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Liquid fructose in pregnancy exacerbates fructose-induced dyslipidemia in adult female offspring

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Abstract

Fructose intake from added sugars correlates with the epidemic rise in metabolic syndrome and related events. Nevertheless, consumption of beverages sweetened with fructose is not regulated in gestation. Previously, we found that maternal fructose intake produces in the progeny, when fetuses, impaired leptin signaling and hepatic steatosis and then impaired insulin signaling and hypoadiponectinemia in adult male rats. Interestingly, adult females from fructose-fed mothers did not exhibit any of these disturbances. However, we think that, actually, these animals keep a programmed phenotype hidden. Fed 240-day-old female progeny from control, fructose- and glucose-fed mothers were subjected for 3 weeks to a fructose supplementation period (10% wt/vol in drinking water). Fructose intake provoked elevations in insulinemia and adiponectinemia in the female progeny independently of their maternal diet. In accordance, the hepatic mRNA levels of several insulin-responsive genes were similarly affected in the progeny after fructose intake. Interestingly, adult progeny of fructose-fed mothers displayed, in response to the fructose feeding, augmented plasma triglyceride and NEFA levels and hepatic steatosis *versus* the other two groups. In agreement, the expression and activity for carbohydrate response element binding protein (ChREBP), a lipogenic transcription factor, were higher after the fructose period in female descendants from fructose-fed mothers than in the other groups. Furthermore, liver fructokinase expression that has been indicated as one of those responsible for the deleterious effects of fructose ingestion was preferentially augmented in that group. Maternal fructose intake does influence the adult female offspring's response to liquid fructose and so exacerbates fructose-induced dyslipidemia and hepatic steatosis.

Keywords: Fructose; Pregnancy; Fetal programming; Fructokinase; Dyslipidemia

1. Introduction

In the last few decades, obesity, metabolic syndrome and diabetes have escalated to epidemic proportions in many countries worldwide. Fructose, present in added sugars such as sucrose and high-fructose corn syrup, has been linked to obesity and metabolic syndrome [1–3]. In fact, it has been reported that fructose can induce leptin resistance as well as virtually all the features of metabolic syndrome in rats, whereas glucose intake does not [4]. Moreover, liquid fructose (10% wt/vol) causes hypertriglyceridemia and fatty liver, without modifying plasma glucose and insulin levels [5]. Clinical studies also implicate fructose as a cause of metabolic syndrome. Thus, a recent report has shown that consumption of fructose-sweetened beverages, but not glucose-sweetened beverages, with usual diet could induce postprandial hypertriglyceridemia in young subjects [6]. 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 [7].

On the other hand, experimental and epidemiological studies demonstrate that metabolic events during prenatal and postnatal development modulate metabolic disease risks in later life [8]. This phenomenon is called fetal programming of adult health [9]. Since feeding conditions likely constitute one of the most influential parameters on the health of the adult [10], diet manipulation in mothers during critical developmental periods has been used to identify their contribution to the development of obesity and diabetes

Abbreviations: *C/C*, female adult (261-day-old) offspring of control dams that consumed standard pellet and water without additives for their entire lives, including the last 21 days (from 240 to 261 days of age); *C/F*, female adult (261-day-old) progeny from control dams that consumed standard pellet and water without additives for their entire lives, except for the last 21 days when they ingested water containing 10% fructose; *F/F*, female adult (261-day-old) offspring from fructose-fed mothers that consumed standard pellet and water without additives for their entire lives, except for the last 21 days when they ingested water containing 10% fructose; *G/F*, female adult (261-day-old) offspring from glucose-fed mothers that consumed standard pellet and water without additives for their entire lives, except for the last 21 days when they ingested water containing 10% fructose.

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in adult offspring [11]. Thus, although the connection between a greater intake of fructose-containing beverages and the increase in the prevalence of obesity, cardiovascular diseases, *etc.* [12,13] has been established, consumption of those beverages and fruit juices is allowed during gestation and/or lactation.

In fact, when we investigated the effects of a low-fructose intake (10% wt/vol) throughout gestation in mothers and their fetuses [13], 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. Furthermore, we have demonstrated that the fructose intake throughout gestation has long-term consequences on the offspring. Thus, we concluded that the maternal intake of fructose throughout gestation produces impaired insulin signaling, hypoadiponectinemia and other features of metabolic syndrome such as hyperinsulinemia and a slight accretion of hepatic triglycerides in male adult progeny [14]. Interestingly, female progeny born of fructose-fed mothers showed none of these characteristics.

Nevertheless, several disturbances, such as diabetic dyslipidemia and cardiovascular disease associated with diabetes mellitus type 2, seem to be more prevalent in females [15]. Furthermore, when adult rats have been subjected directly to fructose, female animals display a higher metabolic burden produced by fructose ingestion than male rats. Thus, 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 [12]. Likewise, women, but not men, exhibit an association between fructose consumption and an increased risk of type 2 diabetes mellitus [16]. However, human evidence indicating sex differences in acute metabolic responses to fructose consumption suggests that females may be somewhat protected against fructose-induced hypertriglyceridemia [17].

Therefore, the findings observed in our previous study were unexpected. It was the male progeny that displayed a more detrimental response than their female counterparts to the maternal fructose [14]. However, 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 [9]. Another report showed how a high-fructose diet (60%) in rats produced almost no physiological or biochemical differences between control and fructose-fed groups, and only the subsequent exposure of those animals to a high-fat diet led to exacerbated weight gain in fructose-fed *versus* control animals [18]. Furthermore, a recent study has reported that some effects of the mother's sucrose consumption can be detected when their offspring reach adulthood and are themselves given access to sucrose solution [19]. Thus, it is possible that postnatal fructose nutrition could bring all those metabolic abnormalities induced in adult male progeny by the fructose-fed fetal programming [14] to the fore in the female descendants.

In order to investigate this possibility, we studied fatty acid and glucose metabolism in livers and plasma of adult female rats born from control, fructose- or glucose-fed mothers, after themselves receiving fructose as a liquid solution (10% wt/vol in drinking water) for 3 weeks.

2. Materials and methods

2.1. 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±1°C). 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 [13]. 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*. On the 21st day after delivery, the lactating mothers were removed to stop the suckling period, and pups were separated by gender and kept feeding on a standard rat chow diet (see above) and water without any additives. At 240 days of age, a part of the female progeny was randomly separated to be subjected to the protocol explained below. In order to minimize the "litter effects", animals within each experimental group were born to different dams. First of all, they were weighed and an aliquot of plasma was obtained from the tail. Then, vaginal smears were carried out to confirm that all the female rats were at the same period of the cycle. Later, independently from which experimental group of mothers to which they had been born, they were kept on solid pellets and supplied with drinking water containing 10% (wt/vol) of fructose. Thus, three experimental groups were formed: C/F, F/F and G/F, the first letter indicating whether the mothers had been supplied during pregnancy with tap water (C, control) or water containing a carbohydrate (F: fructose; G: glucose) and the second letter indicating the period with fructose (F), when they were adults. When the progeny were 261 days old, they were decapitated at 10 a.m. and blood was collected using tubes containing Na_2 EDTA. Prior to sacrifice, food was removed at 8 a.m. The period with fructose was selected to last 21 days (from 240 to 261 days of age) in order to be the same as that used with pregnant rats [13], since pregnancy in the rat lasts 3 weeks. Liver was immediately removed, placed in liquid nitrogen and kept at −80°C until analysis. Samples were then centrifuged, and plasma was stored at −80°C until processed for glucose, insulin, leptin and other determinations. For some determinations, a fourth experimental group was used, C/C: female progeny from control mothers supplied with water without any additives when adult [14]. Plasma 17β-estradiol concentrations were similar between the four groups, indicating that all the female rats had been sacrificed at the same period of the cycle.

2.2. Determinations

Plasma aliquots were used to measure glucose (Spinreact, Girona, Spain), NEFA (nonesterified fatty acids) (Wako, Neuss, Germany) and triglycerides and uric acid (Spinreact) by 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 kit for rats (Biovendor, Brno, Czech Republic, and Millipore, Bedford, MA, respectively). A chemiluminiscent microparticle immunoassay (Architect) was used for the quantitative determination of estradiol in plasma (Abbott, Ireland).

Two-hundred milligrams of frozen liver was homogenized into 2.5 ml of 0.9% NaCl and was used for lipid extraction following the Bligh and Dyer method [20]. Aliquots of lipid extracts were dried and the remaining residue was weighed. Ten microliters of these homogenates was used to determine triglycerides as mentioned above.

2.3. 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, 10× 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 as follows: 5′-GAAGGCAAGATGGGTCACCAGCAGC-3′ and 5′-CAGGGTAGACAGTTGGTTTCATTGGG-3′ for Rps29 (BC058150). Primer sequences for liver-carnitine palmitoyl transferase-I (L-CPT-I), phosphoenol pyruvate carboxykinase (PEPCK) and peroxisome proliferator-activated receptor α (PPARα) were obtained from Atlas RT-PCR Primer Sequences (Clontech, California, USA). The other primer sequences were designed with Primer3 software [21]. Samples were analyzed in duplicate on each assay. Amplification of nonspecific 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.

2.4. Western blot analysis

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

Table 1
Body weight and plasma analytes of 240-day-old female progeny from fructose- or glucose-supplemented and control mothers.

	Control	Fructose	Glucose
Body weight (g)	295.6 ± 8.3	319.8 ± 5.3	303.5 ± 10.4
Glucose (mg/dl)	144.0 ± 5.1	145.9 ± 4.4	132.2 ± 7.0
Insulin (μg/L)	0.19 ± 0.07	0.56 ± 0.13	0.29 ± 0.08
Triglycerides (mg/dl)	44.8 ± 6.2	55.9 ± 10.4	47.7 ± 3.7
NEFA (mM)	0.47 ± 0.07	0.52 ± 0.05	0.51 ± 0.06
Cholesterol (mg/dl)	68.4 ± 2.2	63.2 ± 3.0	64.1 ± 3.4

Data are means ± S.E., from five to six litters. Different letters indicate significant differences between the groups ($P<.05$). NEFA: nonesterified fatty acids.

from Santa Cruz Technologies, except that for insulin receptor substrate IRS2 which was obtained from Cell Signaling (Danvers, MA, USA).

2.5. Statistical analysis

Results were expressed as means ± S.E., except where indicated, from five to six litters. Treatment effects were analyzed by one-way analysis of variance. When treatment effects were significantly different ($P<.05$), means were tested by Tukey multiple range test, using a computer program SPSS (version 15). When the variance was not homogeneous, a *post hoc* Tamhane test was performed.

3. Results

3.1. Liquid fructose throughout gestation exacerbates fructose-induced dyslipidemia in adult female progeny

As mentioned in the Introduction, in our previous report [14], we found that maternal fructose intake in pregnancy provokes impaired insulin signal transduction and hyperinsulinemia in adult male progeny. On the contrary, adult female rats from fructose-fed mothers did not exhibit any of these disturbances [14]. Therefore, in the present study, before subjecting female adult offspring to 21-day exposure of liquid fructose, we wanted to confirm that progeny from control, fructose- and glucose-mothers showed similar values in plasma analytes. As shown in Table 1, fructose intake throughout pregnancy did not produce any change in the body weight of the adult female progeny at 240 days of age as compared to the other two groups. Glycemia showed no differences between female offspring from fructose-fed mothers compared to the progeny from control and glucose-fed mothers. Although plasma insulin levels tended to be higher in the animals from fructose-supplemented rats, the differences between the three groups were not significant (Table 1). Plasma triglyceride, NEFA and cholesterol concentrations were also similar in the female rats from carbohydrate-fed mothers with respect to control values (Table 1).

Table 3
Expression of hepatic proteins involved in the insulin and leptin signaling pathways of 261-day-old fructose-fed female progeny from fructose-supplemented (F/F) or glucose-supplemented (G/F) and control mothers (C/F).

	C/F	F/F	G/F
IRS1 (a.u.)	1.65 ± 0.06[a]	1.42 ± 0.21[a]	0.82 ± 0.11[b]
IRS2 (a.u.)	0.99 ± 0.21	1.21 ± 0.39	1.12 ± 0.25
Akt (a.u.)	0.75 ± 0.10	0.62 ± 0.07	0.61 ± 0.08
SOCS-3 (a.u.)	1.01 ± 0.05	0.88 ± 0.09	0.87 ± 0.04

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 means ± S.D. of values from five to six animals. Values not sharing a common letter are significantly different ($P<.05$).

As shown in Table 2, 21-day exposure to liquid fructose did not affect the body weight, since there were no differences between fructose-fed female progeny and the control group (C/C). Fructose-supplemented rats clearly showed a marked increase in the ingestion of liquids and a reduction in the amount of ingested solid food in comparison to the C/C group (Table 2). Thus, the total amount of ingested energy was different between fructose-fed rats and control (C/C) group (68.1 ± 1.2, 90.3 ± 7.0, 99.9 ± 0.1 and 85.7 ± 1.4 kcal/rat/day for C/C, C/F, F/F and G/F, respectively; $P<.05$, C/C *vs.* the other three groups). Nevertheless, when the daily energy intake was divided by body weight [22], there were no differences between the groups (data not shown). Interestingly, for the three groups of fructose-fed females (C/F; F/F, G/F), around 48% of the total amount of energy was acquired from fructose, a similar value to that previously reported for females [12]. Compared with the C/C group, the three groups of female rats that received fructose showed higher liver weight, with no differences in the weight of lumbar adipose tissue (Table 2).

Glycemia showed no differences between the four experimental groups. However, female offspring supplemented with fructose showed higher levels of plasma insulin, although it did not become significant, in comparison to the control group (C/C) (Table 2). Leptinemia did not show any differences in the female rats supplemented with fructose with respect to control values. In comparison to the control group (C/C), fructose-supplemented rats showed an increase in adiponectin levels that was significant as compared to C/F and F/F rats (Table 2). Therefore, the leptin/adiponectin ratio (LAR) tended to be lower in the three groups of rat supplemented with fructose *versus* the control values (Table 2). Plasma lactate concentrations were higher in female rats

Table 2
Body weight, food and liquid ingestion and plasma analytes of 261-day-old fructose-fed female progeny from fructose-supplemented (F/F) or glucose-supplemented (G/F) and control mothers (C/F).

	C/C	C/F	F/F	G/F
Body weight (g)	324.4 ± 12.4	322.5 ± 12.7	338.5 ± 6.6	314.8 ± 11.5
Chow consumption (g/rat/day)	18.1 ± 0.3[a]	13.3 ± 0.6[b]	13.6 ± 0.5[b]	11.8 ± 0.4[b]
Liquid consumption (ml/rat/day)	22.8 ± 3.6[a]	116.0 ± 7.7[b]	114.7 ± 4.2[b]	106.0 ± 0.5[b]
Liver weight (g/kg BW)	27.8 ± 0.6[a]	32.6 ± 1.0[b]	34.7 ± 0.7[b]	32.3 ± 1.0[b]
Lumbar adipose tissue weight (g/kg BW)	9.4 ± 1.1	12.0 ± 0.8	11.0 ± 1.4	10.7 ± 1.5
Glucose (mg/dl)	158.4 ± 7.7	180.3 ± 12.5	185.6 ± 10.0	160.6 ± 4.3
Insulin (μg/L)	0.84 ± 0.13	1.33 ± 0.23	1.48 ± 0.37	1.90 ± 0.37
Glucose/insulin	7.37 ± 1.02	5.42 ± 0.75	5.56 ± 1.34	3.37 ± 0.48
Leptin (ng/ml)	10.2 ± 1.4	12.8 ± 2.1	13.4 ± 3.0	9.9 ± 1.9
Adiponectin (μg/ml)	35.9 ± 5.3[a]	70.4 ± 7.7[b]	59.5 ± 5.1[b]	48.5 ± 5.5[ab]
LAR (ng/μg)	0.240 ± 0.037	0.178 ± 0.008	0.183 ± 0.023	0.196 ± 0.053
Lactate (mg/dl)	31.2 ± 4.2	39.9 ± 4.0	36.3 ± 3.2	37.1 ± 3.6
Uric acid (mg/dl)	3.52 ± 0.35	5.62 ± 1.03	6.52 ± 1.06	5.60 ± 1.08

Data are means ± S.E., from five to six litters. C/C: Control female offspring of control dams. BW: Body weight. LAR: leptin/adiponectin ratio. For glucose/insulin ratio, glucose is in milligrams per deciliter and insulin is in microunits per milliliter. Different letters indicate significant differences between the groups ($P<.05$).

supplemented with fructose in comparison to C/C group, although it did not become significant. Similar findings were recorded for uricemia that, as expected, was increased by fructose feeding (Table 2).

In our previous report [14], adult male descendants from fructose-fed rats showed an impaired insulin signal transduction in comparison to rats born from control and glucose-fed mothers. However, adult female offspring did not exhibit any differences in the insulin signaling pathway between the three groups. In order to detect any possible alteration at the molecular level in the hepatic insulin signaling pathway of female rats born of control, fructose- and glucose-supplemented mothers, after being subjected to 21 days of liquid fructose, several components of the insulin signal transduction pathway were determined. As shown in Table 3, fructose did not produce any change in either the amount of IRS2 or Akt proteins between the three groups of female-supplemented with fructose. For the IRS1 protein levels, only the G/F group showed less amount of that protein in comparison to the other two groups. Thus, the glucose/insulin ratio was lower in the three groups of adult female rats supplemented with fructose in comparison to the control group (C/C), the lower value being for the G/F group (Table 2). However, as shown later, this situation did not affect the response to the insulin of hepatic gene expression.

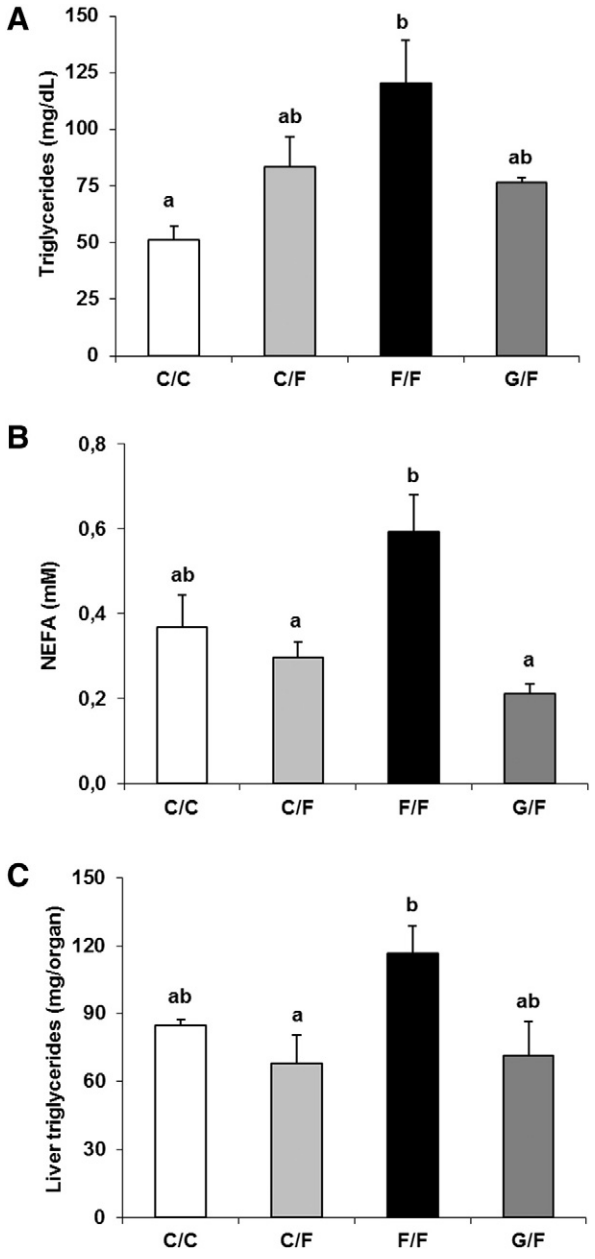

Fig. 1. Liquid fructose in gestation exacerbates fructose-induced dyslipidemia in adult female progeny. (A) Plasma triglycerides, (B) nonesterified fatty acids (NEFA) and (C) liver triglycerides of fructose-fed female adult progeny from control (C/F, light-gray bar), fructose-supplemented (F/F, black bar) and glucose-supplemented (G/F, dark-gray bar) mothers. C/C: Control female 261-day-old offspring from control pregnant rats (empty bar, C/C). Data are means ± S.E. from five to six litters. Values not sharing a common letter are significantly different ($P<.05$).

On the other hand, our previous studies had shown that progeny from fructose-fed mothers were leptin resistant when they were fetuses [13] and then, at 3 months of age, male descendents from fructose-supplemented rats were also hyperleptinemic [23]. Therefore, in order to detect any possible alteration in the hepatic leptin signaling pathway of the female offspring after being subjected to 21 days of liquid fructose, protein levels of suppressor of cytokine signaling-3 (SOCS-3) were determined. As shown in Table 3, fructose did not produce any change in the amount of SOCS-3 protein between the three groups of adult female-supplemented with fructose, consistent with the no differences found in leptinemia between those groups (Table 2).

Altogether, these findings would indicate that maternal fructose intake does not seem to affect fructose-induced effects on glucose metabolism in the progeny. Interestingly however, that was not the case for lipid metabolism. Compared with the C/C group, only the F/F group had a higher serum triglyceride concentration (Fig. 1A). Moreover, the F/F group displayed a marked increase in plasma NEFA levels (Fig. 1B) after fructose feeding, and it was significantly different in comparison to the other two groups of adult female rats supplemented with fructose (C/F and G/F). A similar profile was found for the triglyceride content in liver. Thus, a slight accretion of hepatic triglycerides was found in F/F group in comparison to the other groups (8.66 ± 0.85, 6.41 ± 1.12, 10.01 ± 1.12 and 7.03 ± 1.41 mg/g of tissue, for C/C, C/F, F/F and G/F, respectively), which became significant when expressed as milligrams per organ (Fig. 1C). That is, the increase observed in the F/F group was significantly different ($P<.05$) in comparison to the C/F and (marginally) significant ($P=.067$) *versus* the G/F group. Since fructose feeding increased liver weight in the three groups in comparison to the C/C group (Table 2), it could be assumed that the effect produced by liquid fructose on the hepatic triglyceride content is clearly specific for the F/F group. Therefore, these findings would confirm that liquid fructose throughout gestation exacerbates fructose-induced dyslipidemia in adult female offspring and predisposes the progeny to develop fructose-induced hepatic steatosis.

3.2. Liquid fructose in pregnancy amplifies fructose-induced ChREBP gene expression and activity in adult female progeny

To confirm a possible alteration in the hepatic fatty acid metabolism of female rats born of fructose-supplemented mothers induced by fructose ingestion when adult, the expression of several genes of the lipogenic and fatty acid catabolism pathways were determined.

Consequently, the mRNA levels of sterol response element binding protein 1 (SREBP1c), a transcription factor controlling fatty acid synthesis in liver [24], turned out to be higher in the female offspring supplemented with fructose, although not significantly, in comparison to the control values (C/C) (Fig. 2A). However, since insulinemia was also augmented in these three groups *versus* C/C group (Table 2) and it is known that SREBP1c is a positively regulated gene [25], SREBP1c expression changes seem to be an insulin-mediated effect rather than a direct effect of fructose. Consistent with this, the levels of mRNA for genes whose expression is under negative control by insulin [25] [PEPCK and glucose-6-phosphatase (G6pc)] were decreased in the

three groups of female descendants supplemented with fructose (C/F, F/F and G/F) *versus* C/C group (Fig. 2B and C), although this was only significant for the PEPCK gene.

Regarding the hepatic expression of transcription factors and enzymes involved in fatty acid catabolism, the mRNA levels for PPARα were significantly lower in fructose-supplemented female rats in comparison to the control group (C/C) (Fig. 2D). In accordance with that, postnatal fructose ingestion produced a nonsignificant decrease in the mRNA gene expression for L-CPT-I and PPARγ coactivator 1α (PGC1α) (a transcriptional coactivator for fatty acid oxidation) and a marked and significant diminution in uncoupling protein 2 (UCP2), in comparison to the control values (C/C) (Fig. 2E–G). These findings demonstrate that fructose is able to reduce fatty acid catabolism in liver; however, they were not able to explain why liquid fructose throughout gestation exacerbates fructose-induced dyslipidemia and provokes hepatic steatosis in adult female offspring (Fig. 1).

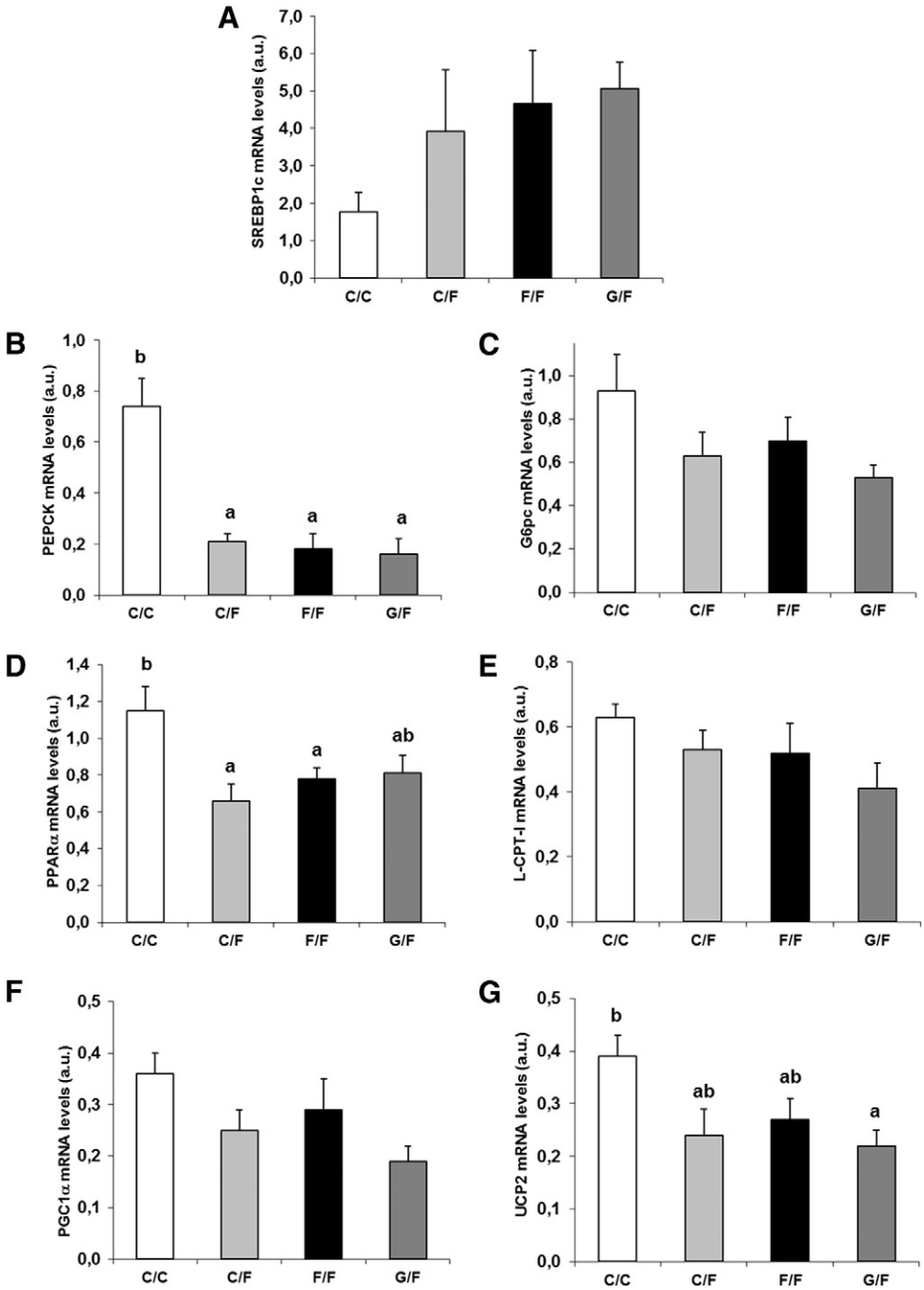

Fig. 2. Hepatic gene expression in fructose-fed female adult progeny from control, fructose- and glucose-fed pregnant rats. Liver (mRNA) expression of fructose-fed female adult progeny from control (C/F, light-gray bar), fructose-supplemented (F/F, black bar) and glucose-supplemented (G/F, dark-gray bar) mothers. C/C: Control 261-day-old female offspring from control pregnant rats (empty bar, C/C). Relative hepatic levels of specific mRNA for both insulin-induced genes: (A) the transcription factor SREBP1c and insulin-repressed genes: (B) PEPCK and (C) G6pc. Relative hepatic levels of specific mRNA for (D) the transcription factor PPARα and its target genes: (E) L-CPT-I, (F) PGC1α and (G) UCP2. 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 means ± S.E. from five to six litters. Values not sharing a common letter are significantly different ($P<.05$).

Interestingly, an increased expression of the carbohydrate response element binding protein (ChREBP), a key lipogenic transcription factor, was observed specifically (Fig. 3A), although not significantly, in the F/F group *versus* the other three groups (C/C, C/F and G/F). Furthermore, the expression of the liver pyruvate kinase (L-PK) gene [26], which is directly under the control of ChREBP transcriptional activity, was only significantly augmented in F/F group compared with the control group (C/C) (Fig. 3B). A similar profile was found for the lipogenic and ChREBP-regulated genes, such as acetyl-CoA carboxylase (ACC) (Fig. 3C) and fatty acid synthase (FAS) (Fig. 3D). That is, in comparison to the control (C/C) group, the F/F group showed a higher increase, although this was only significant for the ACC gene. Finally, the expression of fructokinase (FK), a ChREBP target gene, was also increased in the F/F group, being significantly different *versus* the C/C and C/F groups (Fig. 3E). This enzyme helps us to metabolize fructose in liver, generating uric acid as a final product [27] and, accordingly, uricemia should parallel FK gene expression, as occurred in the present results (Fig. 3E and Table 2). These results would suggest that maternal fructose intake exacerbates fructose-induced dyslipidemia and esteatosis by affecting ChREBP activity.

4. Discussion

In a previous study [13], we reported an impaired hepatic transduction of the leptin signal and hepatic steatosis in fetuses from fructose-fed pregnant rats. We then speculated whether these metabolic disturbances could be responsible for a developmental programming of the progeny. Thus, we found that the intake of a fructose-containing diet by the pregnant mother rat provoked in 90-day-old male progeny and, later, in 261-day-old male progeny: hypoadiponectinemia, hyperinsulinemia and impaired insulin signaling [14,23]. Further, these results turned out to be fructose specific since fetus and male offspring from glucose-fed pregnant rats did not display any features of metabolic syndrome. Interestingly, female progeny born to fructose-fed mothers showed none of these characteristics [14,23]. In accordance with that, 240-day-old female progeny used in the present study showed, before being subjected to the fructose-feeding period, similar values of plasma analytes (Table 1), independently of whether they were born from control, fructose-fed or glucose-fed mothers.

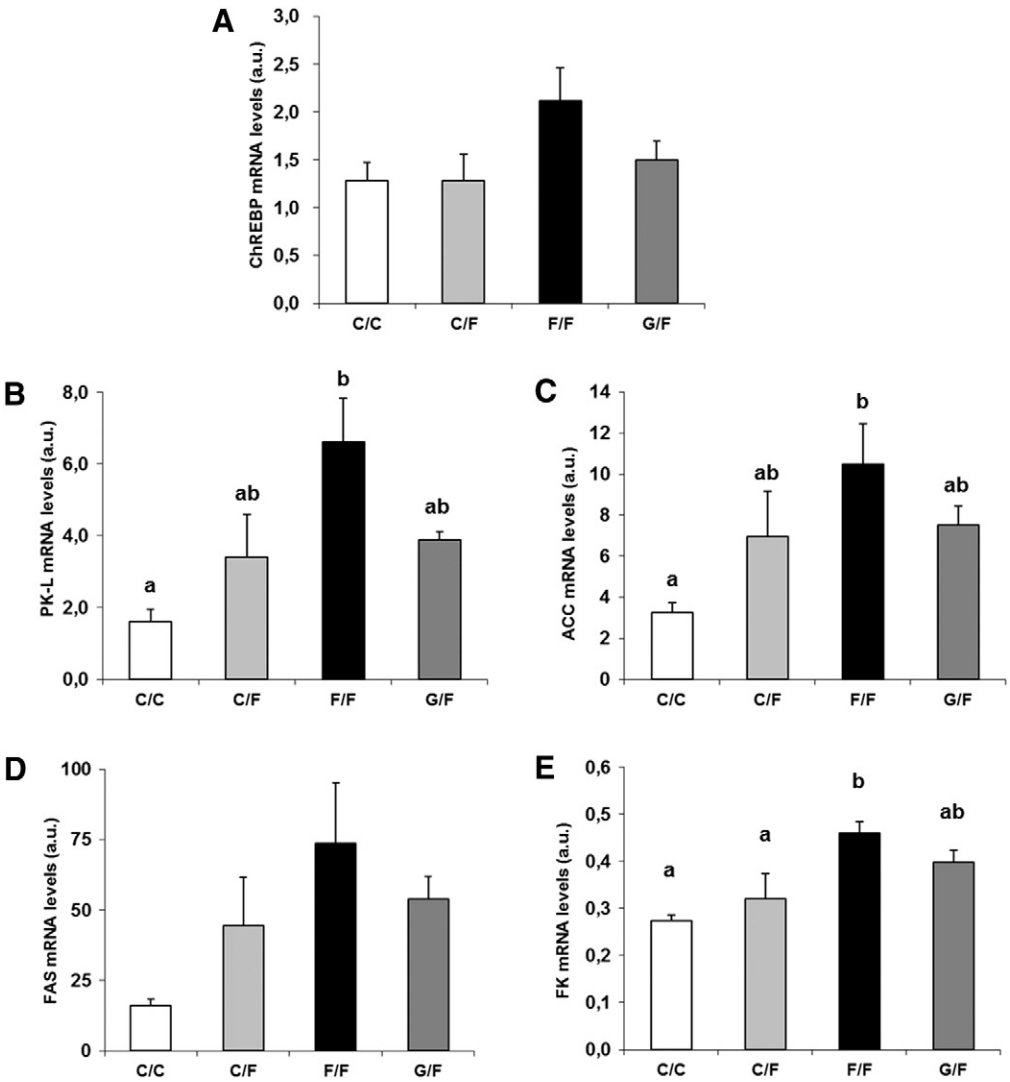

Fig. 3. Liquid fructose in gestation exacerbates fructose-induced ChREBP expression and activity in adult female progeny. Liver (mRNA) expression of fructose-fed female adult progeny from control (C/F, light-gray bar), fructose-supplemented (F/F, black bar) and glucose-supplemented (G/F, dark-gray bar) mothers. C/C: Control 261-day-old female offspring from control pregnant rats (empty bar, C/C). Relative hepatic levels of specific mRNA for (A) the transcription factor ChREBP and its target genes: (B) L-PK, (C) ACC, (D) FAS and (E) liver FK. 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 means ± S.E. from five to six litters. Values not sharing a common letter are significantly different ($P<.05$).

The predictive adaptive responses hypothesis does foresee that the rats whose mothers were fed a high-sucrose diet (HSD) should exhibit a more favorable metabolic profile in the presence of an HSD in their adult life. However, several reports showed that maternal high-sucrose (or fructose) feeding did not lead to a (predictive adaptive) protection from most carbohydrate-induced metabolic derangements in the progeny [19,28,29]. However, these three studies were carried out using only male descendants, and the "two-generation" (F2) exposure to the carbohydrate was chronic: from weaning [28,29] or for 48 days from 91 days of age [19]. Furthermore, in all these studies, a second carbohydrate was not used in parallel to demonstrate that the effects observed were specifically induced by maternal fructose (or sucrose) intake. Therefore, as we had not found any metabolic disturbances in female progeny born from fructose-supplemented mothers [14,23] and in order to provoke, in these animals, a programmed phenotype [9,28], if any, we subjected female progeny from control, fructose- and glucose-fed mothers to a short fructose-feeding period (3 weeks) to determine if the maternal intake influences the offspring's response to a fructose liquid solution (10% wt/vol in drinking water), when adult.

In accordance with a previous report [12], adult females of the three experimental groups (C/F, F/F and G/F) receiving fructose as a liquid solution exhibited higher modifications in insulinemia than in leptinemia, as compared to the control group (C/C). However, in contrast to that report, although the glucose/insulin ratio tended to be diminished by fructose ingestion, insulin sensitivity remained unalterable in the three groups. It is possible, since it has been proposed that adiponectin levels are positively related to insulin sensitivity [30,31], hyperadiponectinemia, found here in female progeny subjected to liquid fructose, could be preserving the insulin sensitivity in the livers of these animals. In fact, the insulin signal transduction of these animals was not affected: (a) the pIRS2/IRS2 ratio was not modified between F/F and C/C groups (data not shown) and (b) IRS2 and Akt proteins amounts were similar between the three fructose-supplemented groups (C/F, F/F and G/F). In agreement with this, the mRNA levels for several insulin-responsive genes (SREBP1c, PEPCK and G6pc) [25] were modified in female progeny that had received liquid fructose for 3 weeks. Interestingly, we described in two previous reports that insulin is a robust negative regulator of PPAR and its target genes expression [32,33]. Accordingly, the lower mRNA levels of PPARα, L-CPT-I, PGC1α and UCP2 found in the three groups of fructose-fed female rats in comparison to the control group (C/C) would be more related to their higher insulinemia than a direct effect of fructose ingestion.

Interestingly, the fructose-induced expression of the lipogenic transcription factor SREBP1c and the fructose-repressed expression of PPARα and its target genes (CPT-I, PGC1α and UCP2), which are related to hepatic fatty oxidation, did not correspond to the profile found here in plasma and liver lipids, which was clearly maternal intake dependent.

However, the fructose-induced expression of the lipogenic genes (ChREBP and its target genes, FAS and ACC) mirrored the profile obtained in fructose-fed female progeny for plasma and liver lipids. In accordance to this, ChREBP has been directly implicated as a key factor in the fructose-induced effects on lipid metabolism [34]. In the present work, fructose was able to induce ChREBP expression and activity (measured as FAS and ACC gene expression) mostly in female progeny of fructose-supplemented dams (F/F), as compared to C/C. On the other hand, whereas the control of the expression of ACC and FAS is shared by SREBP1c and ChREBP, the expression of L-PK gene is directly under the control of ChREBP transcriptional activity [35]. Accordingly, the L-PK gene expression was induced in fructose-fed adult female, although this being significant only for the F/F group *versus* the control group (C/C). This increased L-PK gene expression could activate the glycolysis pathway, supplying carbons to the lipogenesis. Thus, the fructose induction observed in the hepatic mRNA expression of these lipogenic genes in females from fructose-fed mothers (F/F) would explain their more pronounced accumulation of hepatic triglycerides and their dyslipidemia.

Finally, the profile of FK gene expression, that is also a ChREBP target gene [36], resembled those described for ACC, FAS and L-PK. Thus, only the F/F group displayed a significantly augmented FK expression in comparison to the C/C and C/F groups. FK is a key enzyme controlling fructose metabolic handling and, as has been pointed in several reports, one of the responsible factors for the deleterious effects of fructose ingestion. Thus, Vilá *et al.* (2011) proposed that the increased metabolic burden presented by female rats ingesting liquid fructose was due to the fact that fructose induced a much higher expression of FK in the livers of female rats than in those of male rats [12]. Moreover, steatohepatitis induced by a high-fat and high-sucrose (Western) diet was prevented in FK knockout mice [22]. Interestingly, when we determined the liver FK gene expression in female progeny that had not received fructose feeding when adult (those rats used in our previous report [14]), we found that females rats born to fructose-fed mothers showed a higher value of FK (0.274 ± 0.012, 0.300 ± 0.023 and 0.215 ± 0.023 a.u. for female offspring from control, fructose- and glucose-supplemented dams, respectively; $P<.05$, fructose *vs.* glucose). Thus, as previously reported, FK induction establishes a vicious cycle that progressively increases the deleterious effects of fructose on liver metabolism [37]. Therefore, it is not surprising that the interference of FK liver activity has been proposed as a therapeutic approach to prevent detrimental metabolic effects of fructose consumption [38]. Furthermore, Lanaspa *et al.* (2012) proposes that uric acid, by up-regulating FK expression, amplifies the lipogenic effects of fructose [27], and it was precisely in fructose-fed females from fructose-fed dams where uricemia turned out to be higher ($\times 1.60$-, $\times 1.85$- and $\times 1.59$-fold, for C/F, F/F and G/F groups, respectively, *versus* the control group, C/C).

Finally, the most prominent result found here is that liquid fructose in pregnancy exacerbates fructose-induced dyslipidemia in adult female offspring. Further, whereas elevations in plasma levels and liver triglycerides are two effects typically observed in fructose-fed rats [5,12], augmented plasma NEFA levels are not. Related to this, it has been demonstrated that chronically elevated plasma NEFA cause insulin resistance and have emerged as a major link between obesity, the development of metabolic syndrome and atherosclerotic vascular disease [39]. As such, a reduction in elevated plasma NEFA has been proposed as an important therapeutic target in obesity and type 2 diabetes [40]. Thus, the common and worldwide frequent ingestion of beverages containing fructose by adults, including reproductively active individuals, reinforces the notable clinical relevance of the observations obtained in the present study. Altogether, our reports (including the present study) and those from other authors [5,12–14,23,37], a drastic reduction in the consumption of fructose-sweetened beverages, more importantly during pregnancy, should be recommended to curtail the epidemic rise of metabolic syndrome in the human population.

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