Year : 2019

NUTRITIONAL DETERMINANTS OF METABOLIC DISEASES IN HUMANS

Surowska Anna

Surowska Anna, 2019, NUTRITIONAL DETERMINANTS OF METABOLIC DISEASES IN HUMANS

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_635D730749F67

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Département de Physiologie

NUTRITIONAL DETERMINANTS OF METABOLIC DISEASES IN HUMANS

Thèse de doctorat ès Sciences de la Vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

Anna SUROWSKA

Diplômée en Nutrition Humaine et Sciences de la Consommation
Université d'Agriculture de Varsovie (SGGW)

Jury

Prof. Brigitta Danuser, Présidente
Prof. Luc Tappy, Directeur de thèse
Prof. Yves Boirie, expert
Dr Frédéric Preitner, expert

Lausanne 2019

Remerciements

La vie est jalonnée d'étapes. Le nouveau défi que je m'étais posé s'achève, et c'est grâce au soutien de nombreuses personnes que j'ai pu mener la présente thèse à son terme.

Je voudrais en particulier adresser un grand remerciement à mon directeur de thèse, le Professeur Luc Tappy, pour son aide précieuse. Je suis ravie d'avoir travaillé en sa compagnie, car outre son appui scientifique, il a toujours été présent pour me soutenir et me conseiller au cours de l'élaboration de ma thèse. Je le remercie également pour sa gentillesse, sa disponibilité, et pour ses nombreux encouragements.

J'exprime ma gratitude aux membres de mon jury de thèse qui ont bien voulu être examinateurs.

Je remercie toutes les personnes avec qui j'ai partagé ces années de thèse. Spécialement à mes collègues du laboratoire pour leur travail et encadrement, ainsi que toutes les personnes extraordinaires et passionnées rencontrées au Département de Physiologie.

J'exprime ma gratitude à toutes les personnes, à travers le monde, que j'ai eu la chance de rencontrer durant mes recherches. Ces échanges m'ont enrichie tant au niveau professionnel que personnel.

Ce travail a abouti aussi grâce aux nombreuses collaborations, particulièrement avec le personnel du Service d'Endocrinologie, diabétologie et métabolisme du CHUV, et du Département de Recherche Clinique de l'Inselspital à Berne, mais également le personnel de l'Oxford Centre for Diabetes, Endocrinology and Metabolism, du Centre de Recherche en Nutrition Humaine Rhône Alpes de l'Université Lyon1, et du Smart Food Center de l'Université de Wollongong, Australie.

Je tiens à remercier particulièrement mes amis pour toutes nos discussions et leurs conseils qui m'ont accompagnée tout au long de mon cursus et qui ont contribué à alimenter ma réflexion.

Mes derniers remerciements vont à ma famille et à mes proches qui m'ont inconditionnellement apporté leur soutien dans tout ce que j'ai entrepris.

Summary

A high fructose intake, mainly consumed with products containing added sugars, is currently suspected to be responsible for an increase in the global prevalence of obesity and related metabolic diseases. This suspicion rests on several short-term studies showing that a high-fructose intake negatively impacts cardio-metabolic risk factors in healthy volunteers. Some studies however report that fructose's harmful metabolic effects can be partially prevented by other dietary or life-style related factors. Each of the two studies included in this PhD thesis aimed to investigate the effects of a candidate factor. The first of them, bariatric surgery, is considered as the most effective treatment for grade III obesity, and is known to markedly improve obesity-associated metabolic alterations. In the first study, we assessed whether Roux-en-Y gastric bypass surgery altered postprandial fructose kinetics and *de novo* lipogenesis, with a special focus on intestinal *de novo* lipogenesis and on blood lipid profiles. Our results indicate that this surgical procedure does not induce any fructose malabsorption, but drastically decreases postprandial hyperlipemia. The latter effect was observed without any decrease in intestinal *de novo* lipogenesis, however. Second, several studies have also shown that a high-protein intake was associated with beneficial effects on body weight, glucose homeostasis, and, more recently, on intrahepatic fat concentration in obese or in healthy subjects during short-term overfeeding experiments. In the second study, we assessed in healthy volunteers whether the short-term effects of saccharose overfeeding was modulated by the dietary protein and lipid intake. Our results indicate that the same excess saccharose and total energy intake caused a five-fold larger increase in intrahepatic fat content when associated with a low-protein, high-lipid diet than with a high-protein, low-lipid diet.

Résumé

Il est soupçonné qu'une consommation excessive de fructose, principalement présent dans notre alimentation sous forme de sucres ajoutés, pourrait être responsable de la récente augmentation de la prévalence mondiale d'obésité et des maladies métaboliques. Ceci repose sur de nombreuses études d'intervention qui montrent qu'une suralimentation en fructose influe négativement sur les marqueurs de risque métabolique et cardiovasculaire. Pourtant, certaines études démontrent aussi que les effets négatifs du fructose peuvent être partiellement atténués par divers facteurs, alimentaires ou liés au mode de vie. Les études effectuées dans le cadre de cette thèse avaient pour but de préciser l'effet de certains de ces facteurs. La chirurgie bariatrique est actuellement considérée comme la méthode le plus efficace pour le traitement de l'obésité de degré III. De surcroit, elle est susceptible d'améliorer les anomalies métaboliques associées à l'obésité. Dans une première étude, nous avons évalué si le bypass gastrique selon Roux-en-Y altérait la cinétique postprandiale du fructose et la lipogenèse *de novo*. Une attention particulière a été portée à la lipogenèse intestinale *de novo* et aux éventuelles conséquences de sa modification sur les concentrations sanguines de lipides. Les résultats indiquent que le bypass gastrique n'entraîne pas de malabsorption de fructose, mais diminue l'excursion postprandiale de triglycérides, et ce malgré une lipogenèse intestinale préservée. Il a aussi été rapporté à plusieurs reprises, qu'une augmentation de l'apport protéique pouvait être associé à une perte de poids, une amélioration de l'homéostasie du glucose et, plus récemment, la diminution de la quantité de graisse stockée dans le parenchyme hépatique chez l'obèse ou dans des modèles expérimentaux de suralimentation chez le volontaire sain. Dans une seconde étude, nous avons donc évalué si les effets d'une surcharge de courte durée en saccharose variait en fonction du contenu en protéines et lipides de l'alimentation. Les résultats obtenus démontrent que, à même surcharge en saccharose et en énergie totale, le stockage de lipides intrahépatique est 5 fois plus important en présence d'une

alimentation pauvre en protéines et riche en lipide qu'en présence d'une alimentation hyper-protéinée pauvre en lipides.

Table of Contents

Glossary

Acetyl-CoA	Acetylcoenzyme A
Acyl-CoA	Acyl-coenzyme A
ApoB	Apolipoprotein B
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
BA	Bile acids
BMI	Body mass index
BP	Blood pressure
bw	Body weight
CCK	Cholecystokinin
CO2	Carbon dioxide
Ctr	Control
CVD	Cardiovascular diseases
DGAC	Dietary Guidelines Advisory Committee
DHAP	Dihydroxyacetone phosphate
DNL	*De novo* lipogenesis
E	Energy
EAA	Essential amino acids
EFSA	European Food Safety Authority
FFA	Free fatty acids
FGF19	Fibroblast growth factor 19
FXRs	Farnesoid X receptors
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-I
GLUT	Glucose transporter
GLUT2	Glucose transporter 2
GLUT5	Fructose transporter 5
HbA1c	Glycated hemoglobin concentration
HFCS	High fructose corn syrup
HP-LF	High-protein/low-fat diet
IHCL	Intrhepatocellular lipids
IL	Interleukin
IMCL	Intramyocellular lipids
LP-HF	Low-protein/high fat diet
MIDA	Mass isotopomer distribution analysis
MRS	Magnetic resonance spectroscopy
NAFLD	Non-alcoholic fatty liver diseases
NHANES	National Health and Nutrition Examination Survey
P	Phosphate
PL	Protein, lipid diet
PLFG	Protein, lipid, fructose, and glucose diet
PPARG	Peroxisome proliferator activated receptor gamma
RCT	Randomized control trial
RYGB	Roux-En-Y gastric bypass
SAT	Subcutaneous adipose tissue
SGLT1	Sodium-dependent glucose transporter
SSB	Sugar-sweetened beverages
TG	Triglycerides
TRL	Triglyceride-rich lipoprotein
UA	Uric acids
USDA	United States Department of Agriculture
VAT	Visceral adipose tissue
VLDL-TG	Very-low density lipoproteins bound triglycerides
W	Watt
WHO	World Health Organization
WM	Maintenance diet

Context

General philosophical introduction on nutrition, health, and well-being

Equilibrated, balanced, and harmonious are synonyms that are used to describe a successful life and happiness by Thomas Merton, an American poet, Trappist monk and theologian[1]. This "balance" concerns all aspects of our life, but especially one that is essential to survival and structures daily activity: eating. Eating behavior, which is connected to and influenced by social context, cultural values, and personal preferences, can function as an overall indicator of health (1). The perception of "good health" over time was and still is, strongly reflected in the "body image". Disequilibrium related to food intake and lifestyle habits may generate syndromes such as "binge eating" disorder, excessive fat accumulation and its associated comorbidities, or anorexia nervosa and bulimia. These disorders may have an extreme impact on body weight, ranging from exaggerated leanness to obesity.

The prevalence of overweightness and obesity in adults has tripled worldwide since 1975 (2). As a consequence, obesity prevalence is currently even greater than 50% of the population in some countries (3). However, the obese condition is not a new one and was already encountered several thousand years ago. Curious archeological discoveries may give another view on the excess body weight problem. There is a series of sculptures found across Europe, which represent female shapes, known in archeology as "Venus figurines", created from the lower to upper Paleolithic epoch. Interestingly, these figurines represent corpulent female forms. The Venus of Willendorf (Figure 1) created about 30000 years before calendar era (BCE) is considered as the oldest icon of obesity (4).

[1] "Happiness is not a matter of intensity but of balance and order and rhythm and harmony" by Thomas Merton in an essays published in 1955 titled "No Man is an Island", chapter called "Being and Doing".

Figure 1. Venus of Willendorf, made 30,000 BCE. Source: Seshadri K.G. (4).

This observation suggests that obesity was present from early prehistory and not just from the last century. Putting to one side cultural concepts of beauty, recent research has demonstrated significant health risks associated with fatness. Today the popular desire for health and a slim appearance, juxtaposed with the increasing prevalence of obesity, makes it important to find the reasons and solutions for the worldwide increase in adult body weight.

For whom, where, and when did fructose become a modern problem?

The main factor contributing to obesity is an imbalance between energy intake and expenditure, but other risk factors, such as genetics, socioeconomic factors, lifestyle choices and the quality of foods or dietary macronutrient composition, that is, carbohydrate, fat, and protein, may play a role. Previously, in the 1990s, fat intake was considered as the main "culprit" or predictor of body fat. However, since the 2000s, the focus has shifted toward the role of sugar intake in the pathogenesis of obesity (5).

One of the reasons that sugar is so prevalent in modern diets lies in the development of low-cost sugar production methods. The price of sugar production spiked during the 1970s and 80s, which prompted the development of alternatives to traditional cane and beet sugars. The USA was and still is the highest worldwide producer of corn (6), and the corn wet milling industry was looking for new applications for cornstarch manufacturing at this time. The creation of liquid sweetener, enzymatically produced from corn, called high fructose corn syrup (HFCS), offered a successful alternative to sugar in the USA.

In the early 1970s, this alternative to sucrose was initiated in the USA. Today, HFCS is widely used in beverages and the food industry mainly in North America, but also in some countries of Europe and Asia (7). Corn syrup brings several advantages over sucrose production. First, it has a lower production cost compared to sugars from cane or beet (8). Second, the similar sweetening power of HFCS to sucrose leads to the easy replacement of sucrose in industrial products. Additionally, HFCS brings functional advantages, like moisture and microbial growth control, extending the shelf-life of baked goods, and water control in a frozen system of alimentary products (9).

As industrialization increased in the USA, food products became widely available on the market. Between 1970 and 2002 the size of the food portions increased between 2- to 8-fold (10). In result, total individual food consumption increased by approximately 500 kcal/day per

capita (11). The availability of caloric sweeteners (cane and beet sugars, corn sweeteners, edible syrups, and honey) in the USA between 1966 and 1999 increased from 51kg/year per capita to 69kg/year per capita. Since 2000, added sugars as a fraction of daily caloric intake have decreased slightly, but still exceeded dietary recommendations (12). Consumption of sugar-sweetened beverages (SSB) has also increased, and total energy intake for soft drinks rose from 2.8% in 1977 to 7% in 2001 (13).

The stark observation of increased obesity and expanded utilization of HFCS in the USA, mostly in the sugar-sweetened beverage (SSB) sector, triggered a world-wide debate of identifying the causes of weight gain in the population. In 2004, the assumption was made that overconsumption of HFCS and in particular its fructose component, which has more lipogenic potential than glucose, "may be an important contributor to the epidemic of obesity"(14). It was further suggested that SSBs may make an important contribution to an increased total energy intake.

Sugar consumption today represents between 10% and 20% of daily energy (E) intake in North America (15). However, in the National Health and Nutrition Examination Survey (NHANES), between 2003 and 2006, it was observed that 30% of the population consumed even more than 25% of energy from added sugars (16). The global average in 2007 showed that consumption of fructose as a part of sweeteners corresponded to 65g/day per capita (17).

Interestingly, while HFCS is widely used in the USA, its use remains low in other part of the world. However, in the same period, obesity prevalence increased in Europe, Asia, and Australia, where HFCS is little or not used (18). Independently, overweight and obesity as defined by body mass index (BMI) (calculated from the weight and height of an individual (kg/m^2)) in children and adolescents correlated with that of adults, which increased between 1975 and 2016. Surprisingly, from 2000 until 2016 a plateauing of BMI in children and

adolescents was observed in many high-income countries. However, BMI started to increase in other parts of the world, and even accelerated, e.g., in Asia (19).

The role and contribution of sugars and, in particular, fructose, in the current obesity epidemic, and the adverse effects on human health that they may cause, will be discussed in the following chapters. Moreover, corrective factors in imbalanced behaviors seem to be important. In situations of overfeeding with sugar, could some factors have a protective effect on metabolic disorders? In extreme situations due to obesity, when surgical procedures are involved, how do they affect the normal metabolism of fructose?

In the present work I will focus on two aspects:

1. Effect of Roux-En-Y gastric bypass (RYGB) on fructose metabolism.
2. Effect of dietary protein content on fructose-induced deposition of intrahepatic fat and dyslipidemia.

Chapter I Introduction

Sugars

The terms “sugars” or “simple sugars” are commonly used to describe simple carbohydrates like monosaccharides (glucose, fructose, galactose) and disaccharides (sucrose, lactose, maltose). Sugars occur naturally in foods or are added during food preparation and industrial processing (20). High sugar consumption has been proposed to be a cause of increased body weight (21), dental caries (22), and cardiovascular risk (23). The mechanism responsible for sugar-induced body weight gain may be that the sweet flavor, which gives the particular, pleasant taste, and hedonic properties of sugars favor the overconsumption of sweet foods and beverages (24).

In particular, sugar-sweetened beverages are proposed to contribute heavily to the epidemic of obesity by adding directly extra energy to the diet (25). Moreover, the hypothesis that sugar is the main factor responsible for obesity has been challenged on the basis that there is no clear evidence that added sugars or any other nutrients have a unique role in the obesity problem or any other health disorders (26).

It is generally recognized that, besides genetic predisposition, the overconsumption of energy and low physical activity are mandatory factors to promote an excess energy balance and cause obesity and its associated health problems. Whether this stems from one single macronutrient like fructose, or occurs as a consequence of excess calories from any macronutrient class is still controversial, and the debate is ongoing. Nonetheless, many national and international dietary guidelines proposed to reduce sugar intake, mainly based on the observation that it represents an important source of calories, it is a dispensable nutrient, and its major dietary sources in western diets (SSBs and confectionary) have relatively low nutritional quality. Some of these recommendations are briefly summarized below.

a) Added sugars

"Added sugar" is the term proposed in 2000 by the United States Department of Agriculture (USDA) to define sugars not naturally found in foods, but which are added during industrial food processing and in home preparation (27). It includes, among others: HFCS, white and brown sugar, raw sugar, malt syrup, maple syrup, honey, and crystal dextrose. This definition excludes all natural sugars present in fruits, vegetables, and their juices or purees, and sugars from dairy products (28), but includes fruit juice concentrate (20).

"Added sugars" is a term that is referred to in different health guidelines. Indeed, this term is used in the Dietary Guidelines Advisory Committee (DGAC) in the United States (29), in Nordic countries (30), and many other countries and organizations, like the European Food Safety Authority (EFSA) (20). Recommendations for added sugar consumption in these guidelines are less than 10% of total energy intake (E).

b) Free sugars

The definition of "free sugars" was initially proposed by the World Health Organization (WHO) in 2003. Free sugars represent all mono- and disaccharides added to food and beverages through manufacturing or home preparation. This term includes sugars naturally present in honey, syrups, fresh fruit juices, and their concentrates (31). However, sugars naturally present in whole fruits, vegetables (cooked or dried), and sugars present in dairy products are excluded (32). WHO recommendations for free sugars correspond today to less than 10% of E, and a conditional recommendation limit of less than 5% of E (31). "Non-milk extrinsic sugars" (NMES), is another term that was in use in the UK until recently (until SACN report 2015), which is almost synonymous with "free sugars," and also excludes lactose provided in dairy products (20).

c) Natural sugars

Naturally occurring sugars (such as sucrose, fructose, glucose) are present in plants, within cell walls of fruits, vegetables, and berries. Moreover, lactose present naturally in dairy milk and products also belongs to this group. The WHO guidelines do not refer to the natural sugar intake, because there is no reported evidence of adverse effects when consuming these types of sugars (32).

However, in the UK a similar term exists, "intrinsic sugars", which refers to sugars that are an integral part of unprocessed food and naturally enclosed in the cellular structure of food, except milk sugar, lactose (33). In contrast, "extrinsic sugars" are defined as all sugars not present in the cells and includes lactose from milk. To distinguish sugars provided from extrinsic and milk sources, the term "non-milk extrinsic sugars" was established, which corresponds to the term "added sugars" in the US. Figure 2 resumes a visual representation of the different terms that are used to describe sugars.

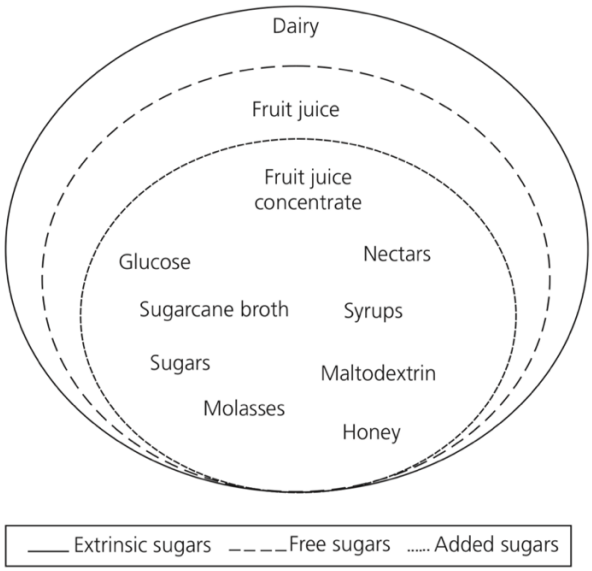


Figure 2. Different sugars terms. Source: Scapin et al., 2017, (34).

None of the governmental organizations base their recommendations on the upper limit of total sugar intake, which includes added sugars and sugars provided from natural sources, except

France, where an upper limit is 100g sugars per day with an emphasis on promoting the consumption of fruits and vegetables (35). Furthermore, the lack of harmonization and unified definitions of added, free, and total sugar intake may be confusing for consumers. Additionally, in the report provided by European Food Safety Authority (EFSA) (36), authors confirm that available evidence is insufficient to provide a unique and directly causal role of sugar intake with health effects, that is, impaired glucose tolerance and insulin sensitivity, increased serum lipids, and cardiovascular risk factors, increased body weight, type 2 diabetes, and caries (36).

Fructose

Fructose is a monosaccharide naturally present in its free form in fruits, some vegetables, honey, and in natural maple and agave syrups. All national and international dietary guidelines recommend a large consumption of fruits and vegetables, suggesting that fructose from fruits may exert beneficial effects on human health (37). Conversely, fructose consumption from added sugar was proposed to have toxic effects and has even been compared to alcohol abuse (38). These contradictory statements may be very confusing for the general public.

It is important to note, that fructose is rarely consumed in its pure form as a sweetener and is mainly ingested as a part of complex foods with other macro- and micronutrients and fiber. Most commonly, fructose is bound with another monosaccharide, glucose, in the same proportion (50%-50%) and is co-ingested in the form of the disaccharide called sucrose, or more popularly known as “table sugar” or “white sugar.” Sucrose is naturally present in fruits and vegetables and is industrially produced from sugar cane and beets. Fructose is 1.2 times sweeter than sucrose and more sweet than most other natural sugars (9).

Fructose is also present in caloric sweeteners, that is, in high fructose corn syrup (HFCS), which is a mixture of free monosaccharides: fructose and glucose. This syrup is obtained through an industrial process by extraction of starch from corn and then hydrolysis to glucose.

The immobilized isomerase reactors of glucose to fructose allow obtaining of equilibrium fructose concentration of 42%. The next step of chromatographic separation technology yielded low glucose to fructose ratio, providing 90% of fructose syrup. HFCS 90% is then mixed with HFCS 42% to obtain HFCS 55% (39). Generally two types of HFCS are used in the food industry. The first type is HFCS-55, which contains 55% fructose, 42% glucose, and 3% glucose polymers, and is mainly used in soft drink production. Its sweetness is very similar (99%) to sucrose and it was designed to serve as a substitute for sucrose in sugar-sweetened soft drinks. The second type is HFCS-42 (42% fructose, 53% glucose, and 5% glucose polymers), which has a lower sweetness (92%) attributed to its lower fructose concentration. This type of HFCS is mainly used in processed foods, baked goods, and some beverages. In general, the fructose to glucose ratio of HFCS is close to the ratio that is found in sucrose (50%-50%), which means that the proportion of sugars released during digestion of sucrose and HFCS is similar (40).

Daily total fructose intake less than 50g is considered as moderate, between 50g and 100g per day as a high intake, and more than 100g per day is excessive intake (41). Fructose is mainly consumed with glucose (i.e., sucrose, HFCS), which means that a moderate daily intake of fructose will represent 100g/day of sucrose (20% total energy intake calculated for a total energy intake of 2000 kcal/day).

a) Fructose absorption

From the perspective of chemical structure and physiological effects, fructose molecules are indistinguishable by source. Therefore, any specific physiological effect of a fructose-containing food has to be determined by the food matrix. For instance, natural sugars in fruits are ingested together with vitamins, minerals, antioxidants, and fiber naturally present in these products. Moreover, it was shown that the form in which fruits are consumed (whole fruit vs

juice) may indeed have an impact on satiety and energy intake of the meal. Whole fruits have a larger satiating effects than isocaloric fruit juices (42). In general, consumption of whole fruits has been associated with a lower risk of cardiovascular diseases (43), type 2 diabetes (44), and obesity (42). This protective effect of fruits and vegetables, despite their sugar content, may be due to various mechanisms. The high fiber content of whole fruits accounts for an increased satiety, and slower digestion and absorption of sugars as compared to fruit juices (45). Furthermore, fruits and vegetables are rich in antioxidants (including vitamins C, E, β carotene, and flavonoids), which may stimulate the immune system and have an impact on cholesterol metabolism and blood pressure (46).

b) Enteric metabolism

Fructose presented in the gut is generally delivered with consumed sucrose or HFCS and rarely in free form. Ingested sucrose (disaccharide) is degraded by the intestinal enzyme sucrase, which releases fructose and glucose molecules. Absorption of these monosaccharides then takes place in the duodenum and jejunum (proximal small bowel) and involves specific transporters (Figure 3). At the brush-border membrane of the lumen, glucose is transported into enterocytes by the sodium-dependent glucose transporter (SGLT1). The SGLT1 transports glucose molecules together with sodium ions and relies on the electrochemical gradient and concentration of Na^+ regulated by a Na^+/K^+ ATP-ase situated on the basolateral membrane. From the intracellular compartment to the bloodstream, glucose is transported by a facilitated glucose transporter GLUT2. It has been also proposed that in the presence of a high concentration of glucose, the GLUT2 transporters may be recruited in the lumen membrane to facilitate glucose passage (47).

SGLT1 is used for absorption, not only of glucose, but also of galactose. In contrast, fructose is absorbed from the gut lumen through a specific fructose transporter GLUT5 (or SLC2A5),

located at the apical pole of enterocytes. This transporter allows a facilitated diffusion of fructose, independent of Na^+ absorption. Both fructose and glucose are then transferred into the bloodstream via the same facilitated transporter, GLUT2. Fructose absorption is slower than that of glucose, and the rate of fructose appearance in the blood is correlated with the number of GLUT5 transporters in the membrane.

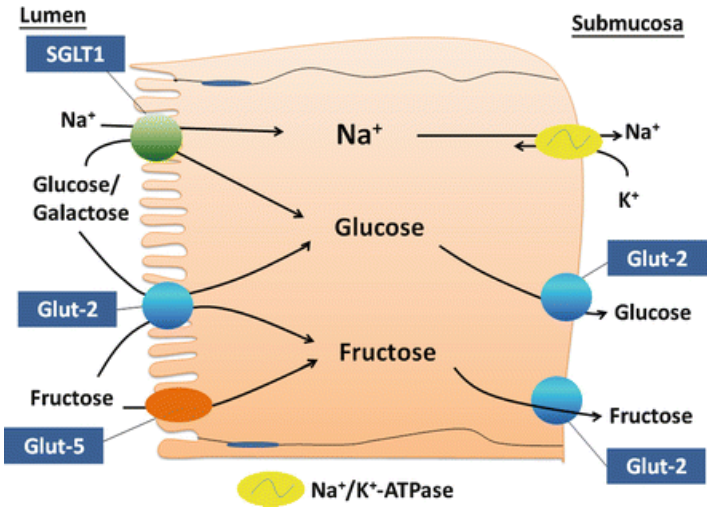


Figure 3. Absorption of monosaccharides in the intestine. Source: Sitrin, 2014, (48).

It was shown that, in healthy humans, the capacity to absorb free fructose varied widely between individuals, with a range from less than 5g to more than 50g (49). In many individuals, with a high fructose load (e.g., >25g), some fructose will not be absorbed in the small intestine, and proceed to the colon where it can exert an osmotic effect, or be fermented by colonic bacteria with the concomitant production of hydrogen gas (50). Under such conditions, increased intestinal gas production may affect intestinal motility and cause gastrointestinal pain, thus eliciting symptoms resembling irritable bowel syndrome. Free fructose alone is particularly poorly absorbed however compared to other hexoses (51). However, in typical Western diets, fructose is mainly ingested in the form of sucrose (fructose bonded with glucose) or as an HFCS (a mixture of free molecules of glucose and fructose) and is better absorbed

than free fructose in healthy individuals (52). Indeed, the presence of glucose (49), galactose (53) and certain amino acids (54) may increase fructose absorption. It was also observed that a chronic, high fructose intake increases the expression of GLUT5, leading to increased absorption of fructose (55).

c) Hepatic metabolism

Unlike glucose, fructose absorbed in the bloodstream cannot be directly metabolized by most cells of the body and first needs to be converted to other metabolites (glucose, lactate or fatty acids), mainly in the liver, which expresses specific enzymes for fructose metabolism. A small portion of ingested fructose may also be metabolized in other splanchnic organs (small intestinal mucosa, kidney cortex), where the same fructose metabolizing enzymes are also expressed. However, the quantity of fructose metabolized outside of the liver remains unknown.

The initial steps for fructose metabolism in the liver differ markedly from glucose. After having been taken up by liver cells, glucose is metabolized to glucose 6-phosphate (P) by glucokinase (hexokinase IV), which is characterized by a high Km[2] (lower affinity) for glucose, and hence glucose metabolism is dependent on glucose concentration. Further down the glycolytic pathway, phosphofructokinase catalyzes the conversion of fructose 6-P to fructose 1,6-diphosphate. This enzyme is a key control point for glycolysis, and is potently inhibited by increased intracellular citrate and ATP (56).

In contrast, the initial steps for fructose metabolism, or fructolysis, are catalyzed by three specific enzymes: fructokinase (ketohexokinase), aldolase type B, and a triokinase. The first step is phosphorylation by ATP to fructose 1-P, which is catalyzed by the enzyme fructokinase.

[2] Km corresponds to the concentration of substrate, which leads the enzyme to obtain half V_{max}. A high Km indicates a low affinity for the substrate and means a high concentration of substrate is needed to achieve maximum reaction velocity.

This enzyme is specific for fructose and is characterized by a low Km, which allows rapid metabolism of fructose in liver cells (57). Then, an aldolase B (liver aldolase) converts fructose 1-P into two trioses: glyceraldehyde and dihydroxyacetone-phosphate. Glyceraldehyde is then converted into glyceraldehyde phosphate by a third enzyme, a triokinase. Dihydroxyacetone-P (DHAP) and glyceraldehyde-3-P are normal glycolytic intermediates that can then be further processed into pyruvate (Figure 4).

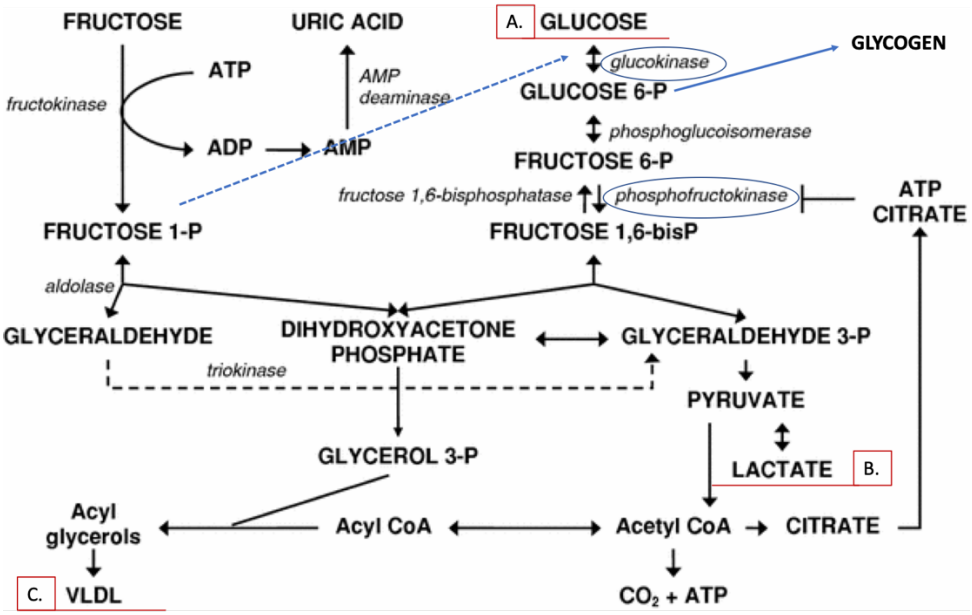


Figure 4. Metabolism of fructose and glucose and major products of fructose metabolism, A) glucose, B) lactate, C) hepatic VLDL-TG secretion, " ⋯▶ " accumulation of fructose 1-P increase hepatic glycogen synthesis. Source: Tran et al., (58) modified.

Aldolase B deficiency (found in the liver, kidneys, and small intestine) is a rare inborn error of metabolism in which fructose consumption may irreversibly damage the liver and kidney (59). Inability to metabolize fructose 1-P provokes an accumulation of this molecule in the liver cells, a consumption of intracellular ATP, an acute intracellular energy crisis, and acute liver and renal dysfunctions.

Of major importance, fructolysis bypasses key regulatory steps of glycolysis at the level of phosphofructokinase. In addition, fructolysis, unlike glycolysis, is not regulated by insulin or glucagon. As a consequence of this, ingestion of large amounts of fructose may lead to the

unregulated generation of large amounts of glyceraldehyde-3-P and DHAP, which will then be substrates for various metabolic pathways (Figure 4) (60):

A. glucose production (gluconeogenesis): trioses-phosphate may join the gluconeogenesis pathway to glucose 6-P, and either be released as glucose in blood (about 50% of puree fructose) or stored as glycogen in the liver (15%) (61, 62).
B. lactate production: triose phosphate may be metabolized into pyruvate and then into lactate by lactate dehydrogenase (about 25% (61)).
C. lipid synthesis: both fructose and glucose can be converted into pyruvate, acetyl-CoA and then fatty acids via the *de novo* lipogenesis pathway (DNL), but fructose is more efficient in activating DNL and in stimulating hepatic VLDL-TG secretion than glucose. However, the conversion of fructose carbons into fatty acids represents quantitatively a minor pathway for fructose disposal (1-5%) (62-64).

It was proposed that the preferential pathways used for fructose metabolism are oxidation and/or lactate production, because these pathways do not require any energy consumption (60, 65). In contrast, gluconeogenesis and DNL require the hydrolysis of considerable amounts of ATP, and hence may be used only when oxidation and lactate production have reached their maximal levels (66).

In summary, fructose and glucose metabolism have many similarities, sharing several common metabolic steps; however, fructose appears to be mainly metabolized in the liver and due to the high affinity of fructolytic enzymes with fructose. The first enzyme of fructose metabolism, fructokinase, is four times more active than glucokinase and thus results in the faster metabolism of fructose than glucose (57). Moreover, fructokinase has no negative feedback mechanisms, which means that all fructose entering a liver cell is rapidly phosphorylated to fructose-1-P. A high fructose load in cells may produce intracellular phosphate depletion,

which can result in harmful effects due to uric acid secretion and other downstream byproducts of metabolism, i.e., fatty acids and lactate. One effect of these metabolites is an impaired glucose uptake. In contrast, glycolysis is a highly regulated pathway with two levels of regulation. Fructose metabolism bypasses these regulated steps of glycolysis and is directly catalyzed by glucokinase and phosphofructokinase (Figure 4), which are inhibited by their products: citrate, ATP, and glycogen (indirectly).

Hepatic fructose metabolism may further impact on glucose metabolism. Indeed, it was observed that small amounts of oral fructose may have a positive impact on postprandial glucose levels (67). Fructose may also play the role of regulator of liver glucose uptake, through fructose 1-P indirectly increasing the activity of glucokinase and hepatic glycogen synthesis (Figure 4) (68, 69).

Effects of fructose on metabolic disease risk factors

The potential adverse health effects of fructose have recently been widely presented in the media. Consumption of fructose is proposed to be a key factor in the development of metabolic diseases due to its particular metabolism. Moreover, consumption of sugar-sweetened beverages (SSBs) has been directly associated with increased body weight (70). Products rich in fructose have also been proposed to be linked with the development of metabolic syndrome, which corresponds to a cluster of metabolic alterations such as obesity, impaired glucose tolerance or insulin resistance, dyslipidemia, and hypertension (71, 72). The potential mechanisms by which fructose may cause these adverse metabolic effects will be briefly summarized below. The primary topics of the present work will be: a) glucose homeostasis, b) lipid profile, and c) ectopic lipids, which are quantifiable levels that can indicate the disorders that are also the main topics of my previously published studies. Below, selected literature will be reviewed, using a classification for total fructose intake proposed by Livesey et al., (41):

- Moderate intake: ≤ 50g/day (10%E calculated for 2000kcal/day)
- High intake: > 50-100g/day (100g, 20%E)
- Very high (excessive) intake: > 100g/day

a) Glucose homeostasis

Glucose homeostasis consists of maintaining an adequate but not excess level of blood glucose, which corresponds to a narrow range of 4 - 6 mmol/l in fasting conditions, and under 7.8 mmol/l in fed conditions (73). The balanced action of pancreatic gluco-regulatory hormones is largely responsible for maintaining blood glucose. Insulin, secreted by the beta-cells of pancreatic islets, is stimulated by the increased blood glucose concentrations occurring after ingestion of a meal. Insulin removes glucose from the bloodstream primarily by stimulating its uptake into insulin-dependent tissue, such as skeletal muscle and adipose tissue. In contrast, glucagon is produced by the alpha-cells of pancreatic islets when blood glucose is low, i.e., between meals and during the overnight fasting period. Glucagon stimulates endogenous (hepatic) glucose production (glycogenolysis and gluconeogenesis) to prevent hypoglycemia (74, 75).

Blood glucose homeostasis can be altered by changing total dietary energy intake, the partition of total daily energy into several meals, and/or the relative intake of carbohydrates (starch and sugars), and type of sweeteners consumed (76). In healthy humans, seven days on a eucaloric[3] diet with low (10%E) and high (25%E) sucrose intake has been shown to not change fasting plasma glucose and does not appear to impact insulin sensitivity (77). Of special interest, it was observed that a small amount (7.5g) of fructose added to glucose beverages improved glucose tolerance in patients with type 2 diabetes without increasing their blood insulin concentration (87). The mechanism proposed for this beneficial effect on blood glucose levels

[3] Eucaloric diet: kcal from diet corresponds to kcal burned, which means that energy intake and expenditure are equal.

was the increased presence of fructose's metabolite fructose 1-P, which increased glucokinase activity, and is considered as a key regulator of glycolysis. This pathway may contribute to the improvement of glucose homeostasis by increasing hepatic glucose uptake and lowering glucose production (88). This indicates that small amounts of fructose may have catalytic effects to improve glucose utilization, mainly by enhancing glucokinase activation in liver cells independently of changes in insulin secretion.

The effects of fructose were tested in diabetics, overweight and obese patients who consumed isocaloric[4] diets containing fructose, glucose or sucrose (78). The amount of fructose was in the range between 25g and 104g per day. The postprandial blood glucose and insulin responses were lower, which indicated improved glucose homeostasis, after the fructose diet than those with sucrose or glucose. In patients with type 2 diabetes a very high dose of fructose (160g/day) exchanged for other carbohydrates did not increase fasting glucose and insulin levels (79), but decreased glycated hemoglobin concentration (HbA_{1c}). This indicator reflects the average daily blood glucose concentration (80). A similar effect was seen in another study, where type 2 diabetic patients consumed isocaloric diets containing either 20% fructose or 19% sucrose. No effect on blood glucose levels was observed after each diet (81). It is therefore suggested that in the short and middle terms (1 week to 52 weeks) fructose within an isocaloric diet does not harm glucose homeostasis, and may even improve glucose levels in diabetic patients.

Due these observations, fructose was initially considered for use as a substitute for sucrose in the diet of diabetic patients. However, it was observed that despite these acute beneficial effects during isocaloric feeding, fructose may cause adverse effects on glucose homeostasis under some conditions which will be briefly reviewed here.

[4] Isocaloric diet: the same or similar energy provided each day from moderate intake of macronutrients (carbohydrates, fats, proteins).

Overfeeding of healthy humans with an excess 15% total energy as fructose for four weeks, slightly increased fasting plasma glucose levels but did not change whole body insulin sensitivity as assessed by a hyperinsulinemic euglycemic clamp (82). In contrast, another study assessed the effects of graded doses of fructose (15%, 30%, and 40% of energy added) (83), and reported that high doses (30% and 40%) significantly increased hepatic glucose production, corresponding to some degree of hepatic insulin resistance. In another study, the diet of healthy volunteers was supplemented during three weeks with sweet beverages containing fructose in low (40g/day) and high (80g/day) doses (84). It was observed that even low amounts of fructose increased fasting glucose levels and caused hepatic insulin resistance. However, another study, in which healthy volunteers were supplemented with 150g/day fructose or glucose per day for four weeks, reported similar effects on hepatic insulin sensitivity with both sugars, suggesting that these effects were not to be specifically attributed to fructose (85).

Interestingly, the effect of fructose may differ according to gender. Overfeeding with fructose (35%E) for six days increased fasting blood glucose in both males and females, but increased fasting insulin concentration in men only (86). These results may indicate that hepatic insulin resistance is more likely to occur in men under this condition than in women.

Therefore, these results suggested that in the short and middle terms, hypercaloric, high fructose diet may impair glucose homeostasis.

b) Lipid homeostasis

Fructose stimulates more *de novo* lipogenesis (DNL) than other hexoses. In this pathway, acetyl-CoA produced from carbohydrate catabolism is reconverted into fatty acids (Figure 5) and by this property may lead to, e.g., hyperlipidemia (89). It has been well documented that high fructose intake may increase blood triglycerides (TG) concentration in healthy (90-93),

overweight subjects, and patients with type 2 diabetes mellitus (90, 94-96). This effect may be associated with deleterious long-term consequences, as elevated fasting and postprandial plasma TG are considered to be independent predictors of cardiovascular diseases (CVD) (97).

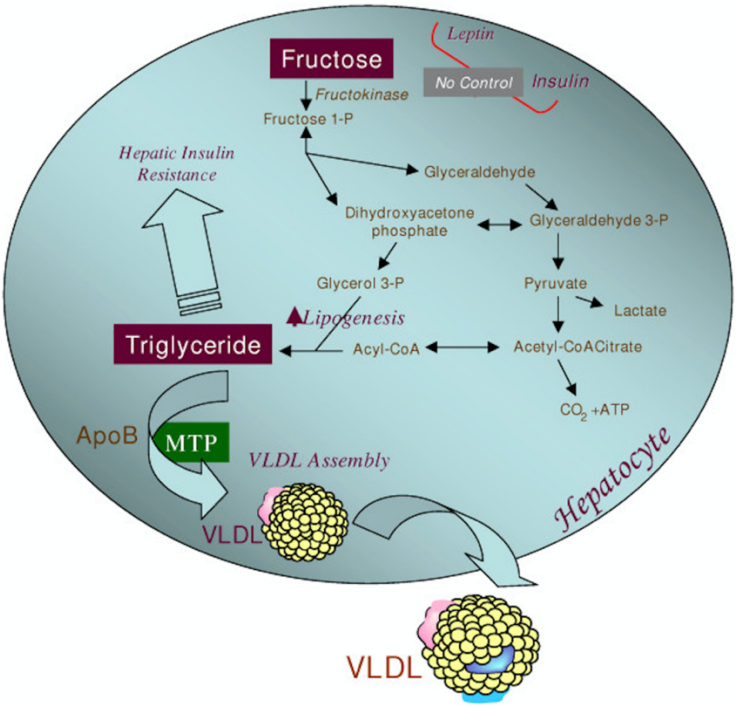


Figure 5. Hepatic fructose metabolism and *de novo* lipogenesis pathway. Source: Adeli et al., (71).

Mechanisms by which high fructose intake may play a role on lipid profiles include providing large amounts of hepatic triose-phosphate, and increased unregulated source acetyl CoA, which can fuel the *de novo* lipogenesis pathway in the liver (Figure 5). Increased synthesis of intrahepatic lipids lead in turn to their deposition within liver cells, which may contribute to cause hepatic insulin resistance (98), and in the long term lead to the development of non-alcoholic fatty liver diseases (NAFLD) (99). High fructose intake can also stimulate the secretion of very low-density lipoprotein (VLDL-TG) and associated apolipoprotein B (apoB) (60, 91) and decreased VLDL-TG clearance (63, 100). Moreover, fructose intake activate adipose tissue lipoprotein lipase less than glucose (101), which in consequence may decrease triglyceride clearance (63). It was also proposed that high fructose intake may be linked with

an increased expression of lipogenic enzymes in the liver (61, 98) and can inhibit hepatic lipid oxidation (78).

The effects of dietary fructose on blood lipids appear to be dose-dependent, and are observed with amounts > 50g/d (41). There is some controversy on whether this effect is specific for fructose, since some studies reported similar effects with glucose (63, 102). However, other studies did not report the same observations. An acute, moderate intake of a fructose drink, compared to glucose and sucrose drinks, showed no significant changes in plasma TG, but increased total cholesterol (103). Also, ingestion of isocaloric loads of fructose, glucose or other sweeteners (HFCS and sucrose) all led to similar increases in blood TG, however, without a significant difference between them (104).

The activity of *de novo* lipogenesis from carbohydrates in normal adults on a typical Western diet[5] (105) appears to be very low (1-2% fractional DNL in fasting and 5% in fed states) (106). Overfeeding with high sucrose or high glucose (50%E) however increases DNL activity markedly, but to the same extent with both sugars (107). In contrast, high fructose intake, corresponding to 25% of energy in weight maintaining diets in healthy non-obese participants consumed over nine days increased DNL significantly compared to the same diet, but with complex carbohydrates (108). Additionally, it was shown that increased DNL and VLDL-TG may be more important in subjects with NAFLD than for healthy individuals in a study that tested an isocaloric diet for 12 weeks with high sucrose intake (26%E) (109).

Effects of fructose on blood lipids have been assessed with both solid foods containing fructose, and fructose drinks, with somewhat differing results. In a study in which a diet containing 7.5–21% fructose from solid foods was compared to the same diet containing carbohydrates, a modest rise was observed (110). In another similar study, a diet containing

[5] Western diet: characterized by highly transformed food rich in fat, protein, refined grains, and lower in fruits and vegetable intake.

fructose (15-100g/day) but eliminating glucose or sucrose in solid or beverages, did not observe any increase of postprandial blood lipid levels. In contrast, a study which compared a 25%E from fructose in sugar-sweetened beverages added to an *ad libitum*[6] diet increased fasting and postprandial TG, and also hepatic fractional DNL (100).

The effect of fructose on lipid profiles may depend on many factors, including the amount of fructose intake, duration of consumption, gender, and health status of patients. Hypertriglyceridemia-induced by high fructose intake is mostly observed with hypercaloric diets, which may suggest a combined effect, among others, of high fructose intake and an excess of energy. It therefore appears that high fructose intake along with high total energy may induced hyperlipidemia and insulin resistance.

c) Ectopic lipids

Overconsumption of energy will in the long term cause an increase in body weight (and an increase in fat > lean body mass) and may lead to obesity. Excess energy is primarily stored as fat in adipose tissue, but small amounts of fat may nonetheless be deposited in other tissues, which do not normally contain lipid droplets to any large extent. Ectopic fat is defined as triglycerides stored in such organs that are not physiologically adapted for fat storage, like the liver, muscles, pancreas, and kidneys (111). Deposition of ectopic fat may have an impact the metabolic activity and/or function of organs. More specifically intrahepatic and intramyocellular fat have been associated with insulin resistance, in the liver or muscle, respectively (112).

There are currently two proposed mechanisms for ectopic lipid deposition. The first proposed mechanism is that ectopic lipid deposition occurs when subcutaneous adipose tissue (SAT) storage becomes saturated or appears dysfunctional, as during a long period of positive energy

[6] *Ad libitum* diet: Corresponds to habitual, "free feeding" diet of participant.

balance (113). A second mechanism proposes that carbohydrate overfeeding, and more specifically carbohydrate overfeeding with mono- or disaccharides (sucrose, glucose, and fructose), may provide an important load of trioses-phosphate as precursors of acetyl-CoA for DNL. In turn, DNL can upregulated VLDL-TG secretion and ectopic VLDL-TG extraction. Additionally, uncontrolled high fructose metabolism may provoke postprandial hypertriglyceridemia, which can increase visceral adipose deposition and ectopic fat (114).

Intrahepatocellular lipid (IHCL) concentration

Fructose consumption has been proposed to play a causal role in the development of obesity. In turn, obesity is associated with nonalcoholic fatty liver diseases (NAFLD). Less than 5% of the fat concentration in hepatocytes is considered as "normal". Higher than 5% is defined as steatosis, the first step of NAFLD (115). Accumulation of fat in the liver is the result of an imbalance between the overall intrahepatocellular TG influx (triglyceride-rich lipoprotein uptake and DNL) and their removal from hepatocytes.

In healthy subjects fructose intake as an 18% excess of energy during four weeks did not change IHCL deposition compared to the isocaloric diet with less than 20g/day of fructose (82). In contrast, in healthy subjects, it was observed that higher dose of dietary fructose content (25% and 35%) associated with a hypercaloric diet may significantly increase the liver fat content as early as after one week of the diet (91, 116). However, similar results on the liver were also observed for glucose overfeeding (30% and 35%E) (83, 93). Additionally, a hypercaloric, high-fructose diet with the addition of high fat almost doubled hepatic fat deposition compared with fructose alone (92).

Overweight subjects increased non-significantly hepatic lipids to the same extent when consuming isocaloric diets with 25% total energy as fructose versus as glucose (117). However, in the same subjects, significantly increased of IHCL was observed after both fructose and

glucose, when were provided at 25% of energy excess (117). Other studies (109) have shown that intrahepatic lipids were higher when subjects consumed weight-maintenance diets with high (26%) vs low (6%) sugar content (non-milk extrinsic sugars) during 12 weeks: It also reported that, with the high sugar diet, IHCL increased to a larger extent in subjects with NAFLD than in healthy subjects (109). In contrast, overweight subjects who reduced their sugar intake by replacing their usual sugar-sweetened beverages by artificially sweetened drinks showed a decrease in IHCL concentration and a loss of body weight within 12 weeks (118).

Various mechanisms can be involved in ectopic lipid deposition in the liver: 1) increased DNL; 2) increased adipose tissue lipolysis and liver FFA uptake, and/or from the diet; 3) decreased hepatic ketogenesis and/or fatty acid oxidation; 4) decreased VLDL-TG secretion, presented in Figure 6 (119).

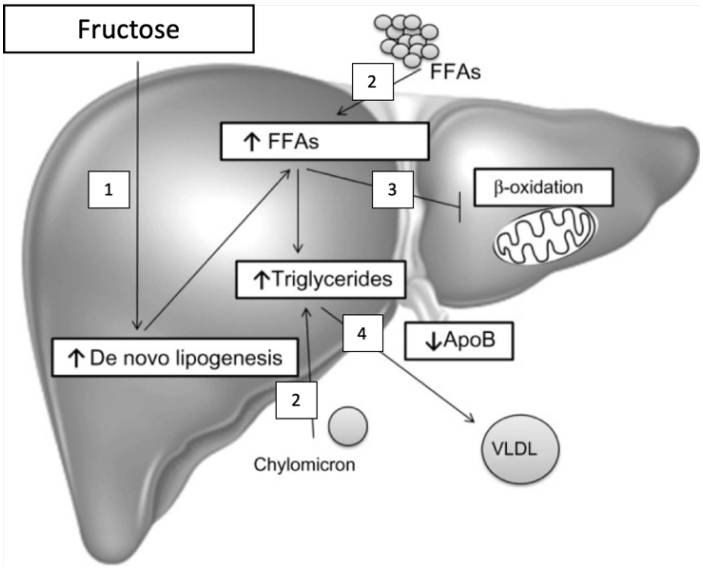


Figure 6. Regulatory mechanisms of lipid accumulation in the liver. Source, Berlanga et al., (120) modified.

In obese patients with NAFLD, it was observed, using oral stable isotopes ($^{13}C_1$-sodium acetate, 1,2,3,4 $^{13}C_4$-potassium palmitate, and $^{2}H_{31}$-glyceryl-tripalmitin), that peripheral fatty acid as well DNL contribute to the accumulation of hepatic fat and lipidic profiles (121). It was

shown that fructose markedly increased hepatic DNL and triacylglycerol production, which can then be stored in the liver, be oxidized, or be secreted in the blood in the form of VLDL-TG.

When taking experimental conditions carefully into account, it appears that experimental studies demonstrated adverse effects only when fructose was provided as part of an hypercaloric diet, while under weight-maintaining diets, adverse effects were observed only in subjects with pre-existing metabolic alterations, such as insulin resistance. In conclusion, we observed that:

- Calories overconsumption (as sugar, fructose, or fat) enhances fructose effect on IHCL in healthy (91, 92, 116) and in overweight subjects (109, 117).
- There was no carbohydrate-specific effect (fructose vs. glucose) of hypercaloric diet intake on IHCL in both healthy or overweight subjects (83, 93, 117).
- There is a dose depends effects associated with hypercaloric diet. No increased of liver fat content was observed in healthy subjects at 18% of the excess of energy as fructose (82). However, significantly increase of IHCL was seen in the healthy subject at 25% and 35% of fructose excess (91, 116).
- Even without calorie overconsumption, high sugar intake increases IHCL in overweight subjects with NAFLD (109).
- The effects of fructose intake on IHCL is also dependent on pre-existing metabolic disorders. A high sugar intake in patients with NAFLD increased IHCL more than in healthy subjects (109). In contrast, a reduction of sugar intake in overweight subjects reduced IHCL and body weight more than in normal weight subjects (118).

Intramyocellular (IMCL) lipid concentration

There are only a few studies that consider the effect of fructose intake on intramyocellular lipids (IMCL). An addition of high fructose (25% and 35%) to an isocaloric diet over seven days increased IMCL in healthy participants (91, 116) and in offsprings of type 2 diabetic patients (91). However, high fructose (35%) and high glucose (35%) overfeeding during one week in healthy volunteers showed that both monosaccharides may increase IMCL (93). Moreover, a significantly higher increase was observed after glucose intake compared to fructose. These results may reflect the different metabolic pathways used by these two monosaccharides. Compared to fructose, which is primarily metabolized in splanchnic organs, a major part of glucose is directly metabolized in muscle (123).

Moderate overfeeding with 15%E from fructose over four weeks did not show changes in IMCL in healthy volunteers (82). Similarly, when fructose was consumed in the form of sucrose and HFCS in weight maintenance diets, at the levels of 8%, 18%, and 30% over ten weeks, it did not increase lipid deposition in muscle (122).

The above described metabolic effects: altered glucose homeostasis, increased lipid profile, and ectopic fat deposition, induced by fructose are a main focus of the present work. However, fructose may also induce other harmful effects, which are briefly described below.

d) Other metabolic diseases

Blood pressure

A direct link between fructose intake and hypertension was shown in a study (124) that used data collected from the National Health and Nutrition Examination Survey in the USA (NHANES), between 2003 and 2006. It was observed, in healthy adults without a history of hypertension, that a fructose intake ≥74g per day (obtained from self-reported diet

questionnaire) in the form of table sugar and/or HFCS was associated with a higher blood pressure than in subjects with fructose intake less than 74g/day. In contrast, a metanalysis of randomized control trials (RCTs) having involved fructose interventions showed that replacement of other dietary carbohydrate with 9-25% total energy as fructose was associated with no change on blood pressure (125). Only with excessive doses of 200g fructose per day (40%E) was an increase of ambulatory blood pressure observed (126). However, an acute fructose intake showed moderate increased of blood pressure (BP) (127, 128). Moreover, elevated uric acid measure was suggested to be a mediator of the effect of fructose on BP (129).

Uric acid

Results from a national survey (NHANES-III) in the USA, performed between 1988-1994, concluded that consumption of sugar-sweetened beverages was associated with elevated serum uric acids (UA) compared to artificially sweetened, calorie-free beverages (130). Similarly, a hypercaloric diet with the addition of high fructose (35%E) for seven days, increased uric acid levels in healthy (91, 93) and type 2 diabetes patients (91). In contrast, a meta-analysis (131) that studied fructose exchange of 5% to 33% energy for other carbohydrates in an isocaloric diet, did not show an impact on UA levels.

An acute dose of 26.7g of fructose, administrated in the form of beverages, only slightly increased levels of plasma uric acid (132). In obese patients, however, acute fructose (at 30%E) intake in the form of beverages, with an isocaloric diet, increased uric acid with significantly higher responses observed in women participants than in men (96). Additionally, fructose-induced hyperuricemia was observed in patients with metabolic syndrome, and more precisely with the presence of hypertriglyceridemia and insulin resistance (133).

Chapter II - Aims and hypotheses

A survey of the literature suggests that conclusions about the potential contribution of fructose to the development of metabolic disorders strongly depends on the type of experimental design that was used.

Experimental design of studies

The previous section illustrated that studies reported often divergent effects of fructose on most of the metabolic parameters considered. Thus, small amounts of fructose in acute administrations were shown to have beneficial effects on postprandial glucose homeostasis, while larger doses and longer exposure were sometimes, but not invariably associated with altered glucose homeostasis. Part of these discrepancies may be related to the very large variation of study designs, ranging from acute administration to medium-term isocaloric substitution, to controlled overfeeding and/or supplementation of habitual diet.

In intervention studies, the metabolic effects induced by fructose may differ according to:

- study population (i.e., age, gender, health status, and BMI) and individual characteristics of volunteers
- duration of intervention
- fructose intake
- total energy ingested
- co-ingested ingredients

I will therefore briefly try to separately address the effects of fructose when administered a) as a single, acute load, b) chronically when included in an isocaloric diet, or c) in a hypercaloric diet, and d) when administered chronically as a fixed controlled supplement while the rest of the diet remains "*ad libitum* diet".

a) Acute fructose administration

Acute fructose (50g) compared to glucose or sucrose leads to lesser increase in glycemia (lower glycemic index) in healthy and diabetic patients (134). In addition, catalytic loads (7.5g) decrease glucose-induced hyperglycemia (potentiation of glucokinase and glycogen storage) (67). However, the same amount of fructose (50g) may significantly modulate plasma lipids, compared to the same amount of glucose and sucrose (103). On the other hand, fructose drinks corresponding to 25% of total energy, when included in a weight-maintaining diet, caused a greater increase of postprandial plasma TG concentration (monitored over a 24-hour period) than isocaloric glucose drinks (104). Another study showed that ingestion of a mixed meal with a 0.75 g/kg free fructose load increased postprandial TG concentration, DNL, and as well VLDL-TG (63). Similarly, a study with a liquid mixed diet containing 0.5g/kg body weight of fructose, compared with the same amount of fructose and glucose together, increased significantly higher plasma TG concentration, but not VLDL-TG (135).

In summary, an acute fructose load does not increase blood glucose, but does enhance postprandial blood TG through DNL and impaired postprandial TG clearance (63) compared to glucose or sucrose.

b) Isocaloric, low and moderate sugar diet

An isocaloric diet is defined as containing the same amount of energy daily, but with different macronutrient composition. Sometimes an isocaloric diet is meant to mean a weight-maintenance diet (WM), i.e., consuming the amount of energy corresponding to the energy expenditure. Within a WM diet, macronutrient distribution should comply with dietary guidelines. In the experimental design of isocaloric diets, the amount of fructose largely replaces sucrose or starch, and in specific cases replaces fat content, or in other cases fructose or glucose is provided in normal moderate doses.

Many studies of short duration (a few days to six weeks) performed on healthy subjects in whom fructose replaced starch at very low to moderate (5% and 25%E) intake levels did not show effects on body weight (136), postprandial TG levels (137), NAFLD (138), or on uric acid concentration (131). Beneficial effects on glucose control (79) and BP (125) were observed when fructose (7-25%E) intake was associated with an isocaloric diet. Some studies, however, reported that a higher dose in WM diet of free fructose at 25%E (108) and 30%E (139) may increase plasma TG and DNL. Meta-analyses (140) bring similar observations that high doses of fructose at 25%E compared to glucose, may raise more importantly postprandial TG, and uric acid.

In type 2 diabetic patients, however, isocaloric exchange with a dose of >60g per day was shown to increase TG levels modestly (110). On the other hand, improvement of glycemic control was observed in this type of patient, where glucose was replaced by fructose (25g–137g/day) (79).

It seems that in the isocaloric diet, fructose in general not induce adverse metabolic effects and even may have some beneficial impacts. In contrast, some very high amounts of fructose intake, even in weight-maintenance diets, may provoke adverse effects. The exact amount of a harmful dose of fructose intake is not known and may depend on individual predispositions.

c) Hypercaloric, high sugar diet

Hypercaloric diets provide an abundance of energy, in excess of personal needs. Imbalance of energy intake and expenditure may provoke the accumulation of energy, mainly in the form of fat and less in the form of lean mass. Many human short-term studies have compared the effect of a hypercaloric diet with a supplementation of fructose to that of a weight-maintenance diet, or to that of a similar hypercaloric diet with glucose supplements, in healthy, obese, and diabetic subjects. Already a moderate excess of fructose intake (1.5g/kg body weight) over four

weeks leads to increased plasma TG and glucose concentrations, but without ectopic lipid deposition or insulin resistance (82). In contrast, higher doses of fructose overfeeding (3g/kg and 3.5g/kg body weight) increased fasting plasma triglycerides, VLDL-TG, and ectopic lipids, and caused hepatic insulin resistance in healthy (91, 141) and healthy relatives of patients with type 2 diabetes mellitus (91). A metanalysis of hypercaloric studies concluded that fructose supplementation, at doses corresponding to 25% total energy or higher, resulted in an increase of fasting and postprandial triglyceride (137). Another meta-analysis observed that hypercaloric fructose intake (18-33%) may raise fasting insulin and may hence impact on development of hepatic insulin resistance (142).

Hypercaloric studies comparing fructose to glucose supplementation in healthy subjects reported that both sugars caused similar increases in ectopic fat deposition, but that fructose stimulated more DNL than glucose (93, 117). Additionally, it was observed that fructose-induced DNL in obese subjects was enhanced compared to lean subjects (107). Moreover, ectopic lipid deposition as well as muscle lipid accumulation is strongly associated with insulin resistance (143) and is common in diabetes type 2 and patients with NAFLD (144). It was observed that already, short term overfeeding of healthy volunteers with high fructose (3g/kg bw) intake induced dyslipidemia and hepatic insulin resistance (98).

High fructose intake with a hypercaloric diet was associated with increased uric acid concentration (131), and BP (145), but also with an increase of whole body weight (136) and especially visceral adipose tissue deposition (100).

In summary, it is not well known if an excess of energy intake or fructose *per se* may have metabolic consequences, or maybe it is the synergic effect of both components. Overall, the amount of fructose intake combined with overfeeding and in prolonged periods may play a role in the development of adverse effects.

d) *Ad libitum* diet with high sugar intake

In a fructose intervention with *ad libitum* diet, subjects are instructed to consume an exact amount of fructose or glucose or type of food, but remain free to choose the other foods they consume and their amount. They may be given instructions (such as: maintain your diet as usual), but their actual food intake is not monitored, and hence the intervention is likely to modify their usual dietary intake.

In some *ad libitum* studies, (100, 146), there was an increase in body weight, visceral fat, impaired glucose tolerance, and decreased insulin sensitivity when fructose was added to the diet. In other studies (82, 147), there was no effect on body weight, suggesting that additional fructose intake was compensated by a reduction of other macronutrients' intake in the *ad libitum* diet. In both conditions, however, there were alterations of blood lipids, which may contribute to IHCL deposition.

These observations suggest that fructose's effects may be related, not only to total energy intake, but also to changes in protein and fat intake in habitual diet, induced by additional fructose consumption. Over a prolonged period, these changes may induce variations in body weight and risk of metabolic syndrome, as well as cardiovascular diseases.

Summary

In summary, there are many discrepancies between studies. Some variances are attributable to experimental design. Prospective studies show an association between sugar intake and risk of disease, but have not proved causality. This may be due to fructose effects *per se* but also is indirectly mediated by effects on body weight, dietary patterns or other lifestyle factors.

Outcome of RCTs depend on the dose of fructose: A small dose may be beneficial due to catalytic effects; large doses are associated with adverse effects. The metabolic effects also depend on overall energy balance. Finally, there are other lifestyle parameters, which

significantly modulate effects of fructose and are often not taken into consideration. These parameters consist of physical activity and dietary factors such as coffee, fish oil, and protein intake, but also bariatric surgery; all these aspects are described below.

Fructose may exert multiple metabolic effects, which can have an impact on the development of metabolic diseases. In the studies presented in this PhD thesis, I will focus on three potentially adverse effects of fructose: alterations of glucose homeostasis; abnormal plasma lipid concentration; and ectopic fat deposition. A brief review of the literature discussed above is summarized in the Appendix, Table 1, with a focus on these three main metabolic outcomes.

Factors influencing the effects of fructose

There is some evidence that the harmful metabolic effects of a high fructose intake may be partially prevented to some extent by some other dietary factors or by lifestyle. The selection of dietary factors and parameters are briefly described below.

a) Selected factors

Physical activity

Physical activity, in general, is associated with many beneficial health effects, among others: the general prevention of overweight and obesity, hypertension, dyslipidemia, glucose intolerance and insulin resistance (148). Furthermore, it has been shown that many of the adverse effects of a high fructose diet may be prevented by physical activity.

The healthy volunteers during four days consumed a weight-maintenance diet containing a high fructose intake of 200g per day (corresponding to 30% of total energy intake), which significantly increased total triglycerides. However, this effect on lipoprotein metabolism was completely eliminated when subjects exercised two times per day during 30min on an ergometric bicycle at a power output of 125W (139). In another study, healthy volunteers had

their usual *ad libitum* diet supplemented with 75g of fructose per day, during 2 weeks. This supplementation was accompanied with either very low (<4500 steps/day) or high (>12500 steps/day) physical activity (149). Like in the previous study, very low physical activity with an excess of fructose intake resulted in increased postprandial TG and VLDL-TG concentration, and increased physical activity seems to prevent these effects. Additionally, it was observed that the mixed meal of high fructose (0.75g/kg bw) and high fat-diet (0.5g/kg bw), may induce postprandial lipemia in healthy volunteers. However, performing the acute resistance exercise during 95min the evening prior to the high fructose, high fat meal significantly decrease TG concentration due to this meal (150).

Coffee - Polyphenols

Polyphenols are an abundant group of micronutrients naturally present in plants, herbs, vegetables, fruits, nuts, and seeds. They are a large group, consisting of four major classes: flavonoids, lignans, phenolic acids, and stilbenes; in total over 500 different polyphenols are known. They are characterized by antioxidant and anti-inflammatory proprieties, which are recognized in the prevention of degenerative diseases, i.e., cardiovascular (151).

Coffee is one of the most widely consumed beverages worldwide, and their consumption was associated with lower risk of type 2 diabetes (152, 153), and beneficial impact on metabolic syndrome and obesity (154). Not only coffee with caffeine but also decaffeinated coffee was associated with lower risk of type 2 diabetes (155) when consumed two or more times per day (156). This observation suggests that other components than caffeine may be involved in the positive effects of coffee. There is more than one mechanism proposed by which coffee may exert its protective effects. It was observed that coffee consumption decrease pro-inflammatory biomarkers (interleukin (IL)-1 b, IL-6) of type 2 diabetes (157). On the other hand, specific coffee components, like chlorogenic acid, also found in fruits and vegetables, may play a role

through the gut and liver. In the gut may alter incretin secretion (GIP, GLP-1) and glucose absorption. In the liver, it may impact glucose production through decreased hepatic glucose-6-phosphate (158).

One of the first and quickly appearing adverse effects observed after consumption of high fructose was increased of fatty liver deposition (91), and decreases of hepatic insulin sensitivity (83). It was observed that patients with fatty liver disease, who increased consumation of coffee, significantly decreased the risk of development of fibrosis (159). However, in healthy volunteers, four cups of coffee during 14 days did not prevent IHCL accumulation induced by short time fructose (4g/kg body weight daily) overfeeding. In contrast, lipid oxidation was significantly increased and positive effects were observed on hepatic insulin resistance (152).

Fish oil - Polyunsaturated fatty acids (omega 3)

Supplementation with fish oil, rich in omega-3, may have some moderated improvements on glycemia and insulin sensitivity in patients with type 2 diabetes mellitus without inducing hypertriglyceridemia. Moreover, omega-3 was observed to have some protective effects against cardiometabolic diseases, by decreasing triglycerides concentration (160, 161). Fish oil added to the *ad libitum* diet increased basal lipid oxidation, which over time may have some improvements in the regulation of fat metabolism (162). During 28 days, supplementation with fish oil leads to significantly increased serum in omega-3 but does not increase plasma TG, compared to the control diet (98). Previously, it was observed that supplementation of a normal diet with 3g/kg body weight per day of fructose during six days increased significantly fasting TG concentration. When supplementation with fish oil was combined with high fructose intake, during 6 days, plasma TG concentration was significantly lower compared to supplementation with fructose alone (98). It seems that the addition of fish oil abolished the effect of fructose on plasma TG.

b) Gastric Surgery - RYGB

In the most severe cases of obesity, diet and physical activity often fail to induce long-lasting weight loss, and bariatric surgery may be indicated. Roux-en-Y gastric bypass (RYGB), is presently considered as the most effective surgical procedure for weight loss. RYGB surgery allows an individual to reduce excess weight by more than 50% in the majority of cases, and to maintain weight loss over extended periods of time (up to 15 years) in many patients (163). With RYGB procedure, the stomach is divided into a very small proximal pouch with a capacity of about 10-20 ml, and a larger, distal gastric "remnant". The proximal small pouch accommodates ingested nutrients, and is directly anastomosed to the mid-jejunum. The remaining distal stomach, duodenum and proximal jejunum are therefore bypassed and re-anastomosed "in Y" to allow for the delivery of pancreatic and biliary secretions (164) (Figure 5).

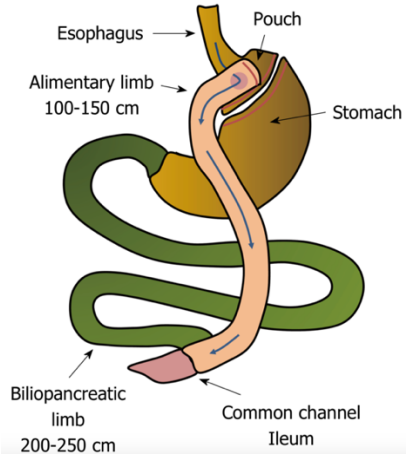


Figure 5. Source: Anatomical changes in gastrointestinal tract RYGB (165).

After RYGB, food intake is in part limited by the small size of the remnant gastric pouch (restrictive component). It however appears that accelerated nutrient transit, and early secretion of gastro-intestinal hormones, may directly signal food intake inhibition in the brain. Finally, due to the fact that approximately 100 cm of dueodenum and jejunum are bypassed, some nutrient malabsorption may be present as well (malabsorptive component), presented in Figure

6. A side-effect of this component is the frequent occurrence of malabsorption of vitamins, and minerals after RYGB.

Due to the bypass of gastric segments primarily involved in carbohydrate absorption, and late mixing with pancreatic secretion, one may have expected that carbohydrate absorption would have been impaired after RYGB. This is however not the case, and glucose absorption after a glucose load is substantially accelerated compared to non-operated controls (166, 167).

In contrast, postprandial triglycerides and chylomicron-TG responses were completely blunted in RYGB patients compared to the non-operated control group after a standardized, solid breakfast intake (168). These results may be due to delayed or suppressed intestinal lipid absorption after the short intestinal circuit in RYGB (169). Another suggestion proposes faster digestion and absorption, but also enhanced clearance of TG-rich lipoprotein (168), which was indicated by earlier secretion bile acids (BA). BA are known for their favoring lipid digestion and absorption (170). Increased BA may cause stimulation of GLP-1 secretion and impact lipid homeostasis, and FGF19, which may stimulate liver lipid oxidation (171).

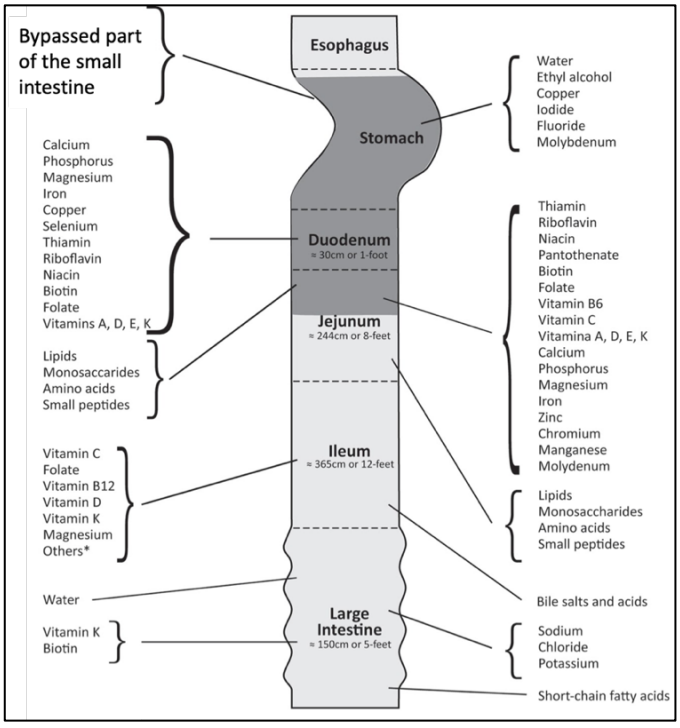


Figure 6. Nutrient absorption in the digestive tract and RYGB impact. Source: Gropper and Smith, 2016.

c) Protein intake – Amino Acid

Recently, it was also shown that protein intake may significantly reduce hepatic lipid deposition induced by overfeeding with a high-fat diet in healthy volunteers (172). Additionally, a 4-week supplement with 60g per day of whey protein, for obese female patients who otherwise consumed their normal, *ad libitum* diet, significantly reduced intrahepatic fat and fasting plasma triglycerides concentration (173). Moreover, in healthy volunteers, a hypercaloric diet with high fructose intake (3g/kg body weight) significantly increased also hepatic fat deposition, but this diet combined with an essential amino acid supplementation (around 20g per day), was shown to blunt this effect of fructose (141).

In subjects receiving a supplement of fructose while the rest of the diet was left *ad libitum*, there was a significant decrease in carbohydrates, fat, but also protein ($1.8 \pm 3.4\%$) intake (147). Given the protective effects of protein supplements on fructose-induced metabolic risk, one may wonder whether a reduction in protein intake favors metabolic risk, and contribute to fructose's adverse effects.

The mechanisms involved in the protective effect of high protein intake during overfeeding are not identified yet. On one hand, fractional hepatic DNL was unchanged by essential amino acid supplementation; on the other hand, VLDL-TG secretions were significantly increased. This may suggest that protein may increase VLDL-TG secretion, thus reducing hepatic lipid storage (141). Additionally, it was observed that a high amount of protein added to a high-fat diet, compared to high-fat diet only, increased the expression of peroxisome proliferator–activated receptor γ (PPARG). PPARG is a receptor mostly expressed in adipose tissue, and plays a role in the regulation of fatty acid storage (adipogenesis); activation of PPARG also prevents insulin resistance and preserves glucose homeostasis (172). Finally, it was observed that secretion of bile acids is enhanced with a high protein diet, and bile acids may in turn activate lipid

oxidation in the liver by activating bile acid receptors, farnesoid X receptors, (FXRs) (172, 174).

Summary

This brief overview of the literature suggests that some effects induced by fructose are modulated by other dietary and non-dietary factors. These aspects should also be considered in nutritional recommendations and be highlighted in the prevention of obesity.

The aim of my PhD thesis was therefore to assess two specific conditions for which one could postulate that fructose metabolism and its long-term consequences would be altered:

1. Bariatric surgery
2. High protein intake

Chapter II Aims and hypotheses

The aim of the present PhD work was to investigate whether and how the effects of fructose on cardio-metabolic risk factors were modulated by other digestive or nutritional parameters. We selectively tested whether:

Study 1. Roux-en-Y gastric bypass surgery altered postprandial fructose kinetics, *de novo* lipogenesis (with a special focus on intestinal *de novo* lipogenesis), and blood lipid profiles. Due to this surgical procedure, the part of intestine areas involved in fructose absorption, and then in the gut *de novo* lipogenesis, is bypassed.

Study 2. The effects of a short-term high-fructose diet were modulated by the concomitant dietary protein content. Several rapports suggest that a high dietary protein intake may have a protective effect on hepatic lipids deposition.

Study I.

Title: Effects of roux-en-Y gastric bypass surgery on postprandial fructose metabolism.

This randomized controlled study was performed on eight patients, 12-17 months after RYGB and on eight control (Ctrl) subjects, matched for age, BMI, and sex. Each participant was studied after ingestion of a protein and lipid meal (PL) and after ingestion of a protein, lipid, fructose, and glucose meal with labeled ^{13}C-fructose (PLFG). Postprandial blood glucose, fructose, lactate, apolipoprotein B48 (apoB48), and triglyceride concentrations were measured. In addition, isotopic-based methods were used to assess the relative fructose disposal pathways (i.e., oxidation, gluconeogenesis, lactic acid production, or *de novo* lipogenesis).

Specific hypotheses: RYGB, may impact gut fructose metabolism by decreasing fructose absorption, intestinal gluconeogenesis, and *de novo* lipogenesis, which may have an impact on postprandial glucose and TG plasma levels.

Personal contribution: Analyzed data and prepared the draft of the manuscript.

Manuscript I.

Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Effects of Roux-en-Y Gastric Bypass Surgery on Postprandial Fructose Metabolism

Anna Surowska[1], Sara De Giorgi[1]*, Fanny Theytaz[1], Vanessa Campos[1], Leanne Hodson[2], Nathalie Stefanoni[1], Valentine Rey[1], Philippe Schneiter[1], Martine Laville[3], Vittorio Giusti[4], Laure Gabert[3], and Luc Tappy[1,4]*

Objective: Fructose is partly metabolized in small bowel enterocytes, where it can be converted into glucose or fatty acids. It was therefore hypothesized that Roux-en-Y gastric bypass (RYGB) may significantly alter fructose metabolism.

Methods: We performed a randomized clinical study in eight patients 12-17 months after RYGB and eight control (Ctrl) subjects. Each participant was studied after ingestion of a protein and lipid meal (PL) and after ingestion of a protein+lipid+fructose+glucose meal labeled with ^{13}C-fructose (PLFG). Postprandial blood glucose, fructose, lactate, apolipoprotein B48 (apoB48), and triglyceride (TG) concentrations, ^{13}C-palmitate concentrations in chylomicron-TG and VLDL-TG, fructose oxidation ($^{13}CO_2$ production), and gluconeogenesis from fructose (GNGf) were measured over 6 hours.

Results: After ingestion of PLFG, postprandial plasma fructose, glucose, insulin, and lactate concentrations increased earlier and reached higher peak values in RYGB than in Ctrl. GNGf was 33% lower in RYGB than Ctrl ($P = 0.041$), while fructose oxidation was unchanged. Postprandial incremental areas under the curves for total TG and chylomicrons-TG were 72% and 91% lower in RYGB than Ctrl ($P = 0.064$ and $P = 0.024$, respectively). ApoB48 and ^{13}C-palmitate concentrations were not significantly different.

Conclusions: Postprandial fructose metabolism was not grossly altered, but postprandial lipid concentrations were markedly decreased in subjects having had RYGB surgery.

Obesity (2016) **24**, 589–596. doi:10.1002/oby.21410

Introduction

Glucose homeostasis is markedly improved in insulin-resistant patients after Roux-en-Y gastric bypass (RYGB) surgery. This effect is in part independent of body weight loss and may involve early absorption of dietary carbohydrates together with enhanced secretion of gut peptides stimulating insulin secretion (1-3). RYGB also corrects dyslipidemia in patients with obesity (4,5), but the mechanisms responsible for improved lipid homeostasis remain largely unknown. Recent observations indicate that blunted postprandial hypertriglyceridemia occurs together with earlier and enhanced rises in plasma bile acid concentrations, suggesting that dietary lipid absorption is indeed accelerated after RYGB (3,6).

In healthy subjects, dietary sugars have long been known to stimulate both gluconeogenesis and *de novo* lipogenesis (7,8). Proximal small bowel enterocytes express the fructose-metabolizing enzymes fructokinase and aldolase B, as well as gluconeogenic and lipogenic enzymes (9,10), and hence contribute to these processes (11). We therefore hypothesized that gut fructose metabolism may be altered in RYGB patients, and that a decrease of fructose absorption, intestinal gluconeogenesis, and *de novo* lipogenesis may contribute to lower postprandial glucose and triglyceride (TG) concentrations. To assess this hypothesis, we monitored postprandial plasma glucose, fructose, lactate, and TG concentrations and ^{13}C isotopic enrichment after ingestion of a meal containing protein, lipid, glucose, and ^{13}C-labeled fructose. To better evaluate the specific effects elicited by sugars, plasma glucose, lactate, and TG concentrations were also measured in the same participants after ingestion of the lipid + protein part of the meal without sugar.

[1] Department of Physiology, University of Lausanne, Lausanne, Switzerland [2] Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK [3] Centre De Recherche En Nutrition Humaine Rhône Alpes, Centre Européen De Nutrition Pour La Santé, Hospices Civils De Lyon, Université Lyon 1, Pierre Bénite, France [4] Service of Endocrinology, Diabetes and Metabolism, Lausanne University Hospital, Lausanne, Switzerland. Correspondence: Luc Tappy (luc.tappy@unil.ch)

Funding agencies: This work was supported by grants 320030-138428 and 320030-135782 from the Swiss National Foundation for Science to Luc Tappy. Leanne Hodson is a British Heart Foundation Intermediate Fellow in Basic Science.

Disclosure: Luc Tappy has received financial support from Nestlé SA, Switzerland, and Ajinomoto Co. Inc., Japan, for studies unrelated to the present study. The other authors declared no conflict of interest.

Author contributions: L.T.: designed the study; F.T., S.D.G., and V.G.: recruited participants and performed the tests; A.S. and V.C.: analyzed other data and performed the statistical analysis; L.G., M.L., L.H., P.S., and V.R. performed all mass spectrometry measurements; N.S.: performed clinical chemistry measurements; F.T., S.D.G., and L.T.: drafted the manuscript; and all authors revised the manuscript.

*A.S. and S.D.G. contributed equally to this work.

Received: 24 August 2015; **Accepted:** 26 October 2015; **Published online 25 February 2016. doi:10.1002/oby.21410**

Materials and Methods

Subjects inclusion

Eight subjects with former obesity (five women, three men) having undergone RYGB 12 to 17 months earlier were included in this study. All were treated with intramuscular vitamin B12 every 2-3 months and with daily oral multivitamin supplements. None was receiving insulin, antidiabetic agents, lipid-lowering drugs, or antihypertensive therapy. Eight healthy age-, weight-, and gender-matched nonoperated subjects were recruited as a control group (Ctrl). All volunteers were nonsmokers, drank less than two servings of alcoholic beverages daily, and had low to moderate habitual physical activity. The experimental protocol was approved by the Commission d'éthique pour la recherche humaine de l'Etat de Vaud, Switzerland, and all participants provided an informed written consent. The experimental protocol was registered at clinicaltrials.gov, NCT02160379.

Experimental protocol

Participants were studied on two different occasions according to a randomized cross-over design. On each occasion, they received a controlled weight maintenance diet providing 1.5 times basal energy requirements calculated with the Harris-Benedict equation containing 55% carbohydrate (35% complex carbohydrate and 20% sucrose), 15% protein, and 30% fat during 3 days. On the fourth day, subjects came to the Clinical Research Center of Lausanne University Hospital at 7:00 am in the fasting state and underwent a metabolic test with the ingestion of one of the two following test meals:

A. Protein + lipid + fructose + glucose (PLFG) meal containing 11.5 kcal/kg body weight, 0.3 g/kg lipid (from cream), 0.3 g/kg whey protein (Sponser, Wollerau, Switzerland), 0.5 g/kg fructose labeled with 1% U-$^{13}C_6$-fructose (Cambridge Isotope Laboratories, Tewksbury, MA), and 0.5 g/kg glucose.
B. Protein + lipid (PL) meal corresponding to the PLFG meal in which glucose and fructose were omitted. It contained 7.8 ± 0.01 kcal/kg body weight, 0.3 g/kg lipid, and 0.3 g/kg protein.

The order of administration of the two meals was randomized, and a washout period of 3-10 weeks was allowed between metabolic tests.

Each metabolic test included a 2-h fasting period and a 6-h postprandial period. On arrival, subjects were asked to void their bladder, the collected urine was discarded, and all urine was thereafter collected for determination of the urinary urea nitrogen excretion rate. Subjects were weighed, and their body composition was assessed by bio-electrical impedancemetry (Imp Df 50; ImpediMed, Pinkenba, Australia). They were then transferred to a bed, and a venous catheter was inserted into an antecubital vein of one arm and was used for blood sampling. A second catheter was inserted into an antecubital vein of the other arm and a primed-continuous infusion of tracer amounts of 6,6-$^2H_2$-glucose (Cambridge Isotope Laboratories, Cambridge, MA; bolus 2.8 mg/kg, continuous infusion 40 μg/kg/min) was administered through this catheter throughout the metabolic test to calculate whole-body glucose rates of appearance (GRa) and of disappearance (GRd) (12). Total carbon dioxide production (VCO_2) was monitored throughout the experiment by open circuit indirect calorimetry (Quark RMR, version 9.1b, Cosmed, Rome, Italy). Blood samples were collected immediately before starting the 6,6-$^2H_2$-glucose administration ($T = -120$ min) and after 90 min and 120 min spent in fasting conditions ($T = -90$ and 0 min); thereafter, subjects consumed their test meal over a 15-min period ($T = 0$ min), during which indirect calorimetry was briefly interrupted. Blood samples were then collected at $T = 30$, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min. Hormones, metabolite concentrations, and plasma 6,6-$^2H_2$-glucose were measured at each time point, while ^{13}C-lactate, ^{13}C-glucose enrichments, and ^{13}C-palmitate concentrations in chylomicron ($S_f > 400$) and in VLDL (S_f 20-400) subfractions were measured at times $T = 0$, 30, 60, 120, 180, 240, and 360 min after PLFG. ^{13}C-palmitate enrichment of chylomicron- and VLDL-TG is an indicator for lipogenesis *de novo*, respectively, in the intestine and in the liver. Breath samples were collected for the measurement of $^{13}CO_2$ isotopic enrichment at $T = -120$, −60, 0, 60, 120, 180, 240, 300, and 360 min after the PLFG meal.

Analytical procedures

Plasma glucose, TG, cholesterol, high-density lipoprotein (HDL)-cholesterol, and lactate and urinary urea were measured by enzymatic methods (Randox Laboratories, Crumlin). Insulin and glucagon were assessed by radioimmunoassay (Millipore, Billerica, MA). Plasma apolipoprotein B (apoBtot) and apolipoprotein B48 (apoB48) were measured by ELISA using kits from Shibayagi, Shibukawa, Japan, and R & D Systems, Abingdon. Plasma fructose concentrations and plasma 6,6-$^2H_2$-glucose isotopic enrichment were measured by gas chromatography-mass spectrometry (GC-MS). For the measurement of fructose concentration an internal standard of 2.3 μm 1,2,3 $^{13}C_3$ D-fructose was added to 250 ml plasma or urine prior to derivatization. Plasma samples were thereafter deproteinized using the $ZnSO_4$-$Ba(OH)_2$ method (13), partially purified over anion- and cation-exchange resins, and derivatized with acetic anhydride and pyridine. Samples were then dried under a stream of nitrogen and resuspended in 60 μl ethyl acetate. One μl of it was analyzed by GC-MS (Agilent Technologies, Santa Clara, CA) in electron impact mode. The fructose concentration in samples was determined from the ratio of *m/z* 277 to *m/z* 275 by means of an unlabeled pure fructose standard curve. Plasma 6,6-$^2H_2$ glucose enrichment was measured with the chemical ionization mode with selective monitoring of *m/z* 333 and *m/z* 331. Plasma ^{13}C-glucose isotopic enrichment was measured by gas-chromatography-isotope ratio mass spectrometry (GC-C-IRMS), as described (14). Plasma lipoprotein subfractions were separated by ultracentrifugation, and fractions $S_f > 400$ (chylomicrons) and S_f 20-400 (VLDL and chylomicron remnants) were isolated. Fatty acid methyl esters (FAMEs) from chylomicron- and VLDL-TG were isolated, and their ^{13}C enrichment was measured by GC-C-IRMS, as described (15). Tricosanoic acid methyl ester was used as an isotopic enrichment standard, and a quality control sample (certified standard of eicosanoic acid FAME; Department of Geological Sciences, Indiana University, Bloomington, IN) was run with each set of samples.

Calculations

A. GRa was calculated with 6,6-$^2H_2$-glucose as:

$$\text{GRa (mg/min)} = \frac{F\text{-}pV\left[\frac{G1+G2}{2}\right] * \left[\frac{E2\text{-}E1}{t2\text{-}t1}\right]}{\frac{E1+E2}{2}}$$

where F is the 6,6-$^2H_2$-glucose infusion rate (mg/min), p is the pool fraction, set at 0.65, V is the glucose distribution volume,

TABLE 1 Characteristics of study participants

	RYGB before surgery	RYGB after surgery	Ctrl	*P* value, RYGB before surgery vs. after surgery	*P* value, RYGB before surgery vs. Ctrl	*P* value, RYGB after surgery vs. Ctrl
Age (years)	37.7 ± 2.0	38.9 ± 3.0	38.8 ± 3.1			
Time elapsed since RYGB (months)	–	14.0 ± 0.6	–			
Body weight (kg)	120.5 ± 8.8	80.2 ± 7.2	86.9 ± 7.3	<0.0001*	0.0004*	0.124
BMI (kg/m^2)	44.2 ± 2.0	28.9 ± 2.0	29.2 ± 2.4	<0.0001*	<0.0001*	0.660
Body fat at inclusion (%)	–	29.3 ± 2.8	31.9 ± 3.9	–	–	0.442
Systolic blood pressure (mm Hg)	–	115.5 ± 4.4	126.3 ± 5.1	–	–	0.016*
Diastolic blood pressure (mm Hg)	–	72.3 ± 2.3	76.6 ± 3.8	–	–	0.172
Heart rate (beats/min)	–	72.5 ± 4.7	74.8 ± 2.7	–	–	0.700
Fasting blood glucose (mmol/L)	7.6 ± 0.9	4.7 ± 0.1	4.7 ± 0.1	0.019*	–	0.838
Fasting insulin (pmol/L)	80.0 ± 18.6[a]	61.7 ± 6.3	62.2 ± 4.7	0.753	0.399	0.952
Fasting total cholesterol (mmol/L)	5.1 ± 0.5	3.8 ± 0.3	4.3 ± 0.2	0.043*	0.050*	0.158
Fasting HDL-cholesterol (mmol/L)	1.4 ± 0.18	1.0 ± 0.03	0.9 ± 0.07	0.033*	0.056	0.369
Fasting blood triglycerides (mmol/L)	1.7 ± 0.31	0.8 ± 0.06	1.1 ± 0.17	0.010*	0.016*	0.076
Fasting uric acid (mmol/L)	–	0.31 ± 0.02	0.37 ± 0.06	–	–	0.273

Data are expressed as mean + SEM; *$P < 0.05$.
[a]Results were available for four participants.

set at 0.2, G is the glucose concentration (mg/l), E is the 6,6-$^2H_2$-glucose isotopic enrichment (mol% excess), and t is the time of collection (min) (16). Parameters were set assuming that kinetics for ^{13}C-glucose appearance in blood after ingestion of a ^{13}C-labeled glucose load apply to the ingestion of a ^{13}C-labeled fructose load as well (17).

B. Gluconeogenesis from fructose (GNGf) was calculated from ^{13}C-glucose appearance (^{13}CGRa):

$$GNGf = \frac{\left(\mathrm{GRa} * \left[\frac{^{13}\mathrm{CG(t1)}+^{13}CG(t2)}{2}\right] + p * V * \left[\frac{G(t1)+G(t2)}{2}\right] * \left[\frac{^{13}CG(t2)+^{13}CG(t1)}{t2-t1}\right]\right)}{^{13}C\text{-}fructose}$$

where ^{13}CG is the plasma ^{13}C-glucose isotopic enrichment and ^{13}C-fructose is the meal ^{13}C-fructose isotopic enrichment (at% excess).

C. Fructose oxidation (Fox) was calculated as:

$$\mathrm{Fox(mg/min)} = 180 * \frac{^{13}CO2(tx)}{^{13}C\text{-}fructose(tx)} * \frac{VCO2(tx)}{22.29 * 6 * 0.8}$$

where $^{13}CO_2$ is the breath CO_2 isotopic enrichment (atom% excess), VCO_2 is the total CO_2 production (l/min), and tx is the time of collection; 22.29 ml CO_2 was assumed to correspond to 1 mmol CO_2; 6 mmol CO_2 correspond to 1 mmol = 180 mg fructose; 0.8 is the recovery factor of $^{13}CO_2$ in breath.

D. Nonoxidative fructose disposal (NOFD) was calculated as:
NOFD (mg/360 min) = (ingested fructose (g)) − (fructose oxidation, cumulated between 0 and 360 min (g)).

E. Energy expenditure and net substrate oxidation were calculated using the equations of Livesey and Elia (18), assuming that total nitrogen excretion was equal to (urinary urea nitrogen excretion)/0.85 (19).

F. Sugar-induced postprandial hypertriglyceridemia was calculated as:

$$\mathrm{iAUC{-}TG_{PLGF}} - \mathrm{iAUC{-}TG_{PL}}$$

Statistical analysis

The normality of data was checked with Shapiro-Wilk tests for all parameters analyzed, and non-normally distributed data were log-transformed before statistical analysis. The effect of meal (PL and PLFG) on all variables measured at different time points was assessed by two-way ANOVA with interaction, with time and group (Ctrl and RYGB) as independent variables. All variables were also reduced to a single value by calculating their average or cumulated postprandial values (gluconeogenesis from fructose, fructose oxidation, net substrate oxidation) or their incremental area under the curve values (iAUC: plasma concentrations of metabolites or hormones) over the 360-min postprandial period prior to t-tests with Bonferronni's correction. All statistical analyses were performed using STATA version 10 (Stata Corp, College Station).

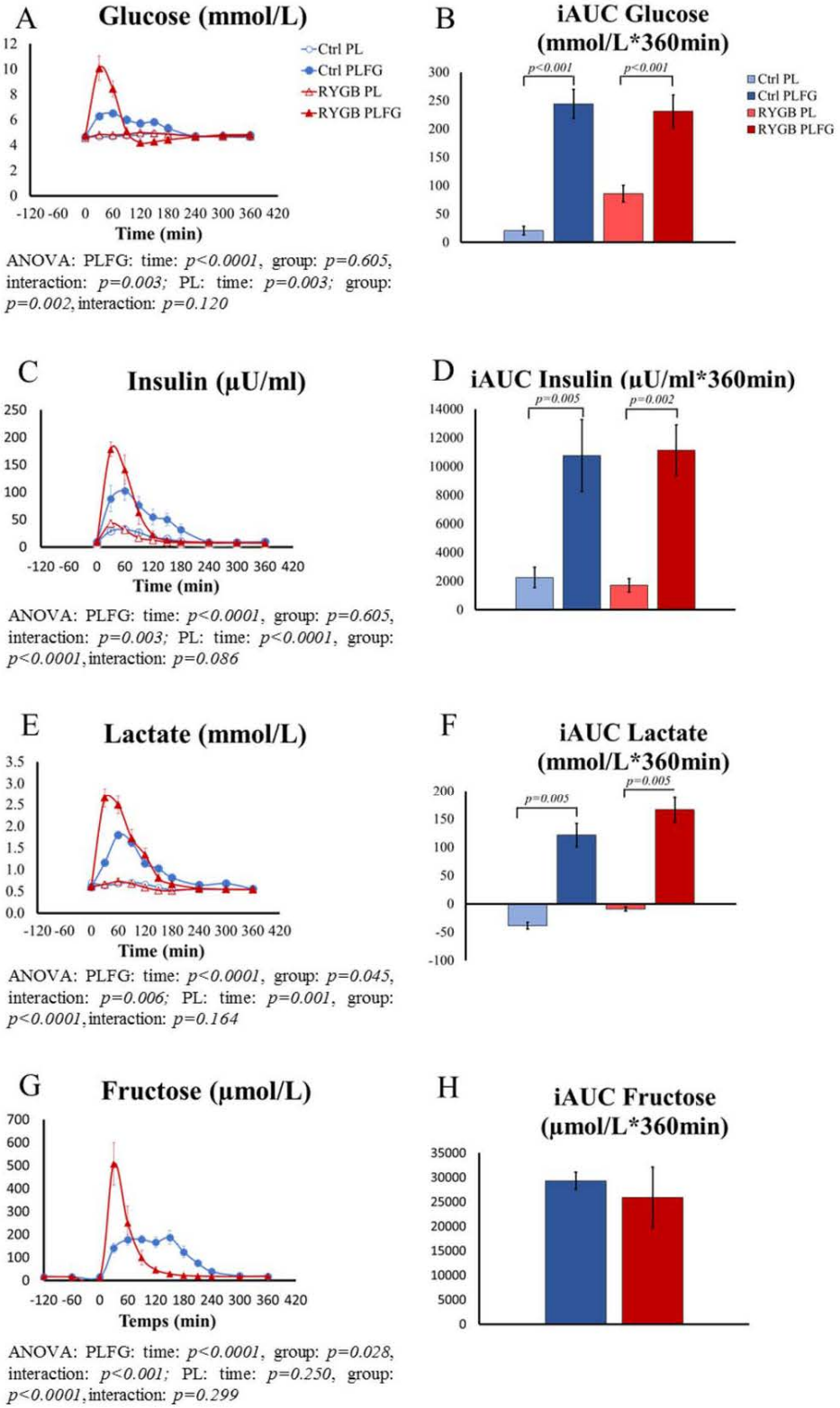


Figure 1 Plasma glucose, insulin, lactate, and fructose responses to meal ingestion. The time course for these parameters is presented on the left side of the figure (panels A, C, E, and G) and their corresponding iAUCs on the right side (panels B, D, F, and H). Data are reported as mean + SEM for $n = 8$. The test meal was given at $T = 0$.

TABLE 2 Different metabolic pathways of fructose at the end of the postprandial period (6 h)

	Ctrl PLFG	RYGB PLFG	*P* value
Fructose oxidation (g/6 h)	11.5 ± 0.5	12.4 ± 1.1	0.474
Fructose stored (g/6 h)	27.0 ± 2.4	23.7 ± 1.6	0.266
GRa (g/6 h)	71.0 ± 4.7	68.1 ± 3.6	0.639
GRd (g/6 h)	71.4 ± 4.9	67.5 ± 3.6	0.524
GNGf (g/6 h)	11.2 ± 1.1	7.6 ± 1.2	0.041*

Data are expressed as mean + SEM; *$P < 0.05$.

Results

Body weight, body fat mass, waist circumference, and fasting plasma glucose, insulin, total TG, total cholesterol, and HDL-cholesterol concentrations in RYGB subjects before and after surgery and in Ctrl at inclusion are shown in Table 1. RYGB decreased body weight, BMI, fasting blood glucose, fasting total cholesterol, HDL-cholesterol, TG, and uric acid (all $P < 0.05$) compared to pre-surgery values. There were no statistically significant differences between RYGB and Ctrl, except for lower systolic blood pressure ($P = 0.016$) in RYGB after surgery.

After ingestion of PLFG, plasma glucose, fructose, insulin, and lactate concentrations increased rapidly and peaked earlier in RYGB than in Ctrl, but they declined rapidly thereafter (Figure 1). There were no significant between group differences for total iAUCs (Figure 1). Plasma glucose iAUC $_{(0\text{-}120\ min)}$, calculated for the initial 2-h postprandial period (time 0-120), was higher in RYGB than in Ctrl (270.7 ± 28.4 vs. 158.0 ± 19.1 mmol/l*120 min, $P = 0.001$). iAUCs $_{(0\text{-}120\ min)}$ for insulin (10,857.4 ± 1619.5 vs. 7869.9 ± 1718.5 μU/ml*120 min, $P = 0.589$) and fructose (23,606.2 ± 5136.4 vs. 15,068.4 ± 789.3 μmol/l*120 min, $P = 0.166$) were not statisically different. Thirty percent of the ingested fructose was oxidized to CO_2 in the Ctrl group and thirty-four percent in RYGB over the 6-h postprandial. (P = ns). These estimates correspond to the sum of fructose oxidized in splanchnic tissues and of whole-body oxidation of glucose and lactate produced from fructose. Nonoxidative fructose disposal was also not different in RYGB (65.6%) and Ctrl (70.2%). Assuming that fructose absorption was essentially complete in both groups within the postprandial period, this figure corresponds to fructose carbons stored within the body. Total glucose appearance and disappearance calculated over the 360-min postprandial period were similar in RYGB and Ctrl subjects. GNGf was slightly but significantly lower (33%) in RYGB compared to Ctrl (Table 2).

In Ctrl subjects, plasma, chylomicron-, and VLDL-TG concentrations and plasma apoB48 concentrations increased significantly after ingestion of PL, while plasma apoBtot was not significantly altered (basal: 5.4 ± 1.0 μg/ml, average postprandial 7.4 ± 1.0 μg/ml, P = ns). Ingestion of PLFG slightly but nonsignificantly increased plasma chylomicron- and VLDL-TG above values observed after PL (Figure 2D, F). The concentration of ^{13}C-palmitate in chylomicron- and VLDL-TG increased progressively after PLFG, to reach a peak between 180 and 240 min after meal consumption (Figure 3).

Compared to Ctrl subjects, the postprandial increases in plasma, chylomicron-, and VLDL-TG were completely inhibited in RYGB subjects (Figure 2C, E). In contrast, apoB48 concentrations increased after both PL and PLFG and peaked earlier in RYGB than Ctrl subjects. Incremental apoB48 AUCs observed in RYGB and Ctrl showed no significant differences (Figure 2H). The increases in ^{13}C-palmitate concentrations in chylomicron and VLDL were not significantly different in RYGB and Ctrl subjects (Figure 3).

Discussion

Several previous studies have documented that the rate of absorption of an oral glucose load is increased after RYGB, resulting in early, enhanced postprandial blood glucose peaks. This altered kinetics of glucose absorption is thought to be secondary to an early postprandial appearance of nutrients in the jejunum and is associated with an enhanced secretion of glucagon-like peptide 1, postprandial hyperinsulinemia, and an increase in postprandial glucose clearance (1,20,21). Vertical sleeve gastrectomy is associated with similar changes in postprandial glycemic responses as RYGB (22,23), which strongly suggests that accelerated delivery of nutrients to the distal small bowel, rather than bypassing the duodenum and the initial portion of the jejunum, may be instrumental in improving glucose homeostasis after bariatric surgery.

Our major novel observations are that: (1) blood fructose concentration peaked earlier after RYGB, but that its relative disposal into gluconeogenesis, lactate production, and *de novo* lipogenesis was not markedly altered; and (2) the increase in postprandial plasma TG concentrations elicited by ingestion of a fat-containing mixed meal, with or without sugars, was nearly abolished after RYGB.

Ingestion of a fructose-containing meal was associated with an early postprandial increase of blood fructose concentrations after RYGB. The total iAUC of blood fructose was similar to that observed in Ctrl, suggesting that RYGB did not induce gross fructose malabsorption. The addition of tracer amounts of ^{13}C-fructose to the meal allowed us to gain further insights into the metabolic pathways used for fructose metabolism in RYGB patients. The rate of appearance of ^{13}C-labeled glucose, which provides an estimate of the portion of fructose carbons converted into glucose and released into the blood, was slightly, but significantly decreased after RYGB. Since both proximal small bowel enterocytes and liver cells can convert fructose into glucose, one may speculate that bypassing the proximal small bowel may indeed decrease gluconeogenesis due to low fructokinase and aldolase B expression in distal than in proximal small bowel enterocytes (9). Alternatively, this may be due to an increased proportion of newly synthesized glucose entering the hepatic glycogen synthesis pathway rather than being released into the blood. Finally, since the kinetics of blood ^{13}C-glucose were markedly accelerated after RYGB, this may merely reflect an underestimation of true glucose synthesis by the one compartment model equations used in our calculation. Whatever the explanation, integrated blood fructose response, total glucose appearance, and postprandial fructose oxidation were not different in RYGB and Ctrl, and the rate of ^{13}C-glucose appearance was only decreased by 2-3 g, corresponding to less than 10% of the ingested fructose load. Taken together this indicates that RYGB was not responsible for major fructose malabsorption.

Our data allow comparing postprandial TG responses after ingestion of test meals containing the same amount of fat with and without

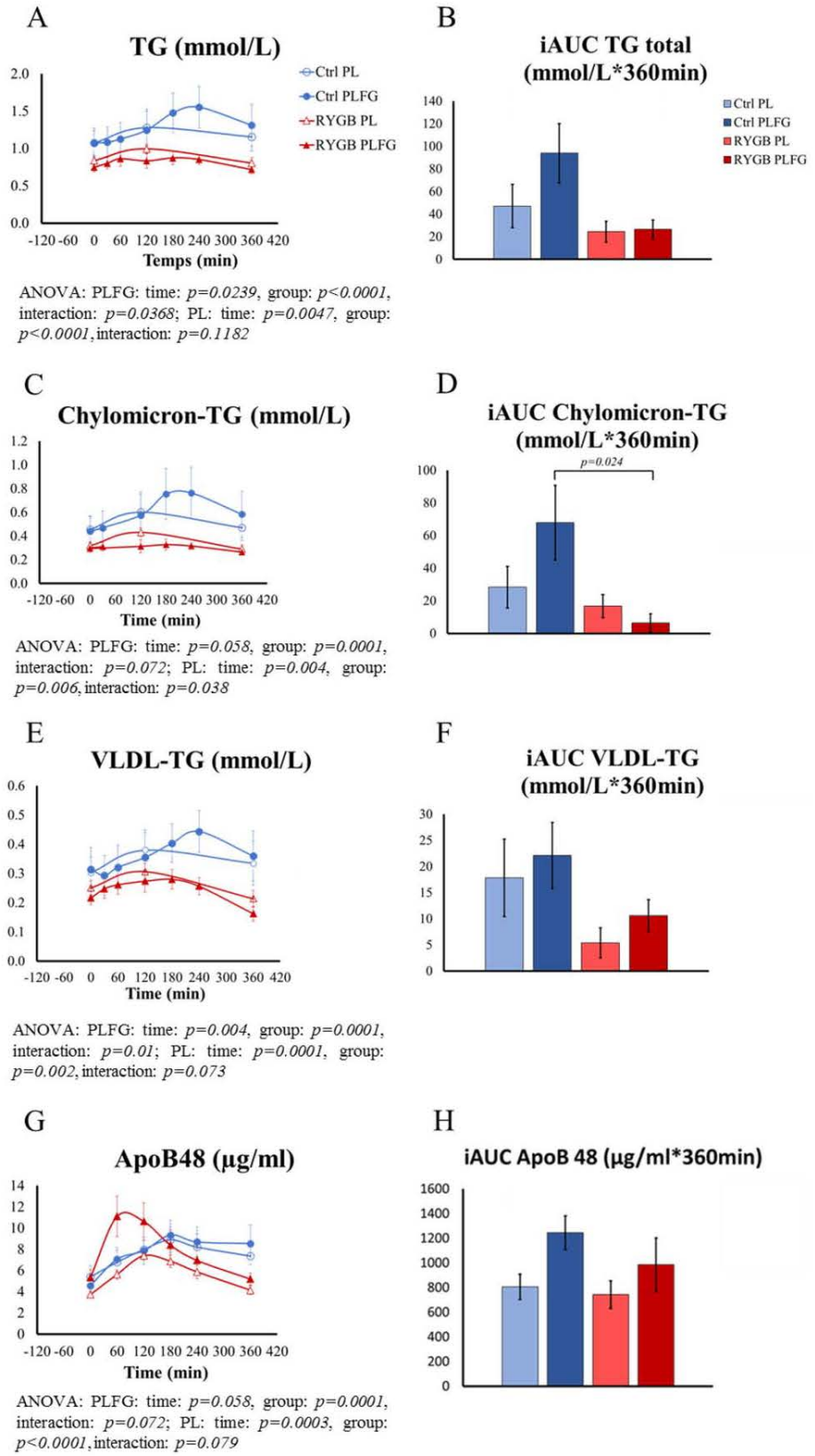


Figure 2 Plasma (**A**) TG , (**C**) chylomicron-TG, (**E**) VLDL-TG, and (**G**) plasma ApoB48 responses to test meal ingestion and (**B**, **D**, **F**, and **H**) their corresponding iAUCs. Data are reported as mean ± SEM for $n = 8$. Interaction – time × group.

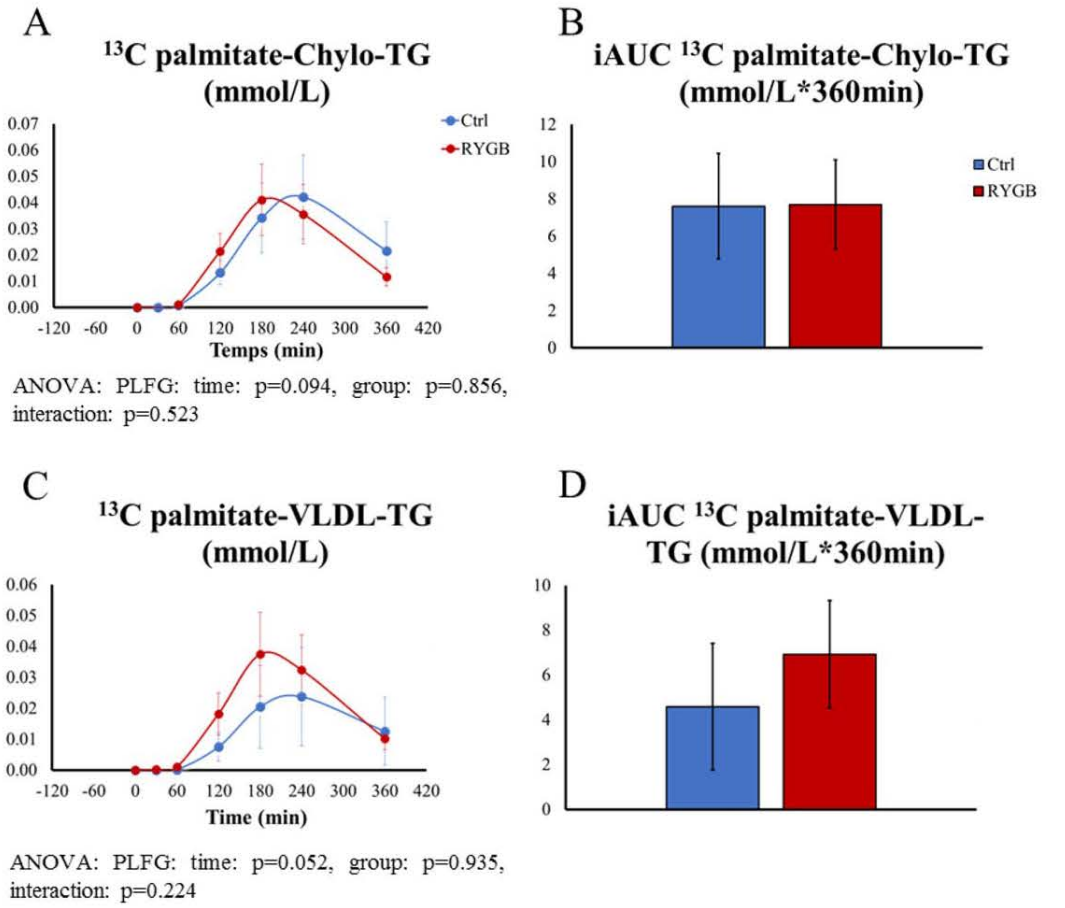


Figure 3 Plasma (**A**) ^{13}C-palmitate-chylomicrons and (**C**) ^{13}C-palmitate-VLDL concentrations and (**B**, **D**) their corresponding iAUCs. Data are reported as mean + SEM for $n = 8$. Interaction – time × group.

sugars and hence provide original information on the sugar-fat interactions in RYGB. In Ctrl subjects, coingestion of sugars enhanced postprandial blood TG concentrations above values observed after ingestion of lipids and proteins alone. Several reports have previously documented that dietary sugar enhanced postprandial hypertriglyceridemia, and this effect has been attributed to stimulation of hepatic (24) or intestinal (25) *de novo* lipogenesis and to an inhibition of blood TG clearance (26-28). The concentration of ^{13}C-palmitate in chylomicron- and VLDL-TG increased progressively after ingestion of ^{13}C-fructose labeled meals, thus providing unequivocal evidence that a portion of fructose carbons were converted into fatty acids in the immediate postprandial period. Furthermore, the appearance of ^{13}C-palmitate in chylomicron-TG was consistent with intestinal lipogenesis contributing to this process. Our data do not allow a quantitative estimate of *de novo* lipogenesis, however, because the cellular ^{13}C enrichment of acetyl-CoA, which provided building blocks for fatty acid synthesis, was not measured.

Interestingly, the postprandial increase in plasma TG and chylomicron-TG were completely inhibited after both PL and PLFG in RYGB subjects (Figure 2A, C). This may have suggested that intestinal lipid absorption was delayed or suppressed after RYGB. In contradiction with this hypothesis, postprandial plasma apoB48 concentrations increased in both RYGB and Ctrl subjects, and peak values after PLFG occurred earlier in RYGB The increase in ^{13}C-palmitate concentrations in chylomicron-TG was also very similar between RYGB and Ctrl. Taken together, these observations strongly suggest that intestinal secretion of chylomicrons and *de novo* lipogenesis from fructose were not inhibited after RYGB, and hence that blunted plasma TG responses were rather due to an increased clearance rate of TG-rich lipoproteins. This conclusion is in line with our recent observation that plasma TG postprandial responses to ingestion of a mixed meal were paradoxically blunted after gastric bypass while earlier increases in plasma bile acids and FGF19 were enhanced (29,30) indicative of accelerated lipid absorption. Here, we extend this observation by showing that increased TG clearance after RYGB also abolishes sugar-induced hypertriglyceridemia in spite of a maintained *de novo* lipogenesis from fructose. The mechanisms responsible for an increased TG clearance after RYGB are yet to be elucidated. However, it is plausible that RYGB-induced postprandial hyperinsulinemia may be instrumental in enhancing lipoprotein lipase activity in adipose tissue. Whether additional factors are also involved remains to be determined.

This study has several limitations that need to be acknowledged. First, due to low ^{13}C-fructose enrichments in the meals, the mass isotopomer distribution of chylomicron-TG and VLDL-TG palmitate could not be determined, and the enrichment of the acetyl-CoA precursor pool could not be calculated. ^{13}C-palmitate enrichment therefore only provides a qualitative assessment of *de novo* lipogenesis. Second, lipoproteins were separated based on their density, and it was assumed that subfraction $S_f > 400$ corresponded to chylomicrons of intestinal origin; however we cannot exclude that this fraction may also have contained some large, buoyant VLDL secreted by the liver. Third, the PLFG meal contained more total energy than the PL meal, and hence differences between postprandial responses may be related to total energy load. Fourth, usual diet composition, alcohol intake, and physical exercise of participants were not recorded. Finally, this was a cross-sectional study, which cannot assess the time course of postprandial changes after RYGB.

Conclusion

Our present data show that fructose absorption and the major pathways used for fructose disposal are not markedly altered after RYGB. In contrast, postprandial TG responses are markedly blunted, without evidence for gross fat malabsorption or inhibition of fructose-induced *de novo* lipogenesis. This suggests that postprandial TG clearance is enhanced after RYGB, through mechanisms which remain to be elucidated.

Acknowledgments

We thank the staff of the Department of Physiology of Lausanne and of the Clinical Research Center for their outstanding assistance and all of the volunteers for their participation.

References

1. Nguyen NQ, Debreceni TL, Bambrick JE, et al. Rapid gastric and intestinal transit is a major determinant of changes in blood glucose, intestinal hormones, glucose absorption and postprandial symptoms after gastric bypass. *Obesity* 2014;22:2003-2009.
2. Quercia I, Dutia R, Kotler DP, Belsley S, Laferrere B. Gastrointestinal changes after bariatric surgery. *Diabetes Metab* 2014;40:87-94.
3. Lutz TA, Bueter M. The physiology underlying Roux-en-Y gastric bypass: a status report. *Am J Physiol Regul Integr Comp Physiol* 2014;307:R1275-R1291.
4. Nguyen NT, Varela E, Sabio A, Tran CL, Stamos M, Wilson SE. Resolution of hyperlipidemia after laparoscopic Roux-en-Y gastric bypass. *J Am Coll Surg* 2006; 203:24-29.
5. Padilla N, Maraninchi M, Beliard S, et al. Effects of bariatric surgery on hepatic and intestinal lipoprotein particle metabolism in obese, nondiabetic humans. *Arterioscler Thromb Vasc Biol* 2014;34:2330-2337.
6. De Giorgi S, Campos V, Egli L, et al. Long-term effects of Roux-en-Y gastric bypass on postprandial plasma lipid and bile acids kinetics in female non diabetic subjects: a cross-sectional pilot study. *Clin Nutr* 2015;34:911-917.
7. Jacobsen SH, Bojsen-Moller KN, Dirksen C, et al. Effects of gastric bypass surgery on glucose absorption and metabolism during a mixed meal in glucose-tolerant individuals. *Diabetologia* 2013;56:2250-2254.
8. Bizeau ME, Pagliassotti MJ. Hepatic adaptations to sucrose and fructose. *Metabolism* 2005;54:1189-1201.
9. Mayes PA. Intermediary metabolism of fructose. *Am J Clin Nutr* 1993;58:754S-765S.
10. Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev* 2010;90:23-46.
11. Theytaz F, de Giorgi S, Hodson L, et al. Metabolic fate of fructose ingested with and without glucose in a mixed meal. *Nutrients* 2014;6:2632-2649.
12. Tounian P, Schneiter P, Henry S, Jequier E, Tappy L. Effects of infused fructose on endogenous glucose production, gluconeogenesis, and glycogen metabolism. *Am J Physiol* 1994;267:E710-E717.
13. Petersen KF, Laurent D, Yu C, Cline GW, Shulman GI. Stimulating effects of low-dose fructose on insulin-stimulated hepatic glycogen synthesis in humans. *Diabetes* 2001;50:1263-1268.
14. Tran C, Jacot-Descombes D, Lecoultre V, et al. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. *Br J Nutr* 2010;104:1139-1147.
15. Bickerton AS, Roberts R, Fielding BA, et al. Preferential uptake of dietary fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes* 2007;56: 168-176.
16. Paquot N, Schneiter P, Jequier E, et al. 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.
17. Ferrannini E, Bjorkman O, Reichard GA, et al. The disposal of an oral glucose load in healthy subjects. A quantitative study. *Diabetes* 1985;34:580-588.
18. 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.
19. Sun SZ, Empie MW. Fructose metabolism in humans—what isotopic tracer studies tell us. *Nutr Metab* 2012;9:89
20. Cummings DE, Overduin J, Foster-Schubert KE, Carlson MJ. Role of the bypassed proximal intestine in the anti-diabetic effects of bariatric surgery. *Surg Obes Relat Dis* 2007;3:109-115.
21. Rodieux F, Giusti V, D'Alessio DA, Suter M, Tappy L. Effects of gastric bypass and gastric banding on glucose kinetics and gut hormone release. *Obesity* 2008;16: 298-305.
22. Peterli R, Wolnerhanssen B, Peters T, et al. Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. *Ann Surg* 2009; 250:234-241.
23. Lho Y, le Roux CW, Park HS, et al. Changes in glucose metabolism in vertical sleeve gastrectomy. *Obesity Surgery* 2015;25:2002-2010.
24. Tappy L, Le KA. Does fructose consumption contribute to non-alcoholic fatty liver disease? *Clin Res Hepatol Gastroenterol* 2012;36:554-560.
25. 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;277:31646-31655.
26. 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;22:363-365.
27. Teff KL, Elliott SS, Tschop M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab* 2004;89:2963-2972.
28. Stanhope KL, Bremer AA, Medici V, 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-E1605.
29. Pournaras DJ, Glicksman C, Vincent RP, et al. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. *Endocrinology* 2012;153:3613-3619.
30. Jansen PL, van Werven J, Aarts E, et al. Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery. *Dig Dis* 2011;29:48-51.

Study II.

Title: Effects of Dietary Protein and Fat Content on Intrahepatocellular and Intramyocellular Lipids during a 6-Day Hypercaloric, High Sucrose Diet: A Randomized Controlled Trial in Normal Weight Healthy Subjects.

This randomized, crossover-controlled study performed on twelve healthy young males and females. Participants were studied after a 3-day controlled, weight maintenance (WM) diet providing 100% daily energy needs with 45% starch, 10% sucrose, 33% lipid, 12% protein, and after 6-day hypercaloric diets containing 150% daily energy needs with 29% starch, 34% sucrose, 7% lactose, and with 5% protein and 25% lipid (low-protein/high-fat, LP-HP) or 20% protein and 10% lipid (high-protein/low-fat, HP-LF). Intrahepatic (IHCL) and intramuscular (IMCL) lipid deposition were measured (magnetic resonance spectroscopy, MRS) and energy expenditure (indirect calorimetry) after WM, and again after HP-LF/LP-HF.

Specific hypotheses: High sucrose overfeeding associated with a high-protein, and low-fat, diet would blunt intrahepatocellular and intramyocellular lipid storage compared to the same high-sucrose intake with low-protein, but with high-fat diet.

Personal contribution: Recruitment and screening of volunteers. Participated in preparation of dietary intervention (diet elaboration), meal preparation, and distribution. Performed metabolic tests with nurses of the clinical research center. Data analysis. Preparation of the manuscript.

Manuscript II.

nutrients

MDPI

Article

Effects of Dietary Protein and Fat Content on Intrahepatocellular and Intramyocellular Lipids during a 6-Day Hypercaloric, High Sucrose Diet: A Randomized Controlled Trial in Normal Weight Healthy Subjects

Anna Surowska [1,†], Prasanthi Jegatheesan [1,†], Vanessa Campos [1], Anne-Sophie Marques [1], Léonie Egli [1], Jérémy Cros [1], Robin Rosset [1], Virgile Lecoultre [1], Roland Kreis [2], Chris Boesch [2], Bertrand Pouymayou [2], Philippe Schneiter [1] and Luc Tappy [1,*]

1 Department of Physiology, University of Lausanne, 1005 Lausanne, Switzerland; anna.surowska@unil.ch (A.S.); pira_jegatheesan@hotmail.com (P.J.); vanessacaroline.campos@rdls.nestle.com (V.C.); asophie.marques@gmail.com (A.-S.M.); Leonie.Egli@rdls.nestle.com (L.E.); Jeremy.cros@unil.ch (J.C.); rosset.robin@gmail.com (R.R.); virgile.lecoultre@hibroye.ch (V.L.); philippe.schneiter@unil.ch (P.S.)

2 Department for Biomedical Research, University of Bern and Institute of Diagnostic Interventional and Pediatric Radiology, University Hospital, 3012 Bern, Switzerland; roland.kreis@insel.ch (R.K.); Chris.Boesch@insel.ch (C.B.); bertrand.pouymayou@insel.ch (B.P.)

* Correspondence: luc.tappy@unil.ch; Tel.: +41-21-692-55-41

† These authors contributed equally to this work.

Received: 14 November 2018; Accepted: 15 January 2019; Published: 21 January 2019

Abstract: Sucrose overfeeding increases intrahepatocellular (IHCL) and intramyocellular (IMCL) lipid concentrations in healthy subjects. We hypothesized that these effects would be modulated by diet protein/fat content. Twelve healthy men and women were studied on two occasions in a randomized, cross-over trial. On each occasion, they received a 3-day 12% protein weight maintenance diet (WM) followed by a 6-day hypercaloric high sucrose diet (150% energy requirements). On one occasion the hypercaloric diet contained 5% protein and 25% fat (low protein-high fat, LP-HF), on the other occasion it contained 20% protein and 10% fat (high protein-low fat, HP-LF). IHCL and IMCL concentrations (magnetic resonance spectroscopy) and energy expenditure (indirect calorimetry) were measured after WM, and again after HP-LF/LP-HF. IHCL increased from 25.0 ± 3.6 after WM to 147.1 ± 26.9 mmol/kg wet weight (ww) after LP-HF and from 30.3 ± 7.7 to 57.8 ± 14.8 after HP-LF (two-way ANOVA with interaction: $p < 0.001$ overfeeding x protein/fat content). IMCL increased from 7.1 ± 0.6 to 8.8 ± 0.7 mmol/kg ww after LP-HF and from 6.2 ± 0.6 to 6.9 ± 0.6 after HP-LF, ($p < 0.002$). These results indicate that liver and muscle fat deposition is enhanced when sucrose overfeeding is associated with a low protein, high fat diet compared to a high protein, low fat diet.

Keywords: sucrose overfeeding; hepatic steatosis; intramyocellular lipids; intrahepatocellular lipids; dietary protein content; dietary fat content; energy expenditure; plasma triglyceride

1. Introduction

Consumption of hypercaloric high-fructose or high-sucrose diets can lead to the deposition of fat in ectopic sites such as visceral adipose tissue, the liver (intrahepatocellular lipids, IHCL), skeletal muscle (intramyocellular lipids, IMCL), the heart, and the pancreas [1]. Such ectopic fat deposition has been associated with insulin resistance and increased risk of cardiovascular and hepatic disorders [2,3].

In addition, hypercaloric high-fructose diets have been shown to impair hepatic insulin sensitivity [4,5], to increase fasting and postprandial blood triglycerides [6,7] and uric acid [8] concentrations, and may therefore be associated with a particularly ominous constellation of cardiometabolic risk factors.

Most studies that have documented metabolic effects of fructose or sucrose overfeeding have involved either the addition of fructose or sucrose to a weight maintenance diet, or the substitution of fructose or sucrose for dietary starch. In real life conditions, however, the addition of sucrose to an *ad libitum* diet is expected to impact habitual food consumption and hence to alter both total energy intake and the dietary macronutrient composition. It has indeed been reported that the addition of fructose-sweetened beverages to the spontaneous diet of overweight subjects was associated with a partial suppression of dietary fat and protein intake from solid foods [9]. One may therefore hypothesize that the metabolic effects of overfeeding depend not only on the amount of excess sucrose, but also on how it impacts other dietary macronutrient intake. Dietary sucrose and fat content may have additive effects on IHCL [10]. Interactions between dietary sucrose and protein are also relevant, since dietary protein intake has been shown to modulate overfeeding-induced ectopic lipid storage: in rodents fed a high fructose diet, the increase in IHCL was lower when excess dietary fructose was associated with a high, compared to a low, protein intake [11,12]. Similar observations were reported for humans overfed with lipids and protein compared to lipids alone [13–15], and with fructose and essential amino-acids compared with fructose alone [16]. In addition, a high protein intake is associated with an increase in energy expenditure, and may thus reduce energy storage [17]. We therefore hypothesized that, in normal weight human subjects, a short-term sucrose overfeeding associated with a high-protein, low-fat intake would blunt intrahepatocellular and intramyocellular lipid storage compared to the same sucrose overfeeding associated with a low-protein, high-fat diet. To assess this hypothesis, we carried out a randomized, cross-over controlled trial in 12 healthy male and female subjects. We monitored IHCL and IMCL, postprandial energy expenditure (EE), and blood metabolite concentrations at baseline, i.e. after 3 days on a 10% sucrose weight maintenance diet (WM), and after 6-days overfeeding with 50% extra-energy added as 40% sucrose and 10% lactose with either a high protein-low fat (HP-LF) or a low protein-high fat (LP-HF) content.

2. Materials and Methods

2.1. Subjects

Twelve healthy and non-obese volunteers (6 males, mean age 21 ± 1 years, weight 71.6 ± 2.3 kg, BMI 22.5 ± 0.8 kg/m^2; 6 females mean age 23 ± 1 years, weight 57.3 ± 0.8 kg, BMI 21.2 ± 0.7 kg/m^2) were included in this study. Volunteers were recruited through advertisements posted at the University of Lausanne and the Lausanne University Hospital. All volunteers were sedentary (less than 2 h of strenuous physical activity per week), were nonsmokers, had no lactose intolerance as documented by a lactose hydrogen breath test [18], and did not take any medication, (except for contraceptive agents which were used by all female participants). They all provided informed written consent.

2.2. Experimental Protocol

The experimental protocol was approved by the ethical committee (Commission d'éthique pour la recherche humaine de l'Etat de Vaud, Switzerland), and was registered at clinicaltrials.gov (NCT02168218). All procedures were performed in accordance with the 1983 revision of the Declaration of Helsinki. The primary outcome of the study was whole body protein turnover using labelled leucine, and will be reported separately. IHCL, IMCL and EE, which are the main focus of this paper, were all secondary outcomes. The experimental protocol is presented in Figure 1.

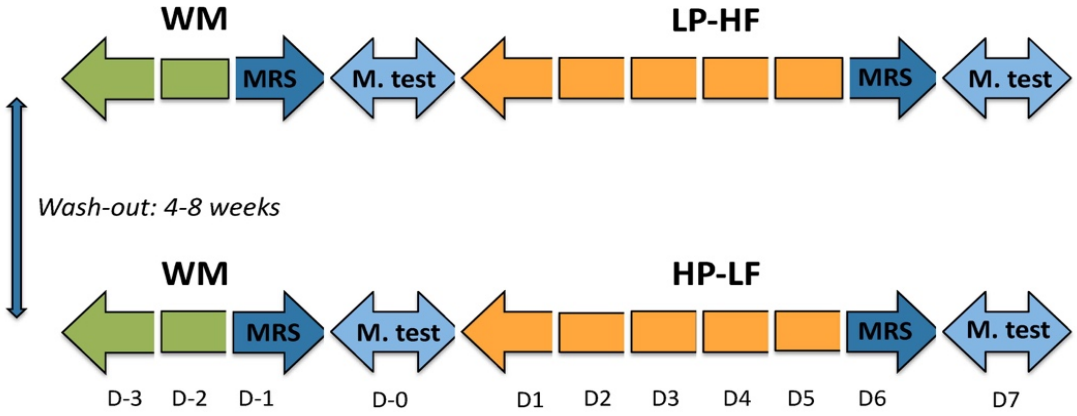


Figure 1. Experimental protocol. Each participant took part in two overfeeding periods according to a randomized, cross-over design. WM: weight maintenance diet, LP-HF: hypercaloric (150% energy requirement high-sucrose, low protein-high fat); HP-LF: hypercaloric (150% energy requirement high-sucrose, high protein-low fat); MRS: magnetic resonance spectroscopy for measurement of IHCL and IMCL; M. test: metabolic test, consisting of measurements of energy expenditure, plasma hormones, and substrate concentrations after ingestion of WM meal providing 40% of total energy requirements (D0), or LP-HF/HP-LF meals providing 60% of total energy requirements.

2.3. Dietary Interventions

All participants were studied on two occasions, each one consisting of a 3-day (D-3–D-1) weight-maintenance (WM), low sucrose diet followed by 6-day of sucrose + lactose overfeeding (D1–D6). On one occasion this overfeeding consisted of a 5% dietary protein and 25% fat content; on the other occasion, it was comprised of 20% dietary protein and 10% fat content. The dietary conditions were applied according to a randomized, cross-over design (Figure 1). Randomization was performed according to a pre-defined sequence, which was generated using R, version 3.0.1. (R Foundation for Statistical Computing, Vienna, Austria). The intervention was not blinded due to the nature of the drinks consumed. The two interventions were separated by a washout period of four to eight weeks.

WM diets were prepared from market foods and provided 100% of energy requirements (estimated from basal energy expenditure, calculated with the Harris-Benedict equation, times a physical activity level of 1.5). Food intake was partitioned into 3 meals/day and 2 snacks/day. It contained 45% of total energy as starch, 10% as sucrose, 33% as lipid, and 12% as protein and 22.6 $\pm$ 0.9 g dietary fiber/day; beverages were provided *ad libitum* as water. Overfeeding was attained by adding an extra 50% energy to the weight-maintenance energy requirements, in the form of six drinks per day. Drinks were prepared with skimmed milk and sucrose for the HP-LF condition or with water, lactose, and sucrose for the LP-HF condition, and had a volume of 218 $\pm$ 52 ml each. Solid diets were adjusted to obtain the same total energy (150% energy requirement): starch (29%), sucrose (34%) and lactose (7%) in both diets, with 20% protein (2.7 g/kg/day) and 10% fat in HP-LF or 5% protein (0.8 g/kg/day) and 25% fat in LP-HF. The addition of fat in LP-HF was mainly achieved by the addition of olive oil, butter, sauces, and cereals bars. Water consumption was left *ad libitum*. The detailed compositions of all three diets are shown in Table 1.

During each intervention, participants came to the metabolic unit of the Physiology Department of the University of Lausanne to consume their breakfasts, lunches, dinners, and three supplemental drinks under supervision. Every day, they also received two packages of snacks, together with three supplemental drinks during the overfeeding periods to consume between main meals, and were instructed not to consume any other food or drinks except plain water.

Table 1. Energy content and macronutrient composition of WM, LP-HF and HP-LF.

Diet Composition	WM		LP-HF			HP-LF		
	Solid Diet kcal/day (%)	Beverages kcal/day (%)	Solid Diet kcal/day (%)	Beverages kcal/day (%)	Total LP-HF kcal/day (%)	Solid Diet kcal/day (%)	Beverages kcal/day (%)	Total HP-LF kcal/day (%)
Starch	1061 (45)	-	1054	-	1054 (29)	1043	-	1043 (29)
Sucrose	249 (10)	-	241	965	1206 (34)	246	964	1210 (34)
Lactose	-	-	-	245	245 (7)	-	246	246 (7)
Protein	274 (12)	-	194	-	194 (5)	514	178	692 (20)
Fat	781 (33)	-	886	-	886 (25)	357	12	369 (10)
SFA	263 (34)	-	313	-	313 (35)	184	-	184 (52)
MUFA	280 (36)	-	389	-	389 (44)	102	-	102 (29)
PUFA	202 (26)	-	168	-	168 (19)	55	-	55 (15)
Total kcal	2365	-	2375	1210	3585	2160	1400	3560

WM: weight maintenance diet; LP-HF: high-sucrose, low-protein; HP-LF: high-sucrose, high-protein. Data are expressed as kcal/day; values into bracket represent % of total energy intake. For SFA, MUFA and PUFA, values in () are given as % total fat intake.

2.4. Measurements of IHCL and IMCL

For each intervention, IHCL and IMCL were measured at 4:00 pm on the 3rd day (D-1) on the WM diet ($WM_{LP\text{-}HF}$ and $WM_{HP\text{-}LF}$) and on the 6th day (D6) on the hypercaloric diets (HP-LF and LP-HF). IHCL and IMCL content were determined by ^{1}H-MRS using a clinical 3T MR system (Verio, Siemens Medical, Germany) using methods similar to those described previously for IMCL [19,20] and for IHCL [21]. For the latter, quantification was based on the unsuppressed water signal corrected for transverse relaxation (characterized by the T_2 value) as determined in each subject individually. Since T_2 values were found to be significantly different before ($WM_{LP\text{-}HF}$, $WM_{HP\text{-}LF}$) versus after the diets (LP-HF, HP-LF), but did not differ between diets (LP-HF vs. HP-LF), individually averaged T_2 values for pre- and post-diet sessions were used for IHCL quantification. Results were expressed as mmol/kg ww.

2.5. Metabolic Tests

On days following IHCL and IMCL measurements (D0 and D7), participants were asked to arrive in the fasting state at the Metabolism, Nutrition and Physical Activity Research Center of the Department of Physiology of the University of Lausanne at 7:00 am for a metabolic test (schema shown in Figure 2). They had performed a 24-h urine collection the day before.

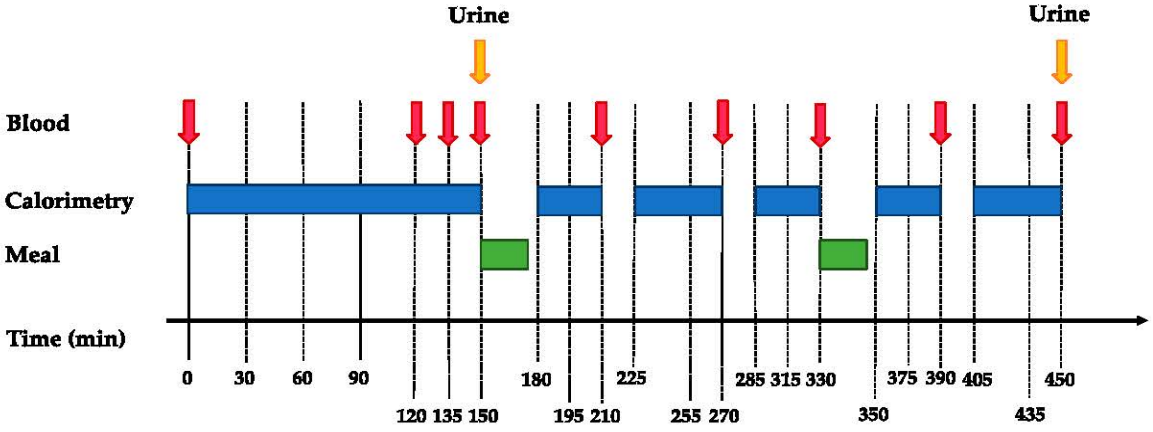


Figure 2. Schema of metabolic tests at D0 and D7.

This metabolic test aimed at comparing their fasting and postprandial energy expenditure, plasma hormones, and substrate profiles during periods of weight maintenance and overfeeding. At their arrival, participants were asked to void and discard their urine. They were then weighed and transferred to a bed where they remained in a semi-recumbent position for the next 7.5 h. A catheter was inserted into an antecubital vein for blood collection. Subjects remained fasted for the initial 2.5 h.

Four fasting blood samples and a urine collection were obtained during this period. Thereafter, they received two meals, one at 150 min and the second one at 330 min. Meal composition corresponded to the current intervention (i.e., WM on D0 and either HP-LF or LP-HF on D7). The sum of these two meals contained 40% (30% in first and 10% in the second meal) of total daily energy intake, which corresponded to 40% of energy requirements with WM, and to 60% of daily energy requirements during overfeeding periods (HP-LF and LP-HF). Postprandial blood samples were collected at the times 210 min, 270 min, 330 min, 390 min, and 450 min. Respiratory gas exchanges were monitored throughout the experiment by open-circuit indirect calorimetry (Quark RMR, version 9.1b, Cosmed, Rome, Italy), except for brief interruptions during meals. A second urine collection was obtained at the end of the test (time 450 min). Energy expenditure (EE) was calculated using the equations of Livesey and Elia [22].

2.6. Analytical Procedures

Plasma glucose, triglycerides (TG), lactate, and urine urea were measured by enzymatic methods (Randox Laboratories, Crumlin, County Antrim, UK). Plasma fructose concentrations were measured by GC–MS apparatus (Agilent Technologies, Santa Clara, CA, USA) [23]. Insulin and glucagon were assessed by radioimmunoassays (Millipore, Billerica, MA, USA). Plasma lipoprotein subfractions were separated by ultracentrifugation [24].

2.7. Statistical Analysis

All results are expressed as means ± SEMs. Postprandial results for all parameters (except for IGF1 and glucagon, which were determined in fasting conditions at only 2-time points postprandial) were expressed as the incremental area under the curve ($iAUC_{(0\text{-}300\ min)}$), which was obtained using the trapezoidal method by subtracting the fasting value. As a preliminary analysis, the normality of data was checked with Shapiro-Wilk tests for all parameters analyzed. Non-normally distributed data were log-transformed (IHCL, fasting insulin, glucagon, TG, and postprandial glucagon). Two-way ANOVA assessed the effects of overfeeding, protein/fat content (HP-LF vs. LP-HF), and interaction between overfeeding x protein/fat content with repeated measures. Tukey post hoc tests were performed to compare individuals when needed. All statistical analyses were performed using Prism 7 (GraphPad Software, Inc., La Jolla, USA). The number of subjects included in the study was based on a power analysis related to whole body protein turnover (not reported here).

3. Results

The recruitment and follow up of subjects took place between June 2013 and April 2016. All volunteers completed the investigation and reported that they did not take any additional caloric drinks and food during the study. One volunteer was not included in the calculation of postprandial fructose due to missing plasma samples. Two volunteers were excluded from 24 h urinary concentration, excretion, and clearance calculation due to missing urine collections. All other calculations were performed with all 12 volunteers.

3.1. Fasting Condition

Fasting parameters are shown in Table 2. All fasting parameters were not significantly different after $WM_{LP\text{-}HF}$ and $WM_{HP\text{-}LF}$. Body weight increased by 0.7 ± 0.1 kg (males 0.9 ± 0.2 kg, females 0.6 ± 0.1 kg) between D0 and D7 after LP-HF and by 1.4 ± 0.2 kg after HP-LF (males 1.8 ± 0.2 kg, females 0.9 ± 0.1 kg) (for the whole group: $p < 0.001$ for overfeeding, $p > 0.999$ for protein/fat content, $p = 0.009$ for overfeeding × protein/fat content). Fasting EE increased from 1.11 ± 0.06 kcal/min ($WM_{LP\text{-}HF}$) to 1.12 ± 0.05 kcal/min (LP-HF), and from 1.10 ± 0.05 kcal/min ($WM_{HP\text{-}LF}$) to 1.18 ± 0.05 kcal/min (HP-LF), ($p = 0.018$ for overfeeding, $p = 0.126$ for protein/fat content, $p = 0.024$ overfeeding x protein/fat content). Fasting plasma glucose, fructose, lactate, TG, and insulin all increased to the same extent with HP-LF and LP-HF (Table 2). Fasting plasma NEFA decreased to the same extent with

HP-LF and LP-HF. In contrast, fasting glucagon concentration and IGF-1 concentrations increased with HP-LF, but remained stable (glucagon) or slightly decreased (IGF-1) with LP-HF.

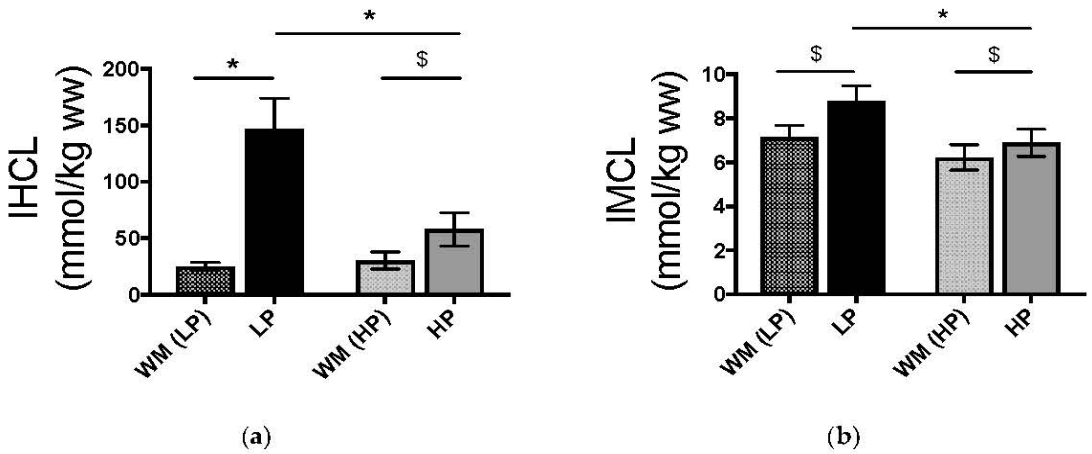
Table 2. Fasting plasma metabolites and hormones concentrations.

Fasting	WM (LP-HF)	LP-HF	WM (HP-LF)	HP-LF	*p* Value		
					Overfeeding	Protein/Fat Content	OxP
Glucose (mmol/L)	4.56 ± 0.07	4.78 ± 0.07	4.46 ± 0.11	4.76 ± 0.09	<0.001	0.444	0.383
Fructose (µmol/L)	25.95 ± 1.41	27.15 ± 1.49	26.35 ± 1.37	28.0 ± 1.32	0.022	0.611	0.750
Lactate (mmol/L)	0.70 ± 0.06	1.22 ± 0.07	0.64 ± 0.04	1.16 ± 0.09	<0.001	0.107	0.935
Uric acid (mmol/L)	0.38 ± 0.02	0.38 ± 0.03	0.39 ± 0.02	0.30 ± 0.02	<0.001	<0.001	<0.001
TG (mmol/L)	0.68 ± 0.07	1.54 ± 0.22	0.66 ± 0.08	1.68 ± 0.19	<0.001	0.429	0.119
NEFA (mmol/L)	0.72 ± 0.05	0.44 ± 0.09	0.77 ± 0.04	0.37 ± 0.07	<0.001	0.779	0.097
Insulin (µU/mL)	8.42 ± 0.83	10.95 ± 1.02	7.82 ± 0.79	11.73 ± 1.54	<0.001	0.744	0.295
Glucagon (pg/mL)	72.42 ± 4.83	72.49 ± 4.94	68.67 ± 4.03	79.27 ± 5.35	0.059	0.276	0.036
IGF-1 (ng/mL)	212 ± 13	176 ± 12	174 ± 18	208 ± 13	0.901	0.712	<0.001

WM: weight maintenance diet; LP-HF: high-sucrose, low-protein; HP-LF: high-sucrose, high-protein. All values are mean ± SEM, $n = 12$. A significant difference in each condition, $p < 0.05$ (2-way ANOVA with repeated measures). OxP: Overfeeding x protein/fat content.

3.2. IHCL and IMCL Concentrations

IHCL and IMCL concentrations after WM and after LP-HF and HP-LF are shown in Figure 3. No statistically significant difference was observed between $WM_{LP\text{-}HF}$ and $WM_{HP\text{-}LF}$. Compared to WM conditions, IHCL and IMCL concentrations increased significantly with both LP-HF and HP-LF overfeeding. However, IHCL increased more importantly with LP-HF than with HP-LF ($p < 0.001$ for effect of overfeeding, $p < 0.001$ for effect of dietary protein/fat content, and $p < 0.001$ for interaction overfeeding x protein/fat content). IMCL also increased more with LP-HF than with HP-LF ($p < 0.001$ for overfeeding, $p = 0.025$ for protein/fat content, and $p = 0.002$ for overfeeding x protein/fat content).



(**a**) (**b**)

Figure 3. Intrahepatocellular (IHCL) (**a**) and intramyocellular (IMCL) lipids (**b**) in response to weight maintaining diet ($WM_{LP\text{-}HF}$ and $WM_{HP\text{-}LF}$) and overfeeding with LP-HF and HP-LF. $n = 12$; significant responses from $WM_{LP\text{-}HF}$ and $WM_{HP\text{-}LF}$ were measured by 2-way ANOVA for repeated measures with interaction. *: $p < 0.001$, interaction overfeeding × protein/fat content. $: $p < 0.005$, Tukey post hoc tests.

3.3. Postprandial Parameters

Postprandial metabolic parameters were not significantly different after WM diets. Postprandial EE and diet-induced thermogenesis were both significantly higher with LP-HF and HP-LF than under their respective WM conditions. Furthermore, EE increased more after HP-LF (from 1.23 ± 0.05

to 1.55 ± 0.06 kcal/min) than after LP-HF (from 1.24 ± 0.05 to 1.41 ± 0.06 kcal/min) ($p < 0.001$ for overfeeding, $p = 0.013$ for protein/fat content, and $p < 0.001$ for overfeeding x protein/fat content).

The postprandial iAUCs for blood metabolites and hormones are shown in Table 3. Postprandial blood glucose did not significantly change with HP-LF and LP-HF compared to their respectively WM conditions. Postprandial fructose, lactate, TG, and insulin iAUC were significantly higher in HP-LF and LP-HF than in the respective WM conditions.

Table 3. Metabolites and hormones at postprandial states.

Postprandial	WM (LP-HF)	LP-HF	WM (HP-LF)	HP-LF	*p* Value		
					Overfeeding	Protein/Fat Content	OxP
iAUC Glucose (mmol/L*300min)	504.0 ± 40.5	495.3 ± 69.2	560.3 ± 43.7	471.4 ± 57.2	0.242	0.616	0.189
iAUC Fructose (mmol/L*300min)	4.2 ± 0.3	30.3 ± 2.9	4.8 ± 0.5	23.4 ± 2.2	<0.001	0.005	0.003
iAUC Lactate (mmol/L*300min)	78.8 ± 12.8	239.8 ± 24.5	92.2 ± 15.1	139.3 ± 15.7	<0.001	0.001	0.001
iAUC TG (mmol/L*300min)	29.3 ± 6.8	121.3 ± 15.3	24.2 ± 8.0	126.7 ± 16.9	<0.001	0.986	0.471
iAUC NEFA (mmol/L*300min)	−162.6 ± 12.5	−86.7 ± 23.2	−173.1 ± 11.0	−76.6 ± 17.2	<0.001	0.984	0.051
iAUC Insulin (µU/ml*300min)	11378 ± 1232	19228 ± 1708	11138 ± 1488	24123 ± 2790	<0.001	0.061	0.028

WM: weight maintenance diet; LP-HF: high-sucrose, low-protein; HP-LF: high-sucrose, high-protein. All values are mean ± SEM, $n = 12$. A significant difference in each condition, $p < 0.05$ (2-way ANOVA, with repeated measures). OxP: Overfeeding x protein/fat content. In the calculation of iAUC fructose ($n = 11$) one volunteer was excluded for reason of missing plasma data.

HP-LF and LP-HF nonetheless differentially altered postprandial insulin, fructose, and lactate concentrations: HP-LF increased postprandial insulin concentrations more than LP-HF, but decreased postprandial fructose and lactate (see Table 3 for detailed statistics). Postprandial plasma uric acid concentration, measured at time 450 min, decreased from 0.38 ± 0.02 (WM) to 0.30 ± 0.02 mmol/L with HP-LF, but increased from 0.38 ± 0.03 (WM) to 0.42 ± 0.04 mmol/L with LP-HF ($p = 0.283$ for overfeeding, $p = 0.001$ for diet, $p < 0.001$ for overfeeding × protein/fat content). Plasma glucagon, measured at time 450 min, increased from 62.6 ± 3.3 to 87.9 ± 8.4 pg/mLwith HP-LF, but did not change with LP-HF: 65.1 ± 4.4 vs. LP-HF: 70.6 ± 4.8 pg/mL, ($p < 0.001$ for overfeeding, $p = 0.026$ for protein/fat content, and $p = 0.001$ for overfeeding x protein/fat content).

24-h urinary excretion and clearance of creatinine and uric acid are shown in Table 4. LP-HF and HP-LF did not significantly change 24-h urinary excretion and clearance of creatinine. HP-LF increased urinary excretion of uric acid and uric acid clearance while LP-HF decreased it. Compared to LP-HF, HP-LF significantly increased urinary creatinine and uric acid clearance; it also increased total 24-h uric acid excretion.

Table 4. 24-h urinary creatinine and uric acid excretion and clearance.

	WM (LP-HF)	**LP-HF**	**WM (HP-LF)**	**HP-LF**	***p* Value**		
					Overfeeding	**Protein/Fat Content**	**OxP**
24-h urinary excretion							
Creatinine (mmol/24h)	13.6 ± 1.8	12.6 ± 1.2	13.0 ± 0.8	12.7 ± 1.1	0.264	0.450	0.638
Uric acid (mmol/24h)	3.5 ± 0.2	3.3 ± 0.2	3.3 ± 0.2	4.1 ± 0.4	0.049	0.238	0.022
Urinary clearance rate							
Creatinine (ml/min)	129.8 ± 9.4	133.7 ± 9.7	135.1 ± 10.8	153.0 ± 13.0	0.279	0.309	0.282
Uric acid (ml/min)	6.9 ± 0.6	6.5 ± 0.6	6.1 ± 0.4	10.0 ± 1.4	0.005	0.015	0.004

WM: weight maintenance diet; LP-HF: high-sucrose, low-protein; HP-LF: high-sucrose, high-protein. All values are mean ± SEM, $n = 10$ as two volunteers were excluded because of missing samples. A significant difference in each condition, $p < 0.05$ (2-way ANOVA, with repeated measures). OxP: Overfeeding x protein/fat content.

4. Discussion

This study was designed to assess whether the consequences of sucrose overfeeding differ according to concomitant changes in daily protein and fat intake. Our main findings were that both HP-LF and LP-HF increased IHCL, IMCL, and blood triglycerides concentrations, but increments were reduced on average by 78% for IHCL and by 59% for IMCL with HP-LF compared to LP-HF.

In addition, fasting and postprandial EE were significantly higher with HP-LF than LP-HF. However, blood triglyceride concentrations were not significantly different with HP-LF and LP-HF. Finally, blood uric acid concentrations were increased with LP-HF, but decreased with HP-LF.

Our experimental design compared the effects of two hypercaloric high sucrose diets, one with a high protein-low fat content and the other with a low protein-high fat content, to that of a weight maintenance control diet. All three diets contained an amount of starch equivalent to approximately 45% total energy requirements, and the two hypercaloric diets contained 150% of daily energy requirements, with about 50% of energy requirements as sucrose, and 7% of energy requirements as lactose. Lactose intake was higher in HP-LF than in WM because of a high milk protein intake and was balanced by lactose addition in LP-HF in order to have equal carbohydrate amounts and composition in both diets. Dietary saturated-monounsaturated and polyunsaturated fatty acid proportions were also different in each diet.

The dietary composition had a profound effect on the amount of ectopic lipids being deposited during overfeeding. HP-LF and LP-HF both increased lipid storage in the liver and muscle, two sites in which ectopic lipid deposition is known to be associated with adverse long-term effects [1]. Several short-term studies had previously documented that excess energy intake from fructose or glucose increased IHCL [10,25,26] and IMCL [26–28]. In our study, this effect was most notable in the liver, where IHCL increased by 542 ± 105% after LP-HF. It was milder in skeletal muscle, where we nonetheless observed a significant increase of +24 ± 3% after LP-HF. In both sites, the increases induced by HP-LF were significantly lower than those induced by LP-HF. Excess energy intake from sugars is thought to increase IHCL by enhancing hepatic *de novo* lipogenesis and inhibiting intrahepatic lipid oxidation [29]. Several hypotheses can be proposed to account for the differential effects of HP-LF and LP-HF. First, LP-HF contained more lipids than HP-LF. Previous experiments have shown that fat overfeeding increases IHCL synthesis from intestinally derived TG-rich lipoprotein particles and/or circulating NEFA [13,30,31]. It has also been shown that fructose and fat have additive effects on IHCL during combined fructose-fat overfeeding [10]. It is therefore likely that, with LP-HF, the high dietary sugar and fat intake had additive effects on IHCL. Second, dietary protein may decrease IHCL independently of dietary fat or energy intake. In support of this hypothesis, a former study reported that IHCL were increased in healthy subjects fed a hypercaloric, high fat diet containing 130% energy requirements. However, the addition of protein to this high fat diet resulted in a similar daily fat and carbohydrate intake, but also in a higher total energy and protein intake with significantly reduced IHCL [13]. The mechanisms by which an increased protein intake may reduce IHCL remain unknown. Inhibition of *de novo* lipogenesis has been postulated [13], but fractional hepatic *de novo* lipogenesis was stimulated to the same extent in healthy subjects overfed with fructose alone or with fructose and proteins [16]. A stimulation of hepatic VLDL-TG secretion and extrahepatic VLDL-TG clearance [16], or a protein-induced increase in plasma bile acid concentrations [13] have also been proposed to play a role. In contrast, no effect of dietary protein intake on IMCL has been reported to our knowledge. Finally, changes in dietary fatty acids composition may modulate diet-induced hepatic fat deposition (reviewed in reference [32]). Hepatic steatosis in animal models is readily produced by consumption of a high saturated fat diet with low PUFA content. In contrast, there is evidence that PUFA or oleic acid supplementation may actually blunt diet-induced hepatic steatosis [32]. In the present study, dietary protein intake in HP-LF was increased through the consumption of skimmed dairy products to avoid an increase in SFA, and dietary fat intake in LP-HF was increased by consumption of vegetable oils (mainly olive oil). As a result, total daily SFA intake was only slightly higher in LP-HF than in HP-LF (34.7 ± 1.5 vs. 20.4 ± 0.9 g/day) while MUFA+PUFA intake was markedly increased. It is therefore unlikely that the higher IHCL observed with LP-HF can be explained by the differences in dietary fat composition.

The postprandial increases in plasma TG concentrations were 5-fold higher with HP-LF and 4-fold higher with LP-HF than with WM. Several studies have reported that fructose and sucrose overfeeding increases fasting and postprandial blood triglyceride by increasing hepatic *de novo* lipogenesis and

VLDL-TG secretion and by decreasing the postprandial clearance of triglyceride-rich lipoprotein particles [27,33,34]. It is therefore likely that an upregulation of lipogenic enzymes with sucrose overfeeding contributed to this hypertriglyceridemia. However, the meals administered during the metabolic tests contained 50% more total energy in overfeeding than in weight-maintenance control conditions, and, therefore, contained also more sucrose and fat, which makes it difficult to sort out the relative role of sucrose and other macronutrients. Globally, the increase in postprandial TG concentrations was not significantly different in HP-LF and LP-HF.

The effect of overfeeding on energy expenditure was also markedly dependent on dietary composition. Postprandial EE increased significantly with both HP-LF and LP-HF, mainly due to the fact that the test meals ingested in both conditions had a caloric content 50% higher than in the control weight-maintenance condition. Postprandial EE increased more with HP-LF than LP-HF. This is most likely explained by the high energy cost of amino-acid metabolism [35].

We also assessed whether dietary composition had significant effects on postprandial blood metabolic markers during overfeeding. The total carbohydrate and sucrose content of meals ingested during the metabolic tests were higher in overfeeding than in the WM control condition, and postprandial increments in blood fructose, lactate, and insulin were accordingly enhanced. Similarly, postprandial NEFA was decreased to lower levels in overfeeding than in WM conditions. However, postprandial blood glucose responses were not significantly altered. Most postprandial parameters were not significantly different in HP-LF and LP-HF overfeeding. However, postprandial glucagon increased more with HP-LF than with LP-HF, as expected due to the well-known stimulation of glucagon secretion by circulating amino-acids after protein ingestion [36]. Surprisingly, blood fructose and lactate concentration increased less with HP-LF than LP-HF. It is possible that the lower lactate concentration was secondary to glucagon stimulating hepatic lactate uptake [37]. The lower fructose response was unexpected, however, and may suggest that hepatic fructose extraction was enhanced when consumed with proteins. Nutrient- or glucagon-mediated changes in portal blood flow may also be implicated [38]. Alternatively, it is possible that gastric emptying was delayed with HP-LF meals, thus accounting for a slower fructose absorption [39]. Finally, compared to WM, postprandial increases in uric acid were higher with LP-HF, but lower with HP-LF, while urinary uric acid excretion and uric acid clearance were significantly increased with HP-LF. This suggests that both HP-LF and LP-HF increased uric acid production, possibly due to the fructose component of sucrose [40], and that an increase in glomerular filtration rate, possibly mediated by glucagon [41], increased uric acid excretion, thus preventing an increase in blood uric acid. Elevated lactate concentrations are also known to impair renal uric acid clearance [42], and it is, therefore, possible that lower lactate concentrations during HP-LF than LP-HF overfeeding also played a role. Our data, however, do not allow accurate comparisons of uric acid production and excretion between HP-LF and LP-HF.

The present study limitations need to be acknowledged. First, we did not include isotopic measurements of *de novo* lipogenesis and VLDL-TG kinetics, and therefore cannot identify the mechanisms by which HP-LF decreased IHCL and IMCL compared to LP-HF. Second, not only total dietary fat intake, but also the proportions of SFA-MUFA-PUFA were different between diets, and we cannot exclude the possibility that this may have impacted IHCL or IMCL storage. Third, in HP-LF condition, dietary protein content was increased by addition of dairy products; whether the observed effects are generic to dietary proteins or specific to dairy products remains to be evaluated. Finally, our study was of short duration and was limited to a small group of healthy male and female subjects, and results may not apply to other subgroups of the population (e.g., overweight subjects or subjects with the metabolic syndrome).

5. Conclusions

In summary, our data indicate that overfeeding with a high sucrose, high protein/low-fat diet markedly reduces ectopic fat accumulation in the liver and muscle, and increases energy expenditure, compared to an isocaloric overfeeding with high sucrose, low protein/high-fat diet. This may be due

to an additive effect of sucrose and dietary fat and/or a protective effect of dietary protein on ectopic fat accumulation.

Author Contributions: Conceptualization, L.E., C.B., R.K., and L.T.; methodology, P.S., C.B., R.K, V.C.; validation, V.C., A.S., P.J.; formal analysis, A.S.; investigation, A.S., P.J., V.C., L.E., A.-S.M., R.K., V.L., R.R., B.P, J.C.; writing—original draft preparation, A.S.; writing—review and editing, all.; visualization, A.S., P.J.; project administration, A.S.; funding acquisition, L.T., C.B., R.K.

Funding: This research was funded by grant from the Swiss National Foundation for science 32003B_156167, and by a grant from the Institute Benjamin Delessert Foundation to P.J.

Acknowledgments: We thank the staff of the Department of Physiology of Lausanne for their great assistance, Shawna McCallin for language editing, and all the volunteers for their participation and commitment.

Conflicts of Interest: L.T. has received research support from Soremartec Italia srl for projects unrelated to this report, and speakers' fees from Soremartec Italia srl, Nestlé AG, Switzerland, and the Gatorade Sport Science Institute, USA. L.E. and V.C. are presently employed by Nestec SA, Switzerland. Other authors declare no conflict of interest.

References

1. Szendroedi, J.; Roden, M. Ectopic lipids and organ function. *Curr. Opin. Lipidol.* **2009**, *20*, 50–56. [CrossRef]
2. Morelli, M.; Gaggini, M.; Daniele, G.; Marraccini, P.; Sicari, R.; Gastaldelli, A. Ectopic fat: The true culprit linking obesity and cardiovascular disease? *Thromb. Haemost.* **2013**, *110*, 651–660. [CrossRef] [PubMed]
3. Britton, K.A.; Fox, C.S. Ectopic fat depots and cardiovascular disease. *Circulation* **2011**, *124*, e837–e841. [CrossRef] [PubMed]
4. Stanhope, K.L.; Havel, P.J. Fructose consumption: Considerations for future research on its effects on adipose distribution, lipid metabolism, and insulin sensitivity in humans. *J. Nutr.* **2009**, *139*, 1236S–1241S. [CrossRef] [PubMed]
5. Aeberli, I.; Hochuli, M.; Gerber, P.A.; Sze, L.; Murer, S.B.; Tappy, L.; Spinas, G.A.; Berneis, K. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: A randomized controlled trial. *Diabetes Care* **2013**, *36*, 150–156. [CrossRef] [PubMed]
6. Stanhope, K.L.; Havel, P.J. Fructose consumption: Potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. *Curr. Opin. Lipidol.* **2008**, *19*, 16–24. [CrossRef]
7. Stanhope, K.L.; Schwarz, J.M.; Keim, N.L.; Griffen, S.C.; Bremer, A.A.; Graham, J.L.; Hatcher, B.; Cox, C.L.; Dyachenko, A.; Zhang, W.; et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J. Clin. Investig.* **2009**, *119*, 1322–1334. [CrossRef]
8. Wang, D.D.; Sievenpiper, J.L.; de Souza, R.J.; Chiavaroli, L.; Ha, V.; Cozma, A.I.; Mirrahimi, A.; Yu, M.E.; Carleton, A.J.; Di Buono, M.; et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. *J. Nutr.* **2012**, *142*, 916–923. [CrossRef]
9. Taskinen, M.R.; Soderlund, S.; Bogl, L.H.; Hakkarainen, A.; Matikainen, N.; Pietilainen, K.H.; Rasanen, S.; Lundbom, N.; Bjornson, E.; Eliasson, B.; et al. Adverse effects of fructose on cardiometabolic risk factors and hepatic lipid metabolism in subjects with abdominal obesity. *J. Intern. Med.* **2017**, *282*, 187–201. [CrossRef]
10. Sobrecases, H.; Le, K.A.; Bortolotti, M.; Schneiter, P.; Ith, M.; Kreis, R.; Boesch, C.; Tappy, L. Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. *Diabetes Metab.* **2010**, *36*, 244–246. [CrossRef]
11. Hamad, E.M.; Taha, S.H.; Abou Dawood, A.G.; Sitohy, M.Z.; Abdel-Hamid, M. Protective effect of whey proteins against nonalcoholic fatty liver in rats. *Lipids Health Dis.* **2011**, *10*, 57. [CrossRef] [PubMed]
12. Chaumontet, C.; Even, P.C.; Schwarz, J.; Simonin-Foucault, A.; Piedcoq, J.; Fromentin, G.; Azzout-Marniche, D.; Tome, D. High dietary protein decreases fat deposition induced by high-fat and high-sucrose diet in rats. *Br. J. Nutr.* **2015**, *114*, 1132–1142. [CrossRef] [PubMed]
13. Bortolotti, M.; Kreis, R.; Debard, C.; Cariou, B.; Faeh, D.; Chetiveaux, M.; Ith, M.; Vermathen, P.; Stefanoni, N.; Le, K.A.; et al. High protein intake reduces intrahepatocellular lipid deposition in humans. *Am. J. Clin. Nutr.* **2009**, *90*, 1002–1010. [CrossRef] [PubMed]

14. Martens, E.A.; Gatta-Cherifi, B.; Gonnissen, H.K.; Westerterp-Plantenga, M.S. The potential of a high protein-low carbohydrate diet to preserve intrahepatic triglyceride content in healthy humans. *PLoS ONE* **2014**, *9*, e109617. [CrossRef] [PubMed]
15. Rietman, A.; Schwarz, J.; Blokker, B.A.; Siebelink, E.; Kok, F.J.; Afman, L.A.; Tome, D.; Mensink, M. Increasing protein intake modulates lipid metabolism in healthy young men and women consuming a high-fat hypercaloric diet. *J. Nutr.* **2014**, *144*, 1174–1180. [CrossRef] [PubMed]
16. Theytaz, F.; Noguchi, Y.; Egli, L.; Campos, V.; Buehler, T.; Hodson, L.; Patterson, B.W.; 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*, 1008–1016. [CrossRef] [PubMed]
17. Bray, G.A.; Smith, S.R.; de Jonge, L.; Xie, H.; Rood, J.; Martin, C.K.; Most, M.; Brock, C.; Mancuso, S.; Redman, L.M. Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: A randomized controlled trial. *JAMA* **2012**, *307*, 47–55. [CrossRef] [PubMed]
18. Eisenmann, A.; Amann, A.; Said, M.; Datta, B.; Ledochowski, M. Implementation and interpretation of hydrogen breath tests. *J. Breath Res.* **2008**, *2*, 046002. [CrossRef]
19. Le, K.A.; 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*, 1374–1379. [CrossRef]
20. Boesch, C.; Kreis, R. Observation of intramyocellular lipids by 1h-magnetic resonance spectroscopy. *Ann. N. Y. Acad. Sci.* **2000**, *904*, 25–31. [CrossRef]
21. Cros, J.; Pianezzi, E.; Rosset, R.; Egli, L.; Schneiter, P.; Cornette, F.; Pouymayou, B.; Heinzer, R.; Tappy, L.; Kreis, R.; et al. Impact of sleep restriction on metabolic outcomes induced by overfeeding: A randomized controlled trial in healthy individuals. *Am. J. Clin. Nutr.* **2019**. [CrossRef] [PubMed]
22. 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. [PubMed]
23. Tran, C.; Jacot-Descombes, D.; Lecoultre, V.; Fielding, B.A.; Carrel, G.; Le, K.A.; Schneiter, P.; Bortolotti, M.; Frayn, K.N.; Tappy, L. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. *Br. J. Nutr.* **2010**, *104*, 1139–1147. [CrossRef] [PubMed]
24. Karpe, F.; Steiner, G.; Olivecrona, T.; Carlson, L.A.; Hamsten, A. Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J. Clin. Investig.* **1993**, *91*, 748–758. [CrossRef] [PubMed]
25. Lecoultre, V.; Egli, L.; Carrel, G.; Theytaz, F.; Kreis, R.; Schneiter, P.; Boss, A.; Zwygart, K.; Le, K.A.; Bortolotti, M.; et al. Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity* **2013**, *21*, 782–785. [CrossRef] [PubMed]
26. Johnston, R.D.; Stephenson, M.C.; Crossland, H.; Cordon, S.M.; Palcidi, E.; Cox, E.F.; Taylor, M.A.; Aithal, G.P.; Macdonald, I.A. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. *Gastroenterology* **2013**, *145*, 1016–1025.e2. [CrossRef]
27. Le, K.A.; Ith, M.; Kreis, R.; Faeh, D.; Bortolotti, M.; Tran, C.; Boesch, C.; Tappy, L. 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. [CrossRef]
28. Ngo Sock, E.T.; Le, K.A.; Ith, M.; Kreis, R.; Boesch, C.; Tappy, L. Effects of a short-term overfeeding with fructose or glucose in healthy young males. *Br. J. Nutr.* **2010**, *103*, 939–943. [CrossRef]
29. Tappy, L.; Le, K.A. Does fructose consumption contribute to non-alcoholic fatty liver disease? *Clin. Res. Hepatol. Gastroenterol.* **2012**, *36*, 554–560. [CrossRef]
30. Donnelly, K.L.; Smith, C.I.; Schwarzenberg, S.J.; Jessurun, J.; Boldt, M.D.; Parks, E.J. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Investig.* **2005**, *115*, 1343–1351. [CrossRef]
31. Kotronen, A.; Yki-Jarvinen, H. Fatty liver: A novel component of the metabolic syndrome. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 27–38. [CrossRef] [PubMed]
32. Ferramosca, A.; Zara, V. Modulation of hepatic steatosis by dietary fatty acids. *World J. Gastroenterol.* **2014**, *20*, 1746–1755. [CrossRef]
33. Teff, K.L.; Elliott, S.S.; Tschop, M.; Kieffer, T.J.; Rader, D.; Heiman, M.; Townsend, R.R.; Keim, N.L.; D'Alessio, D.; Havel, P.J. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial

suppression of ghrelin, and increases triglycerides in women. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2963–2972. [CrossRef] [PubMed]
34. Stanhope, K.L.; Bremer, A.A.; Medici, V.; Nakajima, K.; Ito, Y.; Nakano, T.; Chen, G.; Fong, T.H.; Lee, V.; Menorca, R.I.; 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–E1605. [CrossRef] [PubMed]
35. Tappy, L. Thermic effect of food and sympathetic nervous system activity in humans. *Reprod. Nutr. Dev.* **1996**, *36*, 391–397. [CrossRef] [PubMed]
36. Calbet, J.A.; MacLean, D.A. Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans. *J. Nutr.* **2002**, *132*, 2174–2182. [CrossRef] [PubMed]
37. Ramnanan, C.J.; Edgerton, D.S.; Kraft, G.; Cherrington, A.D. Physiologic action of glucagon on liver glucose metabolism. *Diabetes Obes. Metab.* **2011**, *13* (Suppl. 1), 118–125. [CrossRef]
38. Granger, D.N.; Richardson, P.D.; Kvietys, P.R.; Mortillaro, N.A. Intestinal blood flow. *Gastroenterology* **1980**, *78*, 837–863. [PubMed]
39. Ma, J.; Stevens, J.E.; Cukier, K.; Maddox, A.F.; Wishart, J.M.; Jones, K.L.; Clifton, P.M.; Horowitz, M.; Rayner, C.K. Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care* **2009**, *32*, 1600–1602. [CrossRef] [PubMed]
40. Le, M.T.; Frye, R.F.; Rivard, C.J.; Cheng, J.; McFann, K.K.; Segal, M.S.; Johnson, R.J.; Johnson, J.A. 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. [CrossRef] [PubMed]
41. Ahloulay, M.; Dechaux, M.; Laborde, K.; Bankir, L. Influence of glucagon on gfr and on urea and electrolyte excretion: Direct and indirect effects. *Am. J. Physiol.* **1995**, *269*, F225–F235. [CrossRef] [PubMed]
42. Yu, T.F.; Sirota, J.H.; Berger, L.; Halpern, M.; Gutman, A.B. Effect of sodium lactate infusion on urate clearance in man. *Proc. Soc. Exp. Biol. Med.* **1957**, *96*, 809–813. [CrossRef] [PubMed]

Chapter III Discussion and perspectives

This work intended to provide complementary information to existing knowledge on the interactions between dietary fructose intake and other digestive or nutritional factors.

In grade II obesity, energy intake chronically exceeds daily recommendations. In this condition, dietary therapies often fail to induce long-term weight loss, and RYGB is presently considered to be the most effective treatment. It however induces drastic changes in gastro-intestinal anatomy and physiology. In the first study, we evaluated the impact of these changes on the metabolic fate of an acute fructose load. We had specifically postulated that RYGB would decrease intestinal *de novo* lipogenesis, which normally takes place in the proximal small bowel.

Discussion Study I.

Gastric bypass is known to enhance the speed of nutrients' delivery to jejunum and ileum, and of their gut absorption. This has been documented for carbohydrate and lipids. We further documented in this study that the same is true for fructose. Postprandial fructose concentration increased early after meal ingestion in RYGB patients, but thereafter decreased quickly, suggesting rapid absorption. Similar results were observed for glucose, insulin, and lactate concentration. Additionally, there was no significant difference between RYGB and control group for the total incremental fructose area under the curve, for calculated fructose oxidation, and for fructose storage. Our results confirmed previous observations concerning monosaccharide absorption (166, 175), and show a lack of evidence for malabsorption of fructose in RYGB patients.

Addition of the labeled ^{13}C fructose to the meal allowed us documenting DNL from fructose. ^{13}C palmitate concentration in chylomicrons-TG and VLDL-TG increased similarly in both RYGB and control groups. The concentration of ^{13}C palmitate-chylomicrons-TG reflects the contribution of fructose-induced DNL to intestinal chylomicrons' secretion. No quantitative evaluation could be obtained, however, due to the fact that our protocol did not allow us to measure intestinal or hepatic ^{13}C acetyl-CoA enrichment. Contrary to our hypothesis, we did not observe evidence that intestinal or hepatic DNL were inhibited after RYGB.

This study, allowed us to observe the impact of dietary sugars on postprandial dietary lipid handling after RYGB. It was previously observed that sugars enhance postprandial TG concentration after ingestion of a mixed meal (135). Indeed, we observed higher TG plasma concentration after addition of sugars to the meal in the control group. The mechanisms proposed to be involved are hepatic (176) or intestinal (177) lipogenesis and/or inhibition of TG clearance (90). In contrast, in RYGB patients, postprandial TG and chylomicrons-TG responses were nearly abolished, suggesting that bariatric surgery may simultaneously increase lipid absorption rate and increase their plasma clearance. We also considered the possibility that RYGB may result in some degree of fat malabsorption (178). However, in a previous study we observed that postprandial responses of CCK and bile acids occurred after ingestion of a mixed meal early and with the higher pick in RYGB than in controls, which may rather indicate an enhanced rate of lipid absorption (168). This observation may highlight other possible mechanisms involved in RYGB effects on blood lipid profiles.

Perspectives Study I.

Immediate impact and novel questions

We observed that total fructose absorption after RYGB surgery does not differ compared with the healthy volunteers. This observation suggests that the same amount of fructose carbons

may be available for metabolic pathways, i.e., DNL, in RYGB patients and in non-operated controls. Whether and how the early postprandial hyperfructosemia happens in real-life conditions after RYGB remains unknown. Some cell types have GLUT5 transporters, and may directly take up fructose. It is possible that, at high fructose concentrations, direct fructose metabolism increases in the kidney, or in other non-fructolytic tissues such as the brain, and muscle. What impact that may have on health deserves attention.

Pursuing this line of research

To further the findings of this study I propose running a study in which intestinal *de novo* lipogenesis would be quantitatively assessed by measuring fractional DNL with acetate, and using mass isotopomer distribution analysis (MIDA), together with kinetics of TRL-associated apoB48 with a labelled amino acid. Moreover, running the same study, but with labelled dietary lipids to monitor their absorption and clearance would allow to further investigate the mechanisms involved in the normalization of blood lipids after bariatric surgery.

Discussion Study II.

Several studies indicate that a high sugar intake may induce a different metabolic response according to concomitant changes in other dietary nutrients' intake. In this study, we observed that a high sugar consumption causes intrahepatic fat accumulation, but that this effect is much more important when sugar is consumed with a low-protein, high-fat diet that with a high-protein, low-fat diet. This may suggest that hepatic lipid deposition may be induced by a combination of high-sucrose and high-fat intake. However, a beneficial impact of protein on intrahepatic fat accumulation induced by a high-fat diet was previously shown in our

laboratory, suggesting rather a protective effect of a high-protein intake, independent of excess kilocalories in the diet (172). Various potential mechanisms were proposed:

- The protein may inhibit DNL by increasing hepatic lipid oxidation (179). But we do not observe increased whole-body lipid oxidation; in another study (172), with a similar technique, no DNL change was observed. The protein also increased bile acid secretion, and possibly increase hepatic lipid oxidation (172, 180). This has however not been assessed in our present study.
- Protein-induced hyperglucagonemia (181) may channel fructose carbons into gluconeogenesis and therefore lead to a mirror decrease in DNL. Indeed, in our study, we observed lower systemic fructose concentration and higher glucagon production after an HP-LF diet.
- Protein may stimulate VLDL-TG secretion and increase their clearance, which has been proposed in a previously performed study in our laboratory (141). However, in our study, it was not measured.

Perspectives Study II.

Immediate impact and novel questions

Results from our study indicated that high-protein intake has an immediate positive effect on ectopic fat deposition in situations of overfeeding. In this perspective we can challenge other questions:

- By which mechanisms do proteins or some amino acids abolish ectopic lipid deposition? Can these effects be reproduced by branched-chain amino acid supplements (182) ? Does the type of protein and its source, i.e., protein provided from dairy products, animals, and from plant origins, have a different impact on health effects i.e., reduction of ectopic fat deposition? Moreover, dairy products being rich in calcium, does calcium

intake may play a role in prevented dyslipidemia in overfeeding situation (183) ? Additionally, fermented dairy products were inversely associated with CV risk (184).

- What is a quantitative participation of sugars/fructose in intestinal and hepatic DNL with high-protein compared with low-protein intake?
- What will be an impact of the treatment over a prolonged period?
- What effects of the treatment will be in a different type of population, i.e., overweight persons?
- What does it mean for dietary guidelines; should present recommendations concerning protein intake and type/source be revised?
- What about protein intake in the treatment of NAFLD?
- What does it mean for personalized nutrition? In our study, we observed the divergence between participants concerning ectopic fat deposition. Should personalized amounts of protein depend on IHCL accumulation?

Similar questions are also relevant for other nutrient intake as well. Polyphenols and polyunsaturated fatty acids were shown to inhibit or reduce the metabolic effects of a high-fructose intake. These alimentary factors should be further assessed as natural methods to inhibit the adverse effects of fructose overfeeding.

Pursuing this line of research

To broaden the significance of this study, my next steps will include, for example, the addition of a tracer, i.e., labelled acetate to monitor quantitively DNL to observe how or if high protein may impact directly DNL. A similar study will be performed on a different type of population (i.e., overweight, obese, or with NAFLD). Additionally, I will increase the number of subjects in the study and observe gender differences and the impact of female sex hormones in ectopic lipid deposition.

Conclusions

Metabolic disorders induced by imbalanced diet, rich in fructose, provided with high amounts of sucrose and HFCS, can be reduced to some extent by nutritional and non-nutritional factors. In the first of two studies, we examined the impact of bariatric surgery on fructose metabolism. This study does not confirm our hypothesis that bariatric surgery may alert fructose metabolism and then impact its other metabolic pathways. Despite the major digestive changes induced by the performed surgery, no modulation of total fructose absorption was observed. This suggests that, after RYGB, all fructose molecules are presented in the same amount for different metabolic pathways than those in non-operated patients. Moreover, results of ^{13}C palmitate and apoB48 in both groups indicated similar intestinal lipogenesis from fructose. In contrast, an alteration of postprandial triglyceride concentration was observed in RYGB patients. This observation opens new questions: By which mechanisms can lipid clearance be modified, if we exclude fat malabsorption after bariatric surgery (185)? In the second study, the same high-energy intake with different macronutrient compositions differently impacts the effects induced by a high sucrose (high fructose) intake. This suggests that not only total energy overfeeding but also the macronutrient composition in diet may differently impact the risk of metabolic diseases. Overfeeding with sucrose and high-protein, but with low-fat intake, reduced significantly both IHCL and IMCL concentrations. Moreover, sucrose overfeeding combined with high-fat, but low-protein intake may increase the adverse effects of fructose. In contrast, postprandial TG concentration increased similarly after both conditions. Which mechanisms are involved in these protective effects of high-protein intake, remains, however, to be determined.

Appendix

Table 1. Characteristics of clinical studies evaluating fructose consumption versus other sugars on glucose, lipid and ectopic fat response.

Study		Methods	Test meal	Participants	Duration	Glucose homeostasis	Lipid profile	Ectopic lipids
Acute								
1.	Chong et al., 2007, (63).	Single-blind, randomized, crossover	0.75g/kg bw Fructose or glucose and 0.5g/kg bw palm oil ^{13}C fructose or ^{13}C glucose	14 healthy men, women	6h	Lower insulin after fructose.	TG significantly higher after fructose. DNL higher after fructose.	N/A
2.	Evans et al., 2017, (78).	62 studies	Isoenergetic exchange of glucose or sucrose by fructose (15-100g)	Type 1 and 2 diabetes mellitus	48 min to 24h	Replacement of either glucose or sucrose by fructose resulted in significantly lowered peak postprandial blood glucose. Similar results were obtained for insulin.	No blood TG concentrations raising.	N/A
3.	Jameel et al., 2014, (103).	Randomized, single blinded, controlled cross-over trial	3 different isocaloric sugary drinks: 50g fructose 50g glucose 50g sucrose	14 healthy adult men and women	120 min	The change in fasting glucose and insulin responses was modest with fructose.	Fructose as a sole source of energy modulates plasma lipids: significance increase in HDL-cholesterol with a concurrent increase in LDL-cholesterol. AUC for plasma TG levels however remained unchanged.	N/A
4.	Jeppensen et al., 1995, (186).	Randomized	Dairy cream (40g fat) with fructose 40g Dairy cream (40g fat)	11 healthy men and women	12h	N/A	Fructose increased TG rich lipoprotein (TG, chylomicron).	N/A
5.	Le et al., 2011, (128).	Prospective, randomized, single-blinded, crossover trial	HFCS 68.0 g (39.2 g fructose) 13% higher dose Sucrose 69.4 g (34.6g fructose, beverages	40 men and women	6h	No treatment differences insulin and lactate.	No treatment differences of TG.	N/A

6.	Moore et al., 2001, (87).	Single-blind randomized	7.5g fructose to 75g glucose 75g glucose (OGTT drinks)	5 obese with type 2 diabetes	3h	The addition of small (catalytic) amounts of fructose to a glucose load improves glucose tolerance, without enhancing the insulin response.	Plasma NEFA, blood glycerol, and plasma TG concentrations did not differ.	N/A
7.	Parks et al., 2008, (187).	Randomized, blinded	85g sugars: 85g glucose 43g glucose + 43g fructose (50:50) 21g glucose + 64g fructose (25:75)	6 healthy men and women	24h	Postprandial glucose and insulin not differ between 50:50 and 25:75.	TG serum significantly higher after 50:50 and 25:75 vs 100 DNL grater after 50:50	N/A
8.	Stanhope et al., 2008, (104).	Randomized	25% energy from: Fructose, glucose, sucrose, HFCS Beverages	34 men and women	24h	HFCS-sweetened beverages induced a small increase in the 24-h insulin. Sucrose and HFCS on glucose, leptin, and ghrelin were not different.	Sucrose and HFCS resulted in postprandial TG responses comparable to those induced by fructose.	N/A
9.	Surowska et al., (188).	Randomized	Protein and lipid meal (PL) Protein, lipid, fructose, glucose + ^{13}C-fructose (PLFG)	8 RYGB 8 Matched control	6h	Non-differ in glucose and fructose.	In RYGB, postprandial TG responses are markedly blunted after both PL and PLFG.	N/A
10.	Teff et al., 2004, (102).	Randomized, controlled	30% free glucose 30% free fructose in the form of a beverage	12 normal-weight women	24-h	Fructose lower circulating insulin and leptin concentrations.	Fructose higher ghrelin and TG levels.	N/A
11.	Teff et al., 2009, (96).	Randomized	Mixed nutrient meals with 30% fructose 30% glucose beverages	17 obese men and women	24h	Fructose result in decreased insulin secretion, a reduced diurnal leptin profile.	Fructose increased postprandial TG in obese subjects with insulin resistance.	N/A
12.	Theytaz et al., 2014, (135).	Randomized, crossover study	ProLip ProLip +fructose 0.5g/kg (bw) ProLip +fructose+glucose 0.5g/kg + 0.5g/kg bw +^{13}C fructose	8 healthy men and women	6h	Gluconeogenesis, lactic acid production and both intestinal and hepatic DNL contributed to the disposal of fructose carbons.	Co-ingestion of glucose decreased fructose oxidation and gluconeogenesis and tended to increase ^{13}Cpalmitate concentration in gut-derived chylomicrons, but not in hepatic-borne VLDL-TG.	N/A

Isocaloric diet								
13.	Bantle et al., 2000, (189).	Randomized, balances crossover design	14% fructose +3%glucose 14%glucose +3% fructose Solid food	24 healthy, men, women	6 weeks	Postprandial plasma glucose and serum insulin responses were lower after fructose intake.	Fasting and postprandial plasma TG concentrations were significantly higher in men.	N/A
14.	Black et al., 2006, (77).		*Eucaloric,* WM diet: 10% sucrose (low) 25% sucrose (high) From solid food and beverages	14 healthy men, nondiabetic	7 day	High-sucrose intake as part of a balanced had no detrimental effect on insulin sensitivity. Fasting plasma glucose, serum insulin did not change.	Total and LDL cholesterol were higher after 25% sucrose. HDL cholesterol and fasting TG were similar on the two diets.	N/A
15.	Cozma et al., 2012, (79).	8 trials	Exchange of fructose (4.5-21%) 23–137 g/day for other carbohydrate	Diabetes patients	>7 days	Improves long-term glycemic control without affecting insulin.	N/A	N/A
16.	Egli et al., 2013, (139).	Randomized crossover design	Control 30% energy (~200 g/day) fructose 30% + exercise WM diet	8 healthy men	4 days	Increased fasting glucose.	Increased TRL-TG, apoB48 (exercise prevents the dyslipidemia induced by high fructose).	N/A
17.	Lowndes et al., 2014, (190).	Randomized, prospective, double blinded	*Eucaloric* Sucrose: 10%, 20% HFCS: 10%, 20%	65 overweigh, obese men and women	10 weeks	N/A	No changes in total cholesterol, TG, LDL, ApoB. No diff between sucrose and HFCS.	N/A
18.	Malerbi et al., 1996, (81).	Well-controlled	20% Fructose 9% Sucrose	16 type 2 diabetes	28 days	No adversely affect glycemia, or insulin.	No adversely affect lipemia.	N/A
19.	Schwarz et al., 2015, (108).	Randomized	25% energy Fructose Complex CHO (1-13C) acetate (U-13C) glucose	8 healthy men	9 days	Significantly higher postprandial CHO oxidation. Blunted suppression of EGP by insulin. No significant effects of a high-fructose diet on fasting glucose, or insulin.	Significantly higher postprandial levels of hepatic DNL, TG. No significant effects of a high-fructose diet on fasting DNL, lipids.	Modestly higher liver fat.
20.	Sievenpiper et al., 2009, (110).	16 trials	Fructose exchange: 5-21% energy (20 - 109g/d)	Type 1 and 2 diabetes	> 7 days	N/A	Only a modest TG-raising effect in type 2 diabetes at doses >60 g/day with follow-up of >4 weeks or when the reference carbohydrate is starch modest total cholesterol–lowering effect.	N/A

21.	Sievenpiper et al., 2014, (140)	20 controlled trials	Fructose replacing glucose 7-55% (40-300g/d)	344 participants, normal, overweigh, obese	1-10 weeks	Fructose exchange with glucose reduced glycated blood protein. Non-significant reduction in glucose and insulin.	Fructose increased postprandial TG specially at high doses. Markers of NAFLD are not worse than with glucose.	N/A
22.	Swarbrick et al., 2008, (95).	Controlled	25% fructose beverages Control – complex CHO	7 overweight and obese women	10 weeks	Increased fasting glucose concentrations.	Increased: postprandial TG concentrations, fasting plasma apoB.	N/A
23.	Umpleby et al., 2017, (109).	Randomized, cross-over design	Sugars 26% (high) Sugars 6% (low)	11 men with NAFLD 14 Control	12 weeks	N/A	Men with NAFLD higher VLDL-TG (different fractions) after high and low sugar.	High sugars diet increased liver fat to a relatively greater extent in subgroups of men with NAFLD, compared with controls.
24.	Wang et al., 2013, (137).	14 trials	Fructose 4.5-25% (22.5-125g/day) energy	Diabetes Overweight and obese	> 7 days	No effect in diabetics	Fructose in isocaloric exchange for other carbohydrate does not raise postprandial TG. Postprandial TG raising effect of fructose in overweight/obese.	N/A
Hypercaloric diet								
25.	*Bortolotti et al., 2009, (172).*	*Randomized*	*High fat* *High fat + high protein crossover*	*10 healthy men*	*4 days*	*No diff plasma glucose and insulin*	*TG, VLDL-TG no different*	*Protein significantly blunted IHCL*
26.	Bortolotti et al., 2012 (191).	Randomized	High Fructose 3g/kg High Fructose 3g/kg +prot 1.5g/kg	8 healthy men	6 days	High protein meals increased post-prandial energy expenditure and enhanced fructose-induced gluconeogenesis.	Protein enhanced the plasma TG response. Proteins did not increase lipid oxidation.	N/A
27.	Couchepin et al., 2008, (86).	Randomized	Supplementation: 3.5g fructose /kg fat free mass Control diet with 10% mono-di saccharides	8 healthy men and women	6 days	Insulin resistance in man but not in women.	Significant increased TG but lower in women (higher in female vs mal at baseline).	N/A

28.	Faeh et al., 2005, (98).	Randomized, controlled	Supplementation with: 7.2 g of fish oil fructose 3g/kg bw fish oil+fructose	7 healthy men	6 days	Increased endogenous glucose production. Increased insulin resistance, after 6 days of fructose overfeeding.	Increased hypertriglyceridemia, and increased DNL with fructose overfeeding. Fish oil significantly decreased triglycerides after high-fructose diet and tend DNL.	N/A
29.	Horst, et al., 2016, (142).	29 articles	*Isocaloric* exchange CHO by fructose 26-218g (4-25%) *Hypercaloric* with +25% fructose 40-250g (7-33%)	Normal weigh and obese	7-665 days	In both diets iso hyper promotes hepatic insulin resistance. Not promote muscle or peripheral insulin resistance. Fructose rise fasting HOMA-IR in diabetes, but not in normal weigh, obese or overweigh.	N/A	N/A
30.	Johnston et al., 2013, (117).	Randomized, double blind	*Isocaloric:* +25% fructose n=15 +25% glucose n=17 *ad libitum:* - hypercal +25% fructose - hypercal +25% glucose Beverages	32 healthy overweight men	2 weeks	Isocaloric/hypercaloric: No diff after on gluc or insulin.	Isocaloric on a high-fructose or a high-glucose diet did not develop any significant changes in serum levels of liver enzymes. Hypercaloric both high-fructose and high-glucose diets produced significant increases in these parameters without any significant difference between the 2 groups.	Isocaloric on a high-fructose or a high-glucose diet did not develop any significant changes in hepatic concentration of TGs. Hypercaloric diet yes. Energy-mediated, rather than a specific macronutrient-mediated, effect.
31.	Lê et al., 2009 (91).	Randomized, crossover	+3.5g/kg body wt	16 type 2 diabetes 8 control	7 days	No significant effect of group or significant interaction.	Increased fasting VLDL-TG in healthy and more importantly in diabetes.	Increased IHCL, IMCL in healthy diabetes has higher IHCL concentrations.
32.	Lecoultre et al., 2013, (83).	Randomized	Fructose supplementation: F1.5g n=7 F3.0g n=17 F4.0g n=10 G3.0g glucose n=11 30% fat n=10	55 healthy	6-7 days	Fructose overfeeding did not significantly alter plasma glucose. concentration at any of the doses tested. Insulin levels were unchanged in F1.5 and increased only after F3.	N/A	Incresaed IHCL with glucose and fructose (3.0, but not higher with 4.0) and fat.

33.	McDevitt et al., 2001, (107).	Randomized	+50%: Glucose, +50%: Sucrose Control	8 women: 8 lean 5 obese	4 days	No significant increase of plasma glucose and insulin.	*De novo* lipogenesis increases after overfeeding with glucose and sucrose to the same extent in lean and obese women but does not contribute greatly to total fat balance.	N/A
34.	Nego Sock et al., 2010, (93).	Randomized	+35% fructose +35% glucose	7 healthy men	7 days	No change was observed in fasting glycaemia, insulin.	Both sugars increased plasma TG.	IMCL increased significantly only after the glucose IHCL and VLDL-TG were not different between hypercaloric HFrD and HGlcD
35.	Silbernagel et al., 2010, (85).	Exploratory, prospective, randomized, single-blinded	Supplementation: 150g fructose 150g glucose Beverages	12 men, 8 women healthy normal and overweight	4 weeks	Both decreased insulin sensitivity. High fructose and very high glucose in hyperenergetic diets do not have different effects on insulin resistance and hepatic lipid content.	Very high fructose intake was associated with a marked increase in plasma TAG.	In healthy subjects, fructose and glucose have no majorly different impact on hepatic lipid content. No changes in IHCL and IMCL.
36.	Sobrecases et al., 2010, (92).	Controlled	+35% fructose +30% fat +35% fructose +30%fat	30 healthy men	7 days	Hepatic glucose production did not change, suggesting that intrahepatic lipid content is not directly related to hepatic insulin sensitivity.	Fructose increased VLDL-TG High fat decreased VLDL-TG, Fat + fructose abolishes VLDL-TG	Fat and fructose have additive effects on IHCL, but opposite effects on plasma TG.
37.	Surowska et al., 2019, (116).	Randomized, cross-over, controlled	+50% sucrose +high prot, low fat +50% sucrose +low prot, high fat	12 healthy men and women	6 days	Fasting: glucose and insulin no diff. Postprandial: glucose no diff, insulin higher with high protein.	TG no different after both conditions.	Increased after both diet IHCL, IMCL – higher after protein, abolished after low protein.
38.	Theytaz et al., 2012, (141).	Randomized, crossover	Fructose 3g/kg body wt Fructose 3g/kg + EAA	9 healthy men	6 days	Fasting insulinemia was greater with HFr than with the control diet. Neither glucose production nor gluconeogenesis differed between treatments.	HFr increased VLDL-TG and VLDL-^{13}Cpalmitate. HfrAA did not change VLDL-triglyceride concentrations or VLDL-^{13}C palmitate production.	HFr increased the IHCL content. HfrAA significantly decreased IHCL.

Ad libitum								
39.	Aeberli et al., 2011, (84).	Randomized, controlled, double blind, crossover trial	Sugar-sweetened beverages: 40g fructose medium 80g fructose high 40g glucose medium 80g glucose high 80g sucrose	29 healthy	3 weeks	Elevated fasting glucose concentrations after all interventions (no changes on kcal intake).	No effect of any of the different diets on lipid profile. LDL size decreased during the HF and HS intervention.	N/A
40.	Bravo et al., 2013, (122).	Randomized, prospective, partially blinded, parallel investigation	Sucrose 8%, 18%, 30% HFCS 8%, 18%, 30%	Healthy men and women normal and overweight	10 weeks	N/A	Not find any increase on blood lipid.	No significant change IHCL, IMCL.
41.	Campos et al., 2015, (118).	Randomized	Sugar-sweetened beverages, Artificially sweetened	31 healthy overweight	12 weeks	No significant effect on insulin sensitivity.	No changes on lepidic profile.	Artificially exchange decreased IHCL.
42.	Lê et al., 2006, (82).	Randomized	Moderate supplementation, 1.5g/kg bw	Healthy	4 weeks	Increase a modest but significant rise in fasting glycemia.	Increased plasma TG concentration increase in fasting VLDL-TG.	No increase IMCL, IHCL.
43.	Perez-Pozo et al., 2010, (126).	Randomized, controlled trial	Fructose beverages (200g fructose/day)	74 healthy, overweight men	2 weeks	An increase in serum insulin and HOMA index.	Significant increase in fasting serum triglycerides, a decrease in high-density lipoprotein cholesterol.	N/A
44.	Stanhope et al., 2009, (100).	Double blind, parallel arm	25% Fructose n=17 25% glucose n=15 beverages	Overweight Obese	10 weeks	Fructose decreased glucose tolerance and insulin sensitivity (greater decreases in insulin sensitivity in women than in men).	Fructose increased postprandial TG (more in men than women), fast and post ApoB, LDL in overweight, obese women (increased CVD). Fructose increased DNL, postprandial activation lower LPL (induced post hypertriglyceridemia).	Fructose increased VAT (more in men than women) Glucose increased SAT.
45.	Stanhope et al., 2011, (192).	Randomized	glucose n = 15 fructose n = 17 beverages 25%	Health, Overweight and obese	10 weeks	Fructose beverages: Significantly lower pick of glucose and insulin.	N/A	N/A
46.	Stanhope et al., 2011, (90).	Parallel-arm	25% energy: Fructose n =16 glucose n =16	36 Healthy Overweight	2 weeks	Glucose and insulin responses mainly increased during glucose consumption,	Fructose and HFCS (higher) increased fasting and post TG, LDL, non-HDL-C, ApoB.	N/A

			HFCS n =16	Lean		decreased during fructose consumption, and unchanged during HFCS consumption, HOMA-IR was unchanged.	Glucose increased fasting TG concentrations.	
47.	Stanhope et al., 2015, (193).	Parallel-arm, nonrandomized, double blinded	HFCS 0% n=23 10% n=18 17,5% n=16 25% n=28	85 Healthy Overweight Lean	2 weeks	N/A	Increased as the dose Non-HDL cholesterol, LDL cholesterol, ApoB, postprandial TG.	N/A
48.	Taskinen et al., 2017, (147).	Randomized	75g fructose	71 abdominally obese men	12 weeks	No glucose and insulin after OGTT test.	Fructose feeding aggravated the increases in both total TG and apoB48.	Fructose consumption significantly increased liver fat content and hepatic DNL and decreased levels of beta hydroxybutyrate (indicating decreased hepatic beta oxidation).

References

1. Nordstrom K, Coff C, Jonsson H, Nordenfelt L, Gorman U. Food and health: individual, cultural, or scientific matters? Genes Nutr. 2013;8(4):357-63.
2. Organization WH. Obesity and overweight 2018 [
3. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, 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.
4. Seshadri KG. Obesity: A Venusian story of Paleolithic proportions. Indian J Endocrinol Metab. 2012;16(1):134-5.
5. Drewnowski A. The real contribution of added sugars and fats to obesity. Epidemiol Rev. 2007;29:160-71.
6. United States Department of Agriculture FAS. Grain: World Markets and Trade. 2018.
7. Goran MI, Ulijaszek SJ, Ventura EE. High fructose corn syrup and diabetes prevalence: a global perspective. Glob Public Health. 2013;8(1):55-64.
8. Pablo A. Garcia-Fuentes PLK, Gustavo F. C. Ferreira, editor An estimation of a price model of the high fructose corn syrup industry in the Unites States. Selected Paper prepared for presentation at the Southern Agricultural Economics Association (SAEA) Annual Meeting, Mobile, Alabama, 4-7 February 2017; 2017.
9. White J. Fructose, high fructose corn syrup, sucrose and health2014.
10. Young LR, Nestle M. Expanding portion sizes in the US marketplace: implications for nutrition counseling. J Am Diet Assoc. 2003;103(2):231-4.
11. Young LR, Nestle M. The contribution of expanding portion sizes to the US obesity epidemic. Am J Public Health. 2002;92(2):246-9.
12. United States Department of Agriculture ERS. Food Availability (Per Capita) Data System 2018 [Available from: https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/.
13. Nielsen SJ, Popkin BM. Changes in beverage intake between 1977 and 2001. Am J Prev Med. 2004;27(3):205-10.
14. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr. 2004;79(4):537-43.
15. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. J Exp Biol. 2018;221(Pt Suppl 1).
16. Marriott BP, Olsho L, Hadden L, Connor P. 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(3):228-58.
17. Carla Torres Carvalho ZMS, Nawal Arbex2, Diana Sá, Luciana Corrêa de Souza Rodrigues, Diana Aristotelis Rocha de Sá, Larissa Bianca Paiva Cunha de Sá, Alberto Krayyem Arbex. The Role of Fructose in Public Health and Obesity. Health. 2018;10:434-41.
18. Klurfeld DM, Foreyt J, Angelopoulos TJ, Rippe JM. Lack of evidence for high fructose corn syrup as the cause of the obesity epidemic. Int J Obes (Lond). 2013;37(6):771-3.
19. Collaboration NCDRF. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627-42.

20. Tailane SCAPIN ACF, Rossana Pacheco da Costa PROENÇA. Added sugars: Definitions, classifications, metabolism and health implications. Rev Nutri, Campinas. 2017;30((5)):663-77.
21. 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. 2012;346:e7492.
22. Moynihan PJ, Kelly SA. Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines. J Dent Res. 2014;93(1):8-18.
23. Te Morenga LA, Howatson AJ, Jones RM, Mann J. 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.
24. Jayasinghe SN, Kruger R, Walsh DCI, Cao G, Rivers S, Richter M, et al. Is Sweet Taste Perception Associated with Sweet Food Liking and Intake? Nutrients. 2017;9(7).
25. 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.
26. 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.
27. Services USDoAaUSDoHaH. Dietary Guidelines for Americans. Washington; December 2010.
28. Erickson J, Slavin J. Total, added, and free sugars: are restrictive guidelines science-based or achievable? Nutrients. 2015;7(4):2866-78.
29. Agriculture ARttSoHaHSatSo. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. 2015.
30. Nordic Council of Ministers NCoMS. Nordic Nutrition Recommendations 2012: Integrating nutrition and physical activity. 2014.
31. Christian Lindmeier OLD. WHO calls on countries to reduce sugars intake among adults and children Geneva 2015 [Available from: https://www.who.int/mediacentre/news/releases/2015/sugar-guideline/en/.
32. Guideline: Sugars Intake for Adults and Children. WHO Guidelines Approved by the Guidelines Review Committee. Geneva2015.
33. Cummings JH, Stephen AM. Carbohydrate terminology and classification. Eur J Clin Nutr. 2007;61 Suppl 1:S5-18.
34. Scapin T, Fernandes AC, Dos Anjos A, Proenca R. Use of added sugars in packaged foods sold in Brazil. Public Health Nutr. 2018;21(18):3328-34.
35. Tappy L, Morio B, Azzout-Marniche D, Champ M, Gerber M, Houdart S, et al. French Recommendations for Sugar Intake in Adults: A Novel Approach Chosen by ANSES. Nutrients. 2018;10(8).
36. EFSA Panel on Dietetic Products N, and Allergies (NDA). Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre. 2010.
37. Organization WH. Fruit and Vegetable Promotion Initiative. Geneva; 2003 25–27 August 2003.
38. Lustig RH, Schmidt LA, Brindis CD. Public health: The toxic truth about sugar. Nature. 2012;482(7383):27-9.
39. Hanover LM, White JS. Manufacturing, composition, and applications of fructose. Am J Clin Nutr. 1993;58(5 Suppl):724S-32S.
40. White JS. Fructose, High Fructose Corn Syrup, Sucrose and Health2014.

41. Livesey G, Taylor R. Fructose consumption and consequences for glycation, plasma triacylglycerol, and body weight: meta-analyses and meta-regression models of intervention studies. Am J Clin Nutr. 2008;88(5):1419-37.
42. Flood-Obbagy JE, Rolls BJ. The effect of fruit in different forms on energy intake and satiety at a meal. Appetite. 2009;52(2):416-22.
43. Aune D, Giovannucci E, Boffetta P, Fadnes LT, Keum N, Norat T, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. Int J Epidemiol. 2017;46(3):1029-56.
44. Muraki I, Imamura F, Manson JE, Hu FB, Willett WC, van Dam RM, et al. Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies. BMJ. 2013;347:f5001.
45. Bolton RP, Heaton KW, Burroughs LF. The role of dietary fiber in satiety, glucose, and insulin: studies with fruit and fruit juice. Am J Clin Nutr. 1981;34(2):211-7.
46. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr. 1999;70(3 Suppl):475S-90S.
47. Drozdowski LA, Thomson AB. Intestinal sugar transport. World J Gastroenterol. 2006;12(11):1657-70.
48. The Gastrointestinal System, Gastrointestinal, Nutritional and Hepatobiliary Physiology: Springer Netherlands; 2014.
49. Rumessen JJ, Gudmand-Hoyer E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. Gut. 1986;27(10):1161-8.
50. Gibson PR, Newnham E, Barrett JS, Shepherd SJ, Muir JG. Review article: fructose malabsorption and the bigger picture. Aliment Pharmacol Ther. 2007;25(4):349-63.
51. Groen J. The Absorption of Hexoses from the Upper Part of the Small Intestine in Man. J Clin Invest. 1937;16(2):245-55.
52. Skoog SM, Bharucha AE. Dietary fructose and gastrointestinal symptoms: a review. Am J Gastroenterol. 2004;99(10):2046-50.
53. Kneepkens CM, Vonk RJ, Fernandes J. Incomplete intestinal absorption of fructose. Arch Dis Child. 1984;59(8):735-8.
54. Hoekstra JH, van den Aker JH. Facilitating effect of amino acids on fructose and sorbitol absorption in children. J Pediatr Gastroenterol Nutr. 1996;23(2):118-24.
55. Douard V, Ferraris RP. Regulation of the fructose transporter GLUT5 in health and disease. Am J Physiol Endocrinol Metab. 2008;295(2):E227-37.
56. Tornheim K, Lowenstein JM. Control of phosphofructokinase from rat skeletal muscle. Effects of fructose diphosphate, AMP, ATP, and citrate. J Biol Chem. 1976;251(23):7322-8.
57. Heinz F, Lamprecht W, Kirsch J. Enzymes of fructose metabolism in human liver. J Clin Invest. 1968;47(8):1826-32.
58. Tran LT, Yuen VG, McNeill JH. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem. 2009;332(1-2):145-59.
59. Ali M, Rellos P, Cox TM. Hereditary fructose intolerance. J Med Genet. 1998;35(5):353-65.
60. Mayes PA. Intermediary metabolism of fructose. Am J Clin Nutr. 1993;58(5 Suppl):754S-65S.
61. Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev. 2010;90(1):23-46.
62. Sun SZ, Empie MW. Fructose metabolism in humans - what isotopic tracer studies tell us. Nutr Metab (Lond). 2012;9(1):89.

63. 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.
64. Diraison F, Pachiaudi C, Beylot M. Measuring lipogenesis and cholesterol synthesis in humans with deuterated water: use of simple gas chromatographic/mass spectrometric techniques. J Mass Spectrom. 1997;32(1):81-6.
65. Lecoultre V, Benoit R, Carrel G, Schutz Y, Millet GP, Tappy L, et al. 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(5):1071-9.
66. 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(1):54.
67. Moore MC, Cherrington AD, Mann SL, Davis SN. Acute fructose administration decreases the glycemic response to an oral glucose tolerance test in normal adults. J Clin Endocrinol Metab. 2000;85(12):4515-9.
68. Geidl-Flueck B, Gerber PA. Insights into the Hexose Liver Metabolism-Glucose versus Fructose. Nutrients. 2017;9(9).
69. Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem J. 2008;414(1):1-18.
70. Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. Am J Public Health. 2007;97(4):667-75.
71. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab (Lond). 2005;2(1):5.
72. Miller A, Adeli K. Dietary fructose and the metabolic syndrome. Curr Opin Gastroenterol. 2008;24(2):204-9.
73. Expert Committee on the D, Classification of Diabetes M. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003;26 Suppl 1:S5-20.
74. Goke B. Islet cell function: alpha and beta cells--partners towards normoglycaemia. Int J Clin Pract Suppl. 2008(159):2-7.
75. Roder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Exp Mol Med. 2016;48:e219.
76. Gray A. Nutritional Recommendations for Individuals with Diabetes. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, et al., editors. Endotext. South Dartmouth (MA)2000.
77. Black RN, Spence M, McMahon RO, Cuskelly GJ, Ennis CN, McCance DR, et al. Effect of eucaloric high- and low-sucrose diets with identical macronutrient profile on insulin resistance and vascular risk: a randomized controlled trial. Diabetes. 2006;55(12):3566-72.
78. Evans RA, Frese M, Romero J, Cunningham JH, Mills KE. Fructose replacement of glucose or sucrose in food or beverages lowers postprandial glucose and insulin without raising triglycerides: a systematic review and meta-analysis. Am J Clin Nutr. 2017;106(2):506-18.
79. Cozma AI, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Wang DD, et al. Effect of fructose on glycemic control in diabetes: a systematic review and meta-analysis of controlled feeding trials. Diabetes Care. 2012;35(7):1611-20.
80. American Diabetes A. Standards of medical care for patients with diabetes mellitus. Diabetes Care. 2003;26 Suppl 1:S33-50.
81. Malerbi DA, Paiva ES, Duarte AL, Wajchenberg BL. Metabolic effects of dietary sucrose and fructose in type II diabetic subjects. Diabetes Care. 1996;19(11):1249-56.

82. Le KA, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P, 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.
83. Lecoultre V, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P, et 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.
84. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. Am J Clin Nutr. 2011;94(2):479-85.
85. Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Haring HU, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr. 2011;106(1):79-86.
86. Couchepin C, Le KA, Bortolotti M, da Encarnacao JA, Oboni JB, Tran C, et al. Markedly blunted metabolic effects of fructose in healthy young female subjects compared with male subjects. Diabetes Care. 2008;31(6):1254-6.
87. Moore MC, Davis SN, Mann SL, Cherrington AD. Acute fructose administration improves oral glucose tolerance in adults with type 2 diabetes. Diabetes Care. 2001;24(11):1882-7.
88. Le KA, Tappy L. Metabolic effects of fructose. Curr Opin Clin Nutr Metab Care. 2006;9(4):469-75.
89. Herman MA, Samuel VT. The Sweet Path to Metabolic Demise: Fructose and Lipid Synthesis. Trends Endocrinol Metab. 2016;27(10):719-30.
90. Stanhope KL, Bremer AA, Medici V, Nakajima K, Ito Y, Nakano T, 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(10):E1596-605.
91. Le KA, Ith M, Kreis R, Faeh D, Bortolotti M, Tran C, 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(6):1760-5.
92. Sobrecases H, Le KA, Bortolotti M, Schneiter P, Ith M, Kreis R, et al. Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. Diabetes Metab. 2010;36(3):244-6.
93. Ngo Sock ET, Le KA, Ith M, Kreis R, Boesch C, Tappy L. Effects of a short-term overfeeding with fructose or glucose in healthy young males. Br J Nutr. 2010;103(7):939-43.
94. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev. 2005;63(5):133-57.
95. Swarbrick MM, Stanhope KL, Elliott SS, Graham JL, Krauss RM, Christiansen MP, et al. Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-B concentrations in overweight and obese women. Br J Nutr. 2008;100(5):947-52.
96. Teff KL, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, et al. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. J Clin Endocrinol Metab. 2009;94(5):1562-9.
97. Jonkers IJ, Smelt AH, van der Laarse A. Hypertriglyceridemia: associated risks and effect of drug treatment. Am J Cardiovasc Drugs. 2001;1(6):455-66.
98. 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(7):1907-13.

99. Vos MB, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. Hepatology. 2013;57(6):2525-31.
100. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, 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.
101. Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. J Clin Invest. 1982;69(5):1119-25.
102. Teff KL, Elliott SS, Tschop M, Kieffer TJ, Rader D, Heiman M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. J Clin Endocrinol Metab. 2004;89(6):2963-72.
103. Jameel F, Phang M, Wood LG, Garg ML. Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. Lipids Health Dis. 2014;13:195.
104. 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(5):1194-203.
105. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, et al. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr. 2005;81(2):341-54.
106. Hellerstein MK. Synthesis of fat in response to alterations in diet: insights from new stable isotope methodologies. Lipids. 1996;31 Suppl:S117-25.
107. McDevitt RM, Bott SJ, Harding M, Coward WA, Bluck LJ, Prentice AM. De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women. Am J Clin Nutr. 2001;74(6):737-46.
108. Schwarz JM, Noworolski SM, Wen MJ, Dyachenko A, Prior JL, Weinberg ME, et al. Effect of a High-Fructose Weight-Maintaining Diet on Lipogenesis and Liver Fat. J Clin Endocrinol Metab. 2015;100(6):2434-42.
109. Umpleby AM, Shojaee-Moradie F, Fielding B, Li X, Marino A, Alsini N, et al. Impact of liver fat on the differential partitioning of hepatic triacylglycerol into VLDL subclasses on high and low sugar diets. Clin Sci (Lond). 2017;131(21):2561-73.
110. Sievenpiper JL, Carleton AJ, Chatha S, Jiang HY, de Souza RJ, Beyene J, et al. Heterogeneous effects of fructose on blood lipids in individuals with type 2 diabetes: systematic review and meta-analysis of experimental trials in humans. Diabetes Care. 2009;32(10):1930-7.
111. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav. 2008;94(2):231-41.
112. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, et al. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. Int J Endocrinol. 2012;2012:983814.
113. Britton KA, Fox CS. Ectopic fat depots and cardiovascular disease. Circulation. 2011;124(24):e837-41.
114. Stanhope KL, Havel PJ. Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. Curr Opin Lipidol. 2008;19(1):16-24.
115. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol. 2018;24(30):3361-73.

116. Surowska A, Jegatheesan P, Campos V, Marques AS, Egli L, Cros J, et al. Effects of Dietary Protein and Fat Content on Intrahepatocellular and Intramyocellular Lipids during a 6-Day Hypercaloric, High Sucrose Diet: A Randomized Controlled Trial in Normal Weight Healthy Subjects. Nutrients. 2019;11(1).
117. Johnston RD, Stephenson MC, Crossland H, Cordon SM, Palcidi E, Cox EF, et al. No difference between high-fructose and high-glucose diets on liver triacylglycerol or biochemistry in healthy overweight men. Gastroenterology. 2013;145(5):1016-25 e2.
118. Campos V, Despland C, Brandejsky V, Kreis R, Schneiter P, Chiolero A, et al. Sugar- and artificially sweetened beverages and intrahepatic fat: A randomized controlled trial. Obesity (Silver Spring). 2015;23(12):2335-9.
119. Geisler CE, Renquist BJ. Hepatic lipid accumulation: cause and consequence of dysregulated glucoregulatory hormones. J Endocrinol. 2017;234(1):R1-R21.
120. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. Clin Exp Gastroenterol. 2014;7:221-39.
121. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115(5):1343-51.
122. Bravo S, Lowndes J, Sinnett S, Yu Z, Rippe J. Consumption of sucrose and high-fructose corn syrup does not increase liver fat or ectopic fat deposition in muscles. Appl Physiol Nutr Metab. 2013;38(6):681-8.
123. Kelley D, Mitrakou A, Marsh H, Schwenk F, Benn J, Sonnenberg G, et al. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. J Clin Invest. 1988;81(5):1563-71.
124. Jalal DI, Smits G, Johnson RJ, Chonchol M. Increased fructose associates with elevated blood pressure. J Am Soc Nephrol. 2010;21(9):1543-9.
125. Ha V, Sievenpiper JL, de Souza RJ, Chiavaroli L, Wang DD, Cozma AI, et al. Effect of fructose on blood pressure: a systematic review and meta-analysis of controlled feeding trials. Hypertension. 2012;59(4):787-95.
126. Perez-Pozo SE, Schold J, Nakagawa T, Sanchez-Lozada LG, Johnson RJ, Lillo JL. Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: role of uric acid in the hypertensive response. Int J Obes (Lond). 2010;34(3):454-61.
127. Brown CM, Dulloo AG, Yepuri G, Montani JP. Fructose ingestion acutely elevates blood pressure in healthy young humans. Am J Physiol Regul Integr Comp Physiol. 2008;294(3):R730-7.
128. Le MT, Frye RF, Rivard CJ, Cheng J, McFann KK, Segal MS, et al. 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(5):641-51.
129. Klein AV, Kiat H. The mechanisms underlying fructose-induced hypertension: a review. J Hypertens. 2015;33(5):912-20.
130. Choi JW, Ford ES, Gao X, Choi HK. Sugar-sweetened soft drinks, diet soft drinks, and serum uric acid level: the Third National Health and Nutrition Examination Survey. Arthritis Rheum. 2008;59(1):109-16.
131. Wang DD, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Cozma AI, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. J Nutr. 2012;142(5):916-23.
132. Carran EL, White SJ, Reynolds AN, Haszard JJ, Venn BJ. Acute effect of fructose intake from sugar-sweetened beverages on plasma uric acid: a randomised controlled trial. Eur J Clin Nutr. 2016;70(9):1034-8.
133. Chen LY, Zhu WH, Chen ZW, Dai HL, Ren JJ, Chen JH, et al. Relationship between hyperuricemia and metabolic syndrome. J Zhejiang Univ Sci B. 2007;8(8):593-8.

134. Crapo PA, Kolterman OG, Olefsky JM. Effects of oral fructose in normal, diabetic, and impaired glucose tolerance subjects. Diabetes Care. 1980;3(5):575-82.
135. Theytaz F, de Giorgi S, Hodson L, Stefanoni N, Rey V, Schneiter P, et al. Metabolic fate of fructose ingested with and without glucose in a mixed meal. Nutrients. 2014;6(7):2632-49.
136. Sievenpiper JL, de Souza RJ, Mirrahimi A, Yu ME, Carleton AJ, Beyene J, et al. Effect of fructose on body weight in controlled feeding trials: a systematic review and meta-analysis. Ann Intern Med. 2012;156(4):291-304.
137. David Wang D, Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, et al. Effect of fructose on postprandial triglycerides: a systematic review and meta-analysis of controlled feeding trials. Atherosclerosis. 2014;232(1):125-33.
138. Chiu S, Sievenpiper JL, de Souza RJ, Cozma AI, Mirrahimi A, Carleton AJ, et al. Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials. Eur J Clin Nutr. 2014;68(4):416-23.
139. Egli L, Lecoultre V, Theytaz F, Campos V, Hodson L, Schneiter P, et al. Exercise prevents fructose-induced hypertriglyceridemia in healthy young subjects. Diabetes. 2013;62(7):2259-65.
140. Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, Mirrahimi A. Fructose vs. glucose and metabolism: do the metabolic differences matter? Curr Opin Lipidol. 2014;25(1):8-19.
141. Theytaz F, Noguchi Y, Egli L, Campos V, Buehler T, Hodson L, et al. Effects of supplementation with essential amino acids on intrahepatic lipid concentrations during fructose overfeeding in humans. Am J Clin Nutr. 2012;96(5):1008-16.
142. Ter Horst KW, Schene MR, Holman R, Romijn JA, Serlie MJ. Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: a systematic review and meta-analysis of diet-intervention trials. Am J Clin Nutr. 2016;104(6):1562-76.
143. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology. 2003;144(12):5159-65.
144. Kitade H, Chen G, Ni Y, Ota T. Nonalcoholic Fatty Liver Disease and Insulin Resistance: New Insights and Potential New Treatments. Nutrients. 2017;9(4).
145. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62(10):3307-15.
146. Aeberli I, Hochuli M, Gerber PA, Sze L, Murer SB, Tappy L, et al. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. Diabetes Care. 2013;36(1):150-6.
147. Taskinen MR, Soderlund S, Bogl LH, Hakkarainen A, Matikainen N, Pietilainen KH, et al. Adverse effects of fructose on cardiometabolic risk factors and hepatic lipid metabolism in subjects with abdominal obesity. J Intern Med. 2017;282(2):187-201.
148. Knight JA. Physical inactivity: associated diseases and disorders. Ann Clin Lab Sci. 2012;42(3):320-37.
149. 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(11):2091-8.
150. Wilburn JR, Bourquin J, Wysong A, Melby CL. Resistance Exercise Attenuates High-Fructose, High-Fat-Induced Postprandial Lipemia. Nutr Metab Insights. 2015;8:29-35.
151. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727-47.
152. Lecoultre V, Carrel G, Egli L, Binnert C, Boss A, MacMillan EL, et al. Coffee consumption attenuates short-term fructose-induced liver insulin resistance in healthy men. Am J Clin Nutr. 2014;99(2):268-75.

153. van Dam RM. Coffee consumption and risk of type 2 diabetes, cardiovascular diseases, and cancer. Appl Physiol Nutr Metab. 2008;33(6):1269-83.
154. Nordestgaard AT, Thomsen M, Nordestgaard BG. Coffee intake and risk of obesity, metabolic syndrome and type 2 diabetes: a Mendelian randomization study. Int J Epidemiol. 2015;44(2):551-65.
155. Bhupathiraju SN, Pan A, Malik VS, Manson JE, Willett WC, van Dam RM, et al. Caffeinated and caffeine-free beverages and risk of type 2 diabetes. Am J Clin Nutr. 2013;97(1):155-66.
156. van Dam RM, Willett WC, Manson JE, Hu FB. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. Diabetes Care. 2006;29(2):398-403.
157. Akash MS, Rehman K, Chen S. Effects of coffee on type 2 diabetes mellitus. Nutrition. 2014;30(7-8):755-63.
158. Tunnicliffe JM, Shearer J. Coffee, glucose homeostasis, and insulin resistance: physiological mechanisms and mediators. Appl Physiol Nutr Metab. 2008;33(6):1290-300.
159. Molloy JW, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. Hepatology. 2012;55(2):429-36.
160. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol. 2011;58(20):2047-67.
161. Stark KD, Park EJ, Maines VA, Holub BJ. Effect of a fish-oil concentrate on serum lipids in postmenopausal women receiving and not receiving hormone replacement therapy in a placebo-controlled, double-blind trial. Am J Clin Nutr. 2000;72(2):389-94.
162. Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes Relat Metab Disord. 1997;21(8):637-43.
163. Cummings DE, Overduin J, Foster-Schubert KE. Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution. J Clin Endocrinol Metab. 2004;89(6):2608-15.
164. Suter M, Giusti V, Heraief E, Zysset F, Calmes JM. Laparoscopic Roux-en-Y gastric bypass: initial 2-year experience. Surg Endosc. 2003;17(4):603-9.
165. Kaska L, Sledzinski T, Chomiczewska A, Dettlaff-Pokora A, Swierczynski J. Improved glucose metabolism following bariatric surgery is associated with increased circulating bile acid concentrations and remodeling of the gut microbiome. World J Gastroenterol. 2016;22(39):8698-719.
166. Wang G, Agenor K, Pizot J, Kotler DP, Harel Y, Van Der Schueren BJ, et al. Accelerated gastric emptying but no carbohydrate malabsorption 1 year after gastric bypass surgery (GBP). Obes Surg. 2012;22(8):1263-7.
167. Lingvay I, Guth E, Islam A, Livingston E. Rapid improvement in diabetes after gastric bypass surgery: is it the diet or surgery? Diabetes Care. 2013;36(9):2741-7.
168. De Giorgi S, Campos V, Egli L, Toepel U, Carrel G, Cariou B, et al. Long-term effects of Roux-en-Y gastric bypass on postprandial plasma lipid and bile acids kinetics in female non diabetic subjects: A cross-sectional pilot study. Clin Nutr. 2015;34(5):911-7.
169. Poitou Bernert C, Ciangura C, Coupaye M, Czernichow S, Bouillot JL, Basdevant A. Nutritional deficiency after gastric bypass: diagnosis, prevention and treatment. Diabetes Metab. 2007;33(1):13-24.
170. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009;89(1):147-91.

171. Fu L, John LM, Adams SH, Yu XX, Tomlinson E, Renz M, et al. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. Endocrinology. 2004;145(6):2594-603.
172. Bortolotti M, Kreis R, Debard C, Cariou B, Faeh D, Chetiveaux M, et al. High protein intake reduces intrahepatocellular lipid deposition in humans. Am J Clin Nutr. 2009;90(4):1002-10.
173. Bortolotti M, Maiolo E, Corazza M, Van Dijke E, Schneiter P, Boss A, et al. Effects of a whey protein supplementation on intrahepatocellular lipids in obese female patients. Clin Nutr. 2011;30(4):494-8.
174. Ding L, Yang L, Wang Z, Huang W. Bile acid nuclear receptor FXR and digestive system diseases. Acta Pharm Sin B. 2015;5(2):135-44.
175. Odstrcil EA, Martinez JG, Santa Ana CA, Xue B, Schneider RE, Steffer KJ, et al. The contribution of malabsorption to the reduction in net energy absorption after long-limb Roux-en-Y gastric bypass. Am J Clin Nutr. 2010;92(4):704-13.
176. Yki-Jarvinen H. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. Curr Opin Clin Nutr Metab Care. 2010;13(6):709-14.
177. Haidari M, Leung N, Mahbub F, Uffelman KD, Kohen-Avramoglu R, Lewis GF, 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(35):31646-55.
178. O'Keefe SJD, Rakitt T, Ou J, El H, II, Blaney E, Vipperla K, et al. Pancreatic and Intestinal Function Post Roux-en-Y Gastric Bypass Surgery for Obesity. Clin Transl Gastroenterol. 2017;8(8):e112.
179. Leidy HJ, Mattes RD, Campbell WW. Effects of acute and chronic protein intake on metabolism, appetite, and ghrelin during weight loss. Obesity (Silver Spring). 2007;15(5):1215-25.
180. Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. EMBO J. 2006;25(7):1419-25.
181. Linn T, Santosa B, Gronemeyer D, Aygen S, Scholz N, Busch M, et al. Effect of long-term dietary protein intake on glucose metabolism in humans. Diabetologia. 2000;43(10):1257-65.
182. Yoon MS. The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism. Nutrients. 2016;8(7).
183. Kim J, Hwang JY, Kim KN, Choi YJ, Chang N, Huh KB. Relationship between milk and calcium intake and lipid metabolism in female patients with type 2 diabetes. Yonsei Med J. 2013;54(3):626-36.
184. Thorning TK, Bertram HC, Bonjour JP, de Groot L, Dupont D, Feeney E, et al. Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps. Am J Clin Nutr. 2017;105(5):1033-45.
185. Faraj M, Jones P, Sniderman AD, Cianflone K. Enhanced dietary fat clearance in postobese women. J Lipid Res. 2001;42(4):571-80.
186. Jeppesen J, Chen YI, Zhou MY, Schaaf P, Coulston A, Reaven GM. Postprandial triglyceride and retinyl ester responses to oral fat: effects of fructose. Am J Clin Nutr. 1995;61(4):787-91.
187. Parks EJ, Skokan LE, Timlin MT, Dingfelder CS. Dietary sugars stimulate fatty acid synthesis in adults. J Nutr. 2008;138(6):1039-46.
188. Surowska A, De Giorgi S, Theytaz F, Campos V, Hodson L, Stefanoni N, et al. Effects of roux-en-Y gastric bypass surgery on postprandial fructose metabolism. Obesity (Silver Spring). 2016;24(3):589-96.

189. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. Am J Clin Nutr. 2000;72(5):1128-34.
190. Lowndes J, Sinnett S, Pardo S, Nguyen VT, Melanson KJ, Yu Z, et al. The effect of normally consumed amounts of sucrose or high fructose corn syrup on lipid profiles, body composition and related parameters in overweight/obese subjects. Nutrients. 2014;6(3):1128-44.
191. Bortolotti M, Dubuis J, Schneiter P, Tappy L. Effects of dietary protein on lipid metabolism in high fructose fed humans. Clin Nutr. 2012;31(2):238-45.
192. Stanhope KL, Griffen SC, Bremer AA, Vink RG, Schaefer EJ, Nakajima K, et al. Metabolic responses to prolonged consumption of glucose- and fructose-sweetened beverages are not associated with postprandial or 24-h glucose and insulin excursions. Am J Clin Nutr. 2011;94(1):112-9.
193. Stanhope KL, Medici V, Bremer AA, Lee V, Lam HD, Nunez MV, et al. A dose-response study of consuming high-fructose corn syrup-sweetened beverages on lipid/lipoprotein risk factors for cardiovascular disease in young adults. Am J Clin Nutr. 2015;101(6):1144-54.