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Minireview

The role of glucocorticoid hormones in diet-induced metabolic diseases

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Summary. Excessive fructose intake promotes the development of metabolic syndrome through the deregulation of metabolic pathways in the hypothalamus, liver and adipose tissue, which play crucial roles in metabolic homeostasis by responding to the body's nutritional and energy requirements. Variable amounts and modes of fructose intake have been shown to result in different patterns of expression of metabolic disturbances, which generally include adiposity, insulin and leptin resistance, dyslipidemia and hypertension. We explored the possible mediatory role of glucocorticoid signaling on the effects of two different dietary fructose loads on hypothalamic leptin sensitivity and hepatic and adipose tissue lipid metabolism, which are responsible for the development of signs of metabolic syndrome. Experimental rats were provided with 10% and 60% fructose solutions *ad libitum* over a period of nine weeks. Our results revealed that the applied fructose had different impacts on leptin and glucocorticoid signaling and different consequences on visceral adiposity and hepatic lipid metabolism. Only rats maintained on the high-burden 60% fructose diet accumulated visceral fat through the activation of adipogenic transcription factors and adipogenesis. This was paralleled by diminished glucocorticoid signaling in the adipose tissue and the establishment of the state of hypothalamic leptin resistance. The high-burden dietary fructose triggered hepatic *de novo* lipogenesis and a concomitant inhibition of β oxidation. Consumption of 10% fructose enhanced glucocorticoid signaling and lipolysis in the adipose tissue, creating a circulatory influx of free fatty acids and providing substrates for enhanced β oxidation and triglyceride synthesis in the liver. In summary, our results show that a long-term high dietary fructose load leads to hypothalamic leptin resistance, the development of visceral adiposity and increased hepatic *de novo* lipogenesis. Glucocorticoids regulate adipocyte storage functionality and thus may indirectly contribute to the observed changes in hepatic lipid metabolism, aggravating the metabolic disturbance.

Keywords: fructose, glucocorticoids, hypothalamus, liver, metabolic syndrome, visceral adipose tissue.

Metabolic syndrome: an escalating health threat to modern humanity

Metabolic syndrome is defined as a collection of interconnected physiological, biochemical, clinical and metabolic factors that increase the risk of coronary heart disease (CHD), other forms of cardiovascular atherosclerotic diseases (CVD) and diabetes mellitus type 2 (DMT2) (Huang 2009). These diseases represent major mortality factors and escalating public-health and clinical challenges worldwide in the wake of urbanization, surplus energy intake, increasing obesity and a more sedentary lifestyle (Kaur 2014). Although there is still no consensus regarding the definition of metabolic syndrome, the main metabolic disturbances manifested are overweight/obesity, hyperglycemia, dyslipidemia, hypertension and insulin resistance. The origins of metabolic syndrome could be found in the early 1920s, when a Swedish physician, Kylin, demonstrated the association of high blood pressure with high blood glucose. In the following decades, some authors noted that visceral obesity was associated with the metabolic disturbances characteristic for diabetes, and in 1965, Avogaro and Crepaldi described a syndrome comprised of hypertension, hyperglycemia and obesity. Significant progress in this field was achieved in 1988 when Reaven described "a cluster of risk factors for diabe-

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tes and cardiovascular disease" and named it "Syndrome X" (Reaven 1988). The novelty in his contribution was the introduction of the concept of insulin resistance. For a long time, insulin resistance has been considered the key event in most metabolic derangements. However, Reaven surprisingly excluded obesity from the definition, which was renewed in 1989 by Kaplan, who renamed the syndrome "The Deadly Quartet". In 1992, insulin resistance was acknowledged in the new name of the syndrome, "The Insulin Resistance Syndrome" (Haffner et al. 1992). The first attempt to provide a precise definition of metabolic syndrome was made by the World Health Organization (WHO) diabetes group in 1998, and after several modifications, two of the most adopted definitions (in effect diagnoses) were provided by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) in 2001, and by the International Diabetes Foundation (IDF) criteria published in 2005 (Zimmet et al. 2005). The NCEP ATP III included hyperglycemia/insulin resistance, visceral obesity, atherogenic dyslipidemia and hypertension as key features. This definition, which requires that at least three out of five criteria are met, is the most widely used. It also takes into consideration biochemical laboratory results that are easily accessible to physicians. As far as the IDF definition is concerned, although it relies on the same general criteria as other definitions, it requires obesity but not necessarily insulin resistance to be present. The IDF criteria are widely adopted and used, but are still heavily criticized for the emphasis on obesity rather than insulin resistance. According to this definition, about one-quarter of the world's adult population has metabolic syndrome. Among the different risk factors for metabolic syndrome, such as genetic background, socioeconomic status, sedentary lifestyle, levels of physical activity or smoking, diet assumes a very important place, since the globalization of unhealthy dietary choices has already been positively correlated with the prevalence of the metabolic syndrome and its components (Pitsavos et al. 2006).

Dietary habits as a risk factor for metabolic disturbance

Increased consumption of a high-caloric modern Western diet rich in sugars, trans fats, omega-6 fatty acids (FAs) and branched-chain amino acids is known to contribute to the epidemic of metabolic syndrome. One of the first correlations between nutrition and health was proposed two decades ago when both physicians and scientists associated dietary fat with the rising prevalence of obesity (Golay et al. 1997). Consequently, a low-fat diet was introduced and intensively promoted; however, surprisingly this was not reflected in a decline in obesity. Soon after, new evidence suggested that refined sugars were a very important contributing factor for obesity and metabolic diseases (Malik et al. 2010). Although sugars are naturally occurring sweeteners, humans have not always been the enthusiastic consumers of sugar we are today. Our ancestors were hunters and gatherers and their diet was mainly high in protein, moderate in fat and low in carbohydrates, the main source of which were fruits and berries. Intensive consumption of sugar started to increase about 250 years ago, when it became widely available and at low cost due to colonial trade. Increased sugar consumption has risen dramatically over the past few decades. It is also closely linked with increased consumption of a variety of sweetened beverages since the beginning of 20th century, and in the last 50 years the consumption of sugar-sweetened soft drinks has risen fivefold; at present, it represents more than 40% of the human diet. Importantly, the main source of sugar in beverages is high fructose corn syrup (HFCS), a common sweetener introduced to the food industry in the 1960s because of its high sweetening power, organoleptic properties, ability to confer a long shelf-life and low production cost (Hanover et al. 1993). The actual fructose content in HFCS ranges from 42-55%, and the taste and sweetness of 55% HFCS is equivalent to that of sucrose. The next critical step in sugar overconsumption was related to the introduction of 55% HFCS to the carbonated-beverage industry in the mid-1980s. From that point on, fructose became the predominant sweetener in soft drinks (Walker et al. 2014). The dominance of corn syrup sweeteners (which now represent over 20% of total daily carbohydrate intake) was paralleled by a rising obesity epidemic, which prompted both clinical studies and research on animal models to understand whether dietary fructose intake in beverages poses a significant health risk. Indeed, the correlation between a high fructose diet and dyslipidemia was found in humans (Tappy et al. 2010), while animal studies indicated that dietary fructose can lead to obesity, insulin resistance, dyslipidemia, high blood pressure and type 2 diabetes (Jurgens et al. 2005). However, an important question remained unanswered: what are the underlying molecular mechanisms of the metabolic effects of fructose?

The liver as a key organ involved in fructose metabolism

Fructose is involved in the genesis and progression of metabolic syndrome through the deregulation of signaling and metabolic pathways in the liver and adipose tissue. The liver is considered the main site of the metabolism of fructose, which is readily absorbed and rapidly metabolized there (Tappy et al. 2010). It has been assumed that the adverse effects of fructose can be ascribed to its specific metabolic fate. Namely, although upon absorption in the gastrointestinal tract both glucose and fructose are delivered via the portal vein to the liver, they enter the catabolic pathways differently. Initial fructose cleavage in the liver bypasses the rate-limiting step of glycolysis, the phosphofructokinase-catalyzed conversion of glucose-6-phosphate to fructose 1,6-bisphosphate, so that fructose can continuously enter the glycolytic pathway

independently of energy demands, uncontrollably producing glucose, glycogen, lactate and pyruvate, with further metabolism providing substrates such as acyl-glycerol molecules, the precursors for *de novo* lipogenesis (Tappy et al. 2010). This initial difference between the hepatic metabolism of fructose and glucose prompted the hypothesis that the bypass of key feedback regulatory steps in glucose breakdown can lead to increased FA synthesis and the promotion of triglyceride overproduction after fructose utilization (Miller et al. 2008).

Another important difference between glucose and fructose metabolism is related to their absorption from the gastrointestinal tract. While glucose is transported into cells by the insulin-regulated glucose transporter protein GLUT-4, fructose mainly uses high-affinity GLUT-5 transporters that have a low abundance in most cells (Havel 2005). Taking into account that fructose metabolism is independent of insulin and is directed toward increased FA synthesis, it is reasonable to expect that fructose overconsumption can ultimately lead to ectopic lipid deposition and hepatosteatosis. However, whether fructose overconsumption will lead to hypertriglyceridemia and hepatic steatosis depends on the balance between *de novo* lipogenesis and FA β oxidation. The master transcriptional regulator of hepatic FA oxidation is peroxisome proliferator activated receptor α (PPARα), which upon activation induces the expression of genes involved in mitochondrial and peroxisomal FA oxidation. In addition, PPARα is also involved in peripheral lipid mobilization and hepatic lipid droplet formation (Dalen et al. 2006). The transcriptional activation of target genes by PPARα is crucially dependent on its coactivators, PPARγ-coactivator-1α (PGC 1α) and lipin-1. These three proteins form a nuclear complex that stimulates the expression of genes involved in FA oxidation, including carnitine palmitoyl transferase 1 (CPT1) and medium chain acyl CoA dehydrogenase (Djouadi et al. 1999). On the other hand, the regulation of *de novo* lipogenesis in the liver is controlled by several transcription factors, among which are the sterol response element binding proteins (SREBPs). SREBP-1c specifically regulates hepatic triglyceride synthesis through the expression of several downstream target genes, such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) (Horton et al. 2002). It was previously demonstrated that a high-fructose diet leads to increased expression of FAS in the liver and concomitantly to ectopic lipid accumulation and hypertriglyceridemia. However, the mechanism of this hyperlipidemic effect of fructose remains controversial, since it was reported that only a small percentage of ingested fructose appears to be directly converted to plasma triglycerides (Sun et al. 2012).

Visceral adipose tissue and regulation of energy balance

Apart from the liver as the main site of carbohydrate metabolism, dietary fructose can also contribute to the development of metabolic syndrome through the deregulation of metabolic pathways in the hypothalamus-adipose tissue axis, which has a crucial role in the body's response to nutritional and energy requirements. The most prominent early hallmark of diet-induced dysfunction of the hypothalamus-adipose tissue axis is the development of leptin resistance, a state associated with visceral fat accumulation (Oswal et al. 2010). The hypothalamus is the brain region critically involved in the regulation of food intake, body weight and energy expenditure. Leptin is an anorexigenic hormone produced by the adipose tissue in proportion to its mass. Leptin regulates food intake and hence body adiposity through modulation of the synthesis and secretion of both orexigenic and anorexigenic peptides in the hypothalamic nuclei (Friedman et al. 1998). Leptin signaling is conducted through the long form of the leptin receptor (Ob-Rb), which upon leptin binding, stimulates tyrosine Janus kinase 2 (JAK2) and activates the signal transducer and activator of transcription 3 (STAT3) protein by phosphorylation, dimerization and translocation to the nucleus. However, the leptin signaling pathway is constrained by different feedback inhibitors, such as suppressor of cytokine signaling 3 (SOCS3) whose induction in the hypothalamus is one of several mechanisms that contribute to leptin resistance. Leptin resistance is also related to impaired leptin transport across the blood-brain barrier, as well as to defects in hypothalamic Ob-Rb signal transduction resulting from reduced Ob-Rb, JAK2 and/or STAT3 expression (Myers et al. 2008). In addition, in the leptin resistant state, orexigenic neuropeptide Y (NPY) is likely overexpressed due to the absence of leptin suppressive effects (Friedman et al. 1998).

As mentioned, obesity is strongly associated with hypothalamic leptin resistance and is accompanied by elevated plasma leptin concentrations. However, other alterations in the hypothalamic appetite system that favor the orexigenic pathways, leptin/insulin resistance and hyperphagia should not be overlooked in terms of obesity development and progression. These changes include abnormal hypothalamus-adipose axis circadian rhythms, impaired hypothalamic sympathetic outflow and/or reduced innervation of the adipose tissue, which may also account for a modified fat cell metabolism (Breton 2013).

Adipose tissue was traditionally considered a passive organ for triglyceride storage, but this view has been revised. We now know that adipose tissue is a metabolically active endocrine organ capable of synthesizing a number of biologically active compounds known as adipocytokines. Adipocytokines are hormones and appetite regulation-related peptides implicated in the control of energy balance (Coelho et al. 2013). Some of these factors act as regulators of adipocyte lipid metabolism in an autocrine/paracrine manner, while others, like leptin, act as peripheral endocrine signals that regulate hypothalamic energy homeostasis (Wang et al. 2008).

Adipose tissue is mostly comprised of adipocytes, cells specialized for the storage of triglycerides in the form of lipid

droplets under conditions of energy excess. However, when the stored energy is required, triglycerides are hydrolyzed via lipolytic processes driven by sympathetic innervation. It should be kept in mind that both lipogenesis and lipolysis are metabolic processes that are highly regulated by a complex regulatory network of enzymes and transcription factors. An increase in adipose tissue mass can be accomplished either by proliferation and differentiation of new adipocytes, which is known as hyperplasia, or through triglyceride accumulation in pre-existing or mature adipocytes, which is known as hypertrophy (Smith et al. 2006). Of the many transcription factors involved in the regulation of these processes, PPARγ, is unique in terms of being capable of promoting both mechanisms. Namely, PPARγ can increase the expression of genes that promote FA storage, while repressing genes that induce lipolysis and the release of FFAs from adipocytes (Berthiaume et al. 2004). The complex regulatory network in adipocytes also involves SREBP-1, which belongs to the group of related G-protein coupled receptors and is considered an important factor that assists adipogenesis through stimulation of expression of lipogenic genes such as FAS (Tontonoz et al. 1993).

Fructose-induced metabolic disturbances: where would glucocorticoids fit in?

Glucocorticoids are known for their anti-inflammatory and stress response activities; however, they are also considered as potent modulators of energy metabolism since their excess provokes insulin and leptin resistance, affecting the control of energy handling in the liver, adipose tissue and brain (Di Dalmazi et al. 2012). Although circulating glucocorticoids are not elevated in most metabolic diseases, the development of metabolic pathologies is usually intensified by enhanced glucocorticoid action resulting from increased local regeneration of glucocorticoids. In the liver, this increased local regeneration of glucocorticoids has been identified as a mechanism that favors hepatic fat deposition and increases glucose output (Dallman et al. 2007). Thus far, our research has shown that changes in glucocorticoid prereceptor metabolism in both liver and visceral adipose tissue coincide with metabolic disturbances induced by dietary fructose.

Some evident similarities between metabolic syndrome and Cushing syndrome, including obesity, hypertension and insulin resistance, pointed to glucocorticoids as potential contributors to the pathophysiology of the former. The main regulator of prereceptor metabolism, which is responsible for the local regeneration of active glucocorticoids, is 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), a NADPH-dependent enzyme found in the lumen of the endoplasmic reticulum, whose reductase activity is driven through regeneration of NADPH by hexose 6 phosphate dehydrogenase (H6PDH) (Zhang et al. 2009). Many studies have revealed that tissue-specific dysregulation of 11βHSD1 contributes to the pathogenesis of obesity, hypertension and insulin resistance. For instance, the levels of 11βHSD1 mRNA and protein activity were found to be increased in the adipose tissue of obese humans and in monogenic obesity in rodents (Livingstone et al. 2000; Rask et al. 2001), while others reported increased levels of 11βHSD1 mRNA and protein activity in the liver of insulin-resistant lean mice (Aoki et al. 2001). In addition, transgenic overexpression of 11βHSD1 in rodent adipose tissue causes symptoms of metabolic syndrome without changes in circulating glucocorticoids (Masuzaki et al. 2001). At the molecular level, the effects of locally produced glucocorticoids are mediated by the glucocorticoid receptor (GR), a hormone-dependent transcription regulator, which upon hormone binding undergoes a conformational change that allows for its translocation to the nucleus and the regulation of many target genes (Rajapandi et al. 2000).

In the liver, glucocorticoids are involved in the regulation of gluconeogenesis and lipogenesis, which is mediated through glucocorticoid-target genes, phosphoenolpyruvate carboxykinase (PEPCK) (Cassuto et al. 2005) and lipin-1 (Bou Khalil et al. 2009). However, the role of GR in the adipose tissue is still controversial. It is well known that glucocorticoids promote preadipocyte conversion to mature adipocytes and hyperplasia of adipose tissue, but they are also implicated in adipose hypertrophy. On the other hand, while their gene regulatory actions facilitate lipolysis in adipocytes, their antilipolytic effects have also been demonstrated (Campbell et al. 2011). The most interesting aspect of this controversy is that these hormones concomitantly affect both lipolysis and lipogenesis in adipocytes (Wang et al. 2012). As stimulators of lipogenesis, glucocorticoids can increase the expression of several key pro-adipogenic and pro-lipogenic factors during adipose tissue accumulation, such as PPARγ and SREBP-1 (Galitzky et al. 2013). In addition, both PEPCK and lipin-1 are subjected to GR regulation in adipose tissue; however, in this case PEPCK is involved in the regulation of lipid metabolism through glyceroneogenesis (Saltiel et al. 2001). Conversely, the main glucocorticoid-induced lipolytic mechanism is conducted through the direct transcriptional control of hormone sensitive lipase (HSL). Namely, the enhanced activity of this enzyme contributes to the liberation of FFA from the adipose tissue, thus altering their trafficking to other organs (Campbell et al. 2011).

We explored the molecular mechanisms that lead to metabolic derangements after fructose-containing beverage consumption on fructose-fed male Wistar rats. In this review, we summarize the findings after the application of different amounts of fructose (10% and 60% solutions) over a period of nine weeks. The rationale behind the chosen fructose concentrations was that 10% fructose solution approximates sugar beverage consumption in the Western diet, whereas 60% fructose aggravates some metabolic syndrome indicators, including visceral adiposity and hypertriglyceri-

demia. Indeed, we concluded that different fructose loads were important influences on the metabolic outcome of the fructose diet.

The consumption of a 10% fructose solution led to lipolysis in visceral adipose tissue and to an increased circulatory influx of FFA, thus providing the substrates for enhanced β oxidation and triglyceride synthesis in the liver. These rats also exhibited higher systolic blood pressure and elevated triglycerides, but no body-weight gain (Bursac et al. 2013). On the other hand, rats that drank the 60% fructose solution accumulated visceral fat through the activation of adipogenesis, which was most likely driven by the hyperleptinemia and hypothalamic leptin resistance (Bursac et al. 2014). Also, the higher dietary fructose burden triggered hepatic *de novo* lipogenesis, leading to inhibition of β-oxidation and hypertriglyceridemia (Teofilović et al. 2016). Based on the data obtained on this animal model, we concluded that the intake of a lower concentration of liquid fructose aggravated metabolism, while the higher fructose load led to more obvious metabolic disturbances, such as stimulated hepatic *de novo* lipogenesis, visceral adiposity and leptin resistance.

Our main focus is delineated by the proposed triangular interplay between three vertices, respectively represented by glucocorticoids, glucocorticoid prereceptor metabolism and fructose-induced metabolic disturbance. To examine this complex transaction, we analyzed the dynamic changes in the corticosterone levels in the plasma and tissue, the glucocorticoid prereceptor metabolism and GR signaling in the liver, hypothalamus and visceral adipose tissue of the rats that consumed either a 10% or 60% fructose solution. While the 10% fructose-enriched diet did not affect the plasma and hepatic corticosterone levels, it significantly elevated corticosterone concentration in visceral adipose tissue (Fig. 1). The increase in corticosterone concentration in visceral adipose tissue was paralleled by an increase in 11βHSD1, which is in accordance with results from other studies on humans and animal models demonstrating that fructose consumption upregulates the activity of this enzyme (Morton et al. 2004). In addition, 11βHSD1 was shown to be regulated in a tissue-specific manner and fine-tuned to local glucocorticoid concentration independently of its plasma level (London et al. 2009). Since the reductase activity of 11βHSD1 is dependent on the regeneration of NADPH cofactor by H6PDH, the observed simultaneous elevation of both 11βHSD1 and H6PDH is very likely responsible for the increased corticosterone concentration in adipose tissue. Finally, as a result of increased intracellular ligand concentration, activation of the GR was observed. Importantly, these changes in glucocorticoid signaling in the adipose tissue coincided with elevated plasma triglyceride and FFA levels, but without the enlargement of visceral fat (Bursac et al. 2013). These findings corroborate the results from previous *in vitro* and *in vivo* studies suggesting that FFA release from adipose tissue is the consequence of GR activation and increased lipolysis (Campbell et al. 2011). This assumption was confirmed in our study, which revealed an increase in adipocyte HSL mRNA level, which is expected, since GR is a known transcriptional regulator of HSL (Fig. 1). In addition, the activated GR also caused a reduction in PEPCK mRNA in visceral adipose tissue, pointing to limited glyceroneogenesis and suppressed lipogenesis. Interestingly, the 10% fructose diet led to increased 11βHSD1 and H6PDH protein levels in rat liver microsomes, which was not reflected as increased tissue corticosterone. This finding could be explained by the fact that the intrahepatic corticosterone level depends on the balance between its production by 11βHSD1 and its clearance by A-ring reductases. In addition, the observed enhancement of hepatic prereceptor metabolism of glucocorticoids can stimulate the transcription of proinflammatory cytokines; thus the state of inflammation could be the reason for the observed retention of GR in the cytoplasm through NF-κB interference with its intracellular shuttling (Ignatova et al. 2009). Hepatic PEPCK expression was also decreased, which is consistent with other studies (Axelsen et al. 2010), as well as unchanged hepatic GR signaling (Fig. 2). Taking all of these observations into account, we propose that 10% fructose consumption promoted enhanced glucocorticoid signaling in the adipose tissue, which altered the hepatic metabolic phenotype by redirecting plasma FFA uptake from adipose tissue depots to the liver. As shown in Fig. 2, FFA uptake probably stimulated FA oxidation in the liver, which may be considered as an adaptive response aimed at protecting the liver against lipotoxicity. However, the influx of fructose metabolites was most likely responsible for the observed induction of lipogenic enzymes, resulting in an increased secretion of triglycerides rather than in an ectopic accumulation of lipids in the liver and hepatosteatosis (Figs 1 and 2).

Unlike the 10% fructose diet, the 60% fructose-enriched diet was associated with a worsening of the metabolic disturbance, primarily manifesting as hypertriglyceridemia and increased visceral adiposity (Bursac et al. 2014; Teofilović et al. 2016). The amplified effect of the high fructose load was reflected in increased nuclear PPARα, and SREBP-1c proteins and FAS mRNA, together with unchanged PGC-1α and a decreased level of CPT1 in the liver, which led to the inhibition of β-oxidation and more pronounced hypertriglyceridemia (Fig. 3). These findings suggested that the more concentrated fructose solution aggravated the disturbance in lipid metabolism in the liver linked with metabolic changes in the adipose tissue and the hypothalamus. Namely, the long-term *ad libitum* access to 60% fructose solution led to plasma leptin elevation, a reduction in Ob-Rb protein level and an elevation in SOCS3 mRNA in the hypothalamus, suggesting that leptin resistance in these animals was a possible link between fructose overconsumption and increased adiposity. Importantly, the observed hallmarks of leptin resistance were also associated with the upregulated expression of orexigenic NPY in the hypothalamus (Fig. 4). Interestingly,

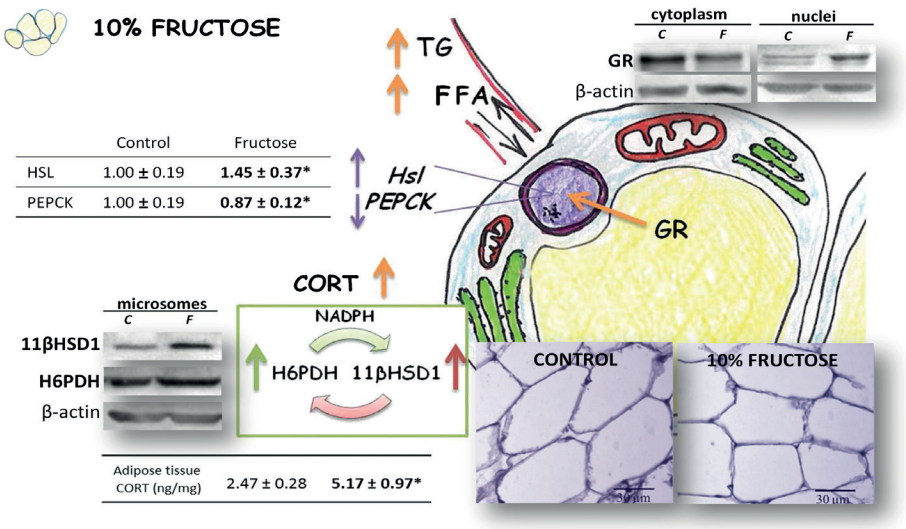


Fig. 1. Schematic overview of changes in glucocorticoid signaling and expression of genes involved in lipid metabolism in visceral adipose tissue of male rats fed a 10% fructose solution. TG–triglyceride, FFA–free fatty acid, CORT–corticosterone, GR–glucocorticoid receptor, 11βHSD1–11beta-hydroxysteroid dehydrogenase type 1, H6PDH–hexose 6 phosphate dehydrogenase, PEPCK–phosphoenolpyruvate carboxykinase, HSL–hormone sensitive lipase, NADPH–nicotinamide adenine dinucleotide phosphate.

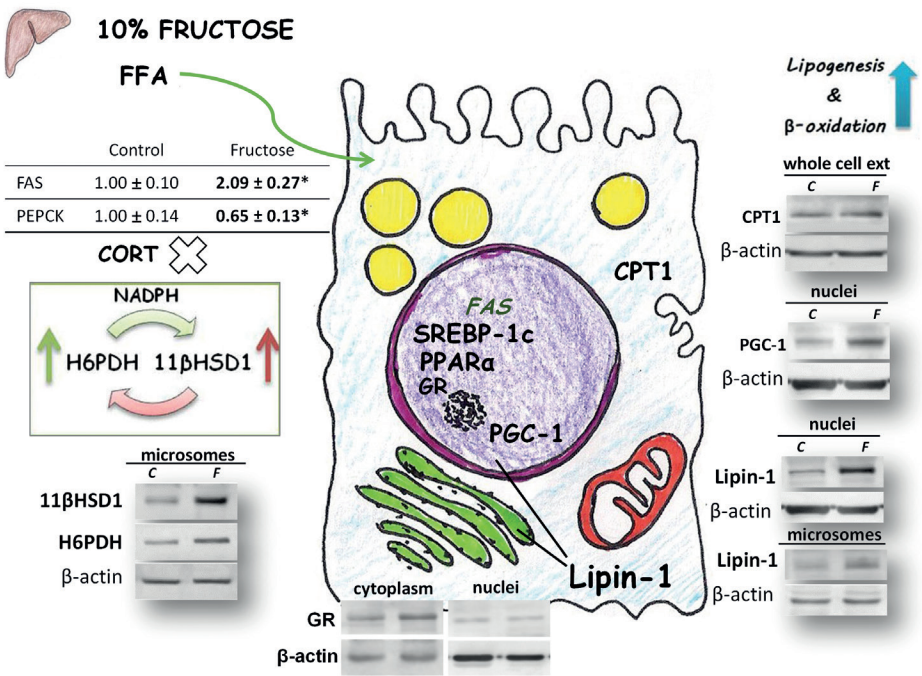


Fig. 2. Schematic overview of hepatic lipid metabolism (β-oxidation and lipogenesis) and glucocorticoid signaling in male rats fed a 10% fructose solution. TG–triglyceride, FFA–free fatty acid, CORT–corticosterone, GR–glucocorticoid receptor, 11βHSD1–11beta-hydroxysteroid dehydrogenase type 1, H6PDH–hexose 6 phosphate dehydrogenase, PEPCK–phosphoenolpyruvate carboxykinase, HSL–hormone sensitive lipase, NADPH–nicotinamide adenine dinucleotide phosphate, PPAR–peroxisome proliferator activated receptor, PGC1α–PPARγ-coactivator-1α, CPT1–carnitine palmitoyl transferase 1, SREBP–sterol response element binding protein, FAS–fatty acid synthase.

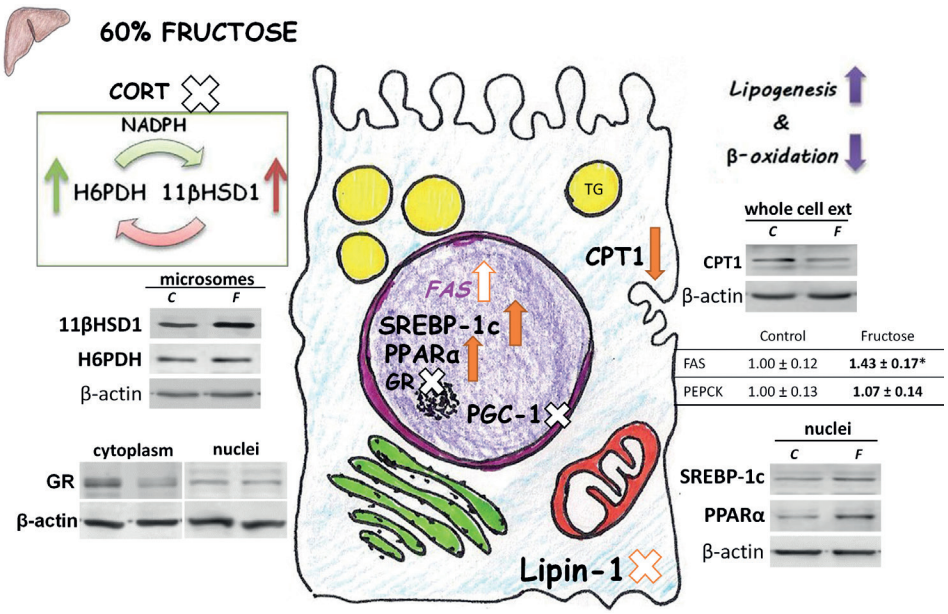
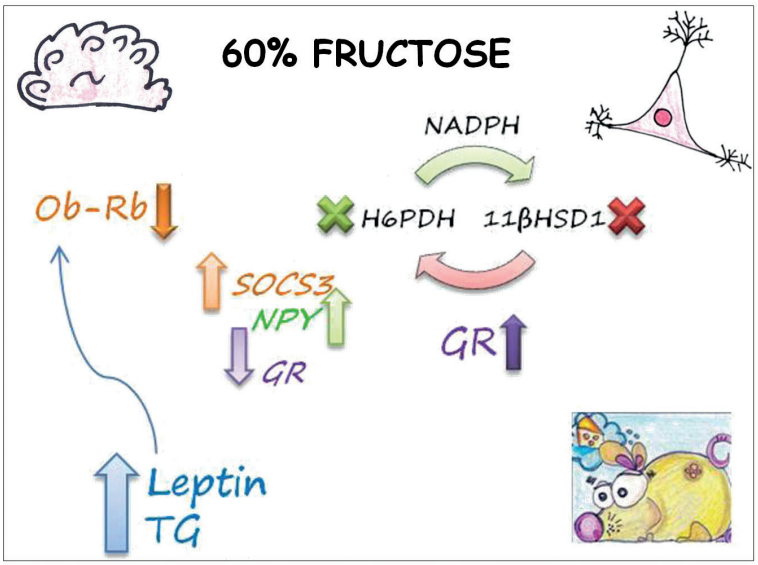
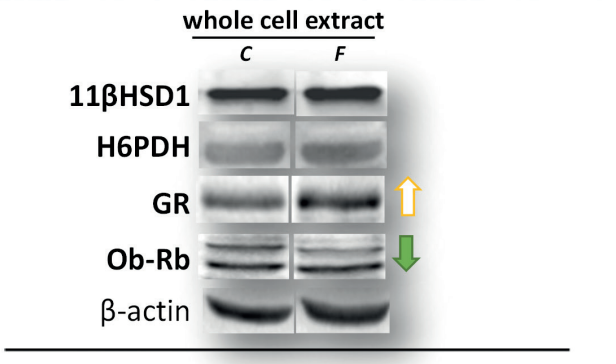


Fig. 3. Schematic overview of hepatic lipid metabolism (β-oxidation and lipogenesis) and glucocorticoid signaling in male rats fed a 60% fructose solution. TG–triglyceride, FFA–free fatty acid, CORT–corticosterone, GR–glucocorticoid receptor, 11βHSD1–11beta-hydroxysteroid dehydrogenase type 1, H6PDH–hexose 6 phosphate dehydrogenase, PEPCK–phosphoenolpyruvate carboxykinase, HSL–hormone sensitive lipase, NADPH–nicotinamide adenine dinucleotide phosphate, PPAR–peroxisome proliferator activated receptor, PGC1α–PPARγ-coactivator-1α, CPT1–carnitine palmitoyl transferase 1, SREBP–sterol response element binding protein, FAS–fatty acid synthase.

the 60% fructose diet did not influence the regeneration of active glucocorticoids through prereceptor metabolism in the hypothalamus, but it significantly increased the level of GR protein (Fig. 4). The parallel increase in hypothalamic NPY mRNA could link the involvement of glucocorticoids in the positive regulation of this orexigenic peptide with the development of central leptin resistance. In this way, enhanced GR signaling in the brain impacts body weight and adiposity. Another important site of glucocorticoid action is visceral adipose tissue. After 60% fructose consumption, while the plasma corticosterone concentration was unchanged, corticosterone was significantly decreased in visceral adipose tissue despite 11βHSD1 protein elevation (Fig. 5). Such an outcome could be due either to the unchanged adipose tissue H6PDH or the enhanced metabolic clearance of active glucocorticoids by α- and β-reductases. As shown in Fig. 5, the decrease in adipose tissue corticosterone was accompanied by a decline in GR protein in both the cytoplasm and nuclei, indicating overall diminishment of glucocorticoid signaling, which coincided with unchanged plasma FFA levels. However, GR activity is not only ligand-dependent. It was previously shown that protein phosphatase 5 is a reciprocal modulator antagonizing the lipolytic actions of GR, while simultaneously promoting the adipogenic actions of PPARγ (Hinds et al. 2011). Since PPARγ plays a key role in linking adipose tissue lipid metabolism to the nutritional state, it is tempting to speculate that fructose excess could contribute to visceral adiposity through enhanced PPARγ activity. As seen in Fig. 5, this hypothesis was confirmed. In addition to PPARγ, several other adipo/lipogenic genes and transcription factors, including SREBP-1, lipin-1 and FAS, were also elevated in the adipose tissue after the 60% fructose diet (Bursac et al. 2014). These changes can be considered as key molecular events that shift changes in the adipose tissue towards adipogenesis, since clearly separated populations of small adipocytes were observed in the visceral adipose tissue of the 60% fructose-fed rats (Fig. 5). Finally, even though the 60% dietary fructose diet induced alterations in the expression of liver glucocorticoid-metabolizing enzymes, the levels of GR and its ligand were not affected (Fig. 3). The pattern of glucocorticoid-regulated proteins expression was similar to that in animals fed with 10% fructose. Hence, we conclude that a fructose diet, independently of the applied concentration, does not change the hepatic GR signaling pathway downstream of prereceptor metabolism. This conclusion is further supported by the finding that the PEPCK mRNA level, which is transcriptionally regulated by GR, was not changed in the liver of fructose-fed animals. It is indicative, however, that fructose-induced metabolic disturbances are more likely associated with alterations in GR level and activity in the hypothalamus and visceral adipose tissue.



Leptin (ng/ml)	2.14 ± 1.36	**4.53 ± 2.49 ****



	Control	60% Fructose
SOCS 3	1.00 ± 0.14	**1.47 ± 0.05 ***
NPY	1.00 ± 0.20	**1.47 ± 0.26 ***

Fig. 4. Markers of hypothalamic leptin resistance and concomitant changes in glucocorticoid signaling in male rats fed a 60% fructose solution. TG–triglyceride, CORT–corticosterone, GR–glucocorticoid receptor, 11βHSD1–11beta-hydroxysteroid dehydrogenase type 1, H6PDH–hexose 6 phosphate dehydrogenase, NADPH–nicotinamide adenine dinucleotide phosphate, SOCS–suppressor of cytokine signaling, NPY–neuropeptide Y, Ob-Rb–long form of the leptin receptor.

Concluding remarks

Our findings indicate that long-term fructose intake leads to hypothalamic leptin resistance and the development of visceral adiposity, but only at a high dietary fructose load. The adiposity-related effects of fructose consumption involve the interplay between leptin and glucocorticoid signaling at the level of central effector pathways that control energy balance. The fructose-induced leptin resistance is a critical component of the obesity-promoting vicious cycle that results in extreme forms of untreatable obesity (Fig. 6, right panel). Although the liver is the central organ of fructose metabolism, the impact of the glucocorticoid signaling pathway on the visceral adipose tissue of fructose-fed rats is predominant since glucocorticoids regulate adipocyte storage functionality and thus may indirectly influence hepatic lipid metabolism (Fig. 6, left panel) and enhance the development of metabolic disturbances.

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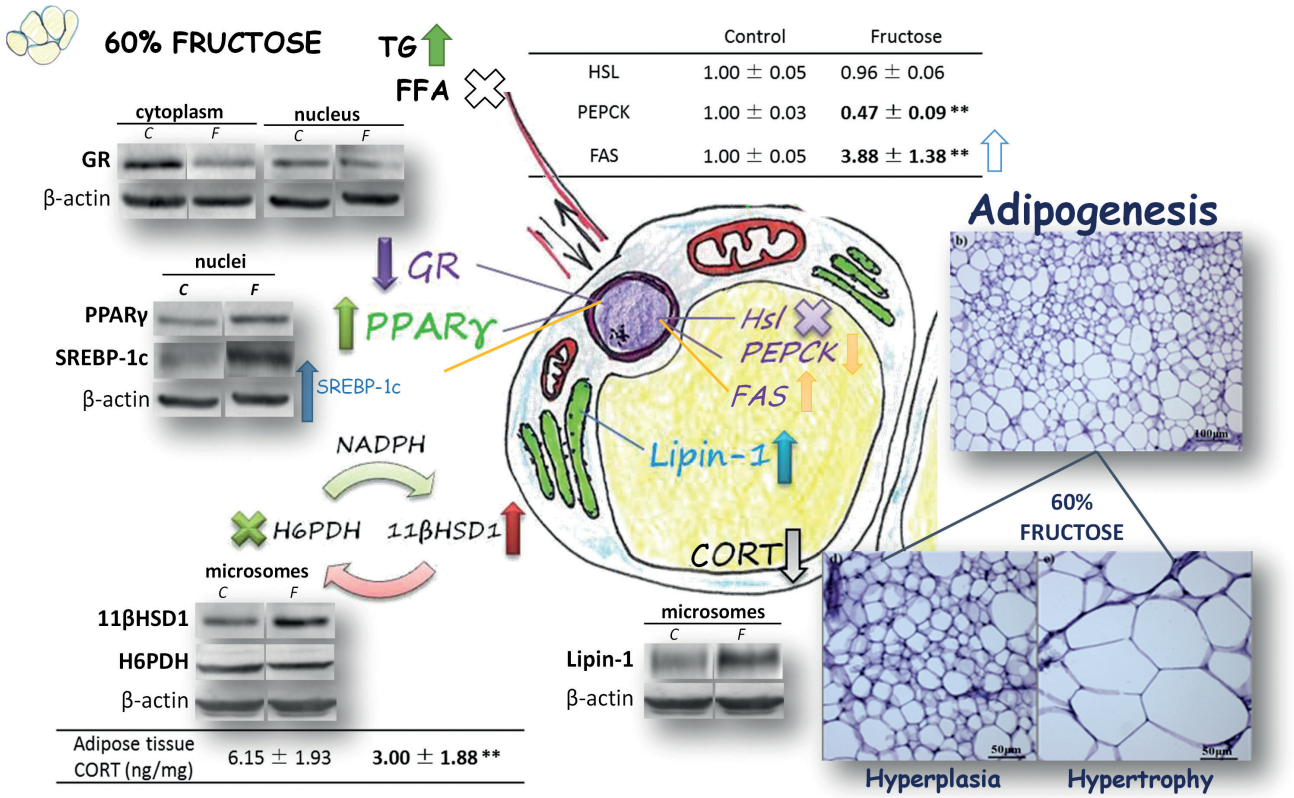

Fig. 5. Histological examination of visceral adipose tissue with a schematic representation of the molecular changes in glucocorticoid signaling and lipid metabolism gene expression in male rats after a 60% fructose diet. TG–triglyceride, FFA–free fatty acid, CORT–corticosterone, GR–glucocorticoid receptor, 11βHSD1–11beta-hydroxysteroid dehydrogenase type 1, H6PDH–hexose 6 phosphate dehydrogenase, PEPCK–phosphoenolpyruvate carboxykinase, HSL–hormone sensitive lipase, NADPH–nicotinamide adenine dinucleotide phosphate, PPAR–peroxisome proliferator activated receptor, SREBP–sterol response element binding protein, FAS–fatty acid synthase.

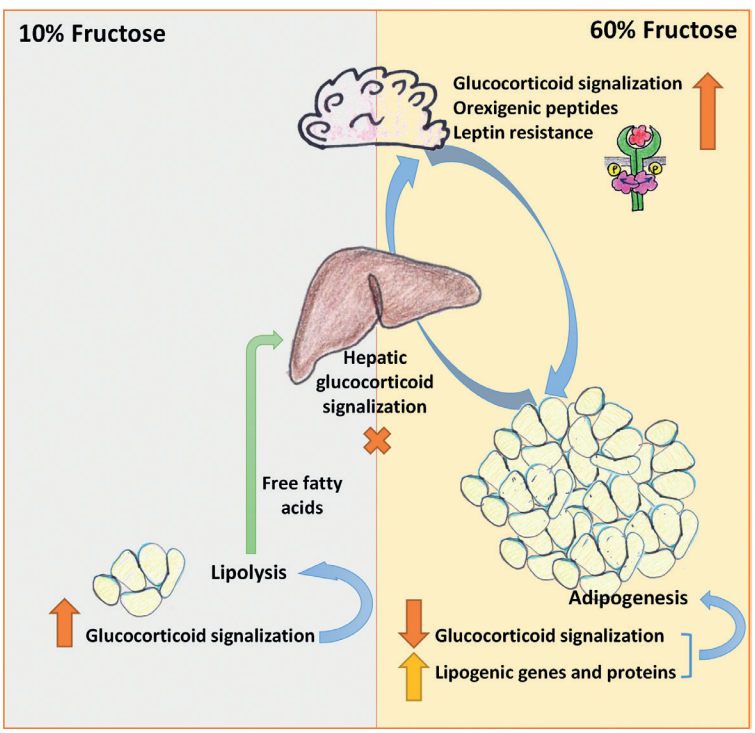

Fig. 6. Summarized overview of different impact of applied fructose diets on glucocorticoid signaling in the hypothalamus, visceral adipose tissue and liver of male rats. Note that the adiposity-related effects of fructose consumption involve the interplay between leptin and glucocorticoid signaling at the level of hypothalamus but only at higher fructose loads. Glucocorticoid signaling pathway in visceral adipose tissue of rats fed with lower fructose concentration may indirectly influence hepatic lipid metabolism through free fatty acid release.

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