FRUCTOSE ONLY IN PREGNANCY PROVOKES HYPERINSULINEMIA, HYPOADIPONECTINEMIA AND IMPAIRED INSULIN SIGNALING IN ADULT MALE, BUT NOT FEMALE, PROGENY

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Abstract

Purpose Fructose intake from added sugars correlates with the epidemic rise in metabolic syndrome and cardiovascular diseases. However, consumption of beverages containing fructose is allowed during gestation. Recently, we found that an intake of fructose (10% wt/vol) throughout gestation produces impaired fetal leptin signaling and hepatic steatosis. Therefore, we have investigated whether fructose intake during pregnancy produces subsequent changes in the progeny, when adult.

Methods Fed 261-day-old male and female descendants from fructose-fed, control or glucose-fed mothers were used. Plasma was used to analyze glucose, insulin, leptin, and adiponectin. Hepatic expression of proteins related to insulin signaling was determined.

Results Fructose intake throughout pregnancy did not produce alterations in the body weight of the progeny. Adult male progeny of fructose-fed mothers had elevated levels of insulin without a parallel increase in phosphorylation of protein kinase-B. However, they displayed an augmented serine phosphorylation of insulin receptor substrate-2, indicating reduced insulin signal transduction. In agreement, adiponectin levels, which have been positively related to insulin sensitivity, were lower in male descendants from fructose-fed mothers than in the other two groups. Furthermore, mRNA levels for insulin-responsive genes were not affected (phosphoenol pyruvate carboxykinase, glucose-6-phosphatase) or they were decreased (sterol response element-binding protein-1c) in the livers of male progeny from fructose-supplemented rats. On the contrary, adult female rats from fructose-fed mothers did not exhibit any of these disturbances.

Conclusion Maternal fructose, but not glucose, intake confined to the prenatal stage provokes impaired insulin signal transduction, hyperinsulinemia, and hypoadiponectinemia in adult male, but not female, progeny.

Keywords: Fructose; pregnancy; fetal programming; metabolic syndrome.

Introduction

In the last few decades, obesity, metabolic syndrome and diabetes have escalated to epidemic proportions in many countries worldwide. Experimental and epidemiological studies demonstrate that metabolic events during pre- and postnatal development modulate metabolic disease risks in later life [1]. This phenomenon is called fetal programming of adult health [2]. Since feeding conditions likely constitute one of the most influential parameters on the health of the adult [3], diet manipulation in mothers during critical developmental periods has been used to identify their contribution to the development of obesity and diabetes in offspring [4]. Given the current worldwide shift toward a Westernized lifestyle, there is an urgent need to address the relationship between the quality and quantity of nutrient intake during pregnancy and/or lactation and the metabolic fate of the offspring [5].

Fructose, present in added sugars such as sucrose and high fructose corn syrup, has been linked to obesity and metabolic syndrome [6-8]. Fructose can induce leptin resistance as well as virtually all the features of metabolic syndrome in rats, whereas glucose intake does not [9]. In fact, rodents fed chronically with high-fructose (60 wt/wt) solid diets develop hypertriglyceridemia and insulin resistance [10]. Likewise, liquid fructose (10% wt/vol) causes hypertriglyceridemia and fatty liver, without modifying plasma glucose and insulin levels [11]. Clinical studies also support fructose as a cause of metabolic syndrome. Thus, overweight subjects that consumed a 25% fructose-based diet developed insulin resistance and postprandial hypertriglyceridemia, unlike subjects given a glucose-based diet [12]. A recent report has shown that consumption of fructose-sweetened, but not glucose-sweetened beverages, with usual diet could induce postprandial hypertriglyceridemia in young subjects [13]. Further, in a 20-year follow-up study, it has been demonstrated that consumption of sugar-sweetened beverages is associated with an increased risk of coronary heart disease [14].

Interestingly, sex-dependent differences in the influence of fructose to induce metabolic diseases have been reported. Thus, women, but not men, exhibit an association between fructose consumption and an increased risk of Type 2 diabetes mellitus. In accordance with this, experimental studies have shown that female rats submitted to fructose have a more detrimental response than their male counterparts. Fructose-fed male rats were resistant to the hepatic effects of leptin, whereas fructose-fed females had no signs of leptin resistance but had hyperinsulinemia and altered glucose tolerance test [15].

Thus, although the connection between a greater intake of fructose-containing beverages and the increase in the prevalence of obesity, cardiovascular diseases, etc [15, 16]

has been established, consumption of those beverages and fruit juices is allowed during gestation and/or lactation. Unfortunately, studies investigating altered maternal nutrition have used quite different experimental designs to determine the role of fructose. Some have used either a combined maternal high fat:high fructose diet approach [17] or a high sucrose amount (63 % wt/wt) in a pelleted diet [18, 19]. Other investigations have used a high amount of fructose either in a solid diet (50 % wt/wt) [20] or in a fructose solution (60 % wt/vol) [21]. Alternatively, some researchers have used lower amounts of fructose. Thus, weanlings from dams ingesting fructose (10% wt/vol) during pregnancy and lactation showed elevated insulin levels [22]. Also, in a recent study, 60-day-old males born from fructose (10% wt/vol) fed lactating mothers showed increased insulin and leptin peripheral levels [4]. However, a fructose solution administered from d1 of pregnancy until posnatal day 10 produced similar levels of plasma leptin at postnatal day 10 in control and fructose-fed groups [23].

In addition, since it has been proposed that the lactational diet seems to be a stronger determinant of offspring fat metabolism than the gestational diet [24], there are very few studies where the timing of intervention in rodents is confined to the prenatal stage. However, in our previous report, we investigated the effects of a low fructose intake (10% wt/vol) throughout gestation in mothers and their fetuses [16], and we found that fetuses from fructose-fed mothers displayed an impaired transduction of the leptin signal, and these findings were not observed in glucose-fed rats. Since there is solid evidence that much of our predisposition to adult illness is determined by the time of birth [25], in the current study we have demonstrated that the fructose intake (10% wt/vol) throughout gestation has long-term consequences on the offspring. Further, since sex-dependent differences have previously been reported both in the response to maternal nutritional manipulations [23, 25] and in the influence of fructose to induce metabolic alterations [15], special attention has been given to determine whether any potential consequence of the treatment differed between female and male offspring.

Material and methods

Animals and experimental design

Female Sprague-Dawley rats weighing 200-240 g were fed *ad libitum*, a standard rat chow diet (B&K Universal, Barcelona, Spain), and housed under controlled light and temperature conditions (12-h light-dark cycle; 22 ± 1ºC). The experimental protocol was

approved by the Animal Research Committee of the University San Pablo-CEU, Madrid, Spain. The experimental protocol to which pregnant rats were subjected was the same as previously reported [16]. Briefly, pregnant animals were randomly separated into a control group, a fructose-supplemented group (Fructose), and a glucose-supplemented group (Glucose) (five to six rats per group). Fructose and glucose were supplied as a 10% (wt/vol) solution in drinking water throughout gestation. Control animals received no supplementary sugar. Pregnant rats were allowed to deliver and on the day of birth, each suckling litter was reduced to nine pups per mother. After delivery, both mothers and their pups were maintained with water and food *ad libitum*. It is remarkable that these animals (mothers and pups) received no subsequent additive in the drinking water. On the 21st day after delivery, the lactating mothers were removed to stop the suckling period and pups were separated by gender. In order to minimize the "litter effects", animals within each experimental group were born to different dams. At 15 weeks of age and, later, at 28 weeks of age, intake of solid food and liquid per cage were recorded daily during three weeks; daily data for each parameter was used to calculate the area under the curve (AUC) values. When the progeny were 261-day-old, rats were decapitated at 10 a.m. and blood collected using tubes containing Na_2-EDTA. Prior to sacrifice, food was removed at 8 a.m. Liver was immediately removed, placed in liquid nitrogen and kept at -80 ºC until analysis. Samples were then centrifuged, and plasma was stored at -80 ºC until processed for glucose, insulin, leptin and other determinations.

Determinations

Plasma aliquots were used to measure glucose (Spinreact, Girona, Spain), NEFA (non-esterified fatty acids) (Wako, Neuss, Germany), glycerol (Sigma Chemical, St. Louis, MO), and triglycerides (Spinreact) by an enzymatic colorimetric tests using commercial kits. Insulin was determined in plasma samples using a specific ELISA kit for rats (Mercodia, Uppsala, Sweden). Leptin and adiponectin were assayed in plasma samples using a specific enzyme immunoassay (EIA) kit for rats (Biovendor, Brno, Czech Republic; and Millipore, Bedford, MA, respectively). A Chemiluminiscent Microparticle Immunoassay (CMIA, Architect) was used for the quantitative determination of estradiol in plasma (Abbott, Ireland).

Two hundred milligrams of frozen liver were homogeneized into 2.5 mL of 0.9% ClNa, and used for lipid extraction following the Bligh and Dyer [26] method. Aliquots of

lipid extracts were dried and the remaining residue weighed. Ten microliters of these homogenates were used to determine triglycerides as mentioned above.

Total RNA preparation and analysis

Total RNA was isolated from liver using Ribopure (Ambion Inc., USA). The 260/280 absorption ratio of all samples was between 1.8 and 2.0. Total RNA was subjected to DNase I treatment using Turbo DNA-free (Ambion Inc., USA), and RNA integrity was confirmed by agarose gel electrophoresis. Genomic DNA contamination was discarded by PCR using primers for ribosomal protein S29 (Rps29), resulting in a negative reaction. Later, cDNA was synthesized by oligo(dT)-primed reverse transcription with Superscript II (Invitrogen, USA). Quantitative real time PCRs (qPCR) were performed using a LightCycler 1.5 (Roche, Germany). The reaction solution was performed in a volume of 20 μl, containing 10 pmol of both forward and reverse primers, 10x SYBR Premix Ex Taq (Takara Bio Inc., Japan) and appropriate nanograms of the cDNA stock. Rps29 was used as a reference gene for qPCR. The sense and antisense primer sequences were: 5'-GAAGGCAAGATGGGTCACCAGCAGC-3' and 5'-CAGGGTAGACAGTTGGTTTCATTGGG-3' for Rps29 (BC058150). Primer sequences for liver-carnitine palmitoyl transferase-I (CPT-I) were obtained from Atlas RT-PCR Primer Sequences (Clontech, CA, USA). The other primer sequences were designed with Primer3 software [27]. Samples were analyzed in duplicate on each assay. Amplification of non-specific targets was discarded using the melting curve analysis method for each amplicon. qPCR efficiency and linearity were assessed by optimization of standard curves for each target. The transcription was quantified with LightCycler Software 4.05 (Roche, Germany) using the relative quantification method.

Western Blot Analysis

Thirty micrograms of different protein fractions from rat livers were subjected to 10% SDS-polyacrylamide gel electrophoresis, as described previously [11]. Briefly, proteins were transferred to Immobilon polyvinylidene difluoride transfer membranes (Millipore, Bedford, MA, USA) and blocked for 1 hr at room temperature with 5% non-fat milk solution in TBS-0.1% Tween-20. Detection was achieved using the enhanced chemiluminescence (ECL) kit for horseradish peroxidase (HRP) (Amersham Biosciences). To confirm the uniformity of

protein loading, the blots were stained with β-actin (Sigma-Aldrich) as a control. The size of detected proteins was estimated using protein molecular-mass standards (Invitrogen, Life Technologies). All antibodies were obtained from Santa Cruz Technologies, except those for insulin receptor substrate-2 (IRS-2) and phospho-protein kinase B (p-Akt), which were obtained from Cell Signaling (Danvers, MA, USA).

Statistical analysis

Results were expressed as means ± S.E., except where indicated, from 5-6 litters. Treatment effects were analyzed by one-way analysis of variance (ANOVA). When treatment effects were significantly different ($P< 0.05$), means were tested by Tukey multiple range test, using a computer program SSPS (version 15). When the variance was not homogeneous, a post hoc Tamhane test was performed. Differences between two groups were analyzed using the Student *t* test.

Results

Ingestion of a 10% wt/vol fructose solution throughout gestation produces hyperinsulinemia and impairs insulin signal transduction in adult male progeny

As shown in Table 1, fructose intake throughout pregnancy did not produce any change in the body weight of the adult male and female progeny. Male and female rats from fructose-fed mothers showed no-differences in the ingestion of liquids and in the amount of ingested solid food, in comparison to the descendants from control mothers, at 15 weeks of age (data not shown) and, later, at 28 weeks of age (Table 1). However, progeny from glucose-fed pregnant rats turned out to be smaller versus the other two groups, the difference being significant in males. Nevertheless, the ingestion of liquids and solid food was similar in comparison to the other two groups (Table 1). Neither male nor female rats showed any differences in the weight of diverse tissues (liver, epididymal and lumbar adipose tissue, and brown adipose tissue), corrected by body weight, between the three groups (data not shown).

Plasma NEFA, glycerol and triglyceride concentrations were similar in the male rats from carbohydrate-fed mothers with respect to control values (Table 1). Similar findings were recorded in female progeny (Table 1). Furthermore, plasma 17β-estradiol concentrations were

similar between the three groups, indicating that all the female rats were sacrificed at the same period of the cycle (Table 1).

As shown in Table 1, triglyceride content in liver of male descendants from fructose-fed rats was slightly higher than in liver of progeny from glucose-fed and control rats. In accordance with this, a hepatic steatosis was also observed in fetuses [16], and then, in 21-day-old suckling pups (data not shown) from fructose-supplemented pregnant rats in comparison to the other two groups. Related to this, hepatic expression of transcription factors and enzymes involved in fatty acid catabolism was diminished in adult male rats from fructose-fed mothers. More precisely, fructose intake in pregnancy produced a significant decrease in male progeny in the mRNA levels for liver-carnitine palmitoyl transferase-I (L-CPT-I) (0.876 ± 0.033, 0.506 ± 0.062, and 0.714 ± 0.029 a.u., for control, fructose and glucose groups, respectively; $P < 0.05$, fructose vs. other two groups), and a marked diminution in PPAR-gamma coactivator 1-alpha (PGC1α) levels, a transcriptional coactivator for fatty acid oxidation, in comparison to the other two groups (0.175 ± 0.013, 0.098 ± 0.019, and 0.137 ± 0.015 a.u., for control, fructose and glucose groups, respectively; $P < 0.05$, fructose vs. control).

In contrast, female descendants from fructose-supplemented rats showed similar values in their triglyceride content to the progeny from control and glucose-supplemented mothers (Table 1).

Glycemia showed no-differences between male offspring from fructose-fed mothers compared to the progeny from control (Figure 1A), and it was lower in the male rats from glucose-fed mothers compared to the offspring from control rats. However, it is remarkable that fructose intake throughout pregnancy significantly modified the insulinemia of the adult male progeny. Thus, male rats from fructose-fed mothers showed higher levels of plasma insulin levels (Figure 1B). Accordingly, insulin sensitivity index (ISI), calculated as previously described [15], showed a decrease in male rats from fructose-fed mothers compared to the progeny from control and glucose-supplemented rats (0.50 ± 0.04, 0.40 ± 0.03, and 0.69 ± 0.10, for control, fructose-fed and glucose-fed rats, respectively; $P < 0.05$, fructose vs. glucose). This finding could be related to a diminished insulin sensitivity in the male progeny born from fructose-fed rats.

On the other hand, insulinemia and glycemia were similar in the female rats from fructose-fed mothers with respect to control values (Figure 1A and 1B). In contrast, plasma

insulin was significantly lower in female animals from glucose-fed pregnant rats in comparison to the other two groups (Figure 1B). Therefore, ISI was found to be similar for the female progeny of control and fructose-fed pregnant rats, and higher in female rats from glucose-fed mothers, versus the other two groups (0.91 ± 0.07, 0.95 ± 0.06, and 1.19 ± 0.08, for control, fructose-fed and glucose-fed rats, respectively; $P < 0.05$, glucose vs. control group).

To confirm a possible alteration at the molecular level in the hepatic insulin signaling pathway of male rats born of fructose-supplemented mothers, several components of the insulin signal transduction pathway were determined. Fructose in pregnancy did not produce any change either in the amount of insulin receptor substrate-1 (IRS-1) protein (data not shown), or in the ratio between the active, phosphorylated form of Akt and the total Akt protein (0.70 ± 0.06, 0.63 ± 0.15, 0.56 ± 0.31 a.u., for control, fructose and glucose groups, respectively), in the liver of adult male progeny. If a normal functioning of the insulin pathway is assumed, the phosphorylated form of Akt should be increased by the hyperinsulinemia observed in these same animals (Figure 1B) but this was not the case. Consequently, in the livers of male descendents of fructose-fed mothers, the ratio between the inactive, Ser-phosphorylated form of IRS-2 and the total IRS-2 protein, was significantly higher than in the other two groups (Figure 1C), confirming a significant reduction in insulin signal transduction.

In accordance with this, the levels of mRNAs for genes whose expression is under negative control by insulin [28] (phosphoenol pyruvate carboxykinase, PEPCK; and glucose-6-phosphatase, G6pc) or positively regulated by insulin (liver pyruvate kinase, LPK), were not modified in the livers of male rats from fructose-fed mothers in comparison to the control rats (Table 2). Moreover, increased plasma insulin levels (Figure 1B) should be associated with increased sterol response element-binding protein-1c (SREBP-1c) expression in livers of male animals from fructose-fed mothers [28], however, the level of mRNA for SREBP1c was significantly reduced in comparison to the control rats (Table 2). Altogether these results confirm an impairment of the insulin transduction pathway in the livers of male progeny from fructose-fed rats.

In contrast to males, livers of female descendents from fructose-supplemented rats showed similar values both in the ratio between the phosphorylated form of Akt and the total Akt protein (0.74 ± 0.16, 0.84 ± 0.30, 0.88 ± 0.33 a.u., for control, fructose and glucose

groups, respectively), and in the ratio between the phosphorylated form of IRS-2 and the total IRS-2 protein (Figure 1C), in comparison to the animals from control and glucose-supplemented mothers. Consequently, the liver mRNA expression of several insulin-responsive genes (PEPCK, G6pc and SREBP1c) was not modified in female rats from fructose-fed mothers versus the control animals (Table 2).

Ingestion of a 10% wt/vol fructose solution throughout gestation provokes hypoadiponectinemia in adult male progeny

Leptinemia did not show any differences in the male rats from carbohydrate-fed mothers with respect to control values (Figure 2A). In the female progeny, although plasma leptin levels tended to be lower in the animals from glucose-supplemented rats, the differences between the three groups were not significant (Figure 2A).

Surprisingly, male progeny from fructose-supplemented rats showed a marked decrease in plasma adiponectin concentrations (Figure 2B), and it was significantly different in comparison to the other two groups. Thus, male progeny from fructose-fed rats presented a significant increase in the leptin/adiponectin ratio (LAR) versus the control values (0.35 ± 0.03, 0.66 ± 0.10, and 0.45 ± 0.05, for control, fructose-fed and glucose-fed rats, respectively; $P < 0.05$, fructose vs. other two groups).

In contrast to males, female offspring from fructose-supplemented rats showed an increase in adiponectin levels which was significant only as compared to control rats (Figure 2B). Therefore, LAR tended to be lower in the progeny from carbohydrate-fed rats, this effect being more pronounced in the progeny from glucose-fed mothers (0.24 ± 0.04, 0.16 ± 0.03, and 0.10 ± 0.01, for control, fructose-fed and glucose-fed rats, respectively; $P < 0.05$, glucose vs. control).

Discussion

In our previous study [16], we reported an impaired hepatic transduction of the leptin signal in the fetuses from fructose-fed, but not glucose-fed, pregnant rats. Since it has been proposed that a period of relative hypoleptinemia (or, in our case, leptin resistance) during development may induce some metabolic adaptations that underlie developmental programming [2], we speculated whether our previous findings [16] could be responsible for a

developmental programming of the progeny inducing some metabolic disturbances, when adult. Thus, we here demonstrate the deleterious effects of the intake of a fructose-containing diet by the pregnant mother rat on key metabolic parameters of male progeny when they reach adulthood. Fructose, only supplemented in pregnancy, provokes hypoadiponectinemia, hyperinsulinemia and impaired insulin signaling in adult male, but not female, progeny.

It is interesting to note that these sex-dependent differences were found in adult animals which, after birth, had received no further carbohydrate additive in the drinking water. Interestingly, other studies have instead shown that when adult rats are submitted directly to fructose, it is the female animals which display a more detrimental response than their male counterparts. In fact, fructose-fed male rats were resistant to the hepatic effects of leptin, whereas fructose-fed females had no signs of leptin resistance but had hyperinsulinemia and altered glucose tolerance test [15].

It has been proposed that adiponectin levels are positively related to insulin sensitivity [29, 30]. Thus, hypoadiponectinemia found in male descendents from fructose-fed rats could be related to the impaired insulin sensitivity observed in the livers of these animals. Low plasma levels of adiponectin could be responsible for elevated levels of serine phosphorylation of IRS-2, which participates in the modulation of insulin resistance. Furthermore, the organism responds to a lower insulin sensitivity with a higher amount of hormone released by the pancreas, resulting in hyperinsulinemia. In a previous report using pregnant rats, we showed that the low levels of adiponectin affected serine phosphorylation of IRS, which was increased, this being at least partially responsible for the insulin resistance [31]. Moreover, treatment of pregnant rats with englitazone, an antidiabetic agent, provoked a significant increase in adiponectin levels, a decrease in serine phosphorylation of IRS and an improvement in the insulin sensitivity [31]. Therefore, findings reported here also indicate a role for adiponectin and serine phosphorylation of IRS-2 in the modulation of hepatic insulin sensitivity in male progeny from fructose-fed mothers. In accordance with this, the mRNA levels for several insulin-responsive genes (PEPCK, G6pc, LPK) were not affected or, even, were decreased (SREBP-1c) in male progeny from fructose-supplemented rats, despite being hyperinsulinemic. A reduction in hepatic SREPB-1 protein and mRNA levels along with elevated serum insulin have previously been reported in fructose-fed adult females [15] and in male offspring from mothers fed a high fructose diet (60%) [21]. Moreover, fatty acid synthase (FAS), a SREBP1c target gene [32], also showed a reduced expression in the livers of male

rats from fructose-fed mothers in comparison to the control rats (data not shown). This reduction observed in the hepatic mRNA expression of these lipogenic genes in males from fructose-fed mothers, could possibly explain why the accumulation of triglycerides in their livers did not reach a significant level (Table 1). In contrast to our findings, in most situations of hyperinsulinemia related to insulin resistance, the stimulating effect of insulin on hepatic lipogenesis is preserved [33]. We do not know the reason for this discrepancy. The effects of insulin signaling attenuation by Ser-phosphorylation of IRS-2 on metabolism are still poorly known [34], however, there are several reports showing that hepatic IRS-2 knockdown can affect either glucose homeostasis or lipid metabolism, or neither of them [32, 35]. It has been assumed that IRS-1 and -2 have complementary functions in the liver, but that both IRS proteins also have distinct individual roles [32]. Thus, insulin can shut down hepatic gluconeogenesis via the IRS-2 pathway and inhibit hepatic fatty acid oxidation through both the IRS-2 and the IRS-1 pathways, which might indicate that there are different levels of sensitivity to the hormone for each insulin-responsive pathway (gluconeogenesis, β-oxidation, lipogenesis, etc) [33, 36]. Thus, fatty acid oxidation may still react to insulin when inhibition of gluconeogenesis is already impaired [33], and that was the case in our study: insulin reduced β-oxidation but it failed to inhibit gluconeogenesis. If a similar situation is occurring for lipogenesis, this requires further investigation.

The presence of hyperinsulinemia in male fructose-fed progeny could indicate leptin resistance at the level of pancreatic islets [37]. Since insulin stimulates leptin production in adipocytes whereas leptin inhibits the production of insulin in pancreatic β-cells, a prolonged elevation of plasma leptin levels would result in dysregulation of the adipoinsular axis and a corresponding failure to suppress insulin secretion [38]. In fact, progeny from fructose-fed mothers were already leptin resistant when they were fetuses [16], and then, at 3 months of age, male descendents from fructose-supplemented rats were hyperleptinemic and showed fasting hyperinsulinemia and a clear impairment in the insulin action [39]. Related to this, it has been reported that adipocytes from male offspring of lactating mothers consuming fructose, spontaneously released more leptin than control rat-derived adipocytes and also displayed an impaired response to insulin stimulation [4]. Therefore, in our study, the male progeny of fructose-fed pregnant rats could present a vicious circle (leptin resistance, hypersecretion of insulin and increasing insulin resistance). As has already been proposed, this situation could accelerate a later development of metabolic and cardiovascular disorders [22, 40].

Our findings agree with a previous report where it was found that 60-day-old males born from fructose (10% wt/vol) fed lactating mothers showed increased insulin and leptin peripheral levels, decreased adiponectin plasma levels and impaired insulin sensitivity [4]. All those alterations were provoked by fructose intake only during lactation. However, in contrast to our study, these disturbances were accompanied by increased food intake and body weight in male offspring [4]. In fact, in the present study, fructose intake only during pregnancy did not produce any alterations in body weight and food intake in the adult male and female progeny at 261 days of age. Moreover, in that study [4], it was not clarified if the pups had access, like their mothers, to the water containing fructose. At the end of suckling period, around day 15 of life, it is known that rat neonates begin to mix milk and pellets and, possibly, also to drink water [41]. It has been reported that the consumption of a carbohydrate-rich diet by pups between postnatal day 4 and 24 produces distorted insulin sensitivity [42]. In the present study, hypoadiponectinemia, hyperinsulinemia, and impaired insulin sensitivity in adult male progeny were provoked by maternal fructose intake exclusively during pregnancy.

Finally, the most prominent result found here is that the intake of just a small amount (10%) of fructose, but not glucose, throughout gestation produces an impaired insulin signaling, hypoadiponectinemia, and other features of metabolic syndrome such as, hyperinsulinemia and a slight accretion of hepatic triglycerides in male progeny. Curiously, female progeny born of fructose-fed mothers showed none of these characteristics. Nevertheless, it has been reported that females born to mothers subjected to undernutrition express a programmed phenotype only in the presence of a high-fat diet, whereas the male progeny manifested it independently of postnatal nutrition [2]. Another interesting report showed how a high fructose diet (60%) in rats induced a “silent” leptin resistance with almost no physiological or biochemical differences (except hypertriglyceridemia) between control and fructose-fed groups [40]. And so, subsequent exposure of those animals to a high-fat diet led to exacerbated weight gain in fructose-fed rats compared to the control animals [40]. Therefore, it is possible that postnatal hypercaloric nutrition could amplify all these metabolic abnormalities induced by the fructose-fed fetal programming, and this deserves further investigation.

The importance of maternal nutrition during critical periods of development has been noted, since maternal feeding can induce aberrant phenotype in offspring irrespective of postnatal nutrition [43]. In fact, one of the most relevant findings of the present study is that the effects of fructose intake here reported had to originate during intrauterine development since

the carbohydrate was administered only during gestation and, moreover, those effects have been observed in the progeny after a long-period of life (261-day-old) without any access to fructose. Thus, the common and worldwide frequent ingestion of fructose containing beverages by young adults, including reproductively active individuals, reinforces the notable clinical relevance of the observations obtained in the present study.

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Figure Legends

Fig. 1 Fructose in gestation produces hyperinsulinemia and affects insulin sensitivity in adult male progeny. Male and female (A) plasma glucose, and (B) insulin of 261-day-old progeny from control (empty bar), fructose- (black bar) and glucose-fed (grey bar) pregnant rats. Data are means ± S.E. from 5-6 litters. (C) Bar plot showing the ratio between the inactive, Ser-phosphorylated form, and the total IRS-2 protein in hepatic samples from 261-day-old progeny from control, fructose-, and glucose-fed pregnant rats. The amount of protein loaded was confirmed by the Bradford method, and the uniformity of protein loading in each lane was assessed by staining the blots with Ponceau S. Values were normalized to β-actin levels and expressed in arbitrary units (a.u.). Each bar represents the mean ± SD of values from 5-6 animals. Values not sharing a common letter are significantly different ($P < 0.05$).

Fig. 2 Fructose in pregnancy produces hypoadiponectinemia in adult male progeny. Male and female (A) plasma leptin, and (B) adiponectin values of 261-day-old progeny from control, fructose- and glucose-fed pregnant rats. Data are means ± S.E. from 5-6 litters. Different letters indicate significant differences between the groups ($P < 0.05$).

Figure 1-Revised version

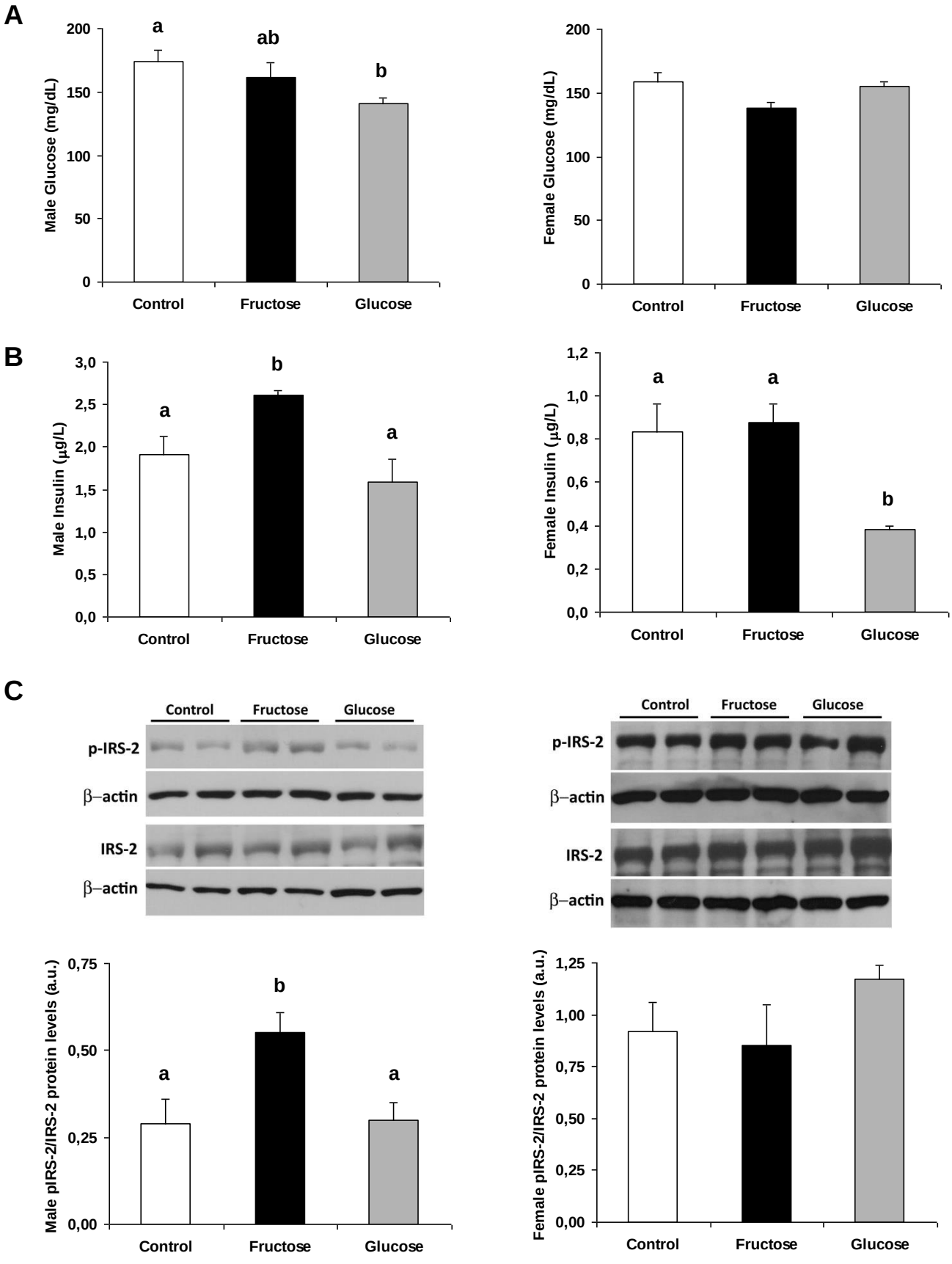

Figure 2

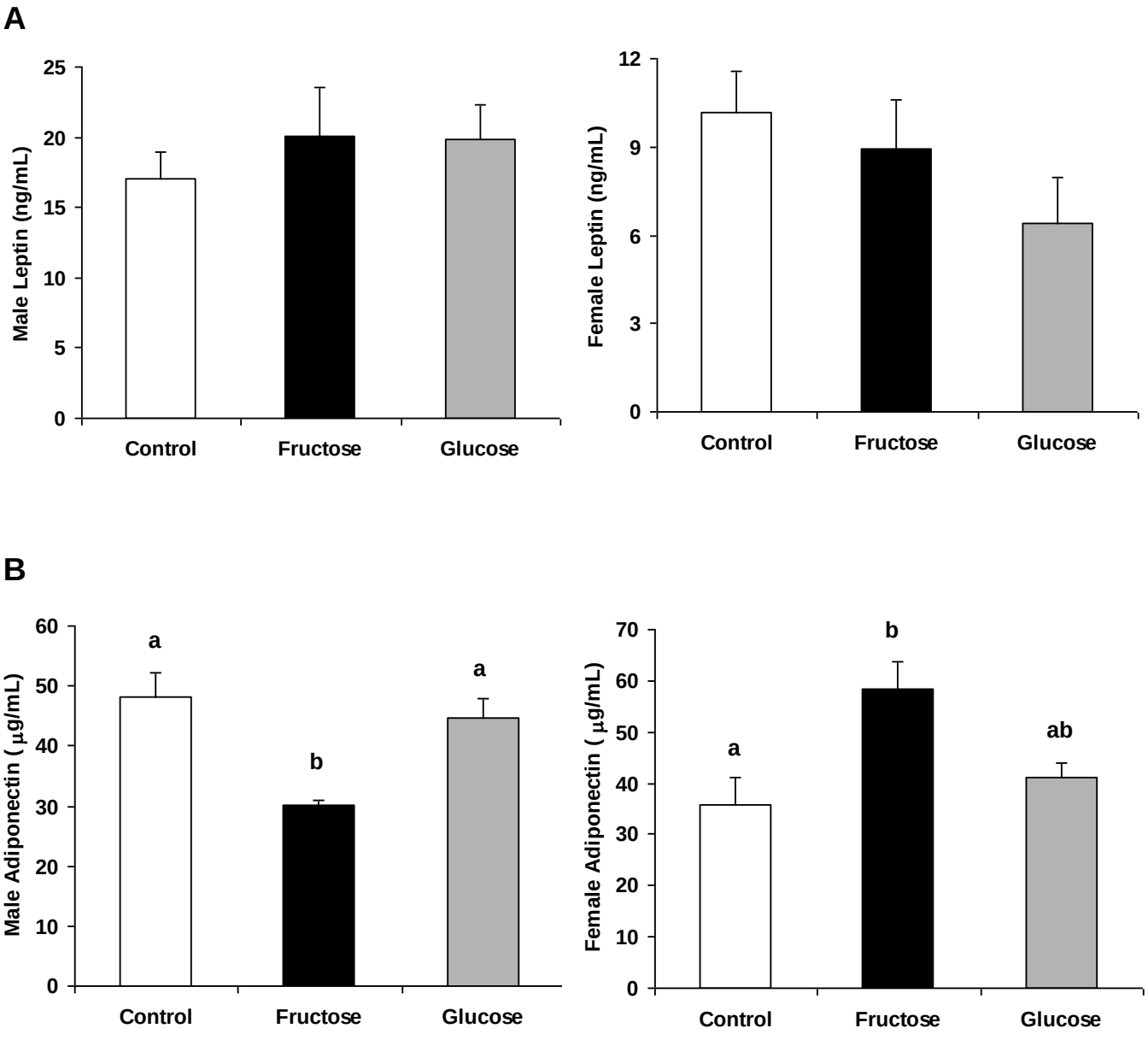
A
Male Leptin (ng/mL)
25
20
15
10
5
0
Control
Fructose
Glucose
Female Leptin (ng/mL)
12
9
6
3
0
Control
Fructose
Glucose
B
Male Adiponectin (µg/mL)
60
50
40
30
20
10
0
a
b
a
Control
Fructose
Glucose
Female Adiponectin (µg/mL)
70
60
50
40
30
20
10
0
a
b
ab
Control
Fructose
Glucose

Table 1

Body weight, plasma analytes and liver triglycerides in male and female adult progeny from fructose- or glucose-supplemented and control mothers

		Male	
	Control	Fructose	Glucose
Body weight (g)	564.5 ± 7.3[a]	558.0 ± 17.4[ab]	508.5 ± 2.6[b]
AUC consumed diet (g/21 days per 2 rats)	1160.0 ± 43.2	1041.8 ± 47.6	1070.5 ± 27.2
AUC ingested liquid (ml/21 days per 2 rats)	1170.7 ± 75.8	1137.6 ± 73.6	1091.0 ± 13.7
Plasma triglycerides (mg/dL)	69.9 ± 2.3	59.3 ± 5.4	60.4 ± 6.4
NEFA (mM)	0.26 ± 0.02	0.27 ± 0.01	0.33 ± 0.03
Glycerol (mg/dL)	3.63 ± 0.38	2.63 ± 0.28	2.96 ± 0.38
Liver triglycerides (mg/mg of protein)	0.096 ± 0.009	0.117 ± 0.009	0.106 ± 0.013
		Female	
	Control	Fructose	Glucose
Body weight (g)	324.4 ± 12.4	309.3 ± 9.3	299.3 ± 8.9
AUC consumed diet (g/21 days per 2 rats)	696.8 ± 104.9	1036.5 ± 190.8	671.5 ± 76.8
AUC ingested liquid (ml/21 days per 2 rats)	919.2 ± 144.2	1251.4 ± 392.4	751.1 ± 130.5
Plasma triglycerides (mg/dL)	51.3 ± 6.2	49.3 ± 3.9	46.4 ± 2.0
NEFA (mM)	0.37 ± 0.07	0.22 ± 0.03	0.36 ± 0.08
Glycerol (mg/dL)	2.94 ± 0.34	2.43 ± 0.15	2.63 ± 0.29
Estradiol (pg/mL)	33.0 ± 9.2	21.2 ± 5.5	28.6 ± 8.7
Liver triglycerides (mg/mg of protein)	0.083 ± 0.006	0.072 ± 0.009	0.091 ± 0.010

Data are M ± SE, from 5-6 litters. Individual AUC values for ingested liquid and solid diet are the mean corresponding to two animals housed in the same cage. Different letters indicate significant differences between the groups ($P < 0.05$). AUC: area under the curve; NEFA: non-esterified fatty acids.

Table 2
Liver (mRNA) gene expression in male and female adult progeny from fructose-supplemented or control mothers

	Male		Female	
	Control	Fructose	Control	Fructose
PEPCK (a.u.)	1.11 ± 0.32	0.59 ± 0.21	0.74 ± 0.11	0.54 ± 0.11
G6pc (a.u.)	0.83 ± 0.15	0.59 ± 0.06	0.93 ± 0.17	0.93 ± 0.03
SREBP1c (a.u.)	4.19 ± 0.42	1.06 ± 0.18**	1.28 ± 0.30	2.28 ± 0.41
LPK (a.u.)	1.76 ± 0.28	1.10 ± 0.16	1.29 ± 0.17	0.88 ± 0.04*

Relative target gene mRNA levels were measured by Real Time PCR, as explained in Materials and Methods, and normalized to Rps29 levels and expressed in arbitrary units (a.u.). Data are M ± SE, from 5-6 litters. Asterisks indicate significant differences versus control (*, $P < 0.05$; **, $P < 0.001$). PEPCK: phosphoenol pyruvate carboxykinase; G6pc: glucose-6-phosphatase; SREBP1c: sterol response element-binding protein-1c; LPK: liver pyruvate kinase.