey proteins (Whey Protein 94; Sponser Sport Food). All of these products are available commercially. Carbohydrate was added as either 5.0 g glucose/kg in GLU, or 4.4 g fructose/kg with 0.6 g glucose/kg in FRU [D-(+)-Glucose and D-(−)-Fructose; Sigma-Aldrich]. Using small amounts of glucose in the fructose meals prevents intestinal fructose malabsorption (22). After MRS2, subjects were instructed to ingest a small sandwich (347 ± 11 kcal containing 0.6 g carbohydrate/kg (mainly starch), 0.1 g fat/kg, and 0.5 g protein/kg) under both conditions, and to continue fasting thereafter (except for water).

Metabolic follow-up (0800–1400 on day 4)

The metabolic effects of the experimental diets were directly assessed on day 4 (0800–1400). Fasting subjects reported to the Clinical Research Center at 0700 and were installed on a bed after a void. A venous cannula was inserted into a forearm vein to allow repeated blood sampling at 0800 (baseline), 0830, 0900, 1000, 1030, 1100, 1200, 1230, 1300, and 1400. Respiratory exchange measurements (indirect calorimetry) were performed continuously in the fasting state (0730–0800) and, after the provision of 3 experimental meals, at 0800, 1000, and 1200. Throughout the test, urine was collected in a container to determine urea nitrogen excretion and the exact time at which urine collection was ended. The exact time elapsed since the initial miction was used to calculate urinary urea excretion rate. At 1400, metabolic follow-up was discontinued, and the subjects were instructed to ingest the last 2 liquid meals (1400 and 1600)

while traveling to the MRS laboratory to repeat measurements (MRS2: 1700–1800 on day 4).

Energy metabolism during standardized endurance exercise (0900–1200 on day 5)

The effect of the experimental diets on energy metabolism were evaluated on day 5 during a prolonged cycling exercise session performed in the fasting state. Upon arrival at the laboratory (0700), subjects were asked to void. They were then weighed, and a cannula was inserted into a forearm vein for repeated blood sampling (0800, 0830, 0900, 0920, 0940, 1000, 1020, 1040, 1100, 1120, 1140, and 1200). After an initial baseline resting period (0800–0900), subjects mounted the ergometer and started cycling at a constant workload for 3 h or until exhaustion (exercise 2: 50% W_{max}, 0900–1200). Subjects had free access to water, but otherwise remained in a fasting state. Their energy expenditure, respiratory exchange ratio, and substrate oxidation were measured by indirect calorimetry. As on the previous day, urine was collected throughout the test to determine protein oxidation.

Analyses

Blood samples were collected in EDTA-coated tubes and centrifuged (10 min; 2800 × *g*; 4°C), and plasma aliquots were stored at −20°C until analyzed. Plasma FFAs, triglycerides, glucose, lactate, and urinary nitrogen were measured by enzymatic methods (RX Monza; Randox Laboratories), and insulin and glucagon were determined by radioimmunoassay with the use of commercial kits (Merck Millipore).

Calculations

Energy expenditure (kilocalories per minute) and net carbohydrate, lipid, and protein oxidations (grams per minute) were calculated from respiratory gas exchanges with the use of standard indirect calorimetry equations (23). Net rates of oxidation measured by indirect calorimetry are a reflection of each nutrient's balance, but it is not possible to specify the metabolic pathways used for each nutrient's oxidation. For example, net carbohydrate oxidation represents the sum of exogenous glucose, fructose, and endogenous glycogen oxidation, without including oxidation of glucose formed from amino acid precursors, which would be included in net protein oxidation (24). Net protein oxidation was calculated while assuming that urinary urea nitrogen represented 85% total nitrogen excretion, which may be overestimated during exercise because of sweat losses. During the postprandial follow-up, net lipid and carbohydrate oxidations were calculated postprandially over 6 h. Net carbohydrate storage was obtained by subtracting cumulated net carbohydrate oxidation over 6 h from total carbohydrate ingested over the same period, assuming complete carbohydrate absorption. This calculation does not include the eventual storage of newly synthesized fat because de novo lipogenesis from glucose or fructose is computed as carbohydrate oxidation by indirect calorimetry equations, and, hence, provides an estimate of net whole body glycogen storage (without, however, differentiating between hepatic and muscle glycogen synthesis) (24). Net IMCL and muscle glycogen synthesis over 24 h were calculated by subtracting MRS1 from MRS2 values.

Statistics

Treatment allocation was determined by random generation of blocks of 4 sequences until completion of the study. A sample size of 8 subjects was calculated (1-β: 80%; $\alpha = 0.05$) to detect an ~35% difference in IMCL recovery between fructose and glucose consumption. Outlier subjects were detected by a Grubb test. Distribution normality and homoscedasticity were verified with the use of Shapiro-Wilk and Bartlett tests and data were log-transformed when appropriate (postprandial test: glucagon, triglycerides, and FFAs; exercise test: lactate and glucagon). Unless otherwise specified, significance was determined with the use of a paired Student's *t* test. Significance of changes over time was determined with the use of mixed-models analysis, with fixed effects of time and condition, and random effects for subject-specific intercepts and slopes. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Pearson's correlation test was used to determine linear associations. Subsequent exercise capacity was evaluated by plotting the cycling time of subjects as a cumulative survival curve, and both conditions were compared with the use of the log-rank test. Analyses were performed with the use of R, version 3.0.3, and the level of significance was set as 2-tailed $P = 0.05$.

RESULTS

The experimental part of the study was completed between March 2013 and March 2014. All subjects reported that they strictly followed the outpatient dietary and physical activity instructions before both conditions. However, 1 subject had highly variable muscle glycogen (FRU: 68.2 mmol/kg WW, compared with GLU: 34.2 mmol/kg WW) and IMCL (FRU: 11.5 mmol/kg WW, compared with GLU: 7.8 mmol/kg WW) concentrations on day 4, and was identified as an outlier (Grubb's test: $P < 0.05$). Furthermore, his muscle glycogen concentration did not increase between day 4 and day 5. Because dietary intake was not directly supervised before MRS1 (day 3), we speculated that this subject did not adhere to the run-in instructions, and he was therefore removed from all analyses. In the remaining 8 subjects, body weight and heart rate during exercise 1 were similar across conditions, as well as day-4 IMCL (FRU: 6.5 ± 1.0 mmol/kg WW compared with GLU: 6.0 ± 0.9 mmol/kg WW; $P = 0.22$) and muscle glycogen (FRU: 32.7 ± 4.0 mmol/kg WW compared with GLU: 35.9 ± 3.1 mmol/kg WW; $P = 0.44$) concentrations.

Follow-up of the metabolic effects of fructose and glucose (day 4, 0800–1400)

Fasting glucose, fructose, insulin, glucagon, lactate, and triglyceride concentrations were not different between FRU and GLU (all $P > 0.05$) (**Figure 2**A–E), but fasting FFA concentrations were lower in FRU than in GLU (0.6 ± 0.0 mmol/L compared with 0.9 ± 0.1 mmol/L, respectively; $P = 0.01$) (Figure 2F). Thereafter, postprandial glucose and insulin responses were lower, and glucagon, lactate, and triglyceride concentrations were higher in FRU than in GLU (all time × condition: $P < 0.01$) (Figure 2A–E). Postprandial FFA concentrations were almost completely suppressed under both conditions (Figure 2F).

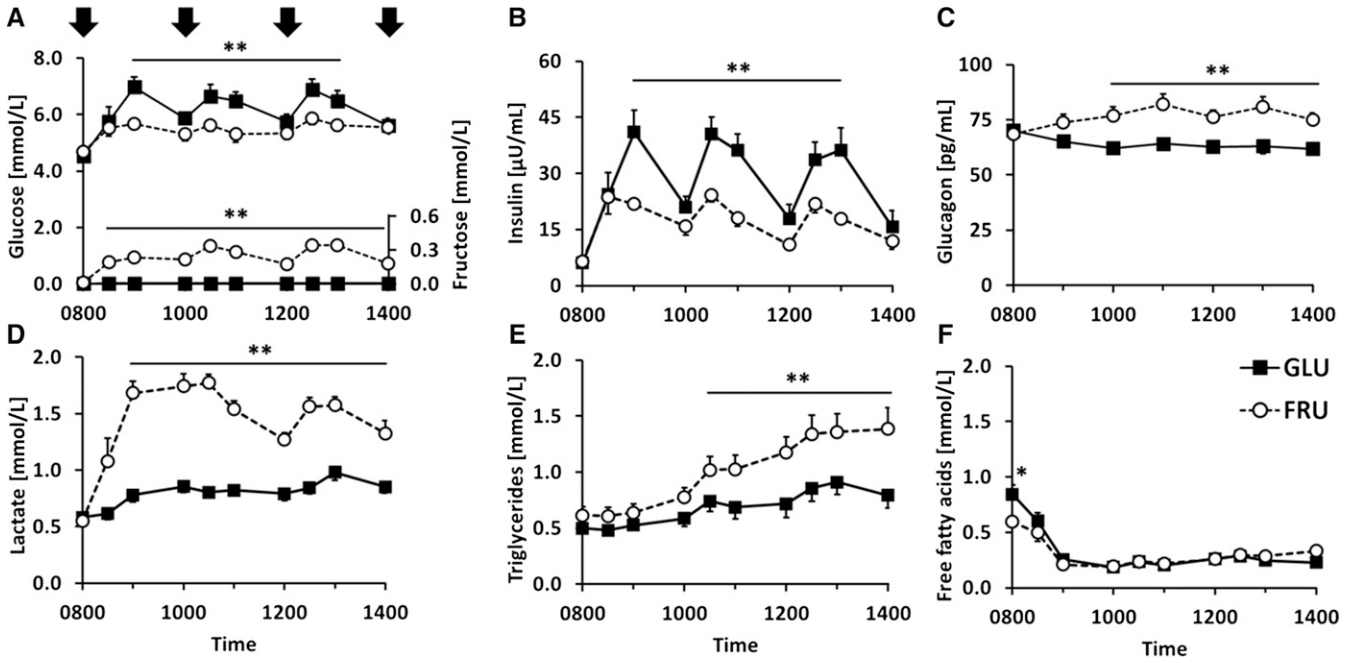

FIGURE 2 Plasma markers during postprandial follow-up (day 4, 0800–1400). Changes over time in plasma glucose (A), insulin (B), glucagon (C), lactate (D), triglyceride (E), and free fatty acid (F) concentrations after ingestion of the meals in FRU and GLU. Values are means ± SEMs, $n = 8$ subjects. Postprandial glucose and insulin responses were lower and glucagon, lactate, and triglyceride concentrations were higher in FRU than in GLU (all time × condition: $P < 0.01$). Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; glucagon, triglyceride, and free fatty acid data were log-transformed for analyses. Metabolic effects of FRU and GLU were compared with the use of a mixed model with time and condition as fixed effects. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Paired contrasts were used to determine differences between conditions at specific time points, $^*P < 0.05$ and $^{**}P < 0.01$. Lines: FRU and GLU significantly different over all covered time points. Arrows: meal ingestion (0800, 1000, and 1200). FRU, fructose condition; GLU, glucose condition.

Fasting resting metabolic rate, respiratory exchange ratio, and substrate oxidation were similar in FRU and GLU (data not shown). Postprandial energy expenditure was higher in FRU than in GLU (567 ± 21 compared with 542 ± 21 kcal/6 h, respectively; $P = 0.03$), corresponding to a higher respiratory exchange ratio (0.81 ± 0.01 compared with 0.78 ± 0.01, respectively; $P < 0.01$), and, hence, to an increased net carbohydrate oxidation (FRU: 47 ± 4 g/6 h compared with GLU: 29 ± 3 g/6 h; $P < 0.01$). Because the same amount of carbohydrate was ingested under both conditions (165 ± 7 g/6 h), carbohydrate balance (accounting for whole-body glycogen storage) was lower in FRU than in GLU (+117 ± 9 compared with +135 ± 9 g/6 h, respectively; $P < 0.01$). In contrast, lipid oxidation was lower in FRU than in GLU (31 ± 2 compared with 35 ± 3 g/6 h, respectively; $P = 0.02$), resulting in a higher lipid balance (FRU: +36 ± 2 g/6 h compared with GLU: +32 ± 2 g/6 h; $P = 0.02$) (**Table 1**). Protein oxidation and balance over 6 h were similar across both conditions (data not shown).

Recovery of muscle energy stores (day 3, 1700, to day 4, 1700)

Two MRS measurements were performed 24 h apart to determine the effects of diet on IMCL and muscle glycogen repletion (**Figure 3**). Both FRU and GLU were effective in raising IMCL concentrations from MRS1 to MRS2, but with no difference between treatments (FRU: +2.4 ± 0.4 mmol/kg WW compared with GLU: +2.0 ± 0.6 mmol/kg WW; time: $P < 0.01$;

TABLE 1
Whole-body substrate balance over the 6-h follow-up (day 4, 0800–1400)[1]

	Intake		Net oxidation		Storage	
	FRU	GLU	FRU	GLU	FRU	GLU
Carbohydrate, g	165 ± 7	165 ± 7	47 ± 4**	29 ± 3**	117 ± 9**	135 ± 9**
GLU	17 ± 1	134 ± 6	—	—	—	—
FRU	117 ± 5	—	—	—	—	—
Other	31 ± 5	31 ± 5	—	—	—	—
Fat, g	67 ± 3	67 ± 3	31 ± 2*	35 ± 3*	36 ± 2*	32 ± 2*

[1] Values are means ± SEMs, $n = 8$ subjects. Intake values correspond to the 3 experimental meals ingested during the postprandial metabolic follow-up. Substrate oxidation data were averaged over the entire 6-h period, and whole-body storage was calculated by difference from intake values. *,**FRU and GLU significantly different as assessed by Student's paired t test, $^*P < 0.05$ and $^{**}P < 0.01$. FRU, fructose condition; GLU, glucose condition.

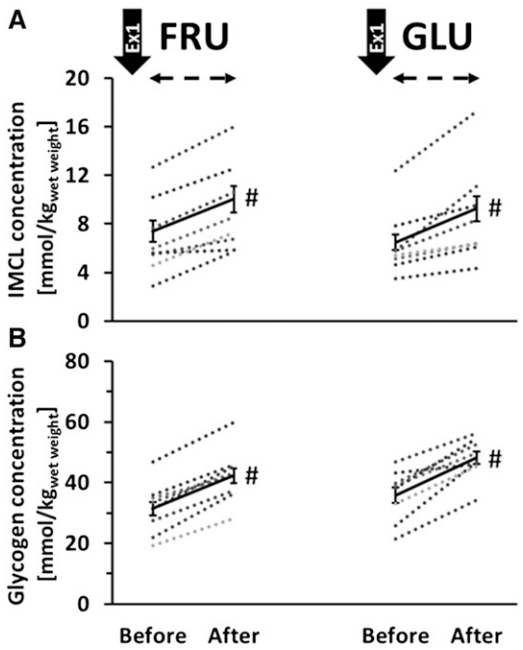

FIGURE 3 Effect of diet on energy storage in vastus muscles. Changes in IMCL (A) and muscle glycogen (B) concentrations before and after FRU and GLU diets. Values are means ± SEMs, $n = 8$ subjects. FRU and GLU were similarly effective in increasing IMCL (time: $P < 0.01$; time × condition: $P = 0.45$) and muscle glycogen (time: $P < 0.01$; time × condition: $P = 0.45$) concentrations. Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; no transformations were necessary for analyses. Metabolic effects of FRU and GLU were compared with the use of a mixed model with time and condition as fixed effects. Models included time and condition interaction, and baseline values were included in the models whenever the goodness of fit was significantly improved. #Before and after dietary intervention significantly different, $P < 0.01$. Ex, exercise; FRU, fructose condition; GLU, glucose condition; IMCL, intramyocellular lipid.

time × condition: $P = 0.45$) (Figure 3A). Muscle glycogen contents were also effectively but similarly raised in FRU and GLU (+10.9 ± 0.9 mmol/kg WW compared with +12.3 ± 1.9 mmol/kg WW, respectively; time: $P < 0.01$; time × condition: $P = 0.45$) (Figure 3B).

To obtain information regarding potent precursors of these stores, we determined linear associations between mean plasma concentrations of circulating substrates (metabolic follow-up) and muscle energy storage (**Figure 4**). Glycogen repletion was associated with plasma glucose under both conditions (GLU: $r = 0.77$ and $P = 0.02$; FRU: $r = 0.84$ and $P < 0.01$) (Figure 4A), and with plasma lactate only in FRU ($r = 0.75$ and $P = 0.03$) (Figure 4B). No other relations were found.

Energy metabolism during subsequent exercise (day 5, 0900–1200)

On day 5, the subjects who fasted overnight, reported to the laboratory for an endurance exercise trial (3 h at 50% W_{max}: 180 ± 10 W). Pre-exercise body weight, glucose, lactate, FFA, triglyceride, insulin, and glucagon concentrations, as well as energy expenditure, respiratory exchange ratio, and substrate oxidation, were all similar in FRU and GLU (data not shown; all $P > 0.05$). In contrast to GLU, in which all subjects cycled for 3 h, 4 of 8 subjects were unable to maintain the target power output after the diet provided in FRU (**Figure 5**), suggesting an impaired endurance exercise capacity (mean completion: 2 h 48 min ± 0 h 05 min; log-rank test: $P = 0.03$) (Figure 5A). Plasma glucose concentrations increased transiently at the beginning of exercise, but then declined progressively under both conditions to reach significantly lower values in FRU than in GLU (time: $P < 0.01$; time × condition: $P = 0.01$) (Figure 5B). Plasma lactate concentrations were initially lower, but increased more during exercise in FRU than in GLU (time × condition: $P = 0.03$) (Figure 5C), whereas FFA concentrations were similarly raised under both conditions (time × condition: $P = 0.80$) (Figure 5D). At the last point completed by all subjects (i.e., at 1120), the lower plasma glucose concentration in FRU than in GLU (3.6 ± 0.3 compared with 4.2 ± 0.1 mmol/L; $P < 0.01$) (Figure 5A) was associated with higher glucagon (183 ± 3 compared with 89 ± 6 pg/mL, respectively; $P < 0.01$) (Figure 5F) and similarly low insulin (4.6 ± 0.4 compared with 4.8 ± 0.3 μU/mL, respectively; $P = 0.48$) (Figure 5E) concentrations, suggesting that endogenous glucose output was impaired in FRU.

During exercise, mean energy expenditure (14.1 ± 0.6 compared with 14.1 ± 0.4 kcal/min, respectively; $P = 0.98$), respiratory exchange ratio (0.83 ± 0.01 compared with 0.84 ± 0.01 g/min, respectively; $P = 0.18$), carbohydrate oxidation (1.62 ± 0.12 compared with 1.70 ± 0.12 g/min, respectively; $P = 0.63$) and lipid oxidation (0.70 ± 0.10 compared with 0.67 ± 0.10 g/min, respectively; $P = 0.29$) were not significantly different between FRU and GLU. Protein oxidation, however, was slightly higher in FRU than in GLU (0.06 ± 0.02 compared with 0.05 ± 0.01 g/min, respectively; $P = 0.03$).

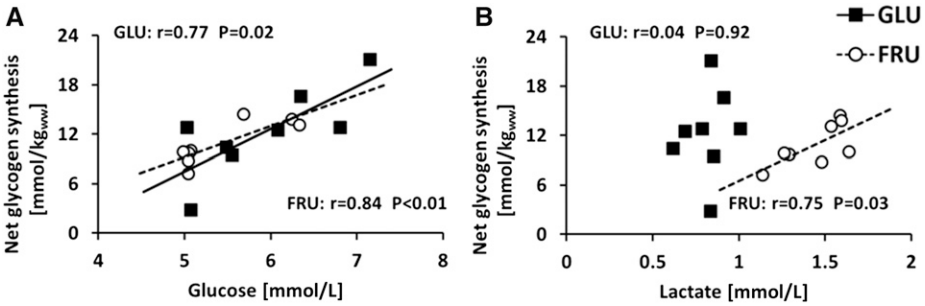

FIGURE 4 Associations between muscle glycogen synthesis and mean glucose (A) and lactate (B) concentrations after the ingestion of FRU or GLU meals. For each condition, $n = 8$ subjects. Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; no transformations were applied. Linear relation between covariates was determined with the use of a Pearson's test, and correlation coefficients with corresponding P values are indicated. FRU, fructose condition; GLU, glucose condition; WW, wet weight.

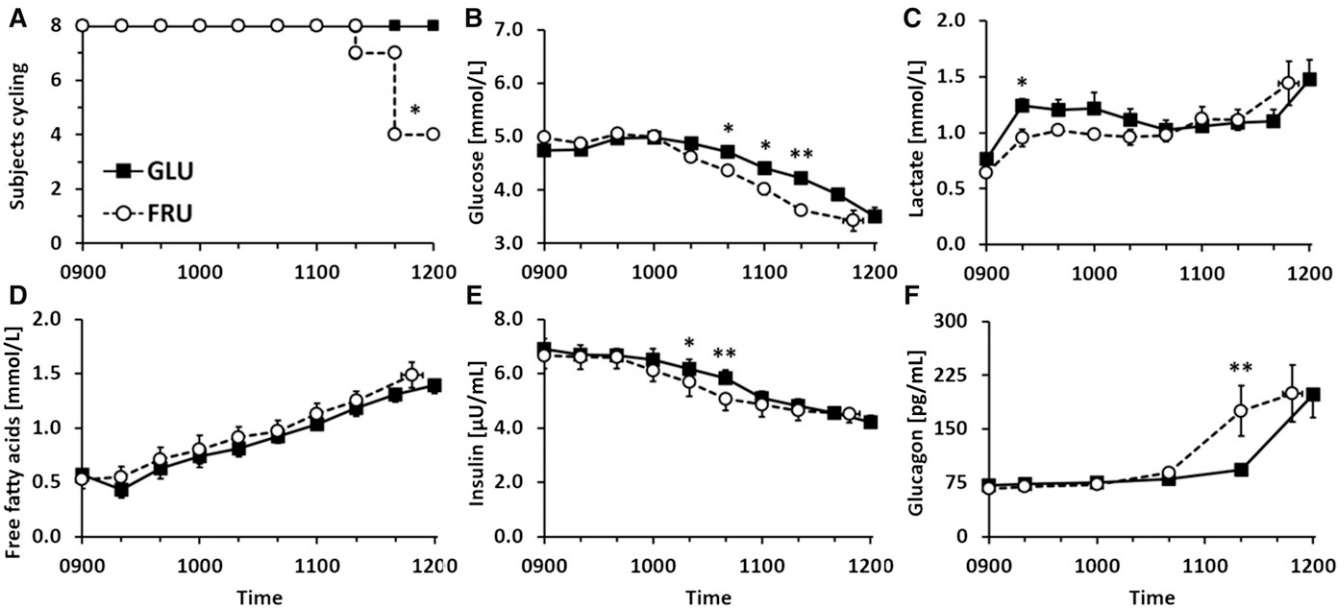

FIGURE 5 Plasma substrates and hormones during subsequent exercise (day 5, 0900–1200). Cumulative curves of subjects still cycling during exercise 2, with survival AUCs compared with the use of the log-rank test; *FRU and GLU significantly different, $P < 0.05$ (A). Changes over time in plasma glucose (B), lactate (C), free fatty acid (D), insulin (E), and glucagon (F) concentrations in subjects who previously consumed FRU or GLU diets. Metabolic effects of FRU and GLU conditions were compared with the use of a mixed-model with time and condition as fixed effects. Time and condition interaction and baseline values were included in the models whenever the goodness of fit was improved. Paired contrasts were used to determine differences between conditions at specific time points; *$P < 0.05$ and **$P < 0.01$. Because several subjects did not complete the exercise session in FRU, all values collected after 1120 are drawn as a single point for FRU ($n = 8$ until 1120; then $n = 4$ under FRU conditions). Values are means ± SEMs, $n = 8$ subjects. Plasma glucose concentrations increased transiently at the beginning of exercise, but then declined progressively under both conditions to reach significantly lower values in FRU than in GLU (time × condition: $P = 0.01$). However, plasma lactate concentrations increased more during exercise in FRU than in GLU (time × condition: $P = 0.03$), whereas free fatty acid concentrations were similarly raised under both conditions (time × condition: $P = 0.80$). This was also associated with a tendency toward higher glucagon response in FRU than in GLU (time × condition: $P = 0.09$) and similarly decreased insulin (time × condition: $P = 0.72$). Distribution normality was visually inspected and checked with the use of a Shapiro-Wilk test; lactate and glucagon data were log-transformed for analyses. FRU, fructose condition; GLU, glucose condition.

DISCUSSION

The aim of this study was to determine how fructose or glucose ingested with mixed meals would contribute to postexercise muscle lipids and, more importantly for subsequent performance, glycogen repletion. We hypothesized that fructose would favor IMCL repletion and alter muscle glycogen synthesis (8, 25). To assess this hypothesis, we studied healthy volunteers after a single session of exercise that decreased IMCL and glycogen concentrations to values close to those reported in the literature under similar conditions (10, 12). Participants thereafter consumed liquid mixed meals that contained glucose or fructose, which elicited postprandial responses consistent with reports that compared fructose- and glucose-based meals in resting humans (15, 17).

Contrary to our hypothesis, fructose- and glucose-containing meals induced comparable IMCL repletion. This occurred despite 1.5-fold higher plasma triglyceride concentrations in FRU than in GLU, suggesting that muscle fatty acid uptake was not dependent on triglyceride-rich lipoprotein concentrations. One explanation could be a higher insulin-induced vasodilation in GLU than in FRU (26), thus compensating for lower triglyceride concentrations by greater muscle blood flow. Alternatively, this may reflect that IMCL synthesis is mainly dependent on whole-body lipolysis and plasma FFA concentrations (27, 28), and that increased concentrations of plasma triglyceride-rich lipoproteins contributed little to this process. Consistent with a prime role of FFAs in IMCL synthesis (11), postprandial FFA concentrations were indeed not different between the 2 conditions. Further specifically designed experiments will be needed to identify the metabolic pathways used for IMCL repletion in FRU.

Both FRU and GLU resulted in similar muscle glycogen repletion. This was unexpected, because plasma glucose and insulin, which are prime determinants of muscle glycogen synthesis, were markedly higher in GLU than in FRU. FRU, but not GLU, increased postprandial fructose concentrations, but to values an order of magnitude lower than plasma glucose concentrations. This, and the lower affinity of muscle hexokinase for fructose than glucose, strongly suggests that plasma fructose was not a major precursor of muscle glycogen (9, 10). The possibility that the small amounts of glucose present in FRU were sufficient to reach a plateau in muscle glycogen repletion is also very unlikely, considering the dose-response relations between glucose loads, postprandial glycemia, and muscle glycogen synthesis (1). Rather, we postulate that the similar muscle glycogen repletion in FRU and GLU was mainly explained by conversion of fructose-derived circulating glucose and lactate. Such muscle glycogen synthesis from lactate has been described in animal models, with considerable between-species variations (29). Muscle glycogen synthesis has further been shown to be stimulated by increased lactate concentrations (30). No similar data are currently available in humans after oral fructose consumption, but it has been reported that lactate contributed to muscle glycogen repletion when fructose was administered intravenously after exercise (31).

Compared with glucose consumption, fructose consumption increased postprandial energy expenditure and net carbohydrate oxidation. As a consequence, whole-body carbohydrate storage, calculated as the difference between ingested and oxidized carbohydrate over the 6 h during which resting indirect calorimetry was performed, was 18 g lower in FRU than in GLU. This provides a reliable estimate of whole-body glycogen storage (24) when carbohydrate absorption is complete. For FRU, this may not hold true because of fructose malabsorption (32). Fructose malabsorption would, however, result in an overestimation of whole-body glycogen storage, and, hence, an underestimated difference in glycogen storage between FRU and GLU. Carbohydrate oxidation was monitored for 6 h under supervised resting conditions, but cannot be extrapolated to the whole 24 h because physical activity, although maintained as low as possible, was certainly higher during the rest of the day. One may nonetheless speculate that whole-body net carbohydrate oxidation remained higher, and, hence, that whole-body glycogen storage was lower in FRU than in GLU. Given that muscle glycogen repletion was similar with the consumption of both sugars, one may then suspect that hepatic glycogen synthesis was somewhat lower with fructose consumption than with that of glucose. This conclusion is at odds with several reports showing that fructose is a better hepatic glycogen precursor than glucose (8, 25, 33), and will need to be confirmed with direct assessment of hepatic glycogen in future studies.

Glucose and fructose also altered metabolic homeostasis differently during a subsequent standardized exercise session. This part of our experimental protocol was mainly exploratory and did not intend to specifically assess hepatic or muscle metabolism. Yet, plasma glucose concentrations decreased more sharply and were significantly lower in the second session of exercise, whereas glucagon increased earlier in FRU than in GLU. This would certainly be consistent with lower hepatic glycogen concentrations and lower hepatic glucose production in FRU, but we cannot discard the fact that other mechanisms, such as increased muscle glucose transport, may occur. Although it was not our aim to evaluate exercise performance, we also observed that one-half of the subjects had to stop exercising in FRU, whereas all subjects were able to complete the exercise in GLU. This strongly suggests that exercise performance may not be adequately maintained by a very high fructose diet administered during postexercise recovery. In this context, our observation of higher plasma glucagon and lactate concentrations in FRU may reflect counterregulatory responses to low endogenous glucose production. Failure to complete the exercise trial may then be related to lower glycemia, which may be associated with lower energy supply to both skeletal muscle and the brain, and, hence, to both impaired muscle performance and increased central fatigue (34). This remains, however, an academic rather than practical issue, because our experimental diets were designed to assess the effects of mixed meals comprising fructose or glucose on muscle energy repletion, but were not aimed at providing practical recommendations for athletes. This would require further studies focused on exercise performance, and including various ratios of fructose to glucose, rather than the individual sugars.

This study presents several limitations. First, we studied trained athletes because of their high turnover of glycogen and IMCLs. However, this population also exhibits high oxidative capacity and insulin sensitivity, both of which could affect muscle energy storage. Second, we did not measure hepatic glycogen, nor did we measure substrate kinetics. Third, we designed liquid meals from commercially available foods and nutrients and administered them as split doses every 2 h for convenience and purposes of accuracy. This could have affected our results, which may not be extrapolated to other nutrient sources. The FRU diet also contained much larger ratios of fructose to glucose than usually consumed. Our aim was, however, not to develop a novel feeding procedure for athletes, but to assess whether fructose specifically affected the metabolic pathways used for muscle energy storage.

In conclusion, this study indicates that pure fructose or glucose ingested together with fat and protein in the 24 h period after a strenuous exercise session leads to similar energy storage in IMCLs and muscle glycogen. Muscle glycogen synthesis was likely fueled in FRU by glucose and lactate derived from fructose and released into the blood by splanchnic organs. Net whole-body glycogen storage was lower, and performance during subsequent exercise was decreased in FRU.

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The authors' responsibilities were as follows—RR, VL, LE, CB, RK, PS, and LT: designed the study; RR, VL, and JC: recruited subjects and performed metabolic tests; ASD and KZ: performed scanner measurements; RR: analyzed the data; RR and LT: drafted the manuscript; LT: had primary responsibility for the final content; and all authors: read and approved the final manuscript. LT previously received research support from Nestlé and Ajinomoto for research unrelated to this trial. None of the other authors reported a conflict of interest related to the study.

REFERENCES

1. Burke LM, Kiens B, Ivy JL. Carbohydrates and fat for training and recovery. J Sports Sci 2004;22:15–30.
2. Cermak NM, van Loon LJ. The use of carbohydrates during exercise as an ergogenic aid. Sports Med 2013;43:1139–55.
3. Hermansen L, Hultman E, Saltin B. Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 1967;71:129–39.
4. Hawley JA, Leckey JJ. Carbohydrate dependence during prolonged, intense endurance exercise. Sports Med 2015;45Suppl 1:S5–12.
5. Blom PC, Hostmark AT, Vaage O, Kardel KR, Maehlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. Med Sci Sports Exerc 1987;19:491–6.
6. Conlee RK, Lawler RM, Ross PE. Effects of glucose or fructose feeding on glycogen repletion in muscle and liver after exercise or fasting. Ann Nutr Metab 1987;31:126–32.
7. Van Den Bergh AJ, Houtman S, Heerschap A, Rehrer NJ, Van Den Boogert HJ, Oeseburg B, Hopman MT. Muscle glycogen recovery after exercise during glucose and fructose intake monitored by 13C-NMR. J Appl Physiol 1996;81:1495–500.
8. Décombaz J, Jentjens R, Ith M, Scheurer E, Buehler T, Jeukendrup A, Boesch C. Fructose and galactose enhance postexercise human liver glycogen synthesis. Med Sci Sports Exerc 2011;43:1964–71.
9. Mayes PA. Intermediary metabolism of fructose. Am J Clin Nutr 1993; 58(5 Suppl):754S–65S.
10. Sun SZ, Empie MW. Fructose metabolism in humans - what isotopic tracer studies tell us. Nutr Metab (Lond) 2012;9:89.
11. Popadic Gacesa J, Schick F, Machann J, Grujic N. Intramyocellular lipids and their dynamics assessed by 1 H magnetic resonance spectroscopy. Clin Physiol Funct Imaging 2016 Feb 11 (Epub ahead of print; DOI: 10.1111/cpf.12346).

12. van Hall G. The physiological regulation of skeletal muscle fatty acid supply and oxidation during moderate-intensity exercise. Sports Med 2015;45Suppl 1:S23–32.
13. Zehnder M, Christ ER, Ith M, Acheson KJ, Pouteau E, Kreis R, Trepp R, Diem P, Boesch C, Decombaz J. Intramyocellular lipid stores increase markedly in athletes after 1.5 days lipid supplementation and are utilized during exercise in proportion to their content. Eur J Appl Physiol 2006;98:341–54.
14. Goldberg IJ, Eckel RH, Abumrad NA. Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways. J Lipid Res 2009;50Suppl:S86–90.
15. Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. Am J Clin Nutr 2008;87: 1194–203.
16. Faeh D, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. Diabetes 2005; 54:1907–13.
17. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr 2007;85:1511–20.
18. Lecoultre V, Benoit R, Carrel G, Schutz Y, Millet GP, Tappy L, Schneiter P. Fructose and glucose co-ingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. Am J Clin Nutr 2010;92: 1071–9.
19. Harris JA, Benedict FG. A biometric study of human basal metabolism. Proc Natl Acad Sci USA 1918;4:370–3.
20. Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput Biol Med 2001;31:269–86.
21. Boesch C, Machann J, Vermathen P, Schick F. Role of proton MR for the study of muscle lipid metabolism. NMR Biomed 2006;19:968–88.
22. Truswell AS, Seach JM, Thorburn AW. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. Am J Clin Nutr 1988;48:1424–30.
23. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. World Rev Nutr Diet 1992;70:68–131.
24. Tappy L, Paquot N, Tounian P, Schneiter P, Jequier E. Assessment of glucose metabolism in humans with the simultaneous use of indirect calorimetry and tracer techniques. Clin Physiol 1995;15:1–12.
25. Fuchs CJ, Gonzalez JT, Beelen M, Cermak NM, Smith FE, Thelwall PE, Taylor R, Trenell MI, Stevenson EJ, van Loon LJ. Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion when compared to glucose ingestion in trained athletes. J Appl Physiol (1985) 2016;120:1328–34.
26. Vollenweider P, Tappy L, Randin D, Schneiter P, Jequier E, Nicod P, Scherrer U. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 1993;92:147–54.
27. Schrauwen-Hinderling VB, van Loon LJ, Koopman R, Nicolay K, Saris WH, Kooi ME. Intramyocellular lipid content is increased after exercise in nonexercising human skeletal muscle. J Appl Physiol 2003; 95:2328–32.
28. Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, Sachinwalla T, Thompson MW. Effect of short-term starvation versus high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit men. Exp Physiol 2006;91:693–703.
29. Fournier PA, Brau L, Ferreira LD, Fairchild T, Raja G, James A, Palmer TN. Glycogen resynthesis in the absence of food ingestion during recovery from moderate or high intensity physical activity: novel insights from rat and human studies. Comp Biochem Physiol A Mol Integr Physiol 2002;133:755–63.
30. McDermott JC, Bonen A. Glyconeogenic and oxidative lactate utilization in skeletal muscle. Can J Physiol Pharmacol 1992;70:142–9.
31. Ahlborg G, Bjorkman O. Splanchnic and muscle fructose metabolism during and after exercise. J Appl Physiol 1990;69:1244–51.
32. Latulippe ME, Skoog SM. Fructose malabsorption and intolerance: effects of fructose with and without simultaneous glucose ingestion. Crit Rev Food Sci Nutr 2011;51:583–92.
33. Nilsson LH, Hultman E. Liver and muscle glycogen in man after glucose and fructose infusion. Scand J Clin Lab Invest 1974;33:5–10.
34. Nybo L. CNS fatigue and prolonged exercise: effect of glucose supplementation. Med Sci Sports Exerc 2003;35:589–94.

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Endurance Training with or without Glucose-Fructose Ingestion: Effects on Lactate Metabolism Assessed in a Randomized Clinical Trial on Sedentary Men

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Endurance Training with or without Glucose-Fructose Ingestion: Effects on Lactate Metabolism Assessed in a Randomized Clinical Trial on Sedentary Men

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Abstract: Glucose-fructose ingestion increases glucose and lactate oxidation during exercise. We hypothesized that training with glucose-fructose would induce key adaptations in lactate metabolism. Two groups of eight sedentary males were endurance-trained for three weeks while ingesting either glucose-fructose (GF) or water (C). Effects of glucose-fructose on lactate appearance, oxidation, and clearance were measured at rest and during exercise, pre-training, and post-training. Pre-training, resting lactate appearance was 3.6 ± 0.5 vs. 3.6 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ in GF and C, and was increased to 11.2 ± 1.4 vs. 8.8 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$ by exercise (Exercise: $p < 0.01$). Lactate oxidation represented 20.6% ± 1.0% and 17.5% ± 1.7% of lactate appearance at rest, and 86.3% ± 3.8% and 86.8% ± 6.6% during exercise (Exercise: $p < 0.01$) in GF and C, respectively. Training with GF increased resting lactate appearance and oxidation (Training × Intervention: both $p < 0.05$), but not during exercise (Training × Intervention: both $p > 0.05$). Training with GF and C had similar effects to increase lactate clearance during exercise (+15.5 ± 9.2 and +10.1 ± 5.9 $mL \cdot kg^{-1} \cdot min^{-1}$; Training: $p < 0.01$; Training × Intervention: $p = 0.97$). The findings of this study show that in sedentary participants, glucose-fructose ingestion leads to high systemic lactate appearance, most of which is disposed non-oxidatively at rest and is oxidized during exercise. Training with or without glucose-fructose increases lactate clearance, without altering lactate appearance and oxidation during exercise.

Keywords: glucose; fructose; lactate; lactate metabolism; substrate oxidation; carbohydrate; exercise

1. Introduction

During moderate and high intensity exercise, muscle energy needs are essentially met by carbohydrate oxidation [1] and muscle performance is dependent on both muscle glycogen and plasma glucose concentrations [2–4]. Plasma glucose entry into skeletal muscle is activated by contraction [5] and depends on systemic glucose appearance, i.e., the sum of endogenous glucose production (hepatic glycogen breakdown and gluconeogenesis) and gut glucose absorption [1,6]. Accordingly, whole-body and muscle glucose oxidation can be enhanced by glucose ingestion that increases plasma glucose appearance and muscle glucose uptake [6]. A portion of glucose is actually made indirectly available through glycolytic lactate production in some muscle fibers, followed by lactate uptake and oxidation in other fibers [7]. These lactate "shuttles" may possibly increase muscle

energy substrates provision when glucose transport and/or glycolytic capacity is saturated. Endurance training has also been well-established to increase the expression of proteins involved in lactate transport and metabolism [8], suggesting that lactate can be a major energy substrate for the trained muscle [7,9]. Alternatively, lactate can also be recycled into glucose (gluconeogenesis), with recent indications that this can be altered in trained subjects [10,11].

The oxidation rate of exogenous glucose increases dose-dependently up to ≈1 g·min^{-1}, but then plateaus at higher glucose ingestion rates [4]. Co-ingestion of glucose and fructose can further increase exogenous [12] and net carbohydrate oxidations [13], and can also improve exercise performance [14]. This has been attributed to glucose and fructose being absorbed through distinct transporter proteins in the apical membrane of enterocytes, thus allowing for a higher rate of total gut carbohydrate absorption [4]. In addition, a substantial portion of ingested fructose is converted into lactate in the liver, to increase plasma lactate concentration and lactate delivery as an energy substrate to working muscle [15,16].

Fructose absorption is poor when it is ingested alone, but is markedly enhanced by glucose co-ingestion [17]. Furthermore, gut fructose transport is potently induced by fructose itself, and fructose absorption increases within a few days upon chronic fructose ingestion [9]. One may therefore suspect that the beneficial effects of ingesting glucose and fructose mixtures during exercise may depend on their chronic use during training. In addition, hyperlactatemia is thought to be instrumental in training-induced increase in lactate clearance by the stimulation of muscle lactate transporters [9]. We therefore postulated that the combined, repeated effects of exercise and glucose-fructose ingestion may significantly impact training-induced adaptation of muscle lactate metabolism. To assess this hypothesis, we enrolled two groups of healthy sedentary males in a 3-week training program during which they consumed glucose-fructose drinks (GF intervention) or plain water as a control (C intervention) during training sessions.

2. Materials and Methods

2.1. Participants

Sixteen healthy young males (mean ± standard error (SEM) age: 25 ± 1 years; weight: 73.2 ± 2.0 kg; body mass index: 22.9 ± 0.4 kg·m^{-2}) completed this study. One additional volunteer dropped out prior to the interventions and was hence removed from all analyzes. At inclusion, all participants were sedentary and low-sugar consumers (exercise: <1 h·week^{-1} and sugar intake: <60 g·day^{-1}) and were asked to maintain their lifestyle with the exception of the supervised exercise sessions prescribed in the study protocol. They were fully informed of the nature and risks involved by the procedures, in accordance with the 1983 revision of the Declaration of Helsinki. All experiments were performed at the Clinical Research Center, Lausanne, Switzerland, after approbation by the local ethics committee. This study was registered at ClinicalTrials.gov database as NCT01610986.

2.2. Study Design

Prior to the 3-week exercise training program, participants underwent two pre-training visits to determine baseline characteristics. Maximal oxygen consumption (VO_{2max}), maximal aerobic workload (W_{max}), and workload eliciting the lactate turnpoint (W_{LT}) were assessed at the first visit. At a second visit 48 h later, plasma glucose and lactate metabolism were investigated at rest and during moderate-intensity exercise when fed glucose-fructose (metabolic evaluation). Participants were then separated into two parallel groups, and both groups performed 15 sessions of supervised moderate-intensity laboratory cycling on an ergometer (60 min each; 5 day·week^{-1}) either with glucose-fructose drinks in GF or water in C. Finally, preliminary visits were repeated post-training (beginning 48–72 h after the last training session) to assess the effect of exercise training with glucose-fructose ingestion on metabolic response to these drinks, at rest and during exercise (Figure 1). To assume comparable glycogen concentrations between interventions, participants filled food diaries and were instructed to repeat dietary intake and physical activity patterns the 48 h prior to each visit.

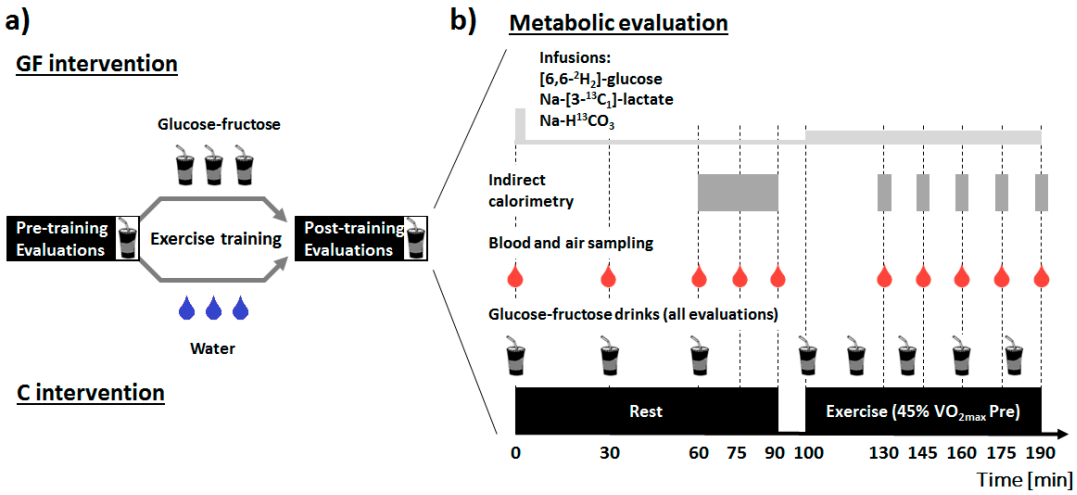

Figure 1. Study design (**a**) and description of the metabolic evaluations (**b**). Drinks containing 19 g glucose and 12 g fructose were administered at time 0, 30 and 60 min at rest, and at 20 min intervals during exercise. Primed-continuous infusions of (6,6-$^{2}H_2$)-D-(+)-glucose and Na-(3-$^{13}C_1$)-L-(+)-lactate were started at time 0, and resting measurements were obtained after 60 min equilibration. Continuous infusion rates were upgraded at the beginning of exercise at time 100 min (see methods for further details). GF: intervention in which glucose-fructose drinks were provided during training sessions; C: control intervention in which plain water was provided during training sessions.

2.3. Incremental Exercise-Testing

Overnight fasted participants performed an incremental test to exhaustion on a cycle ergometer (Ergoselect 100, Ergoline GmbH, Bitz, Germany) pre-training and post-training. Respiratory gas exchanges (SensorMedics Vmax; Sensormedics Corp., Yorba Linda, CA, USA) and heart-rate (Polar S810; Polar Electro Oy, Kempele, Finland) were continuously monitored throughout the test. Briefly, after a resting period of 5 min and a warm-up of 5 min at 40 W, ergometer workload was increased by 25 W every 3 min. As VCO_2 exceeded VO_2, ergometer workload was increased by 25 W every minute until volitional exhaustion. VO_{2max} and W_{max} were determined as previously described [13] and used to determine training intensities. Earlobe blood lactate concentration was measured at the end of each step (Lactate Pro, Arkray, Kyoto, Japan). Participants were then familiarized to the endurance capacity task (pre-training) or could leave the laboratory (post-training).

2.4. Metabolic Evaluations

Participants were instructed to remain sedentary, filled food diaries and had to avoid caffeine, alcohol and ^{13}C-rich foods the 48 h before metabolic evaluations. Overnight-fasted participants reported to the metabolic unit at 0700 h and, after a void, were weighed and installed on a bed. One indwelling venous cannula was then inserted into an antecubital vein for blood sampling. This forearm was then constantly placed under a heating pad to open arteriovenous anastomoses, allowing for accurate determination of substrate exchanges in arterialized venous blood [18]. Another cannula was inserted into a vein of the opposite forearm for the infusion of stable isotopes tracers (Cambridge Isotope Lab., Andover, MT, USA). After background sampling at 0800 h (time = 0 min), a labelled-bicarbonate bolus (Na-H^{13}CO$_3$: 3.05 g) and primed continuous infusions of glucose ((6,6-$^{2}H_2$)-D-(+)-glucose; prime: 2 mg·kg^{-1}; continuous: 0.02 mg·kg^{-1}·min^{-1}) and sodium lactate (Na-(3-$^{13}C_1$)-L-(+)-lactate; prime: 0.4 mg·kg^{-1}; continuous: 0.02 mg·kg^{-1}·min^{-1}) were started. Infusion rates were tripled during exercise to account for increased substrate kinetics.

The metabolic evaluation consisted of a resting period (time = 0–90 min) in which participants remained in the supine position, followed by a continuous exercise session (time = 100–190 min) at 45% pre-training VO_{2max} (i.e., at the same workload pre-training and post-training). This intensity aimed to elicit the greatest effect of endurance-training on lactate metabolic clearance [19]. Glucose-fructose sweetened drinks (193 mL of a 9.8% glucose, 6.2% fructose drink flavored with 2% lemon

juice and 1.17 g·L^{-1} NaCl) were provided at time = 0 then every 30 min at rest, and every 20 min during exercise. Blood and expired air samples were collected at time = 0, 30, 60, 75, and 90 min (rest), then 130, 145, 160, 175 and 190 min (exercise). Energy expenditure and substrate oxidation were measured by open-circuit indirect calorimetry (Quark RMR, Cosmed, Roma, Italia) in the last 30 min of rest and for 5 min intervals during exercise (SensorMedics Vmax; Sensormedics Corp, Yorba Linda, CA, USA). After 190 min, infusions were stopped and participants' urine was collected to estimate protein oxidation.

Twenty-five minutes later, ergometer workload was set at 85% of the current VO_{2max} and participants were asked to cycle at 60 rpm until exhaustion to measure endurance capacity.

2.5. Training Intervention

Starting 48–72 h after the pre-training evaluation, participants entered a supervised endurance-training program of 1 session·d^{-1}, 5 day·week^{-1} over 3 weeks. Each session consisted of 60 min cycling at a constant workload, with intensities set as 50% (sessions 1–3), 55% (sessions 4–6), 60% (sessions 7–9), and 65% (sessions 10–15) of pre-training VO_{2max}. Experimental interventions differed by the drinks provided during sessions: the GF group ingested three 163 mL doses of glucose-fructose drinks provided −20, 0, and +20 min referred to exercise onset, while the C group correspondingly received water. Participants were instructed to have their last meal at least two hours before exercise onset. Earlobe blood lactate concentration was measured at 0, 30 and 60 min (Lactate Pro, Arkray, Japan).

2.6. Analytical Procedures

Arterialized venous blood samples were collected on lithium heparin for measurement of glucose, lactate, fructose, and tracers, with ethylenediaminetetraacetic acid (EDTA)-coated tubes for free fatty acids, triglycerides and insulin or with trasylol-EDTA for glucagon. Plasma was immediately separated by centrifugation (10 min; 2800× *g*; 4 °C), and aliquots were stored at −20 °C until analyzed. Plasma glucose, lactate, free fatty acids, and urinary nitrogen concentrations were determined using a semi-automated clinical chemistry analyzer (RX Monza, Randox Laboratories Ltd., Crumlin, UK). Insulin and glucagon concentrations were obtained by radioimmunoassay using commercial kits (Merck Millipore, Billerica, MA, USA).

Expired air $^{13}CO_2$ isotopic enrichments were obtained by isotope-ratio mass spectrometry (IRMS) (SerCon Ltd., Crewe, UK), as previously described [13]. Gas chromatography-mass spectrometry (GCMS) (Hewlett-Packard Instruments, Palo Alto, CA, USA) was used to measure plasma fructose concentration [13] and plasma ($^{13}C_1$)lactate and ($^{2}H_2$)glucose isotopic enrichments [20,21]. Plasma (^{13}C)glucose enrichments were measured by GC/C/IRMS [22] (Thermo Scientific, Bremen, Germany).

2.7. Calculations

Energy expenditure and substrate oxidation were calculated from respiratory gas exchanges using standard equations [23]. When exceeding VO_2, VCO_2 values were set as corresponding to VO_2 to reflect aerobic metabolism, and $^{13}CO_2$ isotopic enrichment was corrected for bicarbonate retention [24]. Rates of plasma glucose and lactate appearance, disposal, and metabolic clearance were calculated using Steele's equations for non-steady state using a volume of distribution of 180 mL·kg^{-1} for both substrates [25]. Plasma lactate oxidation, non-oxidative lactate disposal (NOLD), and gluconeogenesis from lactate were calculated as:

$$\text{Lactate oxidation} = \frac{\text{lactate disposal} \cdot VCO_2 \cdot {}^{13}CO_2}{F \cdot k \cdot 89.08} \ (\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \quad (1)$$

$$\text{NOLD} = \text{lactate disposal} - \text{lactate oxidation} \ (\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \quad (2)$$

$$\text{Gluconeogenesis from lactate} = \frac{({}^{13}\text{C})\text{glucose} \cdot 6 \cdot \text{glucose appearance}}{({}^{13}\text{C})\text{lactate}} \ (\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) \quad (3)$$

where $^{13}CO_2$ and (^{13}C)glucose represent ^{13}carbon isotopic enrichments in expired CO_2 (atom% excess) and plasma glucose (atom% excess), (^{13}C)lactate is (M+1)lactate isotopic enrichment (mol% excess), F is (3-$^{13}C_1$)lactate infusion rate, k is a correction factor for ^{13}C losses in body pools during substrate oxidation [24] (rest: k = 0.8; exercise: k = 1.0), 89.08 is the molar weight of lactate and 6 is the number of mole of CO_2 per mole of glucose. To minimize tracer assumptions, mean values of the last 30 min of rest (time = 60, 75 and 90 min) and exercise (time = 160, 175 and 190 min) are reported in figures (see results). Lactate turnpoint was obtained by the D-max method [18].

2.8. Statistics

Interventions allocation was determined by random generation of four-sequence blocks. A sample size of 16 participants was estimated (1-β: 90%; $\alpha = 0.05$) to detect ≈15% difference in lactate clearance gain between GF and C. Normality and homoscedasticity were first checked visually, then using Shapiro-Wilk and Bartlett tests. Data were transformed in their square root when appropriate (plasma lactate, fructose, free fatty acids and insulin concentrations, carbohydrate and lipid oxidations, endurance capacity). Baseline values were compared using Student's *t*-tests. Evolving data were analyzed using mixed-models, with training (T) and intervention (I) as fixed effects and random effects for participant-specific intercepts and slopes. The training and intervention interaction (T × I), baseline (B) and exercise (E) effects were included in models whenever improving goodness of fit. Paired contrasts were used to determine differences between pre-training vs. post-training (symbol: #) and rest vs. exercise periods (symbol: $), and unpaired contrasts to compare GF vs. C interventions (symbol: *). Analyses were run on R version 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria). $p < 0.05$ was considered significant. Data are presented as mean ± SEM.

3. Results

3.1. Participants Characteristics and Training Effectiveness

This study was completed between April 2012 and December 2014. All participants reported to have followed dietary instructions, completed every exercise session under investigators' supervision and remained weight-stable throughout the experiments (T effect: $p = 0.66$; T × I effect: $p = 0.54$). Plasma lactate concentration was monitored immediately before and during training sessions. Ingestion of GF increased pre-session lactate as compared to C. Lactate was then increased by exercise but, interestingly, mean concentrations after 30 and 60 min exercise were not different (Figure 2: rest: $p < 0.01$; exercise: both $p > 0.05$).

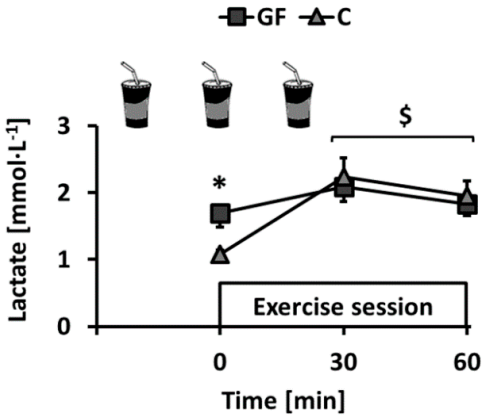

Figure 2. Changes over time of earlobe blood lactate concentration in GF and C groups during training sessions. GF received glucose-fructose drinks and C received water −20, 0, and +20 min relative to exercise onset. Effects of exercise (E) and intervention (I) were compared using a mixed-model analysis. Paired and unpaired contrasts were used to determine differences between rest and exercise (E effect: time = 0 min vs. time = 30–60 min: $: $p < 0.01$) and GF vs. C (I effect: *: $p < 0.05$). Mean ± SEM for $n = 8$ participants in all groups.

Training was effective in both GF and C and increased VO_{2max}, W_{max} and W_{LT} to similar extents (Table 1: all T effects: $p < 0.01$; T × I effects: $p > 0.05$). Consistent with improved conditioning, the fixed workload of the metabolic evaluation corresponded to lower relative exercise intensities post-training (GF and C, respectively 41% and 42% VO_{2max}) than pre-training (45% VO_{2max} by design). Heart rate was decreased similarly in GF and C (all T effects: $p < 0.01$; all T × I effects: $p > 0.05$).

Table 1. Participants' body weight and performance parameters.

	GF Pre	GF Post	C Pre	C Post
Body weight (kg)	73.7 ± 3.0	73.6 ± 2.8	72.9 ± 2.9	73.1 ± 3.0
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	44.3 ± 2.3	48.4 ± 2.0 #	46.4 ± 2.2	49.4 ± 2.1 #
W_{max} (W)	249 ± 20	281 ± 19 #	249 ± 16	287 ± 21 #
W_{LT} (W)	156 ± 15	180 ± 15 #	156 ± 12	181 ± 14 #
Endurance capacity (s)	663 ± 110	1134 ± 163 #	687 ± 177	1455 ± 293 #

Changes of participants' body weight and performance parameters. Baseline values were compared using an unpaired Student's *t*-test. Effects of training interventions were compared using a mixed-model analysis. Paired contrasts were used to determine differences between pre- vs. post-training (T effect: #: $p < 0.01$). GF: glucose-fructose intervention; C: control intervention; Pre: pre-training; Post: post-training; VO_{2max}: maximal oxygen consumption; W_{max}: maximal workload; W_{LT}: workload at lactate turnpoint. SEM: standard error of the mean; Mean ± SEM for $n = 8$ participants in all groups.

3.2. Metabolic Evaluation: Plasma Substrates and Hormones

In overnight-fasted participants, training increased fasting glucose similarly in GF and C (Figure 3a: T effect: $p < 0.01$; T × I effect: $p = 0.69$), but did not affect plasma fructose, lactate, insulin and glucagon concentrations (Figure 3b,c, Figure S1a,b for insulin and glucagon: all effects: $p > 0.05$). Fasting free fatty acids concentration was decreased after training only in C (Figure S1c: T × I effect: $p = 0.02$).

With repeated ingestion of glucose-fructose drinks, glucose, lactate and fructose concentrations (Figure 3a–c) rapidly increased to stabilize in the last part of the resting period (time = 60–90 min). Plasma insulin followed the same time course, whereas glucagon decreased and free fatty acids were decreased below detectable values (Figure S1a–c; all T effect: $p < 0.01$). Regarding the effects of the interventions, plasma glucose was not affected by training (Figure 3a: T effect: $p = 0.86$; T × I effect: $p = 0.56$), while plasma lactate was decreased post-training compared to pre-training in both GF and C (Figure 3b: T effect: $p = 0.02$; T × I effect: $p = 0.18$). Plasma fructose tended to be increased in GF and decreased in C (Figure 3c: T effect: $p = 0.39$; T × I effect: $p = 0.06$) and other parameters were not altered by the interventions (Figure S1a–c: all T and T × I effects: $p > 0.05$).

As compared to rest, exercise then decreased glucose, lactate and insulin and increased fructose and glucagon concentrations (all E effects: $p < 0.01$) to new steady-state values in the last part of the exercise period (time = 160–190 min). However, free fatty acids concentrations remained below the detection limit (Figure S1c: E effect: $p > 0.05$). Plasma lactate was decreased post-training compared to pre-training in both GF and C (Figure 3b: T effect: $p < 0.01$; T × I effect: $p = 0.13$), while plasma glucose, fructose, free fatty acids, insulin and glucagon were unaffected by the interventions (Figure 3a,b, Figure S1a–c for insulin, glucagon and free fatty acids: all effects: $p > 0.05$).

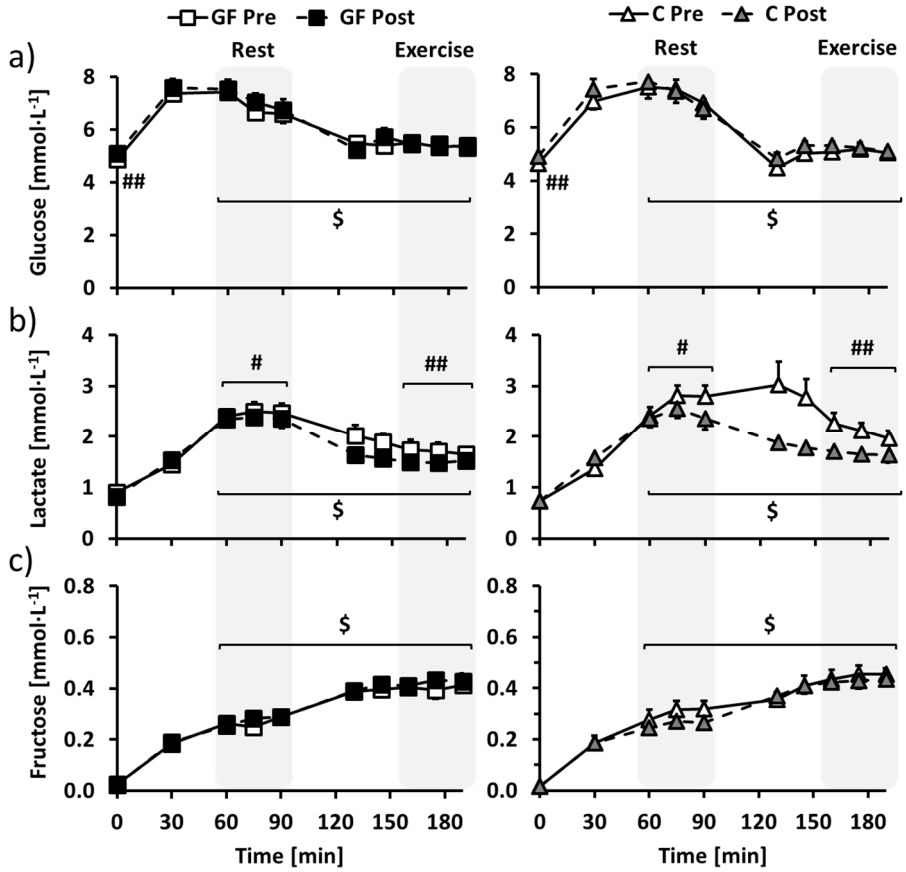

Figure 3. Changes over time of plasma (**a**) glucose, (**b**) lactate and (**c**) fructose concentrations in GF (**left**) and C (**right**) participants during metabolic evaluations. Glucose-fructose drinks were provided both at rest (time = 0–90 min) and during exercise (time = 100–190 min) in all tests. GF pre-training (GF Pre) and C pre-training (C Pre) is indicated in white, GF post-training (GF Post) in black and C post-training (C Post) in grey. Effects of exercise and interventions were compared using a mixed-model analysis. Paired contrasts were used for rest vs. exercise periods (E effect: \$: $p < 0.01$) and pre- vs. post-training (T effect: #: $p < 0.05$; ##: $p < 0.01$). Dashed zones: Measures considered for tracer calculations. Mean ± SEM for $n = 8$ participants in all groups.

3.3. Metabolic Evaluation: Isotopic Enrichments

Glucose, lactate and CO_2 isotopic enrichments (Figure 4) of the last 30 min of rest (time = 60–90 min) and exercise (time = 160–190 min) were selected to determine glucose and lactate metabolisms at rest and during exercise. Mean ($^2H_2$)glucose and (^{13}C)glucose isotopic enrichments were increased from rest to exercise, but were not affected by both GF and C interventions (Figure 4a,b: E effects: $p < 0.01$; all T and T × I effects: $p > 0.05$). At rest, (^{13}C)lactate isotopic enrichment was distinctly affected after GF and C (Figure 4c: T effect: $p = 0.89$; T × I effect: $p = 0.02$), and the difference was no longer significant during exercise (Figure 4c: T effect: $p = 0.61$; T × I effect: $p = 0.18$). $^{13}CO_2$ isotopic enrichment was increased from rest to exercise, without being affected by GF or C (Figure 4d: E effect: $p < 0.01$; all T and T × I effects: $p > 0.05$).

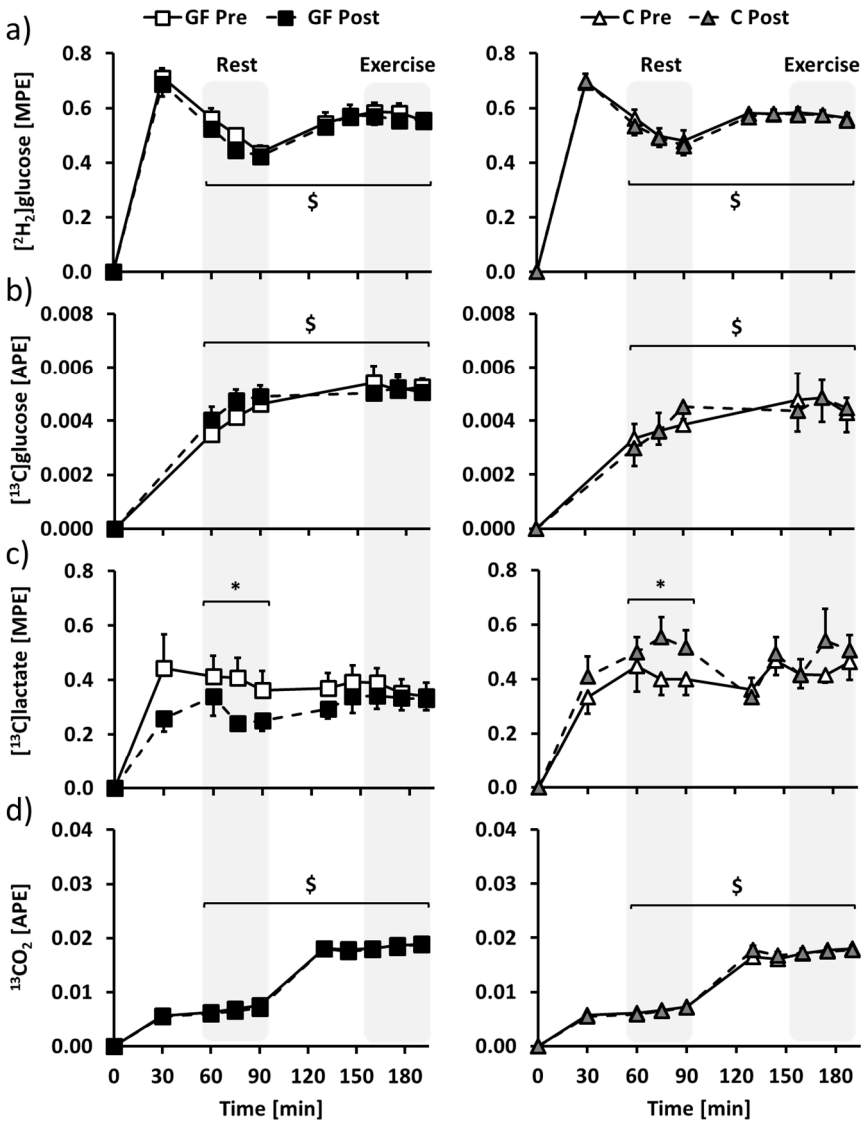

Figure 4. Changes over time of plasma (**a**) ($^2H_2$)glucose, (**b**) (^{13}C)glucose, (**c**) ($^{13}C_1$)lactate and (**d**) expired air $^{13}CO_2$ isotopic enrichments in GF (**left**) and C (**right**) participants during metabolic evaluations. Glucose-fructose drinks were provided in all tests, both during rest (time = 0–90 min) and exercise (time = 100–190 min) periods. GF pre-training (GF Pre) and C pre-training (C Pre) is indicated in white, GF post-training (GF Post) in black and C post-training (C Post) in grey. Effects of exercise and training interventions were compared using a mixed-model analysis. Paired contrasts were used for rest vs. exercise periods (E effect: \$: $p < 0.01$) and training × interventions (T × I effect: *: $p < 0.05$). Dashed zones: Measures considered for tracer calculations. Mean ± SEM for $n = 8$ participants in all groups.

3.4. Metabolic Evaluation: Glucose Metabolism

Summarized as mean values for selected periods of rest and exercise (Table 2), glucose appearance was similar in GF pre-training, GF post-training, C pre-training and C post-training at rest (T effect: $p = 0.19$; T × I effect: $p = 0.99$), then was increased to the same extent by exercise in all evaluations (E effect: $p < 0.01$; T effect: $p = 0.71$; T × I effect: $p = 0.98$). Glucose disposal was also similarly increased by exercise (rest: T effect: $p = 0.19$; T × I effect: $p = 0.62$; exercise: E effect: $p < 0.01$; T effect: $p = 0.38$; T × I effect: $p = 0.90$). Glucose clearance was also increased by exercise as compared to rest and remained constant after training in both GF and C (rest: T effect: $p = 0.70$; T × I effect: $p = 0.74$; exercise: E effect: $p < 0.01$; T effect: $p = 0.78$; T × I effect: $p = 0.86$).

Table 2. Glucose and lactate fluxes in the resting and exercise periods

		GF Pre	GF Post	C Pre	C Post
Glucose appearance ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	5.6 ± 0.3	5.8 ± 0.2	5.1 ± 0.4	5.4 ± 0.4
	Exercise	10.8 ± 0.6 $	10.9 ± 0.5 $	10.5 ± 0.3 $	10.7 ± 0.3 $
Lactate ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	0.5 ± 0.1	0.7 ± 0.1 *	0.3 ± 0.0	0.3 ± 0.1 *
	Exercise	1.2 ± 0.2 $	1.1 ± 0.2 $	0.7 ± 0.1 $	0.7 ± 0.2 $
Other ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	5.1 ± 0.3	5.1 ± 0.2	4.8 ± 0.5	5.1 ± 0.4
	Exercise	9.6 ± 0.6 $	9.8 ± 0.6 $	9.8 ± 0.3 $	9.9 ± 0.3 $
Glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	6.2 ± 0.5	6.4 ± 0.4	5.5 ± 0.6	6.0 ± 0.5
	Exercise	10.8 ± 0.7 $	11.2 ± 0.5 $	10.5 ± 0.3 $	10.8 ± 0.3 $
Glucose clearance ($mL \cdot kg^{-1} \cdot min^{-1}$)	Rest	5.0 ± 0.5	5.0 ± 0.4	4.4 ± 0.6	4.7 ± 0.4
	Exercise	11.2 ± 0.9 $	11.5 ± 0.7 $	11.5 ± 0.6 $	11.5 ± 0.3 $
Lactate appearance ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	3.6 ± 0.5	5.2 ± 0.7 **	3.6 ± 0.4	2.6 ± 0.5 **
	Exercise	11.2 ± 1.4 $	12.1 ± 1.5 $	8.8 ± 0.7 $	8.3 ± 0.9 $
Lactate disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	3.4 ± 0.5	5.0 ± 0.7 **	3.2 ± 0.4	2.5 ± 0.4 **
	Exercise	11.3 ± 1.4 $	12.1 ± 1.5 $	9.1 ± 0.7 $	8.4 ± 1.0 $
Oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	0.7 ± 0.1	0.9 ± 0.1 *	0.6 ± 0.1	0.4 ± 0.1 *
	Exercise	9.7 ± 1.4 $	10.6 ± 1.7 $	7.9 ± 1.0 $	7.3 ± 1.1 $
NOLD ($mg \cdot kg^{-1} \cdot min^{-1}$)	Rest	2.7 ± 0.4	4.1 ± 0.6 **	2.7 ± 0.4	2.0 ± 0.4 **
	Exercise	1.5 ± 0.3 $	1.5 ± 0.4 $	1.2 ± 0.6 $	1.0 ± 0.5 $
Lactate clearance ($mL \cdot kg^{-1} \cdot min^{-1}$)	Rest	17.8 ± 3.0	26.9 ± 4.9 *	16.0 ± 2.6	13.1 ± 2.6 *
	Exercise	75.5 ± 8.7 $	91.0 ± 9.6 $#	47.6 ± 4.7 $	57.6 ± 7.0 $#

Mean values during rest (time = 60–90 min) and exercise (time = 160–190 min) periods of metabolic evaluations performed pre-training (Pre) and post-training (Post). Effects of exercise and training interventions were compared using a mixed-model analysis. Paired and unpaired contrasts were used for rest vs. exercise periods (E effect: $: $p < 0.01$), pre- vs. post-training (T effect: #: $p < 0.01$) and training × interventions (T × I effect: *: $p < 0.05$; **: $p < 0.01$). NOLD: non-oxidative lactate disposal. Mean ± SEM for $n = 8$ participants in all groups.

3.5. Metabolic Evaluation: Lactate Metabolism

Distinctly from glucose metabolism, lactate metabolism was affected by GF and C interventions. Post-training, mean lactate appearance was increased in GF and decreased in C at rest (T effect: $p = 0.56$; T × I effect: $p < 0.01$). Lactate appearance was then significantly increased by exercise and, interestingly, the difference between GF and C interventions was no longer significant during exercise (E effect: $p < 0.01$; T effect: $p = 0.68$; T × I effect: $p = 0.16$). Lactate disposal was also differently affected by GF and C interventions at rest (T effect: $p = 0.41$; T × I effect: $p < 0.01$) and was increased by exercise (E effect: $p < 0.01$) during which differences between interventions were no longer significant (T effect: $p = 0.91$; T × I effect: $p = 0.12$). Lactate clearance followed the same trend at rest in which it was also differently affected by GF and C (T effect: $p = 0.30$; T × I effect: $p = 0.02$) and was then increased by exercise as compared to rest (E effect: $p < 0.01$). During exercise, lactate clearance was interestingly enhanced post-training compared to pre-training, but to similar extents in both GF and C (T effect: $p < 0.01$; T × I effect: $p = 0.53$).

3.6. Metabolic Evaluation: Lactate Disposal

The use of ($^{13}C_1$)lactate allowed to investigate several of its fates. Consistent with the effects of interventions on lactate metabolism, lactate oxidation (T effect: $p = 0.60$; T × I effect: $p = 0.01$), NOLD (T effect: $p = 0.40$; T × I effect: $p < 0.01$) and gluconeogenesis from lactate (T effect: $p = 0.57$; T × I effect: $p = 0.01$) measured during the resting period were all distinctly affected by GF and C interventions. Despite absolute values being affected by the interventions, lactate oxidation still represented a stable proportion of lactate disposal at rest (GF: 20.6 ± 1.0% to 18.6 ± 0.6% vs. C: 17.5 ± 1.7% to 17.7 ± 0.8%; T effect: $p = 0.20$; T × I effect: $p = 0.52$). In contrast, resting gluconeogenesis from lactate represented a larger part of glucose production after GF than after C (GF: 8.2 ± 1.5% to 11.9 ± 1.6% vs. C: 5.7 ± 1.2% to 5.0 ± 1.0%: T effect: $p = 0.21$; T × I effect: $p = 0.01$).

Compared to rest, exercise increased lactate oxidation, decreased NOLD and increased gluconeogenesis from lactate (all: E effects: $p < 0.01$). There were no more significant differences

between GF and C interventions for absolute values of lactate oxidation (T effect: $p = 0.74$; T × I effect: $p = 0.14$), NOLD (T effect: $p = 0.60$; T × I effect: $p = 0.77$) and gluconeogenesis from lactate (T effect: $p = 0.86$; T × I effect: $p = 0.99$). Lactate oxidation represented a significantly higher part (and NOLD a lower part) of lactate disposal during exercise than at rest, yet without effect of GF or C interventions (lactate oxidation: GF: 86.3 ± 3.8% to 87.6 ± 4.9% vs. C: 86.8 ± 6.6% to 87.6 ± 6.6%; E effect: $p < 0$ .01; T effect: $p = 0.99$; T × I effect: $p = 0.83$). Similarly, no training effect was observed on fractional gluconeogenesis from lactate measured during exercise after both GF and C (GF: 10.7 ± 1.9% to 10.4 ± 1.6% vs. C: 7.0 ± 1.2% to 6.8 ± 1.5%; E effect: $p = 0.06$; T effect: $p = 0.87$; T × I effect: $p = 0.97$).

3.7. Metabolic Evaluation: Substrate Oxidation and Exercise Capacity

Carbohydrates provided most of the substrates to be oxidized throughout the evaluations (Table 3). Accordingly, lipid oxidation and protein oxidation (from urinary nitrogen collected during both periods) were low at rest and during exercise. In contrast, energy expenditure, total carbohydrate, lactate and other carbohydrate oxidations were all markedly increased during exercise as compared to the resting period (all E effects: $p < 0.01$).

Table 3. Fuel Selection in the Resting and Exercise Periods of Metabolic Evaluations

		GF Pre	GF Post	C Pre	C Post
Energy expenditure (kcal·min^{-1})	Rest	1.5 ± 0.1	1.5 ± 0.0	1.4 ± 0.1	1.4 ± 0.1
	Exercise	8.3 ± 0.5 $	8.4 ± 0.6 $	8.9 ± 0.5 $	9.0 ± 0.6 $
Protein (mg·kg^{-1}·min^{-1})	Both	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Lipid (mg·kg^{-1}·min^{-1})	Rest	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.2
	Exercise	0.8 ± 0.3	0.5 ± 0.3	0.4 ± 0.2	0.8 ± 0.3
Carbohydrate (mg·kg^{-1}·min^{-1})	Rest	2.8 ± 0.4	2.8 ± 0.3	2.9 ± 0.4	2.8 ± 0.4
	Exercise	27.6 ± 2.4 $	28.5 ± 2.6 $	30.6 ± 1.7 $	30.0 ± 1.7 $
Lactate (mg·kg^{-1}·min^{-1})	Rest	0.7 ± 0.1	0.9 ± 0.1 *	0.6 ± 0.1	0.4 ± 0.1 *
	Exercise	9.7 ± 1.4 $	10.6 ± 1.7 $	7.9 ± 1.0 $	7.3 ± 1.1 $
Other (mg·kg^{-1}·min^{-1})	Rest	2.1 ± 0.4	1.8 ± 0.4	2.4 ± 0.5	2.3 ± 0.4
	Exercise	17.8 ± 1.5 $	17.9 ± 1.4 $	22.7 ± 1.4 $	22.7 ± 1.8 $

Mean values during rest (time = 60–90 min) and exercise (time = 160–190 min) periods of metabolic evaluations performed pre-training (Pre) and post-training (Post). Effects of exercise and training interventions were compared using a mixed-model analysis. Paired and unpaired contrasts were used for rest vs. exercise periods (E effect: $: $p < 0.01$) and training × interventions (T × I effect: *: $p < 0.05$). Mean ± SEM for $n = 8$ participants in all groups.

As reported, at rest, lactate oxidation was increased post-training as compared to pre-training in GF, but not in C (T effect: $p = 0.55$; T × I effect: $p = 0.01$). Yet, this occurred within a stable total carbohydrate oxidation (T effect: $p = 0.75$; T × I effect: $p = 0.61$) and reflected an increasing proportion of carbohydrate oxidation coming from lactate oxidation after GF, but not after C (GF: 30 ± 6% to 38 ± 7% vs. C: 22 ± 4% to 15 ± 2%; T effect: $p = 0.89$; T × I effect: $p < 0.01$).

During exercise, there were no longer differences in lactate oxidation (T effect: $p = 0.73$; T × I effect: $p = 0.11$), total carbohydrate oxidation (T effect: $p = 0.15$; T × I effect: $p = 0.71$), or the fraction of total carbohydrate oxidation as lactate (GF: 35 ± 4% to 36 ± 4% vs. C: 26 ± 3% to 24 ± 3%; T effect: $p = 0.92$; T × I effect: $p = 0.22$) after interventions.

Finally, training with GF was hypothesized to specifically increase endurance capacity at 85% of current VO_{2max}. Yet, time-to-exhaustion was similarly improved by GF and C interventions (Table 1: T effect: $p < 0.01$; T × I effect: $p = 0.19$).

4. Discussion

4.1. Efficiency of the Training Programs

Pre-training, participants' VO_{2max} were in the middle range of normal values [26]. The exercise training programs were effective, and produced a +8% VO_{2max} increase similar to results from

previous studies using a comparable training load [19,27]. Similar to other works comparing the effects of training with or without carbohydrate ingestion [27,28], no difference in performance gain was observed between GF and C.

4.2. Lactate Appearance and Energy Metabolism before Training

The initial metabolic evaluation involved the ingestion of repeated glucose-fructose drinks by both groups of participants at rest and during exercise. Since this visit was performed prior to intervention, results were expectedly similar in GF and C. In all participants, glucose-fructose ingestion increased blood lactate concentration compared to fasting values. Plasma lactate appearance, presumably from fructose in splanchnic tissues [29], but also resulting from glucose/glycogen degradation in various tissues including skeletal muscle, amounted to 3.6 $mg \cdot kg^{-1} \cdot min^{-1}$ in both GF and C. We did not measure fasting lactate appearance, but it was previously estimated as ≈1.4 $mg \cdot kg^{-1} \cdot min^{-1}$ in resting subjects [30]. This is consistent with GF being responsible for a substantial increase in lactate production. Our tracer approach does not allow the relative contributions of glucose and fructose to total lactate appearance to be estimated. Published human reports, however, indicate that intravenous fructose essentially stimulated splanchnic lactate release at rest [31] and during exercise [15], while animal studies suggest that glucose stimulated extra-splanchnic (presumably muscle) lactate production [32].

Gluconeogenesis from lactate was also minimal, most likely because it was inhibited by hyperinsulinemia induced by glucose ingestion [6]. Interestingly, lactate oxidation represented only ≈20% of lactate disposal, and the remaining ≈80% was metabolized non-oxidatively. Since there is no substantial lactate and glucose stores in the human body, this most likely corresponded to liver and/or muscle glycogen synthesis. In support of this hypothesis, we recently reported that post-exercise muscle glycogen resynthesis was quantitatively similar when subjects were fed glucose or fructose, and that lactate concentrations elicited by fructose ingestion were positively correlated with muscle glycogen resynthesis [33].

During exercise, lactate appearance increased to ≈10 $mg \cdot kg^{-1} \cdot min^{-1}$, of which ≈30% may have derived from fructose contribution, according to a previous study using comparable glucose-fructose drinks [13]. Relative to resting conditions, plasma lactate concentrations decreased, reflecting the effect of exercise to increase muscle lactate uptake and hence lactate clearance [30]. In addition, exercise directed ≈90% of lactate appearance toward oxidation, representing a much larger fraction than at rest. This is consistent with previous investigations of lactate metabolism in unfed individuals [10,11,34], confirming that lactate disposal between oxidative and non-oxidative fates is largely dictated by metabolic rate [7], also with glucose-fructose ingestion. We postulate that the transfer of lactate from splanchnic organs to working muscle after glucose-fructose ingestion (i.e., "reverse Cori cycle") is the result of two simultaneous processes: first, an increase of splanchnic lactate production pushed by fructose lacticogenesis increasing intrasplanchnic lactate concentration, and thus lactate efflux; second, an increased muscle lactate uptake pulled by low intramuscular lactate concentration due to continuous lactate removal toward oxidation.

4.3. Evolution of Plasma Lactate Concentration during Training Sessions

There was no detailed metabolic evaluation during training sessions. However, plasma lactate concentration was measured throughout the training program at rest and during exercise. At rest, plasma lactate concentration was higher in the GF group than in the C group, reflecting the well-known increase in plasma lactate induced by fructose ingestion [29,35]. During exercise, interestingly, plasma lactate concentration was similar in both groups, suggesting that the effect of glucose-fructose drinks was minor compared to that of exercise per se [30].

4.4. Lactate Appearance and Disposal after Training

All participants returned at the end of the training program for a second evaluation with glucose-fructose ingestion at rest and during exercise. The resting period revealed differences after

GF and C interventions. Interestingly, unlike C that induced a decrease in lactate metabolism, confirming previous reports [19,34,36], GF differed by increasing lactate appearance. This may derive from an enhanced capacity to digest, absorb and metabolize fructose [29,37] or reflect the increased lactate appearance observed in fasted individuals after a few days of fructose exposure [38]. The mechanisms of such adaptations remain to be elucidated. Lactate clearance was increased in GF only, while lactate oxidative and non-oxidative disposal remained remarkably stable after training. Interestingly, resting lactate metabolism was increased by GF along with an unchanged glucose metabolism and carbohydrate oxidation, consistent with considerations that lactate may be preferred over other substrates for energy or glucose production [7].

In contrast to the resting period, lactate appearance measured during exercise was unaltered by training. Lactate clearance, however, increased by ≈20% in both GF and C, and plasma lactate concentration decreased. This can be attributed to an enhanced expression of muscle lactate transporters and lactate metabolizing enzymes [8]. However, and contrary to our hypothesis, training with glucose-fructose did not potentiate the effects of training alone. This observation is in line with the fact that lactate concentration was similar during training sessions with or without glucose-fructose drinks, and suggests that any additional effect of glucose-fructose ingestion during exercise was minimal compared to the effects of exercise training on lactate metabolism.

4.5. Limitations

First, glucose-fructose drinks were used as a tool to change lactate metabolism through effects on lactate concentration. While measuring blood lactate concentration during training sessions, our experimental protocol did not allow to assess intrasplanchnic and intramuscular lactate concentrations. Second, participants' diet was not entirely controlled during the training period and we cannot ascertain how glucose-fructose drinks modified total sugars intake in GF and C interventions. Third, our choice of tracers did not allow endogenous glucose appearance to be distinguished from exogenous glucose appearance, nor lactate appearance from fructose and from glucose-lactate shuttles. Four, the small sample size may have prevented the detection of still meaningful differences.

5. Conclusions

Ingestion of glucose-fructose drinks increase lactate appearance, metabolism and plasma lactate concentration above fasting values. This lactate is then mainly metabolized non-oxidatively at rest (presumably ending up in glycogen stores [33]) and oxidatively during exercise [13]. After having completed the 3-week training program, the ingestion at rest of glucose-fructose drinks increased lactate appearance and oxidation more in subjects who had received glucose-fructose during sessions than in those who had received water. This suggests that repeated glucose-fructose ingestion during training upregulated fructose absorption and splanchnic lacticogenesis from fructose. During exercise, however, lactate appearance and oxidation remained unchanged compared to pre-training conditions, indicating that neither training nor glucose-fructose consumption had a major impact on splanchnic lacticogenesis from fructose.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/9/4/411/s1, Figure S1: Changes over time of plasma insulin, glucagon free fatty acids concentrations during metabolic evaluations.

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Author Contributions: L.T., V.L. and L.E. designed the study; R.R., L.E., V.L. and J.C. recruited participants and performed metabolic evaluations; V.R., N.S., R.R., V.S., M.L. and P.S. performed analyzes; R.R. and L.T. analyzed data and interpreted results; R.R. and L.T. drafted the manuscript, which was revised by all authors.

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Abbreviations

C	Control intervention in which plain water was provided during training sessions
GF	Intervention in which glucose-fructose drinks were provided during training sessions
VO_{2max}	Maximal oxygen consumption during incremental tests
W_{max}	Maximal workload during incremental tests
W_{LT}	Workload eliciting lactate turnpoint during incremental tests
VO_2	Oxygen consumption
VCO_2	Carbon dioxide production
Na-$H^{13}CO_3$	Sodium bicarbonate with its carbon being a carbon-13
NOLD	Non-oxidative lactate disposal
(6,6-$^2H_2$)-D-(+)-glucose	D-(+)-glucose deuterated twice on 6th carbon
(3-$^{13}C_1$)-L-(+)-lactate	L-(+)-lactate with its 3rd carbon being a carbon-13
NaCl	Sodium chloride
EDTA	Ethylenediaminetetraacetic acid

References

1. Romijn, J.A.; Coyle, E.F.; Sidossis, L.S.; Gastaldelli, A.; Horowitz, J.F.; Endert, E.; Wolfe, R.R. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* **1993**, *265*, E380–E391.
2. Coyle, E.F.; Coggan, A.R.; Hemmert, M.K.; Ivy, J.L. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J. Appl. Physiol. (1985)* **1986**, *61*, 165–172.
3. Cermak, N.M.; van Loon, L.J. The use of carbohydrates during exercise as an ergogenic aid. *Sports Med.* **2013**, *43*, 1139–1155.
4. Jeukendrup, A.E.; Jentjens, R. Oxidation of carbohydrate feedings during prolonged exercise: Current thoughts, guidelines and directions for future research. *Sports Med.* **2000**, *29*, 407–424.
5. Ploug, T.; van Deurs, B.; Ai, H.; Cushman, S.W.; Ralston, E. Analysis of glut4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J. Cell Biol.* **1998**, *142*, 1429–1446.
6. Jeukendrup, A.E.; Raben, A.; Gijsen, A.; Stegen, J.H.; Brouns, F.; Saris, W.H.; Wagenmakers, A.J. Glucose kinetics during prolonged exercise in highly trained human subjects: Effect of glucose ingestion. *J. Physiol.* **1999**, *515 Pt 2*, 579–589.
7. Brooks, G.A. Cell-cell and intracellular lactate shuttles. *J. Physiol.* **2009**, *587*, 5591–5600.
8. Dubouchaud, H.; Butterfield, G.E.; Wolfel, E.E.; Bergman, B.C.; Brooks, G.A. Endurance training, expression, and physiology of ldh, mct1, and mct4 in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **2000**, *278*, E571–E579.
9. Thomas, C.; Bishop, D.J.; Lambert, K.; Mercier, J.; Brooks, G.A. Effects of acute and chronic exercise on sarcolemmal mct1 and mct4 contents in human skeletal muscles: Current status. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2012**, *302*, R1–14.
10. Emhoff, C.A.; Messonnier, L.A.; Horning, M.A.; Fattor, J.A.; Carlson, T.J.; Brooks, G.A. Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold. *J. Appl. Physiol. (1985)* **2013**, *114*, 297–306.
11. Emhoff, C.A.; Messonnier, L.A.; Horning, M.A.; Fattor, J.A.; Carlson, T.J.; Brooks, G.A. Direct and indirect lactate oxidation in trained and untrained men. *J. Appl. Physiol. (1985)* **2013**, *115*, 829–838.
12. Jentjens, R.L.; Jeukendrup, A.E. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *Br. J. Nutr.* **2005**, *93*, 485–492.
13. Lecoultre, V.; Benoit, R.; Carrel, G.; Schutz, Y.; Millet, G.P.; Tappy, L.; Schneiter, P. Fructose and glucose co-ingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. *Am. J. Clin. Nutr.* **2010**, *92*, 1071–1079.
14. Currell, K.; Jeukendrup, A.E. Superior endurance performance with ingestion of multiple transportable carbohydrates. *Med. Sci. Sports Exerc.* **2008**, *40*, 275–281.

15. Ahlborg, G.; Bjorkman, O. Splanchnic and muscle fructose metabolism during and after exercise. *J. Appl. Physiol. (1985)* **1990**, *69*, 1244–1251.
16. Tappy, L.; Rosset, R. Fructose metabolism from a functional perspective: Implications for athletes. *Sports Med.* **2017**, *47*, 23–32.
17. Truswell, A.S.; Seach, J.M.; Thorburn, A.W. Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. *Am. J. Clin. Nutr.* **1988**, *48*, 1424–1430.
18. Goodwin, M.L.; Harris, J.E.; Hernandez, A.; Gladden, L.B. Blood lactate measurements and analysis during exercise: A guide for clinicians. *J. Diabetes Sci. Technol.* **2007**, *1*, 558–569.
19. MacRae, H.S.; Dennis, S.C.; Bosch, A.N.; Noakes, T.D. Effects of training on lactate production and removal during progressive exercise in humans. *J. Appl. Physiol. (1985)* **1992**, *72*, 1649–1656.
20. Tounian, P.; Schneiter, P.; Henry, S.; Delarue, J.; Tappy, L. Effects of dexamethasone on hepatic glucose production and fructose metabolism in healthy humans. *Am. J. Physiol.* **1997**, *273*, E315–E320.
21. Novel-Chate, V.; Rey, V.; Chiolero, R.; Schneiter, P.; Leverve, X.; Jequier, E.; Tappy, L. Role of Na^+-K^+-Atpase in insulin-induced lactate release by skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E296–E300.
22. Sauvinet, V.; Gabert, L.; Qin, D.; Louche-Pelissier, C.; Laville, M.; Desage, M. Validation of pentaacetylaldononitrile derivative for dual ^2H gas chromatography/mass spectrometry and ^{13}C gas chromatography/combustion/isotope ratio mass spectrometry analysis of glucose. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3855–3867.
23. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1983**, *55*, 628–634.
24. Van Hall, G. Correction factors for ^{13}C-labelled substrate oxidation at whole-body and muscle level. *Proc. Nutr. Soc.* **1999**, *58*, 979–986.
25. Steele, R.; Wall, J.S.; De Bodo, R.C.; Altszuler, N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am. J. Physiol.* **1956**, *187*, 15–24.
26. Schneider, J. Age dependency of oxygen uptake and related parameters in exercise testing: An expert opinion on reference values suitable for adults. *Lung* **2013**, *191*, 449–458.
27. Cox, G.R.; Clark, S.A.; Cox, A.J.; Halson, S.L.; Hargreaves, M.; Hawley, J.A.; Jeacocke, N.; Snow, R.J.; Yeo, W.K.; Burke, L.M. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. *J. Appl. Physiol. (1985)* **2010**, *109*, 126–134.
28. De Bock, K.; Derave, W.; Eijnde, B.O.; Hesselink, M.K.; Koninckx, E.; Rose, A.J.; Schrauwen, P.; Bonen, A.; Richter, E.A.; Hespel, P. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *J. Appl. Physiol. (1985)* **2008**, *104*, 1045–1055.
29. Sun, S.Z.; Empie, M.W. Fructose metabolism in humans—What isotopic tracer studies tell us. *Nutr. Metab.* **2012**, *9*, 89.
30. Van Hall, G. Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol.* **2010**, *199*, 499–508.
31. Bjorkman, O.; Gunnarsson, R.; Hagstrom, E.; Felig, P.; Wahren, J. Splanchnic and renal exchange of infused fructose in insulin-deficient type 1 diabetic patients and healthy controls. *J. Clin. Investig.* **1989**, *83*, 52–59.
32. Youn, J.H.; Bergman, R.N. Conversion of oral glucose to lactate in dogs. Primary site and relative contribution to blood lactate. *Diabetes* **1991**, *40*, 738–747.
33. Rosset, R.; Lecoultre, V.; Egli, L.; Cros, J.; Dokumaci, A.S.; Zwygart, K.; Boesch, C.; Kreis, R.; Schneiter, P.; Tappy, L. Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals. *Am. J. Clin. Nutr.* **2017**, *105*, 609–617.
34. Bergman, B.C.; Wolfel, E.E.; Butterfield, G.E.; Lopaschuk, G.D.; Casazza, G.A.; Horning, M.A.; Brooks, G.A. Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol. (1985)* **1999**, *87*, 1684–1696.
35. Mayes, P.A. Intermediary metabolism of fructose. *Am. J. Clin. Nutr.* **1993**, *58*, 754S–765S.
36. Holloszy, J.O. Muscle metabolism during exercise. *Arch. Phys. Med. Rehabil.* **1982**, *63*, 231–234.
37. Douard, V.; Ferraris, R.P. The role of fructose transporters in diseases linked to excessive fructose intake. *J. Physiol.* **2013**, *591*, 401–414.
38. Abdel-Sayed, A.; Binnert, C.; Le, K.A.; Bortolotti, M.; Schneiter, P.; Tappy, L. A high-fructose diet impairs basal and stress-mediated lipid metabolism in healthy male subjects. *Br. J. Nutr.* **2008**, *100*, 393–399.

Manuscript 6

Glucose-Fructose Ingestion and Exercise Performance: The Gastrointestinal Tract and beyond

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ORIGINAL ARTICLE

Glucose–fructose ingestion and exercise performance: The gastrointestinal tract and beyond

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Abstract
Carbohydrate ingestion can improve endurance exercise performance. In the past two decades, research has repeatedly reported the performance benefits of formulations comprising both glucose and fructose (GLUFRU) over those based on glucose (GLU). This has been usually related to additive effects of these two monosaccharides on the gastrointestinal tract whereby intestinal carbohydrate absorption is enhanced and discomfort limited. This is only a partial explanation, since glucose and fructose are also metabolized through different pathways after being absorbed from the gut. In contrast to glucose that is readily used by every body cell type, fructose is specifically targeted to the liver where it is mainly converted into glucose and lactate. The ingestion of GLUFRU may thereby profoundly alter hepatic function ultimately raising both glucose and lactate fluxes. During exercise, this particular profile of circulating carbohydrate may induce a spectrum of effects on muscle metabolism possibly resulting in an improved performance. Compared to GLU alone, GLUFRU ingestion could also induce several non-metabolic effects which are so far largely unexplored. Through its metabolite lactate, fructose may act on central fatigue and/or alter metabolic regulation. Future research could further define the effects of GLUFRU over other exercise modalities and different athletic populations, using several of the hypotheses discussed in this review.

Keywords: *Endurance, exercise, nutrition, performance, metabolism*

Highlights
- Compared to the ingestion of glucose only, ingestion of glucose–fructose mixtures during exercise can improve exercise performance.
- The benefits of glucose–fructose mixtures are related to fructose specific metabolism.
- Originally thought to occur mainly at the level of the gastrointestinal tract, these benefits extend far beyond the intestine, and most likely involve the liver and active skeletal muscles.

Abbreviations

GLU	glucose ingestion
GLUFRU	glucose–fructose ingestion
GLUTs	glucose transporter
I:G ratio	Insulin:glucagon ratio
SGLT-1	sodium–glucose co-transporter isoform 1
VO_{2max}	maximal oxygen consumption

1. Introduction

Carbohydrate ingestion can improve prolonged exercise performance. Typical guidelines recommend ingesting 30–60 g carbohydrate per hour (American Dietetic et al., 2009). While original recommendations essentially suggested glucose-based formulations (GLU) (Coggan & Coyle, 1991), more recent guidelines propose that with increased exercise duration, the optimal intake should not only be increased (up to 90 g h^{-1} carbohydrate during sessions lasting more than 2.5 h), but also that formulations comprising both glucose and fructose (GLUFRU) may optimize performance (Cermak & Van Loon, 2013; Jeukendrup, 2014). Combinations of both monosaccharides have indeed been first shown to specifically increase performance in 2008 (Currell & Jeukendrup, 2008). In this cross-over controlled study, simulated 40 km cycling time-trial performance was measured after an initial 2 h endurance bout. Repeated glucose ingestion significantly

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enhanced performance by +8% relative to non-caloric placebo, with a further +8% improvement observed with a glucose–fructose formulation.

Comparable advantages of GLUFRU over GLU were repeated in several other works using endurance cycling or running, in laboratory and in field conditions. In these experiments, improved aerobic exercise capacity and reduced perceived exertion (Jentjens et al., 2006; Jeukendrup & Moseley, 2010) were often associated with diminished gastrointestinal complaints (Roberts, Tarpey, Kass, Tarpey, & Roberts, 2014; Wilson & Ingraham, 2015). Consequently, GLUFRU was particularly recommended in situations necessitating high carbohydrate ingestion rates ($>1.2\ g\ min^{-1}$) (Jeukendrup, 2010), leading to the promotion of superior formulations for gastrointestinal function and endurance performance.

The effects of ingested carbohydrate on exercise capacity have been traditionally attributed to several mechanisms, including prevention of hypoglycemia and direct muscle oxidation, as well as central stimulatory effects (Coggan & Coyle, 1991). Yet, contrasting with these general effects of carbohydrate ingestion during exercise, how GLUFRU further improve performance remains largely hypothetical. On their way to provide energy during exercise, exogenous carbohydrates pass through the gastrointestinal tract up to intestinal absorption, then are directed to the liver by the portal circulation before being ultimately transferred to working muscles for oxidation. To complement recent literature reviews (Cermak & Van Loon, 2013; Jeukendrup, 2014; Rowlands et al., 2015; Wilson & Ingraham, 2015), the purpose of the present work is to consider mechanisms by which GLUFRU could improve performance as compared to GLU, with a specific focus on processes taking part beyond the gastrointestinal tract.

2. General background

For decades, there has been a wide recognition that low muscle glycogen can impair endurance performance and that endogenous stores can be spared by carbohydrate ingestion (Coyle, Coggan, Hemmert, & Ivy, 1986). Either in solid or liquid forms, carbohydrate intake was mainly considered to enhance performance by providing muscle alternative fuels while diminishing liver and muscle glycogen use (Cermak & Van Loon, 2013), although this may depend on the mode or duration of exercise (Stellingwerff et al., 2007; Tsintzas, Williams, Boobis, & Greenhaff, 1995; Wallis, Dawson, Achten, Webber, & Jeukendrup, 2006). In turn, these metabolic effects led to a wide interest in determining the conditions influencing exogenous carbohydrate oxidization, typically studied by isotopic techniques. Often combined with indirect calorimetry, this method allows a distinction between endogenous and exogenous carbohydrate by monitoring label appearance in circulating metabolites and in expired CO_2 (Tappy, Paquot, Tounian, Schneiter, & Jequier, 1995). Accordingly, carbohydrate ingested at increasing rates was shown to appear in a dose-dependent manner in the systemic circulation and to be accordingly oxidized. However, this effect was also found to plateau at high ingestion rates that can vary with different saccharides (Jeukendrup & Jentjens, 2000). Since the plateaus of glucose or glucose polymers, oxidation (up to $\approx 1\ g\ min^{-1}$) are higher than those of other monosaccharides (fructose and galactose: $\approx 0.4\ g\ min^{-1}$) (Jeukendrup & Jentjens, 2000), mixtures of glucose or glucose-based polymers appeared as the most susceptible carbohydrate to enhance performance.

The factors dictating the plateau in exogenous carbohydrate oxidation during exercise remain unclear. Indirect calorimetry, isotopic labelling of ingested carbohydrates and monitoring of the appearance of the label in expired CO_2 are usually used in conjunction to determine total as well as exogenous carbohydrate oxidation rates. However, even combined, these techniques fall short in identifying oxidation site and which metabolite derived from labelled carbohydrates are ultimately oxidized (Tappy et al., 1995). Hence, they provide little information on factors limiting exogenous carbohydrate oxidation during exercise. Theoretically, these limitations could reside anywhere in the gastrointestinal tract (gastric emptying, digestion and intestinal absorption) or in metabolic processes (intestinal metabolism, hepatic first-pass and release into systemic circulation, muscle uptake and oxidation). One remarkable finding was that exogenous glucose oxidation is not limited by cardiac output, peripheral blood flow and muscle metabolism. Indeed, a peripheral glucose infusion was found to be oxidized up to rates ($\approx 2.5\ g\ min^{-1}$) much higher than the plateau observed with oral glucose (Hawley, Bosch, Weltan, Dennis, & Noakes, 1994). Maximal exogenous glucose oxidation was also considered independent from gastric emptying (Rehrer et al., 1992). Consequently, the limitation was concluded to be located either in the gut or in the liver (Jeukendrup & Jentjens, 2000).

Digestion of glucose polysaccharides into glucose could be argued to limit fuel provision during exercise. However, the fact that short-chain glucose polysaccharides such as maltose and short-chain maltodextrins reached similar maximal oxidation rates as glucose indicated that the factor limiting

maximal exogenous glucose oxidation may not be found within pre-absorptive mechanisms (Jeukendrup & Jentjens, 2000). Consequently, the limitation of carbohydrate oxidation was supposed to stand at the level of intestinal absorption, with the $\approx$1 g min^{-1} plateau being consistent with jejunum glucose absorption kinetics. This hypothesis was primarily based on multiple intestinal segmentations experiments showing limited absorption of concentrated glucose solutions (Shi et al., 1995). Another physiological effect of exercise, decreased splanchnic blood flow, may also limit intestinal absorption capacity. Yet, in absence of invasive direct assessments of glucose flows across the intestinal barrier, the notion that intestinal absorption limits exogenous glucose oxidation during exercise remains a hypothesis.

The plateau in exogenous glucose oxidation may also result from hepatic limitations. Indeed, the necessary route for ingested carbohydrate is to follow portal circulation to the liver, where they can either be stored, metabolized or can pass to the systemic circulation. The liver is also known to play a pivotal role in euglycaemia maintenance through releasing the precise amount of glucose required to match extrahepatic utilization (Moore, Coate, Winnick, An, & Cherrington, 2012). After meals, this results in part of carbohydrate intake being extracted at first-pass while diminishing the amount appearing in the systemic circulation as well as hepatic glucose production. Interestingly, the total systemic glucose appearance allowed by this mechanism (the sum of hepatic glucose production and carbohydrate intake not extracted at first-pass) is thought to be autoregulated at a maximal value close to 1 g min^{-1}, with higher gut absorption fluxes believed to be directed into hepatic glycogen (Moore et al., 2012). How this contributes to the observed plateau in exogenous glucose oxidation remains unknown as of yet. The understanding of the responsible sites may also be complicated by entero-hepatic crosstalk via portal signals (Moore et al., 2012). Hence, the factors responsible for the limitation in exogenous glucose oxidation during exercise remain unclear, but probably not restricted to intestinal glucose absorption.

3. Glucose–fructose formulations

Fructose, of similar chemical formula than glucose, has always been part of the human diet since our hunter-gatherer ancestors who consumed fruits, vegetables and root crops (Tappy & Le, 2010). Fructose oxidation during exercise is lower than that of glucose (Massicotte, Peronnet, Adopo, Brisson, & Hillaire-Marcel, 1994), and pure fructose has been reported to cause gastrointestinal symptoms together with limited intestinal absorption when provided at high rates during exercise (Fujisawa et al., 1993). Consequently, and despite few results showing equivalent oxidation to glucose when ingested before exercise (Decombaz et al., 1985), pure fructose was generally considered of poor interest for exercise performance (Jeukendrup & Jentjens, 2000).

In contrast to pure fructose, formulations comprising both monosaccharides (GLUFRU) can induce interesting effects for endurance performance. This was first reported in a study showing that a mixture of 50 g glucose and 50 g fructose was more efficiently oxidized during exercise than 100 g of pure glucose or pure fructose (Adopo, Peronnet, Massicotte, Brisson, & Hillaire-Marcel, 1994). In following works, very high oxidation of GLUFRU were reported at high carbohydrate ingestion rates (Jeukendrup, 2014), enabling to exceed the GLU plateau of 1 g min^{-1} to reach a maximal 1.7 g min^{-1} (Jentjens, Moseley, Waring, Harding, & Jeukendrup, 2004). The higher oxidation of GLUFRU as compared to GLU alone was no more observed when carbohydrate ingestion rates were lower (Hulston, Wallis, & Jeukendrup, 2009), further suggesting that GLUFRU may overcome the limitation found with single monosaccharides. Several studies also suggested that combining roughly twice as much glucose as fructose led to the highest exogenous carbohydrate oxidation (Jeukendrup & Moseley, 2010) and this ratio was deemed optimal.

Others tried to further define conditions leading to superior GLUFRU oxidation by varying saccharides amounts, and noticed that oxidation was maximal with glucose:fructose ratios close to 1.0 (providing as much glucose as fructose) (O'Brien, Stannard, Clarke, & Rowlands, 2013; Rowlands et al., 2015). This may not come as a surprise considering glucose and fructose abundances in natural products. Indeed, an analysis of saccharides composition, reporting starch content as glucose, from 30 common edible fruits, vegetables and cereals (Figure 1) indicates that the median glucose:fructose ratio is approximatively 1.3 but also that the distribution may be skewed towards higher ratios due to glucose-abundant starchy food items. Excluding starches, saccharides present in sugars can be estimated using free glucose, free fructose and sucrose, considered to act similarly as 1 glucose and 1 fructose monomers (Wallis & Wittekind, 2013). Interestingly, this leads to a median glucose:fructose of 1.0, suggesting that the optimal effects of GLUFRU during exercise may actually mirror the ratios between both monosaccharides in natural foods that were frequently consumed by our hunter-gatherers' ancestors.

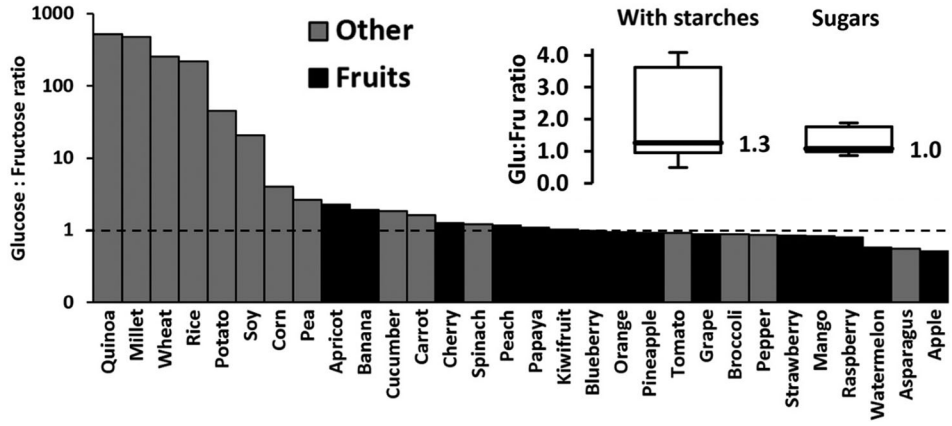

Figure 1. Glucose:fructose ratio in 30 selected fruits, vegetables and cereals. Data originate from United States Department of Agriculture national nutrient database (Retrieved 21 September, 2016, from https://ndb.nal.usda.gov). Only raw products and single varieties per species are included. The glucose:fructose ratio is typically very high in foods such as cereals or tubercles. The predominant energy storage form for vegetal organisms, starches, can indeed contain hundreds to thousands of glucose molecules. In contrast, when the edible part corresponds to leafs, stems or fruits (highlighted in black), the absorbable glucose:fructose ratio is much closer to identity.

4. Effects of GLUFRU in the gastrointestinal tract

GLUFRU ingestion has been classically associated with gastrointestinal benefits compared to pure glucose or fructose (Rowlands et al., 2015). Experiments using hydrogen breath testing indeed showed that fructose malabsorption can be prevented when co-ingested with similar amounts of glucose (Latulippe & Skoog, 2011). Others indicated symptoms of gastrointestinal discomfort to be lower with GLUFRU feedings during exercise as compared to GLU (Jentjens et al., 2006). This can indeed limit exercise performance, and require exploring why the co-ingestion of glucose and fructose may limit these detrimental symptoms.

Fructose may accelerate pre-absorptive carbohydrate delivery. This is illustrated by a faster gastric emptying with fructose and sucrose than glucose, both at rest (Sole & Noakes, 1989) and during exercise (Jeukendrup & Moseley, 2010). This is likely secondary to a more rapid intestinal absorption and thus to a decreased feedback inhibition to the stomach (Weber & Ehrlein, 1998). A few days of fructose exposure were also shown to increase subsequent gastric emptying of fructose-containing solutions (Yau, McLaughlin, Maughan, Gilmore, & Evans, 2014), while dietary fructose is also considered to mediate sucrase-isomaltase expression (Rosensweig & Herman, 1968). Thus, fructose consumption seems to favour carbohydrate delivery to the intestinal mucosa both acutely and chronically. To what extent these mechanisms explain the superior uptake and oxidation of GLUFRU over that of GLU remains unclear, similarly as if this could be used to train the gastrointestinal tract to improve carbohydrate absorption during exercise.

Glucose and fructose co-ingestion may increase total carbohydrate intestinal absorption. This hypothesis (Shi et al., 1995) was based on luminal fructose and glucose absorption occurring through different main transporters (GLUT5 vs. SGLT1). Combining glucose and fructose was then proposed to increase carbohydrate absorption, provided SGLT1 becomes saturated at a given glucose ingestion rate. Accordingly, total carbohydrate absorption was found to be higher with GLUFRU than GLU in proximal sections of the jejunum (Shi et al., 1995), leading to a 'multiple transportable carbohydrate' model whereby improved gut carbohydrate absorption leads to overall increased flux to working muscle (Jeukendrup, 2010).

Despite of the preceding, the improved intestinal function with GLUFRU may also result from other mechanisms. Regulation of glucose and fructose absorptions actually occurs through additional transport proteins (GLUT2, GLUT8-12) in close interaction with GLUT5, SGLT1 and ion channels (Chen, Tuo, & Dong, 2016). GLUT2 was proposed to be particularly key through its translocations both at the apical and basolateral sides of enterocytes (Rowlands et al., 2015) but exact fluxes through each of these transport systems remain unclear. Fructose is also well documented to be partly metabolized in enterocytes (Tappy & Le, 2010), possibly affecting local homeostasis. Consequently, how carbohydrate absorption fluxes are dictated by apical transport, intraenterocellular metabolism and basolateral transport is so far unknown. The difference between

GLUFRU and GLU is thus most likely partly explained by additive effects of glucose and fructose in the gastrointestinal tract, effects that remain to be outlined. Interestingly, there is also evidence of important roles played by the combination of both monosaccharides in organs such as the liver.

5. Effects of fructose and GLUFRU on hepatic metabolism

Distinct effects of exogenous carbohydrate are also to be found in post-absorptive, metabolic processes. In a 1994 study, constant amounts of glucose or fructose (0.8 g min^{-1}) were provided to subjects displaying a wide range of aerobic capacity (Massicotte et al., 1994). Both exogenous glucose and fructose oxidation were directly related to each subject metabolic rate, respectively contributing to 14% and 9% of energy expenditure, as would not be predicted solely by intestinal absorption saturation. Rather, this suggests that glucose and fructose oxidation, and thus their respective delivery into the systemic circulation, are also regulated by post-absorptive metabolism.

Unlike glucose, which is directly used by all body cells, fructose is characterized by a specific, two-step metabolism (Mayes, 1993; Tappy & Le, 2010) in which it is first metabolized in splanchnic organs (particularly the liver), then released as secondary substrates for other organs (Mayes, 1993; Tappy & Le, 2010). First-pass splanchnic fructose extraction is almost complete (>90%, much greater than the ≈33% glucose extraction) (Tappy & Le, 2010), resulting in oral loads eliciting only small (0.3–0.4 mmol L^{-1}), transient increases in systemic fructose concentrations (Rosset et al., 2017). This is also unlikely to be markedly altered during exercise with fructose following the oral route. In one very specific experiment in which splanchnic first-pass was bypassed by using a systemic fructose infusion, 45% fructose was still found to be extracted by splanchnic tissues as measured by arteriovenous difference, with exercising and resting muscles being both responsible for 28% fructose extraction (Ahlborg & Bjorkman, 1990). However, these were supraphysiological conditions in which systemic fructose concentrations (4.8 mmol L^{-1}) could compete for the access to muscle hexokinases, which normally privilege glucose substrate because of a much higher affinity than for fructose, K_m: glucose $\approx 4 \times 10^{-5}$ vs. fructose: $\approx 3 \times 10^{-3}$ mmol L^{-1} (Tappy & Le, 2010). Accordingly, the authors considered that the amount of oral fructose directly metabolized in muscle is minimal during exercise, but that fructose is rather first metabolized into second-hand metabolites later used as energy substrates by working muscles (Ahlborg & Bjorkman, 1990).

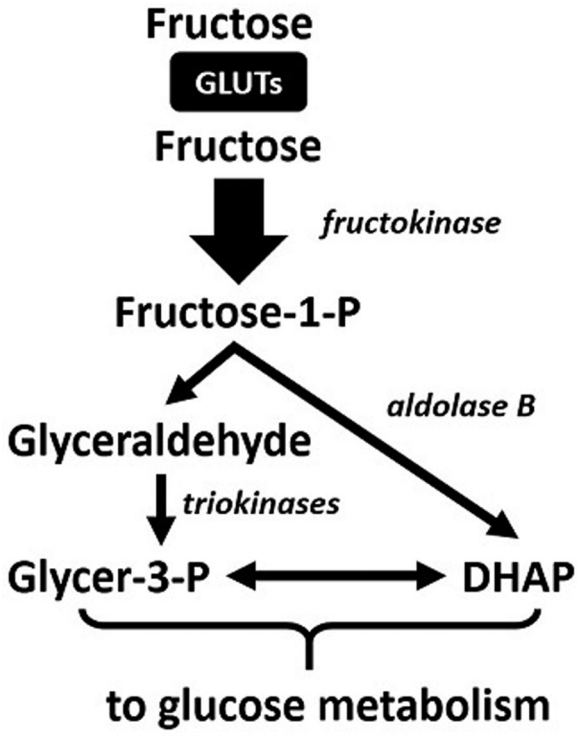

Figure 2. In splanchnic organs, fructolysis generates trioses-P with a build-up of fructose-1-P. Fructose entry occurs through several transporters of the GLUT family (mainly GLUT2, GLUT5). Thereafter, fructose is metabolized through a set of three reactions called fructolysis. Fructolysis begins with the initial phosphorylation of fructose into fructose-1-P by fructokinase. The enzyme responsible for the subsequent step, aldolase B, cleaves fructose-1-P into two three-carbon molecules, dihydroxyacetone-phosphate (DHAP) and glyceraldehyde, later phosphorylated by triokinases to glyceraldehyde-3-P. The two trioses-phosphate Glyceraldehyde-3-P and DHAP are also intermediates of glucose metabolism, and can therefore be directed to several fates. Of note, since the activity of fructokinase is higher than that of aldolase B, active fructolysis results in a rapid build-up of fructose-1-P in fructose-metabolizing splanchnic cells. Symbols: GLUTs: glucose transporters; P: phosphate; Glycer: glyceraldehyde; DHAP: dihydroxyacetone-phosphate.

Fructose splanchnic metabolism can be explained by the expression of a unique set of enzymes directing fructose carbons into a specific, efficient pathway called fructolysis (Figure 2) (Tappy & Le, 2010). After intracellular entry, fructose is rapidly phosphorylated by fructokinase ($K_m \approx 0.1$ mmol L^{-1}) (Tappy & Le, 2010) into fructose-1-phosphate, then cleaved by aldolase B and phosphorylated by triokinases into trioses-phosphate. Importantly, fructokinase displays a higher activity than aldolase B, leading to transiently increased fructose-1-phosphate concentrations during active fructolysis. The subsequent disposal of trioses-phosphate can be variable, and isotopic studies (Sun & Empie, 2012) showed a

large part of a pure fructose load to be converted into glucose (29–54%) and lactate (25–30%) then released into the circulation. Compared to dietary glucose, fructose is known to increase plasma glucose and insulin concentrations to a lower extent, but to induce a sustained increase in plasma lactate concentrations (Decombaz et al., 1985; Rosset et al., 2017). Fructose-containing carbohydrate formulations are also generally reported for their ability to replenish liver glycogen during post-exercise recovery (Décombaz et al., 2011; Gonzalez, Fuchs, Betts, & van Loon, 2016), with a very small proportion of fructose carbons converted into lipids (Sun & Empie, 2012). During exercise, net fructose storage is decreased while fructose oxidation is increased (Egli et al., 2016), however. An understanding of the benefits of GLUFRU over GLU may then require a comparative description of hepatic function during exercise in several nutritional conditions.

The normal action of the liver is to simultaneously consume and produce glucose, with production pathways being quantitatively dominant under fasting conditions (net glucose production). Hepatic glucose production is a complex process deriving from the activation of glycogenolysis and gluconeogenesis (Moore et al., 2012). Both insulin and glucagon (and thus the insulin:glucagon ratio, I:G) control the main regulatory steps of glucose metabolism, so that both glycogenolysis and gluconeogenesis are responsive to glucose concentrations and active in the post-absorptive state. During exercise, net hepatic glucose production is typically increased by changes in the hormonal milieu (rise in catecholamines and drop in the I:G ratio) and in nervous system activity (Moore et al., 2012). Concentrations of metabolic precursors also play a role, with glycogenolysis and gluconeogenesis being respectively enhanced by high glycogen and high availability of substrates such as lactate, glycerol or gluconeogenic amino acids. Lactate is then also consumed and produced, with a net consumption during prolonged exercise (Wasserman, Connolly, & Pagliassotti, 1991). Hence, when exercising in unfed conditions, the liver sustains glucose production partly via lactate consumption (net hepatic lactate production <0) (Figure 3(A)).

Glucose ingestion decreases hepatic glucose production. Indeed, the main consequence of glucose ingestion is to increase plasma insulin which, through elevating I:G ratio, promotes hepatic glucose disposal. By stimulating glycolysis, this also causes an accumulation of fructose-1,6-biphosphate, which represents an important co-activator

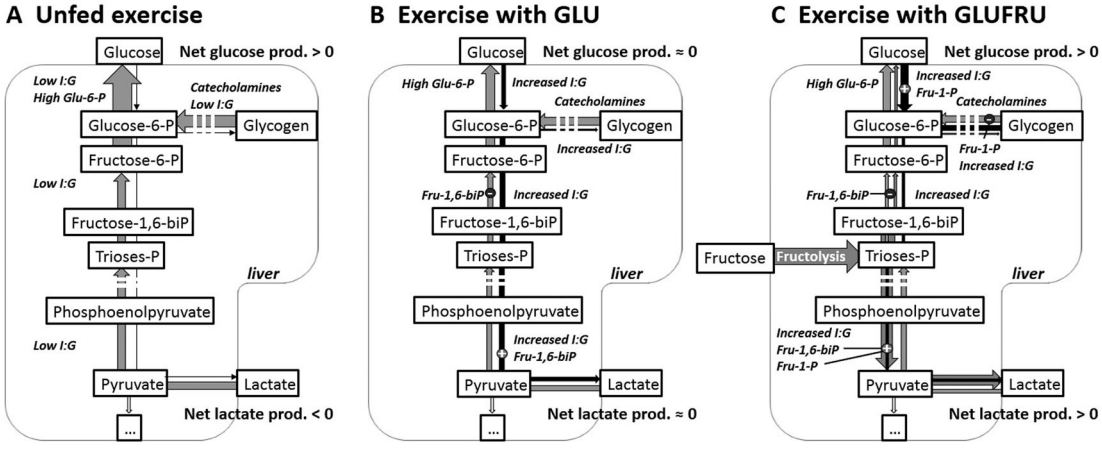

Figure 3. Models of hepatic carbohydrate metabolism during exercise. Exercise typically increases circulating catecholamines and glucagon and decreases insulin concentrations. When unfed (A), this increases Glucose-6-P concentrations through driving glycogen breakdown and gluconeogenesis from three-carbon precursors such as lactate. High Glucose-6-P and low insulin:glucagon ratio then favour glucose production and systemic release. Hence, the liver sustains plasma glucose concentrations and acts as a net glucose producer partly through net lactate uptake (i.e. net lactate production <0) as part of the Cori Cycle. Through increasing insulin and I:G ratio, glucose ingestion during exercise (B) slows glycogenolysis and gluconeogenesis and thus limits Glucose-6-P concentrations. Part of Glucose-6-P can then be directed into lower glycolysis to pyruvate and lactate, with Fru-1,6-biP acting as a co-activator via feed-forward mechanisms. Therefore, glucose ingestion during exercise can partially or totally preserve hepatic carbohydrate metabolism (i.e. both net fluxes ≈ 0). Co-ingested with glucose (C), fructose affects hepatic metabolism by mass effects of its carbons and via allosteric mechanisms. Active fructolysis leads to build-up of Fru-1-P, Trioses-P and Fructose-1,6-biP. Fru-1- P then activates lower glycolysis to pyruvate which, together with Fru-1,6-biP, drives part of fructose carbons into lactate. Fructose carbons can also undergo gluconeogenesis and replenish Glucose-6-P concentrations. Fru-1-P actions to favour hepatic glucose entry and glycogen storage are in balance with the hormonal milieu during exercise. Ingesting glucose and fructose can thus result in both net glucose and lactate productions (i.e. 'reverse Cori cycle') to alter hepatic function as a carbohydrate buffer during exercise. Symbols: Fru: fructose; P: phosphate; I:G ratio: insulin:glucagon ratio.

channelling trioses-phosphate into lactate (Mayes, 1993). Compared to unfed exercise, the overall effect of glucose ingestion during exercise thus favours opposite pathways, glucose consumption and lactate production, and the liver can be viewed as a buffering organ for carbohydrate metabolism (net glucose and lactate productions ≈ 0) (Figure 3(B)).

Compared to fasted and glucose-fed exercise, fructose markedly and complexly affects hepatic metabolism. The mass effects of fructose carbons were specifically visible in 1996 (Paquot et al., 1996). In this work, fructose increased hepatic glucose production by 20% only when provided with simultaneous physiologic hyperglucagonemia (low I:G ratio), indicating that fructose carbons can be diverted to various fates depending on the hormonal milieu. In addition to these mass effects, fructose was also shown, through its intermediate fructose-1-phosphate, to allosterically favour hepatic glucose uptake and storage into glycogen and pyruvate production (Mayes, 1993; Tappy & Le, 2010). Interestingly, glycogen metabolism is also influenced by other actions of fructose and by actual glycogen content (Mayes, 1993; Tappy & Le, 2010), resulting in a complex interplay of opposed effects. This may explain why fructose stimulates both glucose and glycogen (assimilated to diphosphoglucose kinetics) turnovers (Dirlewanger, Schneiter, Jequier, & Tappy, 2000; Tounian, Schneiter, Henry, Jequier, & Tappy, 1994). Whether rapid glucose and/or glycogen turnover could affect substrate availability when metabolic rate is increased during exercise is unknown (Gonzalez et al., 2016). Contrasting with glucose ingestion alone, glucose–fructose ingestion during exercise therefore results in a simultaneous stimulation of hepatic glucose and lactate productions (Figure 3(C)).

The provision of GLUFRU may induce a unique hepatic physiological response. Indeed, a study under highly controlled conditions found that the effect of hyperglycemia/hyperinsulinemia to suppress hepatic glucose production was partly blunted by fructose (Dirlewanger et al., 2000), implying that fructose elevates the point at which the liver switches from net glucose production to net glucose consumption. Interestingly, a study in rats found also that glucose co-ingestion exacerbated the effects of fructose to increase net hepatic lactate production (Underwood & Newsholme, 1965). Whether these specific effects also occur during exercise remain unsettled. However, it may be proposed that glucose and fructose co-ingestion induce a specific form of synergy in which fructose decreases the role of the liver to act as a carbohydrate buffer and in which glucose increases fructose metabolism into lactate, respectively. Similar effects were reported in a 2010 study, in which a glucose:fructose ratio of 1.5 was ingested at a rate of 2.0 g min^{-1} during prolonged exercise at ≈60% VO_{2max}. Compared to an equimolar GLU condition, GLUFRU resulted in systemic glucose and lactate fluxes being increased by respectively ≈10% and ≈30% (Lecoultre et al., 2010), demonstrating that GLUFRU increase circulating carbohydrate availability compared to GLU.

The relative importance of intestinal and hepatic processes contributing to the effects of GLUFRU on exercise performance remains largely unknown. As with GLU, the site limiting GLUFRU oxidation cannot be elucidated without invasive portal assessments. Yet, if the difference in maximal oxidation between GLU and GLUFRU (≈1.2 vs. ≈1.7 g min^{-1}) (Jentjens et al., 2004) was entirely explained for by an improved intestinal absorption, GLU would rapidly yield to carbohydrate accumulation within the gastrointestinal tract. Considering that severe gastrointestinal complaints were observed with as little as 10 g carbohydrate malabsorption (Rumessen, Hamberg, & Gudmand-Hoyer, 1990), a similar difference between GLU and GLUFRU would have been inevitably observed within a period of 20 min. The fact that GLU indeed frequently induced more gastrointestinal distress than GLUFRU, but during typically longer exercise bouts, suggests that intestinal malabsorption was low and likely not solely responsible for the oxidation difference between formulations. Instead, this may further point the importance of the effects of fructose on hepatic metabolism. Interestingly, a study having distinctly labelled both glucose and fructose (Rowlands, Thorburn, Thorp, Broadbent, & Shi, 2008) revealed that total exogenous carbohydrate oxidation was maximal along with an optimal oxidation of fructose, but not of glucose (Rowlands et al., 2015). This may advocate for a specific role of the intermediary metabolite lactate.

6. Effects of GLUFRU through lactate metabolism

Lactate is no longer considered a deleterious waste product, but rather viewed as a carbohydrate substrate shuttled between and possibly within cells or organs (Brooks, 2009). These exchanges are influenced by multiple factors, with a critical role of lactate gradients between plasma/interstitium and cellular compartments (Van Hall, 2010). Depending on conditions, skeletal muscle can revert from net lactate production into net consumption. Interestingly, this can arise from liver damage (Record, Chase, Williams, & Appleton, 1981), indicating a critical role of hepatic lactate exchanges. Accordingly, lactate fluxes measured during exercise by

arteriovenous difference (Ahlborg & Bjorkman, 1990) indicated that fructose infusion caused a net splanchnic lactate production together with net muscle consumption. This interorgan lactate shuttle may be either pushed by fructose metabolism increasing splanchnic blood lactate gradient, or pulled by muscle work increasing blood-muscle lactate gradient.

How lactate provision may affect muscle fuel selection remains largely hypothetical. Lactate is known to be extensively oxidized during exercise and, as compared to glucose, it offers a more immediate conversion into pyruvate while being transported through separate transport systems. A few experiments having infused lactate during exercise noticed that it largely increased lactate oxidation, but however caused a ≈40% decrease in glucose oxidation and did not affect muscle glycogen use (Miller, Fattor, Jacobs, Horning, Navazio, et al., 2002; Miller, Fattor, Jacobs, Horning, Suh, et al., 2002). Interestingly, similar studies at rest indicated that lactate infusion also diminished glucose oxidation in hyperinsulinemia, but only by ≈20% (Paquot et al., 1995) and that lactate infusion was associated with a strong thermogenic effect (Ferrannini et al., 1993). Consequently, if GLUFRU ingestion during exercise also leads to an increased lactate oxidation while incompletely suppressing glucose oxidation, the simultaneous uptake of two different circulating carbohydrate could allow for an increased maximal carbohydrate oxidation.

A recent work having measured glycogen depletion during exercise indicated that both the ingestion of GLUFRU (as sucrose) or GLU during exercise preserved liver glycogen without affecting muscle glycogen use, but that net carbohydrate oxidation was higher with GLUFRU than GLU (Gonzalez et al., 2015). This confirmed other observations of GLUFRU leading to higher carbohydrate oxidation (Roberts et al., 2014) together with increased lactate fluxes (Lecoultre et al., 2010) as compared to GLU. Interestingly, all these works included well-trained endurance athletes. Recent findings show a higher direct lactate oxidation in trained than untrained individuals, the latter relying more on gluconeogenesis to oxidize lactate (Emhoff et al., 2013). This, in turn, raises the hypothesis that GLUFRU may differently affect athletes depending upon their oxidative potential, with athletes exhibiting a high aerobic capacity benefiting more from GLUFRU mixtures than untrained individuals. Interindividual variability in oxidative capacity can be however high, and how this may translate into individualized nutritional recommendations is left to be established.

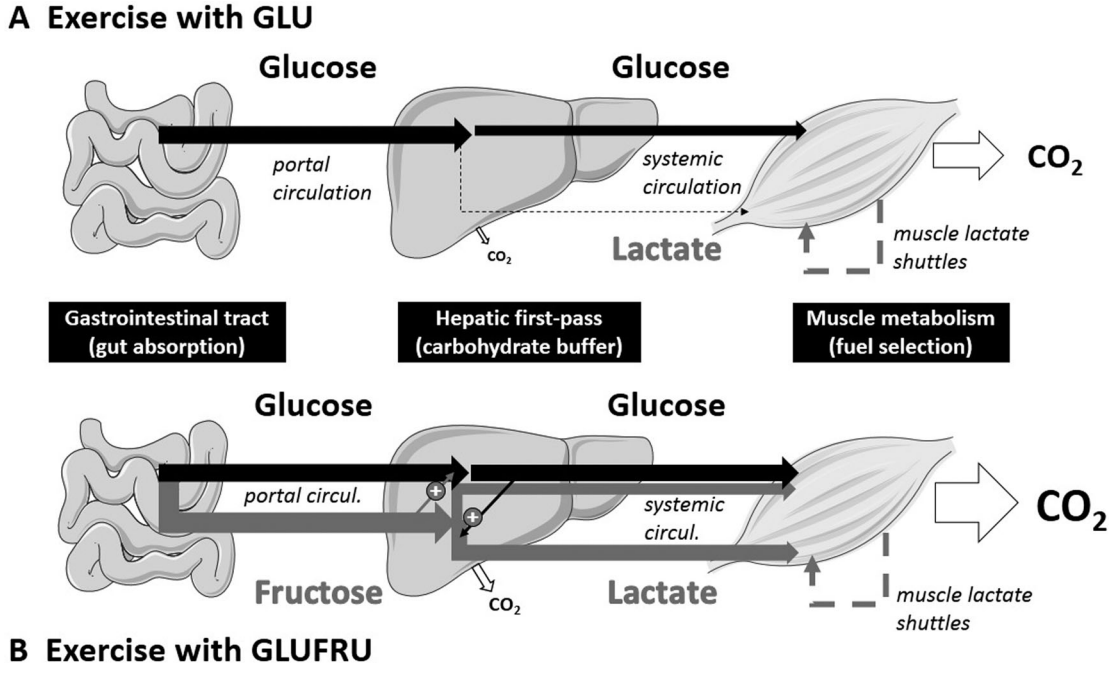

Figure 4. Compared to glucose-based formulations, the simultaneous ingestion of glucose–fructose results in a higher exogenous carbohydrate oxidation. This could be caused by (1) additive effects in the gastrointestinal tract (gut absorption), leading to higher substrate fluxes in the portal circulation, (2) a specific hepatic synergy, in which fructose may limit first-pass glucose extraction, while glucose would increase fructose metabolism into lactate to increase carbohydrate fluxes reaching the systemic circulation (i.e. affecting liver function as a carbohydrate buffer) and (3) the circulation of dietary carbohydrate as both glucose and lactate may affect muscle fuel selection. Symbols: CO_2: carbon dioxide.

The effect of GLUFRU may also vary with exercise modalities. Indeed, most reports of improved exercise performance with GLUFRU over GLU were obtained during exercise at constant or near-constant load, with few experiments involving bursts of high-intensity exercise susceptible to alter lactate metabolism (Triplett, Doyle, Rupp, & Benardot, 2010). Consequently, it remains unclear if the benefits from elevated lactate oxidation are also to be found when lactate fluxes are less stable.

Fructose conversion into glucose and lactate will also likely raise splanchnic metabolic rate, and how this can contribute to the higher oxidation of GLUFRU than GLU is unknown (Tappy, Egli, Lecoultre, & Schneider, 2013). During endurance-type exercise, we thus postulate the observed oxidation profiles to be a complex consequence of processes taking part in the gastrointestinal tract, in the liver and also in muscle (Figure 4). How all processes coordinate to explain the higher exogenous carbohydrate oxidation observed with GLUFRU than GLU remains largely unknown, giving room for further research.

7. Other effects of GLUFRU

Partly replacing glucose by fructose may also have important non-metabolic consequences. Fructose generates a different gluco-incretins profile (Tappy & Le, 2010) and induces different patterns of brain activation (Page et al., 2013) than glucose. Lactate itself may be viewed as a 'lacthormone' acting on metabolic regulation through binding to a specific receptor (Philp, Macdonald, & Watt, 2005). In the brain, this was associated to the neuroprotective and neurostimulatory effects of exercise (Proia, Di Liegro, Schiera, Fricano, & Di Liegro, 2016). Whether glucose combined to fructose and its metabolite lactate may modulate the central component of fatigue was little studied, however. Similarly, lactate was recently considered responsible for some of the adaptations to exercise-training (Proia et al., 2016). How GLUFRU ingestion may contribute to this process remains to be determined, yet.

8. Conclusion

There is accumulating evidence that GLUFRU can increase exercise performance more than GLU ingestion during prolonged exercise. The proposed mechanisms have been largely based on the knowledge of the effects of GLU-based formulations in the gastrointestinal tract. However, glucose and fructose are not only differently sensed, emptied from the stomach and absorbed, but are also differently metabolized. GLUFRU bear the interesting property to distribute carbohydrate energy as both circulating glucose and lactate, providing working muscles with a dual source of carbohydrate. How this modulates muscle fuel selection and interacts with other, non-metabolic effects of fructose and lactate is largely unknown. The similarity between optimal ratios and natural sugars suggest that ingesting glucose with fructose during exercise may mirror human evolutionary adaptation.

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References

Adopo, E., Peronnet, F., Massicotte, D., Brisson, G. R., & Hillaire-Marcel, C. (1994). Respective oxidation of exogenous glucose and fructose given in the same drink during exercise. *The Journal of Applied Physiology*, *76*(3), 1014–1019.

Ahlborg, G., & Bjorkman, O. (1990). Splanchnic and muscle fructose metabolism during and after exercise. *The Journal of Applied Physiology*, *69*(4), 1244–1251.

American Dietetic, A., Dietitians of, C., American College of Sports, M., Rodriguez, N. R., Di Marco, N. M., & Langley, S. (2009). American college of sports medicine position stand: Nutrition and athletic performance. *Medicine & Science in Sports & Exercise*, *41*(3), 709–731. doi:10.1249/MSS.0b013e31890eb86

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *The Journal of Physiology* , *587*(Pt. 23), 5591–5600. doi:10.1113/jphysiol.2009.178350

Cermak, N. M., & Van Loon, L. J. (2013). The use of carbohydrates during exercise as an ergogenic aid. *Sports Medicine*, *43*(11), 1139–1155. doi:10.1007/s40279-013-0079-0

Chen, L., Tuo, B., & Dong, H. (2016). Regulation of intestinal glucose absorption by ion channels and transporters. *Nutrients*, *8*(1), 43. doi:10.3390/nu8010043

Coggan, A. R., & Coyle, E. F. (1991). Carbohydrate ingestion during prolonged exercise: Effects on metabolism and performance. *Exercise and Sport Sciences Reviews*, *19*, 1–40.

Coyle, E. F., Coggan, A. R., Hemmert, M. K., & Ivy, J. L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *The Journal of Applied Physiology*, *61*(1), 165–172.

Currell, K., & Jeukendrup, A. E. (2008). Superior endurance performance with ingestion of multiple transportable carbohydrates. *Medicine & Science in Sports & Exercise*, *40*(2), 275–281. doi:10.1249/mss.0b013e31815adf19

Decombaz, J., Sartori, D., Arnaud, M. J., Thelin, A. L., Schurch, P., & Howald, H. (1985). Oxidation and metabolic effects of fructose or glucose ingested before exercise. *International Journal of Sports Medicine*, *6*(5), 282–286.

Décombaz, J., Jentjens, R. L., Ith, M., Scheurer, E., Buehler, T., Jeukendrup, A. E., & Boesch, C. (2011). Fructose and galactose enhance post-exercise human liver glycogen synthesis. *Medicine & Science in Sports & Exercise*, *43*(10), 1964–1971. doi:10.1249/MSS.0b013e318218ca5a

Dirlewanger, M., Schneiter, P., Jequier, E., & Tappy, L. (2000). Effects of fructose on hepatic glucose metabolism in humans. *American Journal of Physiology – Endocrinology And Metabolism*, *279*(4), E907–E911.

Egli, L., Lecoultre, V., Cros, J., Rosset, R., Marques, A. S., Schneiter, P., … Tappy, L. (2016). Exercise performed immediately after fructose ingestion enhances fructose oxidation and suppresses fructose storage. *American Journal of Clinical Nutrition*, *103*(2), 348–355. doi:10.3945/ajcn.115.116988

Emhoff, C. A., Messonnier, L. A., Horning, M. A., Fattor, J. A., Carlson, T. J., & Brooks, G. A. (2013). Direct and indirect lactate oxidation in trained and untrained men. *Journal of Applied Physiology*, *115*(6), 829–838. doi:10.1152/japplphysiol.00538.2013

Ferrannini, E., Natali, A., Brandi, L. S., Bonadonna, R., De Kreutzemberg, S. V., DelPrato, S., & Santoro, D. (1993). Metabolic and thermogenic effects of lactate infusion in humans. *Journal of Applied Physiology*, *265*(3 Pt. 1), E504–E512.

Fujisawa, T., Mulligan, K., Wada, L., Schumacher, L., Riby, J., & Kretchmer, N. (1993). The effect of exercise on fructose absorption. *American Journal of Clinical Nutrition*, *58*(1), 75–79.

Gonzalez, J. T., Fuchs, C. J., Betts, J. A., & van Loon, L. J. (2016). Liver glycogen metabolism during and after prolonged endurance-type exercise. *American Journal of Physiology – Endocrinology and Metabolism*, *311*(3), E543–E553. doi:10.1152/ajpendo.00232.2016

Gonzalez, J. T., Fuchs, C. J., Smith, F. E., Thelwall, P. E., Taylor, R., Stevenson, E. J., … . van Loon, L. J. (2015). Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *American Journal of Physiology – Endocrinology and Metabolism*, *309*(12), E1032–E1039. doi:10.1152/ajpendo.00376.2015

Hawley, J. A., Bosch, A. N., Weltan, S. M., Dennis, S. C., & Noakes, T. D. (1994). Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects. *Pflugers Archiv European Journal of Physiology*, *426*(5), 378–386.

Hulston, C. J., Wallis, G. A., & Jeukendrup, A. E. (2009). Exogenous CHO oxidation with glucose plus fructose intake during exercise. *Medicine & Science in Sports & Exercise*, *41*(2), 357–363. doi:10.1249/MSS.0b013e3181857ee6

Jentjens, R. L., Moseley, L., Waring, R. H., Harding, L. K., & Jeukendrup, A. E. (2004). Oxidation of combined ingestion of glucose and fructose during exercise. *Journal of Applied Physiology*, *96*(4), 1277–1284. doi:10.1152/japplphysiol.00974.2003

Jentjens, R. L., Underwood, K., Achten, J., Currell, K., Mann, C. H., & Jeukendrup, A. E. (2006). Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat. *Journal of Applied Physiology*, *100*(3), 807–816. doi:10.1152/japplphysiol.00322.2005

Jeukendrup, A. (2010). Carbohydrate and exercise performance: The role of multiple transportable carbohydrates. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(4), 452–457. doi:10.1097/MCO.0b013e328339de9f

Jeukendrup, A. (2014). A step towards personalized sports nutrition: Carbohydrate intake during exercise. *Sports Medicine*, *44* (Suppl. 1), S25–S33. doi:10.1007/s40279-014-0148-z

Jeukendrup, A., & Jentjens, R. (2000). Oxidation of carbohydrate feedings during prolonged exercise: Current thoughts, guidelines and directions for future research. *Sports Medicine*, *29*(6), 407–424.

Jeukendrup, A., & Moseley, L. (2010). Multiple transportable carbohydrates enhance gastric emptying and fluid delivery. *Scandinavian Journal of Medicine & Science in Sports*, *20*(1), 112–121. doi:10.1111/j.1600-0838.2008.00862.x

Latulippe, M. E., & Skoog, S. M. (2011). Fructose malabsorption and intolerance: Effects of fructose with and without simultaneous glucose ingestion. *Critical Reviews in Food Science and Nutrition*, *51*(7), 583–592. doi:10.1080/10408398.2011.566646

Lecoultre, V., Benoit, R., Carrel, G., Schutz, Y., Millet, G. P., Tappy, L., & Schneiter, P. (2010). Fructose and glucose coingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose. *American Journal of Clinical Nutrition*, *92*(5), 1071–1079. doi:10.3945/ajcn.2010.29566

Massicotte, D., Peronnet, F., Adopo, E., Brisson, G. R., & Hillaire-Marcel, C. (1994). Effect of metabolic rate on the oxidation of ingested glucose and fructose during exercise. *International Journal of Sports Medicine*, *15*(4), 177–180. doi:10.1055/s-2007-1021043

Mayes, P. A. (1993). Intermediary metabolism of fructose. *American Journal of Clinical Nutrition*, *58*(Suppl. 5), 754S–765S.

Miller, B. F., Fattor, J. A., Jacobs, K. A., Horning, M. A., Navazio, F., Lindinger, M. I., & Brooks, G. A. (2002). Lactate and glucose interactions during rest and exercise in men: Effect of exogenous lactate infusion. *The Journal of Physiology*, *544*(Pt. 3), 963–975.

Miller, B. F., Fattor, J. A., Jacobs, K. A., Horning, M. A., Suh, S. H., Navazio, F., & Brooks, G. A. (2002). Metabolic and cardiorespiratory responses to "the lactate clamp". *American Journal of Physiology – Endocrinology and Metabolism*, *283*(5), E889–E898. doi:10.1152/ajpendo.00266.2002

Moore, M. C., Coate, K. C., Winnick, J. J., An, Z., & Cherrington, A. D. (2012). Regulation of hepatic glucose uptake and storage in vivo. *Advances in Nutrition: An International Review Journal*, *3* (3), 286–294. doi:10.3945/an.112.002089

O'Brien, W. J., Stannard, S. R., Clarke, J. A., & Rowlands, D. S. (2013). Fructose-maltodextrin ratio governs exogenous and other CHO oxidation and performance. *Medicine & Science in Sports & Exercise*, *45*(9), 1814–1824. doi:10.1249/MSS.0b013e31828e12d4

Page, K. A., Chan, O., Arora, J., Belfort-Deaguiar, R., Dzuira, J., Roehmholdt, B., … Sherwin, R. S. (2013). Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. *JAMA*, *309*(1), 63–70. doi:10.1001/jama.2012.116975

Paquot, N., Schneiter, P., Cayeux, M. C., Chiolero, R., Temler, E., Jequier, E., & Tappy, L. (1995). Effects of infused sodium lactate on glucose and energy metabolism in healthy humans. *Diabete & Metabolisme*, *21*(5), 345–352.

Paquot, N., Schneiter, P., Jequier, E., Gaillard, R., Lefebvre, P. J., Scheen, A., & Tappy, L. (1996). Effects of ingested fructose and infused glucagon on endogenous glucose production in obese NIDDM patients, obese non-diabetic subjects, and healthy subjects. *Diabetologia*, *39*(5), 580–586.

Philp, A., Macdonald, A. L., & Watt, P. W. (2005). Lactate – a signal coordinating cell and systemic function. *Journal of Experimental Biology*, *208*(Pt. 24), 4561–4575. doi:10.1242/jeb.01961

Proia, P., Di Liegro, C. M., Schiera, G., Fricano, A., & Di Liegro, I. (2016). Lactate as a metabolite and a regulator in the central nervous system. *International Journal of Molecular Sciences*, *17*(9). doi:10.3390/ijms17091450

Record, C. O., Chase, R. A., Williams, R., & Appleton, D. (1981). Disturbances of lactate metabolism in patients with liver damage due to paracetamol overdose. *Metabolism*, *30*(7), 638–643.

Rehrer, N. J., Wagenmakers, A. J., Beckers, E. J., Halliday, D., Leiper, J. B., Brouns, F., … . Saris, W. H. (1992). Gastric emptying, absorption, and carbohydrate oxidation during prolonged exercise. *The Journal of Physiology*, *72*(2), 468–475.

Roberts, J. D., Tarpey, M. D., Kass, L. S., Tarpey, R. J., & Roberts, M. G. (2014). Assessing a commercially available sports drink on exogenous carbohydrate oxidation, fluid delivery and sustained exercise performance. *Journal of the International Society of Sports Nutrition , 11*(1), 8. doi:10.1186/1550-2783-11-8

Rosensweig, N. S., & Herman, R. H. (1968). Control of jejunal sucrase and maltase activity by dietary sucrose or fructose in man: A model for the study of enzyme regulation in man. *Journal of Clinical Investigation*, *47*(10), 2253–2262. doi:10.1172/JCI105910

Rosset, R., Lecoultre, V., Egli, L., Cros, J., Dokumaci, A. S., Zwygart, K., … Tappy, L. (2017). Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals. *American Journal of Clinical Nutrition*. doi:10.3945/ajcn.116.138214

Rowlands, D. S., Houltham, S., Musa-Veloso, K., Brown, F., Paulionis, L., & Bailey, D. (2015). Fructose-glucose composite carbohydrates and endurance performance: Critical review and future perspectives. *Sports Medicine*, *45*(11), 1561–1576. doi:10.1007/s40279-015-0381-0

Rowlands, D. S., Thorburn, M. S., Thorp, R. M., Broadbent, S., & Shi, X. (2008). Effect of graded fructose coingestion with maltodextrin on exogenous 14C-fructose and 13C-glucose oxidation efficiency and high-intensity cycling performance. *Journal of Applied Physiology*, *104*(6), 1709–1719. doi:10.1152/japplphysiol.00878.2007

Rumessen, J. J., Hamberg, O., & Gudmand-Hoyer, E. (1990). Interval sampling of end-expiratory hydrogen (H2) concentrations to quantify carbohydrate malabsorption by means of lactulose standards. *Gut*, *31*(1), 37–42.

Shi, X., Summers, R. W., Schedl, H. P., Flanagan, S. W., Chang, R., & Gisolfi, C. V. (1995). Effects of carbohydrate type and concentration and solution osmolality on water absorption. *Medicine & Science in Sports & Exercise*, *27*(12), 1607–1615.

Sole, C. C., & Noakes, T. D. (1989). Faster gastric emptying for glucose-polymer and fructose solutions than for glucose in humans. *European Journal of Applied Physiology and Occupational Physiology*, *58*(6), 605–612.

Stellingwerff, T., Boon, H., Gijsen, A. P., Stegen, J. H., Kuipers, H., & van Loon, L. J. (2007). Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use. *Pflugers Archiv European Journal of Physiology*, *454*(4), 635–647. doi:10.1007/s00424-007-0236-0

Sun, S. Z., & Empie, M. W. (2012). Fructose metabolism in humans – what isotopic tracer studies tell us. *Nutrition & Metabolism*, *9*(1), 89. doi:10.1186/1743-7075-9-89

Tappy, L., Egli, L., Lecoultre, V., & Schneider, P. (2013). Effects of fructose-containing caloric sweeteners on resting energy expenditure and energy efficiency: A review of human trials. *Nutrition & Metabolism*, *10*(1), 54. doi:10.1186/1743-7075-10-54

Tappy, L., & Le, K. A. (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiological Reviews*, *90*(1), 23–46. doi:10.1152/physrev.00019.2009

Tappy, L., Paquot, N., Tounian, P., Schneiter, P., & Jéquier, E. (1995). Assessment of glucose metabolism in humans with the simultaneous use of indirect calorimetry and tracer techniques. *Clinical Physiology*, *15*(1), 1–12.

Tounian, P., Schneiter, P., Henry, S., Jequier, E., & Tappy, L. (1994). Effects of infused fructose on endogenous glucose production, gluconeogenesis, and glycogen metabolism. *Am J Physiol*, *267*(5 Pt. 1), E710–E717.

Triplett, D., Doyle, J. A., Rupp, J. C., & Benardot, D. (2010). An isocaloric glucose-fructose beverage's effect on simulated 100-km cycling performance compared with a glucose-only beverage. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(2), 122–131.

Tsintzas, O. K., Williams, C., Boobis, L., & Greenhaff, P. (1995). Carbohydrate ingestion and glycogen utilization in different muscle fibre types in man. *The Journal of Physiology*, *489*(Pt. 1), 243–250.

Underwood, A. H., & Newsholme, E. A. (1965). Some properties of fructose 1,6-diphosphatase of rat liver and their relation to the control of gluconeogenesis. *Biochemical Journal*, *95*, 767–774.

Van Hall, G. (2010). Lactate kinetics in human tissues at rest and during exercise. *Acta Physiologica*, *199*(4), 499–508. doi:10.1111/j.1748-1716.2010.02122.x

Wallis, G. A., Dawson, R., Achten, J., Webber, J., & Jeukendrup, A. E. (2006). Metabolic response to carbohydrate ingestion during exercise in males and females. *AJP: Endocrinology and Metabolism*, *290*(4), E708–E715. doi:10.1152/ajpendo.00357.2005

Wallis, G. A., & Wittekind, A. (2013). Is there a specific role for sucrose in sports and exercise performance? *International Journal of Sport Nutrition and Exercise Metabolism*, *23*(6), 571–583.

Wasserman, D. H., Connolly, C. C., & Pagliassotti, M. J. (1991). Regulation of hepatic lactate balance during exercise. *Medicine & Science in Sports & Exercise*, *23*(8), 912–919.

Weber, E., & Ehrlein, H. J. (1998). Relationships between gastric emptying and intestinal absorption of nutrients and energy in mini pigs. *Digestive Diseases and Sciences*, *43*(6), 1141–1153.

Wilson, P. B., & Ingraham, S. J. (2015). Glucose-fructose likely improves gastrointestinal comfort and endurance running performance relative to glucose-only. *Scandinavian Journal of Medicine & Science in Sports*, *25*(6), e613–e620. doi:10.1111/sms.12386

Yau, A. M., McLaughlin, J., Maughan, R. J., Gilmore, W., & Evans, G. H. (2014). Short-term dietary supplementation with fructose accelerates gastric emptying of a fructose but not a glucose solution. *Nutrition*, *30*(11–12), 1344–1348. doi:10.1016/j.nut.2014.03.023