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1. Fructose effects on human health: Molecular insights from experimental models

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Abstract. Global changes in dietary habits in the last decades caused an increase of added sugar consumption all over the world, which has been linked to the increasing prevalence of obesity, dyslipidemia, insulin resistance and cardiovascular disease. Fructose is widely used as a sweetener in the food and beverage industry, either as an integrant of the sucrose molecule or as a component of high fructose corn syrups. The consumption of fructose in beverages is especially dangerous, as the process of energy compensation by reduction in the ingestion of other foods does not work equally well with liquid than solid foods. Besides, fructose is the carbohydrate with the highest ability to induce hypertriglyceridemia, due to a marked increase in lipogenesis compared with glucose. In this review we will discuss some of the most recent studies performed in animal models and in humans to investigate the effects of excessive fructose consumption.

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Introduction

Fructose is a natural sugar contained in fruits and honey, and in this form it constitutes a component of a healthy, well-balanced diet. Fructose is also used to sweeten foods and beverages during processing or preparation, and then we refer to fructose as an "added sugar". Usually, fructose is added to foodstuffs as sucrose (table sugar, a disaccharide composed of glucose and fructose) or as high fructose corn syrup (HFCS, a mixture of fructose and glucose at variable proportions, typically 55% fructose/45% glucose). Global changes in dietary habits in the last decades caused an increase of added sugar consumption all over the world, but especially in industrialized countries. As an example, US dietary data show that between 1977 and 1994 the average daily consumption of added sugars increased by 35% [1]. Although these data refer to all caloric sweeteners added to food or drinks, added sugars are considered a surrogate for fructose consumption [2]. The increase in the consumption of added sugars has been linked to several health disturbances, such as obesity, dyslipidemia, insulin resistance and cardiovascular disease [3-6]. Recognizing these deleterious effects to human health, several dietary guidelines from different countries provided recommendations to moderate the intake of added sugars, and possibly as a result of these policies, their consumption has decreased since 1999 [7]. However, sugar consumption is still excessive according to the American Heart Association latest recommendations, which fixed an upper limit of intake for added sugars of 100-150 calories per day [8]. Consistent with this, research on the metabolic effects of the main added sugar, fructose, and the molecular mechanisms involved is warranted. In previous articles we revised scientific evidences, from both animal and human studies, linking fructose consumption to metabolic disturbances, up to 2011 [9,10]. In the present review, we will focus on articles published during 2012 and 2013, as well as on our own results, obtained using liquid fructose-supplemented rats and mice as experimental models. We will also review some of the most recent studies investigating the effects of high fructose consumption in humans.

1. Studies in animal models

Fructose administration to laboratory animals (most commonly rodents) induces metabolic derangements in several tissues -namely liver, adipose tissue, heart and skeletal muscle- so these animals can be used as models to decipher the molecular mechanisms involved in these alterations.

1.1. Fructose effects in the liver

1.1.1. Fructose, fatty liver and mechanisms involved

In an earlier study [11] we demonstrated that fructose administration to male rats (10% w/v in drinking water for 14 days) causes hypertriglycerideamia and hepatic steatosis due the conjunction of two metabolic alterations, an increase in liver lipogenesis and a decrease in hepatic fatty acid β–oxidation. The latter is a pathway that catalyzes the catabolism of fatty acids after they are activated to their acyl-CoA forms by the enzyme acyl CoA synthase (ACS). Recently, Dong et al [12] investigated the role of long chain ACS (ACSL) in hamsters fed a diet containing 60% of fructose for 28 days. Their results showed that the fructose diet specifically reduced ACSL through liver X receptor (LXR) and retinoid X receptor (RXR) signaling, opening the possibility that LXR agonists, by increasing hepatic ACSL expression, could reduce fructose-induced hepatic steatosis.

Creszenzo et al. also found an increase in hepatic de novo lipogenesis in male Sprague Dawley rats fed a fructose-rich diet (30% fructose in solid form) for 8 weeks [13]. The most interesting finding of this study was increased hepatic mitochondrial mass in fructose-fed rats. The authors hypothesize that this would increase the flux of substrates through pyruvate carboxylase and pyruvate dehydrogenase, generating substrates for gluconeogenesis. Increased hepatic gluconeogenesis is one of the classic features of insulin resistance, and in fact we observed a rise in the liver glucose output after a bolus administration of a direct gluconeogenic precursor, such as pyruvate, to 14-day fructose-supplemented rats [14]. However, we were surprised to notice a significant reduction in the expression of two key gluconeogenic enzymes, phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pc), despite a clear impairment in insulin signaling in the livers of these rats. Creszenzo et al proposed that an increase in the expression of enzymes controlling the substrate flux from cytosolic pyruvate to glucose formation could explain enhanced hepatic gluconeogenesis [13]. In fact, we observed an increase in the hepatic expression of the enzymes malate dehydrogenase and malic enzyme in 14-day fructose-supplemented rats, which could explain the increased liver output of glucose in the pyruvate tolerance test, despite the reduced expression of both G6Pc and PEPCK [14].

Several studies analyzed how fructokinase, the first enzyme in the hepatic metabolism of fructose, contributes to the deposition of fat in the liver. Ishimoto et al. [15] demonstrated that mice lacking both fructokinase isoforms (A and C) were protected from the fructose-induced features of

metabolic syndrome (including hepatic steatosis) observed in wild type mice. Moreover, these effects were exacerbated in mice lacking fructokinase A, suggesting that this isoform protects against fructokinase C-mediated metabolic syndrome.

Lanaspa et al [16] used fructokinase knockout mice to evaluate the effects of glucose supplementation (10% w/v in drinking water). Glucose feeding induced fatty liver in wild type but not in fructokinase-deficient animals, showing that this effect was almost entirely mediated by fructokinase. However, fructokinase does not metabolize glucose, therefore the protective effect was attributed to the fact that a significant proportion of the ingested glucose was converted into fructose in the liver. Metabolism of this endogenous fructose is blocked in fructokinase-deficient mice, protecting against fatty liver. More recently, the same research group studied whether fructokinase had also a role in fatty liver induction through a high fat-high sucrose diet (HFHSD) [17]. They observed that wild type mice fed a HFHSD developed more severe hepatic steatosis compared to mice fed a high fat diet. Moreover HFHSD-fed wild type mice showed hepatic inflammation and collagen deposition in the liver, while the development of steatosis from a high fat diet alone did not induce inflammation or fibrosis. Importantly, fructokinase knockout mice fed a HFHSD were completely protected from the development of hepatic inflammation and fibrosis despite similar energy intake compared to wild type mice.

1.1.2. Fructose and hepatic insulin resistance

In animal models, fructose feeding (especially at high percentages in solid form) induces hepatic insulin resistance. We also observed insulin resistance in female, but not male rats, supplemented with 10% fructose in liquid form for 2 weeks [9]. Our preliminary results, after 8 weeks of supplementation in female rats, show hyperinsulinemia and a clear reduction in the expression of insulin receptor substrate (IRS)-2, a major transducer of insulin signaling in the liver (Figure 1). However, plasma glucose levels and ISI were not altered, suggesting that in these animals increased insulin suffices to control plasma glucose levels, masking the underlying insulin resistance (unpublished results). We also observed that mice fed a high fat (western) diet plus 15% liquid fructose (W+F) consumed an equivalent amount of calories compared to mice fed the western diet alone without fructose (W), but only the W+F group exhibited hyperinsulinemia and a reduction in the insulin sensitivity index (ISI) compared to mice fed normal chow [18]. This is in line with the reduced Akt phosphorylation seen only in the W+F group, and points out to a specific effect of fructose, only when it is

given in combination with a high fat diet, on hepatic insulin signaling in mice [18].

We reported previously that carbohydrate response element binding protein (ChREBP) is the main responsible for the increase in hepatic lipogenesis following fructose supplementation in rats [11, 19-21]. Recently, Erion et al studied whether ChREBP is also related to fructose-induced insulin resistance [22]. To this end, they administered a ChREBP specific antisense oligonucleotide to male Sprague-Dawley rats fed a high-fructose diet (60% fructose in solid form). As expected, knockdown of ChREBP led to a decrease in the expression of genes controlled by this transcription factor (lipogenic genes such as ACC, FAS and SCD-1). ChREBP knockdown caused a tendency towards decreased de novo lipogenesis, but these changes did not correlate with a decrease in hepatic triglyceride, and therefore were not associated with improvements in hepatic insulin sensitivity.

1.1.3. Endoplasmic reticulum stress, autophagy and inflammation

It has been suggested that endoplasmic reticulum (ER) stress may play a role in the development of hepatic insulin resistance under conditions of elevated de novo lipogenesis, as occurs with increased fructose consumption. ER stress is characterized by the activation of the unfolded protein response (UPR) signaling, that reduce temporarily the flow of proteins that reach the ER. The UPR is initiated by three transmembrane proteins: IRE1 (inositol-requiring enzyme 1), PERK (protein kinase-like reticulum kinase) and ATF-6 (activating transcription factor-6). A recent study in mice fed a high fat diet (60% from saturated fat) or a high fructose diet (35% fructose in solid form), showed that both diets caused liver steatosis and hepatic insulin resistance, but only the high fructose diet increased de novo lipogenesis, and this effect was coupled with activation of the IRE1 and PERK patways [23]. The same research group demonstrated recently that treatment of the fructose-fed mice with fenofibrate, a PPARα agonist, eliminated fructose-induced hepatic steatosis and insulin resistance while causing an activation of the IRE1 and PERK branches of the UPR [24].

We also studied the effects of fructose feeding (10% w/v in drinking water for 8 weeks) in female Sprague Dawley rats on ER stress pathways [25]. Neither PERK nor ATF-6 branches were affected by fructose, while there was a marked increase in IRE1 phosphorylation, indicative of activation. This is in accordance with our previous results in female rats supplemented with fructose for 2 weeks [9]. However, while in 2-weeks supplemented rats the increase in IRE1 phosphorylation correlated with an increase in the spliced form of X-box-binding protein (XBP)-1 mRNA and nuclear protein [9], this effect is not apparent after 8 weeks of treatment

(Figure 1), suggesting that ER stress is somehow compensated in these animals. It remains to be determined whether longer treatments with fructose maintain or rather exacerbate the ER stress response.

Due to the involvement of ER stress in the metabolic alterations caused by fructose feeding, Ren et al studied the effects of an ER stress inhibitor (4-phenylbutyric acid, PBA) on rats fed a high fructose diet [26]. The authors showed that treatment with PBA significantly reduced hepatic ER stress and improved liver steatosis induced by high-fructose feeding.

Insulin resistance has also been associated to a state of chronic low-grade inflammation derived from the activation of the inflammasome [27]. The inflammasommes are large multimolecular complexes that upon activation by various stimuli result in the processing and maturation of the precursors of the inflammatory cytokines interleukin (IL)-1β and IL-18, via caspase-1. A significant increase in renal nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome has been recently shown in rats fed fructose [28] or high fructose corn syrup [29]. However, it is not known whether the NLRP3 inflammasome is also induced in tissues related to insulin resistance such as the liver, muscle or adipose tissue.

There are also evidences suggesting that autophagy, a pathway that allows the recycling of cellular constituents and facilitates cellular health under ER stress, may play an important role in obesity-induced insulin resistance [30], and recently it has been shown that activated mammalian target of rapamycin (mTOR) suppresses autophagy [31]. Recent results of our research group indicate that fructose markedly increases mTOR activity in the livers of 2- [14] and 8-week supplemented rats. Thus, it is plausible that chronic fructose administration would reduce autophagy via mTOR activation. Our preliminary unpublished results are in line with this hypothesis, as the ratio of active to inactive light chain 3 (LC3B), an indicator of autophagy, seems to be reduced in the livers of 8-week fructose-supplemented female rats (Figure 1).

It has been suggested that leptin, an adipokine mostly produced by white adipose cells, may also regulate autophagy [32]. A recent report showed that long-term (6 months) postnatal high fructose diet (60% in solid form) in Wistar rats did not affect plasma leptin levels, but decreased the mRNA expression of leptin receptors and autophagy-related genes in white adipose tissue, while increasing them in the liver [33]. Our results in rats supplemented with 10% liquid fructose for 8 weeks (non-published) show a significant 1.8-fold increase in plasma leptin levels. However, when we express these results related to white adipose tissue weight, there is no difference between control and fructose-supplemented rats, suggesting that the increase of adipose tissue mass accounts for the observed hyperleptinemia.

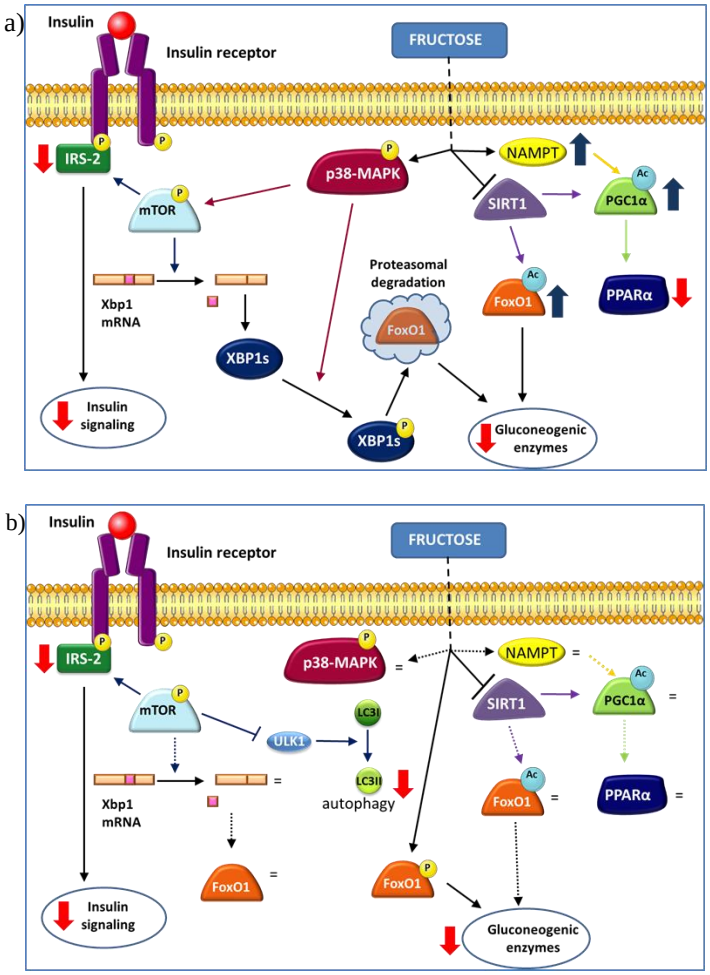

Figure 1. Differential effects of fructose in the livers of female Sprague-Dawley rats supplemented with 10% liquid fructose for a) 2 weeks and b) 8 weeks.

On the other hand, in our recent studies in C57/BL6 mice, we observed a 2.3-fold, 5.5-fold and 7-fold increase in plasma leptin levels after 3 months of feeding a 15% liquid fructose diet, a western diet and western diet plus 15% liquid fructose, respectively (non-published results). In this case, the increase of adipose tissue mass cannot explain the observed increases;

moreover, the mRNA leptin expression in adipose tissue was also increased by the three diets, following the same pattern of stepwise increase as plasma leptin levels.

1.1.4. Progression to non-alcoholic steatohepatitis

The increase of *de novo* lipogenesis and liver steatosis induced by fructose feeding have been related to non-alcoholic fatty liver disease (NAFLD), which is considered the hepatic manifestation of the metabolic syndrome. Accumulation of triglycerides in the liver leads to oxidative stress and expression of proinflammatory cytokines, which results in progression from NAFLD to non-alcoholic steatohepatitis (NASH), characterized by inflammation, hepatocellular injury and hepatic fibrosis. Zhang et al. [34] showed that male Wistar rats fed a high fructose diet (32% fructose) developed hepatic steatosis at 4 weeks, but features of NASH (inflammatory cell infiltration and focal necrosis) appeared only at 8 weeks of fructose feeding. As oxidative stress plays a critical role in the progression of NAFLD to further stages of severity, Zhang et al. examined the role of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which mediates protection against oxidative stress and inflammation by increasing the hepatic expression of antioxidative enzymes. They showed that the amount of Nrf2 in rat hepatic nuclei was increased in rats fed the high-fructose diet, a compensatory response which was not sufficient to overcome the imbalance state of oxidative stress associated to fructose feeding [34].

Inflammation also contributes to the progression of fructose-induced NAFLD into NASH. Zhang et al [34] showed higher plasma tumor necrosis factor alpha (TNFα) levels and increased nuclear factor kappa B (NF-κB) p65 in the nuclei of liver cells of high fructose fed male Wistar rats. Our own study in female Sprague Dawley rats fed fructose for the same length (8 weeks) but to a lower proportion (10%) and supplemented in liquid instead of solid form, showed no clear signs of hepatic inflammation or necrosis [25]. On the contrary, in our study with C57/BL6 mice fed with a high fat diet, alone or combined with 15% liquid fructose, we observed a clear trend towards increase in several markers of hepatic inflammation, such as monocyte chemoattractant protein 1 (MCP-1), as well as in markers of hepatic fibrosis (collagen type 1 expression) [18].

Tsuchiya et al. fed C57BL/6J mice a high-fat, high-fructose diet for 2 to 16 weeks. As expected, they observed that the diet induced hepatic steatosis and insulin resistance, but the most interesting finding was that 2 weeks before the onset of these alterations there was a significant increase in

hepatic non-heme iron content, and a decrease in antioxidant capacity in this organ [35]. The authors of this study suggest that the high-fat high-fructose diet, through causing hepatic iron overload, increases oxidative stress which later results in insulin resistance in the liver.

1.2. Fructose effects on extrahepatic tissues

1.2.1. Heart and cardiovascular system

In addition to effects on hepatic cells, fructose also causes disturbances in other tissues such as the heart and the vasculature. Thus, fructose feeding causes cardiac insulin resistance in ovariectomized female rats, shown by an impairment of Akt/endothelial nitric oxide synthase pathway [36]. The same research group reported that estradiol replacement in this animal model specifically suppresses these effects [37]. The results of this study suggest that the heart of female rats during the reproductive period is partially protected from the negative effects of excessive fructose intake. In fact, estrogen replacement has not always demonstrated a protective role against the detrimental cardiovascular effects of fructose. Thus, Koricanac et al. [38] found that estrogen enhanced the effects of fructose feeding in ovariectomized rats on the distribution and expression of CD36, the most important fatty acid transporter in cardiomyocytes. These effects would reinforce the negative effects of fructose by increasing cardiac fatty acid uptake and utilization, which is characteristic of cardiac insulin resistance [38].

The deleterious effects of an excess of fructose consumption on kidney, endothelium and heart have been associated with systemic hypertension through several mechanisms, among them the generation of uric acid [39]. Hyperuricaemia increases oxidative stress and thus it may promote endothelial dysfunction. Uric acid may also raise systemic blood pressure by activating the renin-angiotensin system and by inhibiting nitric oxide synthase [40]. The role of uric acid has recently been demonstrated by Tapia et al [41]. They showed that blocking the enzyme uricase in rats enhances the alterations induced by fructose on systemic and glomerular blood pressure, as well as on other metabolic alterations (plasma glucose, hepatic triglyceride and oxidative stress).

Akar et al. examined the effects of a diet supplemented with 10-20% HFCS on vascular function in male Sprague-Dawley rats [42]. Consumption of 20% HFCS for 10 weeks increased blood pressure, impaired vascular relaxation to acetylcholine and increased the contractile response of aortas to phenylephrine and to angiotensin II. These effects were related to a

decreased endothelial nitric oxide synthase (eNOS) and sirtuin 1 (SIRT1) expression, together with an increased expression of NADPH oxidase, which caused an increase in superoxide production in the aorta of these animals. Resveratrol supplementation preserved vascular function and increased eNOS and SIRT1 expression in aortas [42]. We also performed some experiments in order to assess the effects of fructose on vascular reactivity, in collaboration with Dr. Rahimian's group from the University of the Pacific (unpublished results). We observed reduced sensitivity to acetylcholine vasodilation in aortas exposed to high fructose concentrations (25 mM). Interestingly, the effect was more pronounced in aortas from male than from female rats. Gender differences in the response of rats to fructose have been already found in our previous in vivo studies [20]. Sexual dimorphism was also observed in mice deficient in the dual-specificity glucose and fructose transporter GLUT8. Thus, DeBosch et al. showed that female mice deficient in GLUT8 exhibited enhanced jejunal fructose uptake, which was related to exacerbated increases in blood pressure in response to high-fructose feeding compared to wild type mice [43]. On the contrary, male GLUT8-knockout mice exhibited lower systolic blood pressure both at baseline and after high-fructose feeding [44].

The renin-angiotensin system plays also an important role in fructose-induced hypertension. Therefore, blocking angiotensin II receptors with losartan was shown to enhance renal cortical vascular responses to adrenergic stimuli, thus reducing fructose-induced hypertension in rats [45]. Similarly, the development of hypertension after 8 weeks of treatment with 60% dietary fructose was prevented by renin inhibition with aliskiren treatment [46].

Recently, it has been proposed that the immune system may also be related to cardiovascular alterations induced by fructose feeding. Thus, in a study performed by Leibowitz et al [47], male Sprague-Dawley rats fed a high-fructose diet (60% fructose) for 5 weeks induced a metabolic-like syndrome associated to vascular oxidative stress, inflammation and reduced IL-10 secretion from T regulatory lymphocytes (Treg). This suggests that despite the total number of Treg is not changed, their function is decreased leading to a reduction in the protective effect of these cells on the development of vascular injury. In this study, however, blood pressure was not altered, suggesting that the effect of Tregs is independent from blood pressure. The lack of effect of fructose feeding on blood pressure is attributed by the authors to a short fructose exposure. In fact, we found a slight increase in blood pressure in female rats after 8 weeks of 10% liquid fructose supplementation (unpublished results).

Insulin resistance and dyslipidemia have also been associated to a proatherogenic state, and therefore an excess of fructose consumption may

promote atherosclerosis development. There are only few studies addressing this problem, and the conclusions are not clear enough, as the animal model used to evaluate atherosclerosis development is a key factor. For example, feeding rats with a high fructose diet for 8 weeks facilitates the development of balloon injury-induced neointimal formation in carotid arteries [48]. However, the formation of atherosclerotic lesions is better assessed in mouse models such as the LDL receptor knock-out mouse ($LDLR^{-/-}$), which develops atherosclerotic lesions similar to humans when challenged with high fat diets. We have studied the effect of fructose supplementation in drinking water (15% w/v), combined or not with a high fat diet, for 3 months in male $LDLR^{-/-}$ mice. Our preliminary results indicate that supplementation with fructose induced atherosclerotic lesions in the aorta, which were much more extensive when fructose was combined with a high fat-diet.

1.2.2. Fructose and adipose tissue

Fructose consumption has been linked to weight gain and obesity, but this is not always apparent in studies using rodents. For example, we have not detected any increase in weight gain in rats supplemented with liquid fructose for 14 days in our previous studies [19-21]. However, our more recent results in female rats receiving 10% liquid fructose for 8 weeks showed not only increased body weight, but also a significant increase in visceral adipose tissue mass (unpublished results). Alzamendi et al. also found an increase in adipose tissue mass in male Wistar rats treated with 10% liquid fructose for 3 weeks [49]. This effect was accompanied by a decrease in the number of cells per gram of adipose tissue, while cell diameter and volume were significantly increased by fructose. Similarly, Creszenzo et al. observed differences in adipocyte number and morphology in male rats fed 30% fructose in solid form for 8 weeks [50]. Thus, the number of intra-abdominal adipocytes was reduced due to an increase in their mean diameter, while the opposite (increased number but decreased adipocyte size) was found for the subcutaneous abdominal depot. Moreover, changes in size correlated with cell function and sensitivity to insulin action, therefore intra-abdominal fat tissue from fructose-fed rats displayed decreased phosphorylated Akt levels, suggesting reduced insulin signaling [50].

In vitro experiments in murine 3T3-L1 cells incubated in standard differentiation medium showed that addition of fructose increased adipogenesis and the expression of PPARγ, C/EBPα and the fructose transporter GLUT4 [51]. The authors were not able to tell whether fructose directly induced this transporter or the results were due to the earlier appearance of GLUT4 due to fructose-induced adipocyte differentiation. Our

own preliminary results in adipose tissue of rats supplemented for 8 weeks with 10% fructose shown an increase, although non-statistically significant, in the mRNA levels of GLUT4, and also of another transporter (GLUT5).

There are some evidences showing an increase in glucocorticoid levels in adipose tissue after high fructose feeding, probably due to the induction of the enzyme 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [52]. Velickovic et al. [53] studied the relationship between glucocorticoids and inflammatory mediators in rat adipose tissue and liver after 9 weeks on a diet containing a 10% fructose solution in drinking water. They observed that the level of corticosterone and of macrophage migration inhibitory factor (MIF) was significantly increased in the adipose tissue, but not in the liver of fructose-supplemented rats. The authors concluded that the glucocorticoid effects predominated over those of MIF, leading to attenuated NF-κB activation and unchanged TNFα expression. Similarly, our recent studies in the adipose tissue of rats supplemented with liquid fructose for 8 weeks showed no increase in inflammatory mediators, such as TNFα and monocyte chemotactic protein-1 (MCP-1). Interestingly, in adipose tissue samples of C57/BL6 mice fed a high fat diet, the addition of 15% liquid fructose produced a more marked increase in these inflammatory markers, specially MCP-1, as well as in markers of fibrosis (collagen type 1 expression), compared with animals fed only the high fat diet [18].

1.2.3. Fructose and skeletal muscle

The importance of skeletal muscle in glucose homeostasis is highlighted by the fact that it is the major site of insulin-mediated glucose uptake in the postprandial state. Moreover, insulin resistance in skeletal muscle has been suggested to be one of the initial events in the establishment of type 2 diabetes [54]. In a recent paper, Benetti et al. showed that feeding mice with 15% HFCS for 30 weeks caused skeletal muscle insulin resistance (shown by the impaired phosphorylation of IRS-1, Akt, and GSK-3β) and inflammation (shown by enhanced expression of NF-κB, iNOS and ICAM-1), and these effects were attenuated by co-administration of a PPAR-δ agonist. The improvement in insulin resistance by the PPAR-δ agonist was attributed, at least in part, to an increase in the expression of GLUT-4 and GLUT-5 transporters in skeletal muscle [55]. In male Wistar rats, 30% fructose feeding in solid form for 8 weeks did not cause any difference in the degree of Akt phosphorylation in skeletal muscle [56]. Neither did we find changes in phosphorylated Akt in female Sprague Dawley rats supplemented with 10% liquid fructose for 8 weeks (non-published results). However, in both models there was a significant decrease when the phospho-Akt levels were

normalised to plasma insulin, suggesting that insulin action was impaired in skeletal muscle from fructose-fed rats. Interestingly, we observed a marked increase in the mRNA expression of GPR 119 in the skeletal muscle of our fructose-supplemented rats. GPR 199 is a G protein-coupled receptor which under activation impairs fatty acid and glucose oxidation by skeletal muscle, a condition that potentiates insulin resistance [57]. Thus, our preliminary results may be in accordance with fructose inducing insulin resistance in this tissue.

2. Epidemiological and clinical studies

Since our last review published in 2012, there have been a number of epidemiological and clinical studies investigating the effects of high consumption of fructose or other sugar-sweetened beverages, in humans. One of these studies aimed to investigate the association between the consumption of sweetened beverages (juices and nectars, sugar-sweetened soft drinks and artificially sweetened soft drinks) and the incidence of type 2 diabetes in a European cohort, the EPIC-InterAct study [58]. In this cohort, composed of men and women, only sugar-sweetened soft drink consumption was associated with an increase in the risk for type 2 diabetes after adjustment for body mass index. This suggests that obesity is not the only nor the main mediator of the association, and that other mechanisms of action might be involved, such as insulin resistance. In a similar study performed in a cohort composed only of women, similar results were obtained, although in this case not only for sugar sweetened beverages but also for artificial sweetened beverages [59]. However, none of these studies specifically assessed the effects of fructose consumption. Aeberli et al. [60] performed a randomized, cross-over trial in 9 healthy, normal-weight male volunteers (aged 21-25 years) who consumed four different sweetened beverages for 3 weeks each: medium fructose (MF) at 40 g/day, and high fructose (HF), high glucose (HG), and high sucrose (HS) each at 80 g/day. The results of the euglycemic-hyperinsulinemic clamps showed that the suppression of hepatic glucose production during insulin infusion was significantly lower after the HF intervention compared with HG, showing a decrease in hepatic insulin sensitivity after fructose consumption. In contrast, no significant differences among diets were seen in insulin-mediated glucose clearance, which is a parameter of whole-body (essentially muscle) insulin sensitivity. This suggests that impaired glucose tolerance by high fructose intake is explained by impaired suppression of hepatic glucose output rather than by muscle insulin resistance, at least with short-term high-fructose diets. However, the authors consider that it is possible that longer periods of

fructose administration could also alter muscle insulin sensitivity, possibly through a progressive deposition of ectopic fat in skeletal muscle. Another important finding was that all diets containing fructose (MF, HF, and HS), caused an elevation in total and LDL cholesterol, but not in triglyceride levels, compared with HG [60]. The lack of effect on plasma triglyceride may be attributed to the relatively low amount of fructose administered, compared to previous studies [61, 62].

The above cited trials were performed in healthy individuals, but it has been claimed that the effects of fructose may be worse in subjects with obesity or other related metabolic diseases. Lewis et al. [63] performed a randomized controlled crossover study in overweight/obese subjects, who completed two 6 week dietary periods (separated by 4 week washout) consisting of two isocaloric diets that differed only in their sucrose content, the low-sucrose diet contributing 5% of total daily energy and the high-sucrose diet 15% of total daily energy. There was no difference between both diets on insulin resistance measured by the hyperinsulinaemic-euglycaemic glucose clamp, suggesting that there is no beneficial effect of a low-sucrose diet on peripheral glucose utilization. However, fasting plasma glucose, which is determined mainly by hepatic glucose production, was higher after the 15% sucrose diet compared to the 5% sucrose diet. Moreover, the results of the oral glucose tolerance test indicated higher insulin levels after the high sucrose diet at comparable levels of glycaemia [63]. These results would suggest that there is indeed some degree of hepatic insulin resistance after the higher sucrose diet.

In another study by Sevastianova et al. [64], overweight subjects were instructed to continue their normal diet and in addition to consume an extra 1000 kcal/day with 98% of energy from carbohydrates (candy, pineapple juice, sugar-sweetened soft drinks, and/or carbohydrate-loading drink) for 3 weeks, and thereafter they were placed on a hypocaloric diet for 6 months. During the carbohydrate overfeeding period, the study subjects gained a 2% of their body weight, which was correlated with a 27% increase in liver fat. The ratio of saturated to essential fatty acids in serum and VLDL triglycerides, taken as an index of de novo lipogenesis, was also significantly increased and correlated with liver fat content. All these changes are reversible by weight loss. The results suggest that an excess of simple sugar intake has a key role in the pathogenesis of NAFLD.

Children and youngsters constitute a population segment in which the prevalence of obesity is increasing, and this has coincided with a large increase in the consumption of sugar-sweetened beverages. Refreshing beverages enriched in fructose actually are the main source of dietary sugars in children. To explore the acute effects of fructose, Jin et al. performed a

2-day crossover feeding study in children with or without NAFLD [65]. Participants were randomly assigned to two nonconsecutive 24-h periods receiving three macronutrient-balanced meals with 33% of total estimated daily calories provided as an isocaloric, sugar-sweetened beverage containing either glucose or fructose, during a 24-h period. The results showed that fructose caused a more marked increase in plasma triglyceride levels than glucose did, both in children with NAFLD and in those without NAFLD, but the increases were greater in children with NAFLD.

DeRuyter et al. [66] conducted a double-blind, randomized intervention study in 641 normal-weight children, who were randomly assigned to receive 1 can per day of a noncaloric, artificially sweetened beverage or a sugar-containing beverage (with 26 g of sucrose, providing 104 kcal). Both beverages tasted and looked essentially the same and were not labeled, to eliminate the effects of psychological cues and socially desirable behavior. The results of the study showed that weight gain, waist-to-height ratio, and fat mass were significantly higher in the group that received the sugar-sweetened beverage. To gain insight into the mechanisms explaining this difference, the same group recently conducted another similar study in children, and concluded that sugar-sweetened and sugar-free beverages produced similar satiety [67]. Therefore when children are given sugar-free instead of sugar-containing drinks they do not feel a need to eat more to compensate for the missing calories, so they gain less weight and accumulate less body fat.

Some clinical studies are intervention studies aimed to evaluate the effects of reducing fructose consumption. For example, Ebbeling et al [68] randomly assigned 224 overweight and obese adolescents who regularly consumed sugar-sweetened beverages to experimental and control groups. The experimental group received a 1-year intervention consisting of home delivery of noncaloric beverages to decrease consumption of sugar-sweetened beverages, with a follow-up for an additional year. At 1 year, increases in body mass index were significantly smaller in the intervention group than the control group, but changes were not sustained at 2 years.

3. Conclusion

Excessive sugar consumption is beginning to be recognized as a huge public health problem worldwide. Some voices have risen about the need to promote policies to control the use of fructose [69], but others consider that public health policies to limit fructose in diet are premature because there is a lack of crucial studies demonstrating the toxicity of fructose [70,71]. Our studies performed in animal models, rats and mice receiving liquid fructose for short periods of time (2 weeks to 3 months) demonstrate that fructose exerts specific effects not

merely dependent on the caloric excess provided. In addition, our results suggest that the metabolic alterations induced by fructose are a dynamic continuum, and some effects that were apparent at short exposure times were not present after longer treatments. Taking into account that excessive fructose consumption in humans is usually a chronic dietary habit, our next challenge is to study the effects of fructose in these animal models after long-term supplementation.

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