

LIQUID FRUCTOSE IN PREGNANCY EXACERBATES FRUCTOSE-INDUCED DYSLIPIDEMIA IN ADULT FEMALE OFFSPRING, by Lourdes Rodríguez, María I. Panadero, Silvia Rodrigo, Núria Roglans, Paola Otero, Juan J. Álvarez-Millán, Juan C. Laguna and Carlos Bocos

Highlights:

- Adult female progeny of fructose-fed mothers hide a programmed phenotype
- Fructose preferentially induces ChREBP activity in females of fructose-fed mothers
- Fructokinase is specially fructose-induced in females born to fructose-fed dams
- Fructose in pregnancy exacerbates fructose-induced dyslipidemia in female offspring

1 1 LIQUID FRUCTOSE IN PREGNANCY EXACERBATES FRUCTOSE-INDUCED
2 2 DYSLIPIDEMIA IN ADULT FEMALE OFFSPRING

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7 7 Lourdes Rodríguez, María I. Panadero, Silvia Rodrigo, Núria Roglans¹, Paola Otero, Juan J.
8 8 Álvarez-Millán², Juan C. Laguna¹, and Carlos Bocos.

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11 11
12 12 Facultad de Farmacia, Universidad San Pablo-CEU, Montepríncipe, Boadilla del Monte, Madrid,
13 13 Spain. ⁽¹⁾ Facultad de Farmacia, Universidad de Barcelona, CIBERobn, IBUB, Barcelona, Spain.
14 14 ⁽²⁾CQS Lab, Madrid, Spain.
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27 15 *Corresponding author:*

28 16 Carlos Bocos; Facultad de Farmacia; Universidad San Pablo-CEU; Urbanización
29 17 Montepríncipe; 28668 Boadilla del Monte, Madrid, Spain. Phone: +34-91-372.47.98;
30 18 FAX: +34-91-351.04.96; E-mail: carbocos@ceu.es
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1 **Abstract**

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

23 *Keywords:* Fructose; pregnancy; fetal programming; fructokinase; dyslipidemia.

1 1 **Abbreviations**

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5 3 *C/C*: female adult (261-day-old) offspring of control dams that consumed standard pellet and
6
7 4 water without additives for their entire lives, including the last 21 days (from 240 to 261
8
9 5 days of age).

10 6 *C/F*: female adult (261-day-old) progeny from control dams that consumed standard pellet and
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12 7 water without additives for their entire lives, except for the last 21 days when they
13
14 8 ingested water containing 10% fructose.

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16 9 *F/F*: female adult (261-day-old) offspring from fructose-fed mothers that consumed standard
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18 10 pellet and water without additives for their entire lives, except for the last 21 days when
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20 11 they ingested water containing 10% fructose.

21 12 *G/F*: female adult (261-day-old) offspring from glucose-fed mothers that consumed standard
22
23 13 pellet and water without additives for their entire lives, except for the last 21 days when
24
25 14 they ingested water containing 10% fructose.

1 **1. Introduction**

2 In the last few decades, obesity, metabolic syndrome and diabetes have escalated to
3 epidemic proportions in many countries worldwide. Fructose, present in added sugars such as
4 sucrose and high fructose corn syrup, has been linked to obesity and metabolic syndrome [1-
5 3]. In fact, it has been reported that fructose can induce leptin resistance as well as virtually
6 all the features of metabolic syndrome in rats, whereas glucose intake does not [4]. Moreover,
7 liquid fructose (10% wt/vol) causes hypertriglyceridemia and fatty liver, without modifying
8 plasma glucose and insulin levels [5]. Clinical studies also implicate fructose as a cause of
9 metabolic syndrome. Thus, a recent report has shown that consumption of fructose-
10 sweetened, but not glucose-sweetened beverages, with usual diet could induce postprandial
11 hypertriglyceridemia in young subjects [6]. Further, in a 20-year follow-up study, it has been
12 demonstrated that consumption of sugar-sweetened beverages is associated with an increased
13 risk of coronary heart disease [7].

14 On the other hand, experimental and epidemiological studies demonstrate that
15 metabolic events during pre- and postnatal development modulate metabolic disease risks in
16 later life [8]. This phenomenon is called fetal programming of adult health [9]. Since feeding
17 conditions likely constitute one of the most influential parameters on the health of the adult [10],
18 diet manipulation in mothers during critical developmental periods has been used to identify
19 their contribution to the development of obesity and diabetes in adult offspring [11]. Thus,
20 although the connection between a greater intake of fructose-containing beverages and the
21 increase in the prevalence of obesity, cardiovascular diseases, etc [12, 13] has been
22 established, consumption of those beverages and fruit juices is allowed during gestation
23 and/or lactation.

24 In fact, when we investigated the effects of a low fructose intake (10% wt/vol)
25 throughout gestation in mothers and their fetuses [13], we found that fetuses from fructose-fed
26 mothers displayed an impaired transduction of the leptin signal, and these findings were not
27 observed in glucose-fed rats. Furthermore, we have demonstrated that the fructose intake
28 throughout gestation has long-term consequences on the offspring. Thus, we concluded that the
29 maternal intake of fructose throughout gestation produces impaired insulin signaling,
30 hypoadiponectinemia, and other features of metabolic syndrome such as, hyperinsulinemia
31 and a slight accretion of hepatic triglycerides in male adult progeny [14]. Interestingly, female
32 progeny born of fructose-fed mothers showed none of these characteristics.

1 Nevertheless, several disturbances, such as diabetic dyslipemia and cardiovascular
2 disease associated with diabetes mellitus Type 2, seem to be more prevalent in females [15].
3 Furthermore, when adult rats have been subjected directly to fructose, female animals display
4 a higher metabolic burden produced by fructose ingestion than male rats. Thus, fructose-fed
5 male rats were resistant to the hepatic effects of leptin, whereas fructose-fed females had no
6 signs of leptin resistance but had hyperinsulinemia and altered glucose tolerance test [12].
7 Likewise, women, but not men, exhibit an association between fructose consumption and an
8 increased risk of Type 2 diabetes mellitus [16]. However, human evidence indicating sex
9 differences in acute metabolic responses to fructose consumption suggests that females may
10 be somewhat protected against fructose-induced hypertriglyceridemia [17].

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

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

2. Material and methods

2.1. Animals and experimental design

1 Female Sprague-Dawley rats weighing 200-240 g were fed *ad libitum*, a standard rat
2 chow diet (B&K Universal, Barcelona, Spain), and housed under controlled light and
3 temperature conditions (12-h light-dark cycle; $22 \pm 1^\circ\text{C}$). The experimental protocol was
4 approved by the Animal Research Committee of the University San Pablo-CEU, Madrid, Spain.
5 The experimental protocol to which pregnant rats were subjected was the same as previously
6 reported [13]. Briefly, pregnant animals were randomly separated into a control group, a
7 fructose-supplemented group (Fructose), and a glucose-supplemented group (Glucose) (five to
8 six rats per group). Fructose and glucose were supplied as a 10% (wt/vol) solution in drinking
9 water throughout gestation. Control animals received no supplementary sugar. Pregnant rats
10 were allowed to deliver and on the day of birth, each suckling litter was reduced to nine pups per
11 mother. After delivery, both mothers and their pups were maintained with water and food *ad*
12 *libitum*. On the 21st day after delivery, the lactating mothers were removed to stop the suckling
13 period, and pups were separated by gender and kept feeding on a standard rat chow diet (see
14 above) and water without any additives. At 240 days of age, a part of the female progeny was
15 randomly separated to be subjected to the protocol explained below. In order to minimize the
16 “litter effects”, animals within each experimental group were born to different dams. First of all,
17 they were weighed and an aliquot of plasma was obtained from the tail. Then, vaginal smears
18 were carried out to confirm that all the female rats were at the same period of the cycle. Later,
19 independently from which experimental group of mothers to which they had been born, they
20 were kept on solid pellets and supplied with drinking water containing 10% (wt/vol) of
21 fructose. Thus, three experimental groups were formed: C/F, F/F, G/F, the first letter
22 indicating whether the mothers had been supplied during pregnancy with tap water (C,
23 control), or water containing a carbohydrate (F: fructose; G: glucose); and the second letter
24 indicating the period with fructose (F), when they were adults. When the progeny were 261-
25 day-old, they were decapitated at 10 a.m. and blood collected using tubes containing $\text{Na}_2\text{-EDTA}$.
26 Prior to sacrifice, food was removed at 8 a.m. The period with fructose was selected to last 21
27 days (from 240 to 261 days of age) in order to be the same as that used with pregnant rats [13],
28 since pregnancy in the rat lasts three weeks. Liver was immediately removed, placed in liquid
29 nitrogen and kept at -80°C until analysis. Samples were then centrifuged, and plasma was
30 stored at -80°C until processed for glucose, insulin, leptin and other determinations. For some
31 determinations, a fourth experimental group was used, C/C: female progeny from control
32 mothers supplied with water without any additives when adult [14]. Plasma 17β -estradiol

1 concentrations were similar between the four groups, indicating that all the female rats had
2 been sacrificed at the same period of the cycle.

3 4 **2.2. Determinations**

5 Plasma aliquots were used to measure glucose (Spinreact, Girona, Spain), NEFA (non-
6 esterified fatty acids) (Wako, Neuss, Germany), and triglycerides and uric acid (Spinreact) by
7 enzymatic colorimetric tests using commercial kits. Insulin was determined in plasma samples
8 using a specific ELISA kit for rats (Merckodia, Uppsala, Sweden). Leptin and adiponectin
9 were assayed in plasma samples using a specific enzyme immunoassay (EIA) kit for rats
10 (Biovendor, Brno, Czech Republic; and Millipore, Bedford, MA, respectively). A
11 Chemiluminiscent Microparticle Immunoassay (CMIA, Architect) was used for the
12 quantitative determination of estradiol in plasma (Abbott, Ireland).

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

17 18 **2.3. Total RNA preparation and Analysis**

19 Total RNA was isolated from liver using Ribopure (Ambion Inc., USA). The 260/280
20 absorption ratio of all samples was between 1.8 and 2.0. Total RNA was subjected to DNase I
21 treatment using Turbo DNA-free (Ambion Inc., USA), and RNA integrity was confirmed by
22 agarose gel electrophoresis. Genomic DNA contamination was discarded by PCR using
23 primers for ribosomal protein S29 (Rps29), resulting in a negative reaction. Later, cDNA was
24 synthesized by oligo(dT)-primed reverse transcription with Superscript II (Invitrogen, USA).
25 Quantitative real time PCRs (qPCR) were performed using a LightCycler 1.5 (Roche,
26 Germany). The reaction solution was performed in a volume of 20 µl, containing 10 pmol of
27 both forward and reverse primers, 10x SYBR Premix Ex Taq (Takara Bio Inc., Japan) and
28 appropriate nanograms of the cDNA stock. Rps29 was used as a reference gene for qPCR.
29 The sense and antisense primer sequences were: 5'-
30 GAAGGCAAGATGGGTCACCAGCAGC-3' and 5'-
31 CAGGGTAGACAGTTGGTTTCATTGGG-3' for Rps29 (BC058150). Primer sequences for
32 liver-carnitine palmitoyl transferase-I (CPT-I), phosphoenol pyruvate carboxykinase

1 (PEPCK), and peroxisome proliferator-activated receptor α (PPAR α) were obtained from
2 Atlas RT-PCR Primer Sequences (Clontech, CA, USA). The other primer sequences were
3 designed with Primer3 software [21]. Samples were analyzed in duplicate on each assay.
4 Amplification of non-specific targets was discarded using the melting curve analysis method
5 for each amplicon. qPCR efficiency and linearity were assessed by optimization of standard
6 curves for each target. The transcription was quantified with LightCycler Software 4.05
7 (Roche, Germany) using the relative quantification method.

8 9 **2.4. Western Blot Analysis**

10 Thirty micrograms of different protein fractions from rat livers were subjected to 10%
11 SDS-polyacrylamide gel electrophoresis, as described previously [5]. Briefly, proteins were
12 transferred to Immobilon polyvinylidene difluoride transfer membranes (Millipore, Bedford,
13 MA, USA) and blocked for 1 hr at room temperature with 5% non-fat milk solution in TBS-
14 0.1% Tween-20. Detection was achieved using the enhanced chemiluminescence (ECL) kit
15 for horseradish peroxidase (HRP) (Amersham Biosciences). To confirm the uniformity of
16 protein loading, the blots were stained with β -actin (Sigma-Aldrich) as a control. The size of
17 detected proteins was estimated using protein molecular-mass standards (Invitrogen, Life
18 Technologies). All antibodies were obtained from Santa Cruz Technologies, except that for
19 insulin receptor substrate-2 (IRS-2) which was obtained from Cell Signaling (Danvers, MA,
20 USA).

21 22 **2.5. Statistical Analysis**

23 Results were expressed as means \pm S.E., except where indicated, from 5-6 litters.
24 Treatment effects were analyzed by one-way analysis of variance (ANOVA). When treatment
25 effects were significantly different ($P < 0.05$), means were tested by Tukey multiple range test,
26 using a computer program SPSS (version 15). When the variance was not homogeneous, a
27 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 both 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).

As shown in Table 2, twenty-one 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 ; 85.7 ± 1.4 Kcal/rat/day, for C/C, C/F, F/F, and G/F, respectively; $P < 0.05$, 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

1 it did not become significant, in comparison to the control group (C/C) (Table 2). Leptinemia
2 did not show any differences in the female rats supplemented with fructose with respect to
3 control values. In comparison to the control group (C/C), fructose-supplemented rats showed
4 an increase in adiponectin levels which was significant as compared to C/F and F/F rats
5 (Table 2). Therefore, the leptin/adiponectin ratio (LAR) tended to be lower in the three groups
6 of rat supplemented with fructose versus the control values (Table 2). Plasma lactate
7 concentrations were higher in female rats supplemented with fructose in comparison to C/C
8 group, although it did not become significant. Similar findings were recorded for uricemia
9 which, as expected, was increased by fructose feeding (Table 2).

10 In our previous report [14], adult male descendants from fructose-fed rats showed an
11 impaired insulin signal transduction in comparison to rats born from control and glucose-fed
12 mothers. However, adult female offspring did not exhibit any differences in the insulin
13 signalling pathway between the three groups. In order to detect any possible alteration at the
14 molecular level in the hepatic insulin signalling pathway of female rats born of control,
15 fructose-, and glucose-supplemented mothers, after being subjected to 21 days of liquid
16 fructose, several components of the insulin signal transduction pathway were determined. As
17 shown in Table 3, fructose did not produce any change in either the amount of insulin
18 receptor substrate-2 (IRS-2) or Akt proteins between the three groups of female-supplemented
19 with fructose. For the insulin receptor substrate-1 (IRS-1) protein levels, only the G/F group
20 showed less amount of that protein in comparison to the other two groups. Thus, the
21 glucose/insulin ratio was lower in the three groups of adult female rats supplemented with
22 fructose in comparison to the control group (C/C), the lower value being for the G/F group
23 (Table 2). However, as shown later, this situation did not affect the response to the insulin of
24 hepatic gene expression.

25 On the other hand, our previous studies had shown that progeny from fructose-fed
26 mothers were leptin resistant when they were fetuses [13] and then, at 3 months of age, male
27 descendants from fructose-supplemented rats were also hyperleptinemic [23]. Therefore, in
28 order to detect any possible alteration in the hepatic leptin signalling pathway of the female
29 offspring after being subjected to 21 days of liquid fructose, protein levels of suppressor of
30 cytokine signaling-3 (SOCS-3) were determined. As shown in Table 3, fructose did not
31 produce any change in the amount of SOCS-3 protein between the three groups of adult

1 female-supplemented with fructose, consistent with the no-differences found in leptinemia
2 between those groups (Table 2).

3 Altogether, these findings would indicate that maternal fructose-intake does not seem
4 to affect fructose-induced effects on glucose metabolism in the progeny. Interestingly
5 however, that was not the case for lipid metabolism. Compared with the C/C group, only the
6 F/F group had a higher serum triglyceride concentration (Figure 1A). Moreover, the F/F
7 group displayed a marked increase in plasma NEFA levels (Figure 1B) after fructose-feeding,
8 and it was significantly different in comparison to the other two groups of adult female rats
9 supplemented with fructose (C/F and G/F). A similar profile was found for the triglyceride
10 content in liver. Thus, a slight accretion of hepatic triglycerides was found in F/F group in
11 comparison to the other groups (8.66 ± 0.85 ; 6.41 ± 1.12 ; 10.01 ± 1.12 ; 7.03 ± 1.41 mg/g of
12 tissue, for C/C, C/F, F/F, and G/F, respectively), which became significant when expressed as
13 mg/organ (Figure 1C). That is, the increase observed in the F/F group was significantly
14 different ($P < 0.05$) in comparison to the C/F and (marginally) significant ($P = 0.067$) versus
15 the G/F group. Since fructose feeding increased liver weight in the three groups in
16 comparison to the C/C group (Table 2), it could be assumed that the effect produced by liquid
17 fructose on the hepatic triglyceride content is clearly specific for the F/F group. Therefore,
18 these findings would confirm that liquid fructose throughout gestation exacerbates fructose-
19 induced dyslipidemia in adult female offspring and predisposes the progeny to develop
20 fructose-induced hepatic steatosis.

21 22 ***3.2. Liquid fructose in pregnancy amplifies fructose-induced ChREBP gene expression*** 23 ***and activity in adult female progeny***

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

28 Consequently, the mRNA levels of sterol response element-binding protein-1
29 (SREBP1c), a transcription factor controlling fatty acid synthesis in liver [24], turned out to
30 be higher in the female offspring supplemented with fructose, although not significantly, in
31 comparison to the control values (C/C) (Figure 2A). However, since insulinemia was also
32 augmented in these three groups versus C/C group (Table 2) and it is known that SREBP1c is

1 a positively-regulated gene [25], SREBP1c expression changes seem to be an insulin-
2 mediated effect rather than a direct effect of fructose. Consistent with this, the levels of
3 mRNA for genes whose expression is under negative control by insulin [25] (phosphoenol
4 pyruvate carboxykinase, PEPCK; and glucose-6-phosphatase, G6pc) were decreased in the
5 three groups of female descendants supplemented with fructose (C/F, F/F, and G/F) versus
6 C/C group (Figures 2B and 2C), although this was only significant for the PEPCK gene.

7 Regarding the hepatic expression of transcription factors and enzymes involved in
8 fatty acid catabolism, the mRNA levels for peroxisome proliferator-activated receptor alpha
9 (PPAR α) were significantly lower in fructose-supplemented female rats in comparison to the
10 control group (C/C) (Figure 2D). In accordance with that, postnatal fructose ingestion
11 produced a non-significant decrease in the mRNA gene expression for liver-carnitine
12 palmitoyl transferase-I (L-CPT-I) and PPAR-gamma coactivator 1-alpha (PGC1 α) (a
13 transcriptional coactivator for fatty acid oxidation), and a marked and significant diminution
14 in uncoupling protein 2 (UCP2), in comparison to the control values (C/C) (Figures 2E-2G).
15 These findings demonstrate that fructose is able to reduce fatty acid catabolism in liver,
16 however, they were not able to explain why liquid fructose throughout gestation exacerbates
17 fructose-induced dyslipidemia and provokes hepatic steatosis in adult female offspring
18 (Figure 1).

19 Interestingly, an increased expression of the carbohydrate response element binding
20 protein (ChREBP), a key lipogenic transcription factor, was observed specifically (Figure
21 3A), although not significantly, in the F/F group versus the other three groups (C/C, C/F and
22 G/F). Furthermore, the expression of the liver pyruvate kinase (L-PK) gene [26], which is
23 directly under the control of ChREBP transcriptional activity, was only significantly
24 augmented in F/F group compared with the control group (C/C) (Figure 3B). A similar profile
25 was found for the lipogenic and ChREBP-regulated genes, such as acetyl-CoA carboxylase
26 (ACC) (Figure 3C) and fatty acid synthase (FAS) (Figure 3D). That is, in comparison to the
27 control (C/C) group, the F/F group showed a higher increase, although this was only
28 significant for the ACC gene. Finally, the expression of fructokinase (FK), a ChREBP target
29 gene, was also increased in the F/F group, being significantly different versus the C/C and
30 C/F groups (Figure 3E). This enzyme helps to metabolize fructose in liver, generating uric
31 acid as a final product [27] and, accordingly, uricemia should parallel FK gene expression, as
32 occurred in the present results (Figure 3E and Table 2). These results would suggest that

1 maternal fructose intake exacerbates fructose-induced dyslipidemia and esteatosis by
2 affecting ChREBP activity.

4. Discussion

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

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

1 In accordance with a previous report [12], adult females of the three experimental groups
2 (C/F; F/F; and G/F) receiving fructose as a liquid solution exhibited higher modifications in
3 insulinemia than in leptinemia, as compared to the control group (C/C). However, in contrast to
4 that report, although the glucose/insulin ratio tended to be diminished by fructose ingestion,
5 insulin sensitivity remained unalterable in the three groups. It is possible, since it has been
6 proposed that adiponectin levels are positively related to insulin sensitivity [30, 31],
7 hyperadiponectinemia, found here in female progeny subjected to liquid fructose, could be
8 preserving the insulin sensitivity in the livers of these animals. In fact, the insulin signal
9 transduction of these animals was not affected: a) the pIRS2/IRS2 ratio was not modified
10 between F/F and C/C groups (data not shown) and b) IRS2 and Akt proteins amounts were
11 similar between the three fructose-supplemented groups (C/F, F/F, and G/F). In agreement with
12 this, the mRNA levels for several insulin-responsive genes (SREBP-1c, PEPCK and G6pc)
13 [25] were modified in female progeny which had received liquid fructose for 3 weeks.
14 Interestingly, we described in two previous reports that insulin is a robust negative-regulator
15 of PPAR and its target genes expression [32, 33]. Accordingly, the lower mRNA levels of
16 PPAR α , L-CPT-I, PGC1 α and UCP2 found in the three groups of fructose-fed female rats in
17 comparison to the control group (C/C), would be more related to their higher insulinemia than
18 a direct effect of fructose ingestion.

19 Interestingly, the fructose-induced expression of the lipogenic transcription factor
20 SREBP1c and the fructose-repressed expression of PPAR α and its target genes (CPT-I,
21 PGC1 α , and UCP2), which are related to hepatic fatty oxidation, did not correspond to the
22 profile found here in plasma and liver lipids, which was clearly maternal intake-dependent.

23 However, the fructose-induced expression of the lipogenic genes (ChREBP and its
24 target genes, FAS and ACC) mirrored the profile obtained in fructose-fed female progeny for
25 plasma and liver lipids. In accordance to this, ChREBP has been directly implicated as a key
26 factor in the fructose-induced effects on lipid metabolism [34]. In the present work, fructose
27 was able to induce ChREBP expression and activity (measured as FAS and ACC gene
28 expression) mostly in female progeny of fructose-supplemented dams (F/F), as compared to
29 C/C. On the other hand, whereas the control of the expression of ACC and FAS is shared by
30 SREBP1c and ChREBP, the expression of liver-PK gene is directly under the control of
31 ChREBP transcriptional activity [35]. Accordingly, the L-PK gene expression was induced in
32 fructose-fed adult female, although this being significant only for the F/F group versus the

1 control group (C/C). This increased L-PK gene expression could activate the glycolysis
2 pathway, supplying carbons to the lipogenesis. Thus, the fructose-induction observed in the
3 hepatic mRNA expression of these lipogenic genes in females from fructose-fed mothers
4 (F/F), would explain their more pronounced accumulation of hepatic triglycerides and their
5 dyslipidemia.

6 Finally, the profile of fructokinase (FK) gene expression, that is also a ChREBP target
7 gene [36], resembled those described for ACC, FAS and L-PK. Thus, only the F/F group
8 displayed a significantly augmented FK expression in comparison to the C/C and C/F groups.
9 Fructokinase is a key enzyme controlling fructose metabolic handling and, as has been
10 pointed in several reports, one of the responsible factors for the deleterious effects of fructose
11 ingestion. Thus, Vilá et al (2011) proposed that the increased metabolic burden presented by
12 female rats ingesting liquid fructose was due to the fact that fructose induced a much higher
13 expression of FK in the livers of female rats than in those of male rats [12]. Moreover,
14 steatohepatitis induced by a high-fat and high-sucrose (Western) diet was prevented in
15 fructokinase knockout mice [22]. Interestingly, when we determined the liver fructokinase gene
16 expression in female progeny that had not received fructose-feeding when adult (those rats used
17 in our previous report [14]), we found that females rats born to fructose-fed mothers showed a
18 higher value of FK (0.274 ± 0.012 ; 0.300 ± 0.023 ; and 0.215 ± 0.023 a.u. for female offspring
19 from control, fructose, and glucose-supplemented dams; $P < 0.05$, fructose vs. glucose). Thus, as
20 previously reported, FK induction establishes a vicious cycle that progressively increases the
21 deleterious effects of fructose on liver metabolism [37]. Therefore, it is not surprising that the
22 interference of fructokinase liver activity has been proposed as a therapeutic approach to prevent
23 detrimental metabolic effects of fructose consumption [38]. Furthermore, Lanaspá et al (2012)
24 proposes that uric acid, by up-regulating FK expression, amplifies the lipogenic effects of
25 fructose [27]. And it was precisely in fructose-fed females from fructose-fed dams where
26 uricemia turned out to be higher (x1.60, x1.85, x1.59 fold, for C/F, F/F and G/F groups,
27 respectively, versus the control group, C/C).

28 Finally, the most prominent result found here is that liquid fructose in pregnancy
29 exacerbates fructose-induced dyslipidemia in adult female offspring. Further, whereas
30 elevations in plasma levels and liver triglycerides are two effects typically observed in
31 fructose-fed rats [5, 12], augmented plasma NEFA levels are not. Related to this, it has been
32 demonstrated that chronically elevated plasma NEFA cause insulin resistance and have

1 emerged as a major link between obesity, the development of metabolic syndrome and
2 atherosclerotic vascular disease [39]. As such, a reduction in elevated plasma NEFA has been
3 proposed as an important therapeutic target in obesity and Type 2 diabetes [40]. Thus, the
4 common and worldwide frequent ingestion of beverages containing fructose by adults,
5 including reproductively active individuals, reinforces the notable clinical relevance of the
6 observations obtained in the present study. Altogether, our reports (including the present
7 study) and those from other authors [5, 12-14, 23, 37], a drastic reduction in the consumption
8 of fructose-sweetened beverages, more importantly during pregnancy, should be
9 recommended to curtail the epidemic rise of metabolic syndrome in the human population.

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22 **References**

- 23 1. Havel PJ (2005) Dietary fructose: Implications for dysregulation of energy
24 homeostasis and lipid/carbohydrate metabolism. *Nutr Rev* 63:133-157.
- 25 2. Tappy L, Lê KA (2010) Metabolic effects of fructose and the worldwide increase in
26 obesity. *Physiol Rev* 90:23-46.
- 27 3. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, Gersch MS,
28 Benner S, Sánchez-Lozada LG (2007) Potential role of sugar (fructose) in the
29 epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney
30 disease, and cardiovascular disease. *Am J Clin Nutr* 86:899-906.
- 31 4. Johnson RJ, Pérez-Pozo SE, Sautin Y, Manitius J, Sánchez-Lozada LG, Feig DI,
32 Shafiu M, Segal M, Glassock RJ, Shimada M, Roncal C, Nakagawa T (2009)

- 1 Hypothesis: Could excessive fructose intake and uric acid cause type 2 diabetes?
2 Endocr Rev 30:96-116.
- 3 5. Roglans N, Vilà L, Farré M, Alegret M, Sánchez RM, Vázquez-Carrera M, Laguna JC
4 (2007) Impairment of hepatic Stat-3 activation and reduction of PPARalpha activity in
5 fructose-fed rats. Hepatology 45(3):778-788.
- 6 6. Stanhope KL, Bremer AA, Medici V, Nakajima K, Ito Y, Nakano T, Chen G, Fong
7 TH, Lee V, Menorca RI, Keim NL, Havel PJ (2011). Consumption of fructose and
8 high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and
9 apolipoprotein-B in young men and women. J Clin Endocrinol Metab 96:E1596-
10 E1605.
- 11 7. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB (2012)
12 Sweetened beverage consumption, incident coronary heart disease, and biomarkers of
13 risk in men. Circulation 125(14):1735-1741.
- 14 8. Koletzko B, Broekaert I, Demmelmair H, Franke J, Hannibal I, Oberle D, et al. EU
15 Childhood Obesity Project (2005) Protein intake in the first year of life: a risk factor
16 for later obesity? The E.U. childhood obesity project. Adv Exp Med Biol 569:69-79.
- 17 9. Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, Breier
18 BH, Harris M (2005) Neonatal leptin treatment reverses developmental programming.
19 Endocrinology 146(10):4211-4216.
- 20 10. Beck B, Richey S, Archer ZA, Mercer JG (2012) Ingestion of carbohydrate-rich
21 supplements during gestation programs insulin and leptin resistance but not body weight
22 gain in adult rat offspring. Front Physiol 3:224.
- 23 11. Alzamendi A, Castrogiovanni D, Gaillard RC, Spinedi E, Giovambattista A (2010)
24 Increased male offspring's risk of metabolic-neuroendocrine dysfunction and overweight
25 after fructose-rich diet intake by the lactating mother. Endocrinology 151:4214-4223.
- 26 12. Vilà L, Roglans N, Perna V, Sánchez RM, Vázquez-Carrera M, Alegret M, Laguna JC
27 (2011) Liver AMP/ATP ratio and fructokinase expression are related to gender
28 differences in AMPK activity and glucose intolerance in rats ingesting liquid fructose.
29 J Nutr Biochem 22(8):741-751.
- 30 13. Rodríguez L, Panadero MI, Roglans N, Otero P, Alvarez-Millán JJ, Laguna JC, Bocos
31 C (2013) Fructose during pregnancy affects maternal and fetal leptin signalling. J Nutr
32 Biochem 24:1709-1716.

- 1 14. Rodríguez L, Panadero MI, Roglans N, Otero P, Rodrigo S, Alvarez-Millán JJ, Laguna
2 JC, Bocos C (2015) Fructose only in pregnancy provokes hyperinsulinemia,
3 hypoadiponectinemia and impaired insulin signaling in adult male, but not female,
4 progeny. *Eur J Nutr*, doi:10.1007/s00394-015-0886-1.
- 5 15. Rydén L, Standl E, Bartnik M, et al (2007) Guidelines on diabetes, pre-diabetes, and
6 cardiovascular diseases: executive summary, the task force on diabetes and
7 cardiovascular diseases of the European Society of Cardiology (ESC) and of the
8 European Association for the Study of Diabetes (EASD). *Eur Heart J* 28:88-136.
- 9 16. Schulze MB, Manson JE, Ludwig DS, et al (2004) Sugar-sweetened beverages, weight
10 gain, and incidence of Type 2 diabetes in young and middle-aged women. *J Am Med*
11 *Assoc* 292:927-934.
- 12 17. Tran LT, Yuen VG, McNeill JH (2009) The fructose-fed rat: a review on the
13 mechanisms of fructose-induced insulin resistance and hypertension. *Molec Cell*
14 *Biochem* 332(1-2):145-159.
- 15 18. Shapiro A, Mu W, Roncal C, Cheng K-Y, Johnson RJ, Scarpace PJ (2008) Fructose-
16 induced leptin resistance exacerbates weight gain in response to subsequent high-fat
17 feeding. *Am J Physiol Regul Integr Comp Physiol* 295:R1370-R1375.
- 18 19. Kendig MD, Ekayanti W, Stewart H, Boakes RA, Rooney K (2015) Metabolic effects of
19 access to sucrose drink in female rats and transmission of some effects to their offspring.
20 *PLoS ONE* 10(7): e0131107.
- 21 20. Bligh EG, Dyer WJ (1959) A rapid method for total lipid extraction and purification.
22 *Can J Biochem Physiol* 37:911-917.
- 23 21. Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist
24 programmers. *Methods Mol Biol* 132:365-386.
- 25 22. Ishimoto T, Lanasa MA, Rivard CJ, Roncal-Jimenez CA, Orlicky DJ, Cicerchi C,
26 McMahan RH, Abdelmalek MF, Rosen HR, Jackman MR, et al (2013) High-fat and
27 high-sucrose (western) diet induces steatohepatitis that is dependent on fructokinase.
28 *Hepatology* 58:1632-1643.
- 29 23. Rodríguez L, Otero P, Panadero MI, Rodrigo S, Álvarez-Millán JJ, Bocos C (2015)
30 Maternal fructose intake induces insulin resistance and oxidative stress in male, but not
31 female, offspring. *J Nutr Metab*, vol. 2015, Article ID 158091.

- 1 1 24. Dentin R, Girard J, Postic C (2005) Carbohydrate responsive element binding protein
2 (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key
3 2 regulators of glucose metabolism and lipid synthesis in liver. *Biochimie* 87(1):81-86.
4 3
5 4
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7 6
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9 8
10 9
11 10
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52 51
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54 53
55 54
56 55
57 56
58 57
59 58
60 59
61 60
62 61
63 62
64 63
65 64
24. Dentin R, Girard J, Postic C (2005) Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. *Biochimie* 87(1):81-86.
25. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS and Goldstein JL (2000) Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol Cell* 6:77–86.
26. Yamashita H, Takenoshita M, Sakurai M, et al (2001) A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proc Natl Acad Sci USA* 98:9116-9121.
27. Lanaspá MA, Sanchez-Lozada LG, Cicerchi C, Li N, Roncal-Jimenez CA, et al (2012) Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. *PLoS ONE* 7(10): e47948.
28. Sedova L, Seda O, Kazdova L, Chylikova B, Hamet P, Tremblay J, Kren V, Krenova D (2007) Sucrose feeding during pregnancy and lactation elicits distinct metabolic response in offspring of an inbred genetic model of metabolic syndrome. *Am J Physiol Endocrinol Metab* 292:E1318-1324.
29. Ching RH, Yeung LO, Tse IM, Sit WH, Li ET (2011) Supplementation of bitter melon to rats fed a high-fructose diet during gestation and lactation ameliorates fructose-induced dyslipidemia and hepatic oxidative stress in male offspring. *J Nutr* 141(9):1664-1672.
30. Elliott SS, Keim NL, Stern JS, Teff KL, Havel PJ (2002) Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr* 76(5):911-922.
31. Laclaustra M, Corella D, Ordovas JM (2007) Metabolic syndrome pathophysiology: The role of adipose tissue. *Nutr Metab Cardiovasc Dis* 17:125-139.
32. Panadero MI, Vidal H, Herrera E, Bocos C (2001) Nutritionally induced changes in the peroxisome proliferator activated receptor- α gene expression in liver of suckling rats are dependent on insulinaemia. *Arch Biochem Biophys* 394(2):182-188.
33. Panadero MI, Herrera E, Bocos C (2005) Different sensitivity of PPAR α expression to nutritional changes in liver of suckling and adult rats. *Life Sciences* 76:1061-1072.
34. Rebollo A, Roglans N, Baena M, Sánchez RM, Merlos M, Alegret M, Laguna JC (2014) Liquid fructose downregulates Sirt1 expression and activity and impairs the oxidation of fatty acids in rat and human liver cells. *Biochim Biophys Acta* 1841:514-524.

- 1 1 35. Denechaud P-D, Bossard P, Lobaccaro J-MA, Millatt L, Staels B, Girard J, Postic C
2 (2008) ChREBP, but not LXRs, is required for the induction of glucose-regulated genes
3 in mouse liver. *J Clin Invest* 118:956-964.
4
5 3
6 4 36. Ma L, Robinson LN, Towle HC (2006) ChREBP-Mlx is the principal mediator of
7 glucose-induced gene expression in the liver. *J Biol Chem* 281:28721-28730.
8
9 5
10 6 37. Vilà L, Rebollo A, Adalsteisson GG, Alegret M, Merlos M, Roglans N, Laguna JC
11 (2011) Reduction of liver fructokinase expression and improved hepatic inflammation and
12 metabolism in liquid fructose-fed rats after atorvastatin treatment. *Toxicol Appl*
13 *Pharmacol* 251:32-40.
14 8
15 9
16 10 38. Khitan Z, Kim DH (2013) Fructose: a key factor in the development of metabolic
17 syndrome and hypertension. *J Nutr Metab*, vol. 2013, Article ID 682673.
18
19 11
20 12 39. Boden G (2008) Obesity and free fatty acids (FFA). *Endocrinol Metab Clin North Am*
21 37(3):635-646.
22
23 13
24 14 40. Boden G, Shulman GI (2002) Free fatty acids in obesity and type 2 diabetes: defining
25 their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin*
26 *Invest* 32(Suppl 3):14-23.
27
28 15
29 16
30 17
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Figure Legends

Fig. 1 Liquid fructose in gestation exacerbates fructose-induced dyslipidemia in adult female progeny. (A) Plasma triglycerides, (B) non-esterified fatty acids (NEFA), and (C) liver triglycerides of fructose-fed female adult progeny from control (C/F, light grey bar), fructose- (F/F, black bar), and glucose-supplemented (G/F, dark grey bar) mothers. C/C: Control female 261-day-old offspring from control pregnant rats (empty bar, C/C). Data are means \pm S.E. from 5-6 litters. Values not sharing a common letter are significantly different ($P < 0.05$).

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 grey bar), fructose- (F/F, black bar), and glucose-supplemented (G/F, dark grey 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 sterol response element-binding protein-1 (SREBP1c); and insulin-repressed genes: (B) phosphoenol pyruvate carboxykinase (PEPCK), and (C) glucose-6-phosphatase (G6pc). Relative hepatic levels of specific mRNA for (D) the transcription factor peroxisome proliferator-activated receptor alpha (PPAR α), and its target genes: (E) liver carnitine palmitoyl transferase-I (L-CPT-I); (F) PPAR gamma coactivator 1-alpha (PGC1 α); and (G) uncoupling protein 2 (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 \pm S.E. from 5-6 litters. Values not sharing a common letter are significantly different ($P < 0.05$).

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 grey bar), fructose- (F/F, black bar), and glucose-supplemented (G/F, dark grey 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 carbohydrate response element binding protein (ChREBP), and its target genes: (B) liver pyruvate kinase (PK-L); (C) acetyl-CoA carboxylase (ACC); (D) fatty acid synthase (FAS); and (E) liver fructokinase (FK). Relative target gene mRNA levels were measured by Real Time PCR, as explained in Materials and Methods, and normalized to

1 1 Rps29 levels and expressed in arbitrary units (a.u.). Data are means \pm S.E. from 5-6 litters.
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3 2 Values not sharing a common letter are significantly different ($P < 0.05$).
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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 M ± SE, from 5-6 litters. Different letters indicate significant differences between the groups ($P < 0.05$). NEFA: non-esterified fatty acids.

Table 2

Body weight, food and liquid ingestion and plasma analytes of 261-day-old fructose-fed female progeny from fructose- (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 M ± SE, from 5-6 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 decilitre and insulin in microunits per millilitre. Different letters indicate significant differences between the groups ($P < 0.05$).

Table 3

Expression of hepatic proteins involved in the insulin and leptin signaling pathways of 261-day-old fructose-fed female progeny from fructose- (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 mean \pm SD of values from 5-6 animals. Values not sharing a common letter are significantly different ($P < 0.05$).

Figure 1

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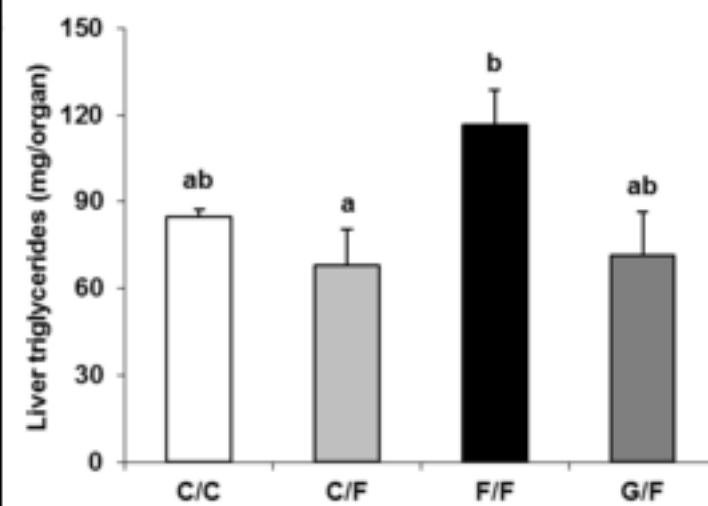
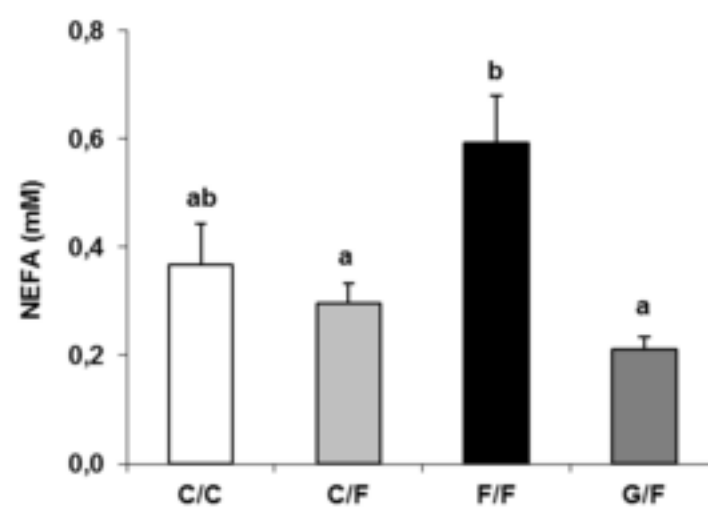
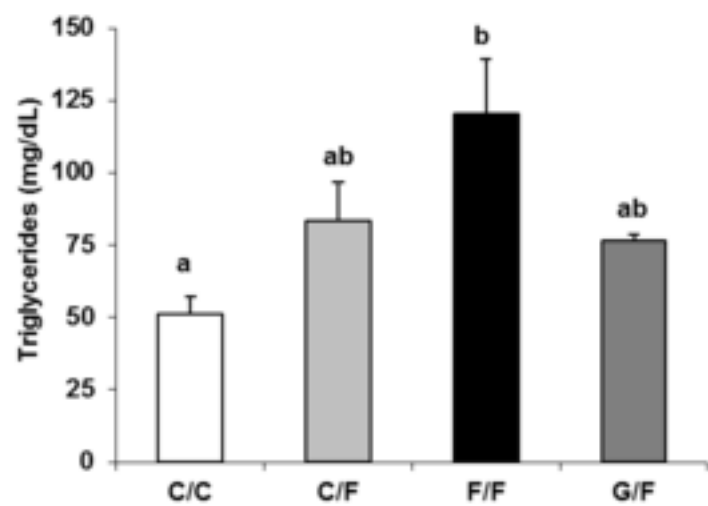


Figure 2

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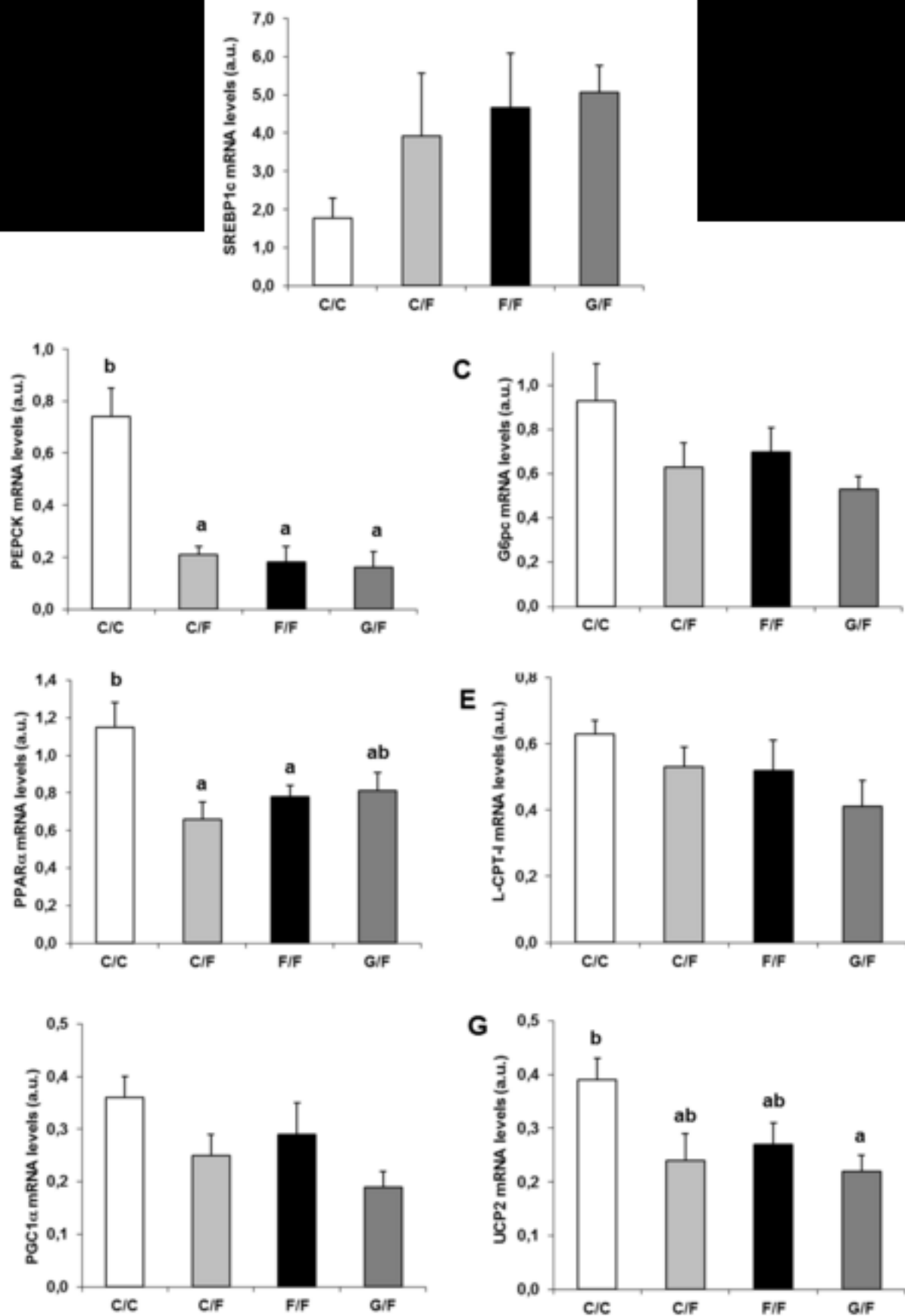


Figure 3
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