Journal of Functional Foods 64 (2020) 103676

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

FGF21-protection against fructose-induced lipid accretion and oxidative stress is influenced by maternal nutrition in male progeny

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ARTICLE INFO

Keywords:
Fructose
Fetal programming
FGF21
Liver
Adipose tissue
Oxidative stress

ABSTRACT

Fructose intake from added sugars correlates with the epidemic rise in metabolic syndrome. Nevertheless, consumption of beverages sweetened with fructose is allowed in gestation. We have studied how adult male progeny from control (C/F), fructose- (F/F) and glucose-fed (G/F) mothers respond to liquid fructose (10% wt/vol), and compared them to the control group (C/C). Interestingly, plasma levels of fibroblast growth factor 21 (FGF21) were augmented in fructose-fed males, mainly in F/F group. FGF21 has proven to be a protective agent against steatosis and oxidative stress. Accordingly, FGF21 protected against fructose-induced lipid accumulation in livers of C/F and F/F males, but not in G/F. However, fructose-induced lipid oxidation was found in the adipose tissue of F/F males due to an impaired response of their antioxidant system. Maternal carbohydrate intake does influence the adult male offspring's response to liquid fructose presumably by affecting the FGF21 function.

1. Introduction

Fructose, present in added sugars such as sucrose and high fructose corn syrup, has frequently been linked to obesity and metabolic syndrome (Johnson et al., 2009). Experimental studies have shown that fructose can provoke features of metabolic syndrome in rats, whereas glucose intake does not (Johnson et al., 2009). Thus, diets containing 10% wt/vol fructose in drinking water induce hypertriglyceridemia and fatty liver (Roglans et al., 2002). However, whereas several trials using fructose and other carbohydrates have reported that no sugar has more deleterious effects than others, numerous clinical studies support fructose as a cause of metabolic syndrome (Stanhope et al., 2011; de Koning et al., 2012; Heden et al., 2014; Chiavaroli et al., 2015). Therefore, debate is assured regarding the relationship between fructose and metabolic syndrome and related diseases.

On the other hand, a phenomenon called fetal programming of adult health (Vickers et al., 2005) indicates that metabolic events during pre- and postnatal development, such as maternal feeding conditions, modulate metabolic disease risk in later life (Koletzko et al., 2005). Thus, although a connection between a high consumption of fructose-containing beverages and the global epidemic of obesity and metabolic syndrome could exist (Vilà et al., 2011; Rodríguez et al., 2013), ingestion of these beverages and fruit juices is still permitted during gestation.

In fact, we have found that fetuses from fructose-fed mothers (Rodríguez et al., 2013) displayed an impaired transduction of the leptin signal and hepatic steatosis. These findings were not observed in fetuses from glucose-fed rats. Furthermore, we have demonstrated that the fructose intake throughout gestation has long-term consequences on the offspring since adult males from fructose-fed mothers exhibited

Abbreviations: C/C, male adult (261-day-old-offspring from 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, male 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, male 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, male 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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https://doi.org/10.1016/j.jff.2019.103676
Received 14 June 2019; Received in revised form 5 November 2019; Accepted 9 November 2019
Available online 16 November 2019

impaired insulin signalling and hyperinsulinemia (Rodríguez et al., 2016a). Interestingly, adult female progeny born of fructose-fed mothers presented an exacerbated response to liquid fructose intake in comparison to females from control and glucose-fed mothers, displaying hypertriglyceridemia and hepatic steatosis (Rodríguez et al., 2016b).

Moreover, it has been demonstrated that fructose is much more reactive than glucose and is able to participate in diverse reactions that generate free radicals (Takagi et al., 1995). In fact, it has previously been demonstrated that short-term administration of fructose to male rats induces changes in many oxidative stress markers as well as in the antioxidant system (Castro, Massa, Del Zotto, Gagliardino, & Francini, 2011; Francini et al., 2010; Rebolledo, Marra, Raschia, Rodríguez, & Gagliardino, 2008) in both liver and adipose tissue. Accordingly, it was not surprising when we found that fructose administration during gestation induced oxidative stress in fetuses (Rodrigo et al., 2016) and then, in the later life of progeny. In fact, we have detected increased plasma levels of protein oxidation products in male progeny from fructose-fed dams (Rodríguez et al., 2015). In accordance with this, Ching, Yeung, Tse, Sit, and Li (2011) showed that a high-fructose diet during gestation and lactation compromises hepatic antioxidant status in adult male offspring (Ching et al., 2011).

In order to investigate whether adult male progeny born of fructose-fed mothers showed a different response to liquid fructose intake in comparison to males from control and glucose-fed mothers, we studied both fatty acid and glucose metabolism in liver and plasma, and the redox homeostasis in liver and adipose tissue of adult male 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 (Rodríguez et al., 2013, 2016b). 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 male 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. 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) fructose. Thus, three experimental groups were formed: C/F, F/F, 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-day-old, they were decapitated at 10 a.m. and blood collected using tubes containing Na_2-EDTA. Prior to sacrifice, food was removed at 8 a.m. 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 (Rodríguez et al., 2013), since pregnancy in the rat lasts three weeks. Liver and adipose tissue were 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. In parallel, a fourth experimental group was used, C/C: male progeny from control mothers supplied with water without any additives when adult (Rodríguez et al., 2016b).

2.2. Determinations

Plasma aliquots were used to measure glucose, lactate, cholesterol (Spinreact, Girona, Spain), NEFA (non-esterified fatty acids) (Wako, Neuss, Germany), and triglycerides (Spinreact) by enzymatic colorimetric tests using commercial kits. Insulin and FGF21 were determined in plasma samples using a specific ELISA kit for rats (Mercodia, Uppsala, Sweden; and R&D Systems, USA, respectively). Leptin was assayed in plasma samples using a specific enzyme immunoassay (EIA) kit for rats (Biovendor, Brno, Czech Republic). Insulin sensitivity index (ISI) was calculated as the ratio 2/[(plasma insulin μM × plasma glucose μM) + 1], as previously described (Rodríguez et al., 2016a).

Two hundred milligrams of liver and 50 mg of adipose tissue were immersed in chloroform:methanol 2:1 plus dibutylhydroxytoluene (BHT), and used for lipid extraction following the Folch method (Folch, Lees, & Sloane Stanley, 1957). Aliquots of lipid extracts were dried and weighed, and the remaining residue redissolved in isopropanol and used to determine cholesterol. The hepatic homogenates were used directly to determine triglycerides and, after deproteinization, to measure glucose, lactate and glycogen.

2.3. Total RNA preparation and analysis

Total RNA was isolated from liver and lumbar adipose tissue using Ribopure (Ambion Inc., USA). Total RNA was subjected to DNase I treatment using Turbo DNA-free (Ambion Inc., USA), and RNA integrity was confirmed by agarose gel electrophoresis. Afterwards, cDNA was synthesized by oligo(dT)-primed reverse transcription with Superscript II (Invitrogen, USA). qPCRs were performed using a Light Cycler 1.5 (Roche, Germany). The reaction solution was carried out in a volume of 20 μl, containing 10 pmol of both forward and reverse primers, 10x SYBR Premix Ex Taq (Takara Bio Inc., Japan) and the appropriate nanograms of the cDNA stock. Rps29 was used as a reference gene for qPCR. The primer sequences were obtained either from the Atlas RT-PCR Primer Sequences (Clontech, CA, USA) or designed using Primer3 software (University of Massachusetts Medical School, MA, USA) (Rozen & Skaletsky, 2000). Samples were analysed in duplicate on each assay. Amplification of non-specific targets was discarded using the melting curve analysis method for each amplicon. qPCR efficiency and linearity were assessed by optimization of the standard curves for each target. The transcription was quantified with Light Cycler Software 4.05 (Roche, Germany) using the efficiency correction method (Pfaffl, 2001).

2.4. Determination of antioxidant enzymes activity

One hundred milligrams of frozen liver or 200 mg of frozen adipose tissue were homogeneized into 0.25 M Tris-HCl, 0.2 M sucrose, and 5 mM dithiothreitol (DTT) pH 7.4 buffer. These tissue homogenates were used to determine the oxidative stress state. The concentration of malondialdehyde (MDA) was measured as a marker of lipid peroxidation using the method previously described (Wong et al., 1987), by measuring the fluorescence of MDA-thiobarbituric acid (TBA) complexes at 515 nm /553 nm excitation/emission wavelengths. As a protein oxidative stress biomarker, carbonyls of protein content were

measured using the method previously described (Levine et al., 1990).

Samples of tissue were also used to determine the contents of reduced glutathione (GSH) and oxidized glutathione (GSSG), using capillary electrophoresis coupled to UV-DAD and following the method previously reported (Maeso, Garcia-Martinez, Ruperez, Cifuentes, & Barbas, 2005).

The activity of the enzyme glutathione reductase was determined by following the oxidation of NADPH to $NADP^+$ cofactor for the reduction of oxidized glutathione, which is proportional to the activity thereof (Goldberg & Spooner, 1987). To measure the activity of glutathione peroxidase, the protocol previously described was used (Gunzler, Kremers, & Flohe, 1974). Catalase activity was studied by the H_2O_2 decomposition caused by the activity of that enzyme (Aebi, 1987). This was done by recording the absorbance maximum of H_2O_2 to 240 nm. Finally, the activity of superoxide dismutase (SOD) was measured using a commercial kit (Spinreact, Spain).

2.5. Statistical analysis

Results were expressed as means ± S.E. Treatment effects were analyzed by one-way analysis of variance (ANOVA). When treatment effects were significantly different ($P < 0.05$), means were tested by Tukey's multiple range test, using the computer program SPSS (version 23). When the variance was not homogeneous, a *post hoc* Tamhane test was performed.

3. Results

In a previous report (Rodríguez et al., 2016a) we found that maternal fructose intake in pregnancy provokes impaired insulin signal transduction and hyperinsulinemia in adult male progeny. Therefore, in the present study, before subjecting male adult offspring to 21 day-exposure of liquid fructose, we wanted to check the state of plasma analytes and body weight of the progeny from control, fructose- and glucose-mothers. As shown in Table 1, fructose intake throughout pregnancy did not produce any change in the body weight of the adult male progeny at 240 days of age as compared to the other two groups. Glycemia showed no-differences between male offspring from fructose-fed mothers compared to the progeny from control and glucose-fed mothers. As previously reported (Rodríguez et al., 2016a), plasma insulin levels tended to be higher in the descendants of fructose-supplemented rats, becoming significantly different versus glucose-fed mothers' progeny (Table 1). Plasma leptin levels paralleled those for insulin, but the differences between the three groups were not significant. Plasma triglycerides and cholesterol concentrations tended to be higher in the progeny from glucose-fed mothers, and similar between male rats from fructose-fed mothers and those from the control (Table 1).

As shown in Table 2, fructose-supplemented rats showed a marked increase in the ingestion of liquids and a reduction in the amount of ingested solid food, with no significant change in body weight after 21-day exposure to liquid fructose (Table 2). Interestingly, this increase in the liquid consumption amount and the reduction in the chow intake were in the same proportion as those previously reported by Vilá et al. (Vilà et al., 2011). Thus, there was no difference in the total amount of ingested energy between fructose-fed rats and control (C/C) group (4289.4 ± 23.1; 4863.9 ± 251.0; 4642.7 ± 481.7; 4528.4 ± 539.3 Kcal/21 days per 2 rats, for C/C, C/F, F/F, and G/F, respectively). Interestingly, for the three groups of fructose-fed males (C/F; F/F, G/F), around 25% of the total amount of energy was acquired from fructose, as previously reported for male rats (Vilà et al., 2011). In fact, similar body weight increase was observed at the end of the 21 days of fructose administration between the three groups, being higher to that found in the control group (C/C) (Table 2).

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

	Control	Fructose	Glucose
Body weight (g)	587.4 ± 20.5	563.7 ± 29.7	563.4 ± 37.6
Glucose (mg/dL)	129.4 ± 2.0	126.0 ± 2.2	136.1 ± 8.1
Insulin (μg/L)	1.21 ± 0.09	1.50 ± 0.25*	0.90 ± 0.14
Leptin (ng/mL)	13.2 ± 2.0	25.7 ± 5.3	17.0 ± 3.6
Triglycerides (mg/dL)	49.8 ± 5.5	43.3 ± 3.8	86.4 ± 17.7
Cholesterol (mg/dL)	62.9 ± 4.4	55.7 ± 4.1	71.7 ± 5.3

Data are M ± SE, from 5 to 6 litters. Asterisk indicates significant differences versus Glucose group (*: $P < 0.05$).

Although glycemia tended to be lower in C/F and F/F groups, no differences were shown between the four experimental groups. While F/F male offspring showed higher levels of plasma insulin, becoming significant in comparison to the control group (C/C) and the G/F rats (Table 2), the insulin sensitivity index (ISI) was similar between C/F and F/F groups in comparison to the control group (C/C) and only the G/F rats showed a higher value than the other groups. Leptinemia did parallel insulinemia, although the differences were not significant (Table 2). Interestingly, even though it is known that fructose intake produces hyperlipidemia (Roglans et al., 2002, 2007), only the C/F group for triglycerides, and C/F and G/F groups for cholesterol and NEFA, had a higher serum concentration (Table 2) versus the control group (C/C), showing that lipidemia of F/F group was not affected by fructose administration. Altogether, these findings indicate that maternal fructose-intake does affect fructose-induced effects on glucose and lipid metabolism in the progeny.

Regarding hepatic gene expression, the mRNA levels of lipogenic genes such as sterol response element-binding protein-1 (SREBP1c), ATP citrate lyase and liver pyruvate kinase (L-PK) and genes of fatty acid catabolism, such as liver-carnitine palmitoyl transferase-I (CPT-I), tended to be positively-regulated and under negative control by insulin, respectively (Table 2), in accordance with the previously reported insulin-mediated effects in these pathways (Dentin, Girard, & Postic, 2005; Shimomura et al., 2000; Rodríguez et al., 2016b). However, carbohydrate response element binding protein (ChREBP), another lipogenic gene, was not affected. On the other hand, changes in genes related to glucose metabolism, such as insulin-like growth factor-binding protein 1 (IGFBP1) and phosphoenol pyruvate carboxykinase (PEPCK), did parallel to glycemia rather than insulinemia (Table 2). Moreover, other genes that have been demonstrated to be affected by fructose intake such as ATP-binding cassette G5 (ABCG5) (Apro, Beckman, Angelin, & Rudling, 2015), peroxisome proliferator-activated receptor alpha (PPARα) and stearoyl-CoA desaturase type 1 (SCD1) (Roglans et al., 2007) showed either a diminution (ABCG5), although not significant, in fructose-fed groups in comparison to the control group (Table 2) or no changes between the four groups (PPAR and SCD1) (Table 2).

Fructose has been demonstrated to stimulate fibroblast growth factor 21 (FGF21) hepatic expression in mice (Fisher et al., 2016) and, moreover, fructose ingestion increases circulating FGF21 levels both in humans and rodents (Fisher et al., 2016; Dushay et al., 2015). Therefore, we determined FGF21 mRNA gene expression in our animal model. As shown in Fig. 1A, fructose-fed male rats showed an increase in hepatic FGF21 expression, this becoming significantly different in males from fructose-fed mothers, in comparison to the control rats (C/C). In accordance with that, plasma FGF21 was clearly augmented in fructose-fed groups, the difference significant being in C/F and F/F groups versus control group (C/C) (Fig. 1B).

FGF21 is a hormone mainly expressed in the liver that functions as a metabolic regulator of glucose, lipid, and energy homeostasis. Its protective role against steatosis and oxidative stress has been proposed in situations that display lipid accumulation and an unbalanced redox state, such as a high fat-high fructose diet (Chukijrungroat, Khamphaya, Weerachayaphorn, Songserm, & Saengsirisuwan, 2017), ethanol

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

	C/C	C/F	F/F	G/F
Body weight (g)	578.3 ± 15.0	602.4 ± 19.4	579.1 ± 31.9	576.7 ± 42.3
Body weight increase (g)	2.58 ± 1.25	14.96 ± 2.19[a]	15.30 ± 5.01	17.01 ± 5.14
Chow consumption (g/21 days per 2 rats)	1140.2 ± 6.1	884.2 ± 71.3	843.4 ± 31.2	947.6 ± 112.8
Liquid consumption (ml/21 days per 2 rats)	1255.3 ± 154.4	3844.2 ± 1297.5	3674.2 ± 911.1	2409,0 ± 287,0
Glucose (mg/dL)	173.6 ± 9.6	145.8 ± 4.2	139.0 ± 5.9	163.2 ± 14.3
Insulin (µg/L)	1.90 ± 0.22	2.10 ± 0.20	2.86 ± 0.18[a,c]	1.26 ± 0.18[b]
ISI	0.50 ± 0.04	0.49 ± 0.02	0.43 ± 0.03	0.66 ± 0.06
Leptin (ng/mL)	17.02 ± 1.93	18.51 ± 2.37	32.79 ± 8.21	23.41 ± 4.12
Triglycerides (mg/dL)	69.9 ± 2.3	83.0 ± 7.2	53.5 ± 4.5[b]	61.9 ± 4.9[b]
Cholesterol (mg/dL)	53.0 ± 3.4	59.4 ± 3.5	48.3 ± 1.6[c]	69.1 ± 5.9
NEFA (mmol/L)	0,26 ± 0,02	0,34 ± 0,02	0,19 ± 0,05[b,c]	0,33 ± 0,02
Lactate (mg/dL)	28.8 ± 6.3	26.3 ± 2.2	30.1 ± 4.4	38.0 ± 4.1
Liver mRNA expression (a.u.)				
SREBP1c	4.19 ± 0.42	2.62 ± 0.48	5.53 ± 0.83[b]	3.83 ± 0.73
ATP citrate lyase	0.86 ± 0.15	0.95 ± 0.11	1.55 ± 0.28	1.11 ± 0.09
LPK	2.70 ± 0.97	3.06 ± 0.40	3.71 ± 0.46	2.38 ± 0.44
CPTI	1.01 ± 0.13	0.90 ± 0.14	0.75 ± 0.09	1.33 ± 0.26
ChREBP	1.81 ± 0.16	1.36 ± 0.26	1.54 ± 0.12	1.42 ± 0.22
PEPCK	1.11 ± 0.32	0.63 ± 0.24	0.52 ± 0.14	1.12 ± 0.20
IGFBP1	3.41 ± 1.49	1.34 ± 0.22	1.49 ± 0.16	2.66 ± 0.42
SCD1	3.34 ± 0.90	3.02 ± 0.50	2.50 ± 0.44	2.86 ± 0.75
PPARα	1.25 ± 0.14	1.36 ± 0.28	1.19 ± 0.17	1.49 ± 0.22
ABCG5	3.33 ± 0.90	1.09 ± 0.23	1.03 ± 0.15	1.30 ± 0.19

Data are M ± SE, from five to six litters. C/C: Control male offspring of control dams. ISI: Insulin sensitivity index. NEFA: Non-esterified fatty acids.
[a] : at least $P < 0.05$ versus C/C.
[b] : at least $P < 0.05$ versus C/F.
[c] : at least $P < 0.05$ versus G/F.

ingestion (Desai et al., 2017), diet-induced obesity (Xu et al., 2009), type 2 diabetes (Chavez et al., 2009), and obesity and metabolic syndrome (Gómez-Sámano et al., 2017; Tanaka et al., 2015). In fact, FGF21 is now considered a stress-responsive hormone in humans and rodents (Gómez-Sámano et al., 2017). Therefore, we measured lipid content, oxidative stress markers and antioxidant enzymes activity in liver of fructose-fed male rats.

As shown in Table 3, liver weight tended to be higher in C/F group and, in fact, fructose ingestion produced an increase in liver weight corrected by the body weight only in the males from control mothers, becoming significantly different in comparison to control group (C/C) and males from glucose-fed mothers. However, total lipids hepatic content was augmented in the G/F group, being significantly different versus the other two groups receiving fructose when adults (C/F and F/F) and, importantly, the same profile was obtained for hepatic triglyceride and total cholesterol contents (Table 3). Curiously, glucose amount decreased and lactate and glycogen contents increased in livers of fructose-fed males from control and fructose-supplemented mothers (C/F and F/F) and it was significantly different in comparison to the control group (C/C) (Table 3). These findings demonstrate that the fructose ingested by the progeny follow diverse pathways (lipid synthesis versus lactate and glycogen production (Herman & Samuel, 2016)) influenced by which carbohydrate had been ingested by their mothers during pregnancy.

Regarding oxidative stress markers, hepatic MDA and protein carbonyls values were not induced by fructose intake and they were even significantly diminished in offspring of fructose-fed mothers (F/F) in comparison to the other three groups (Fig. 2). To further investigate the mechanism behind this unexpected antioxidant effect, we determined several hepatic components of the antioxidant system: glutathione content and catalase, glutathione peroxidase (GPx1), glutathione reductase (GR), and superoxide dismutase activities. As shown in Table 4, whereas catalase, GR and SOD1 were hardly modified, Gpx1 decreased and SOD2 increased significantly by fructose ingestion in male progeny independently of maternal intake. On the other hand, while oxidised glutathione content tended to be diminished in fructose-fed males (data not shown), GSH levels were augmented by fructose intake except in males from fructose-fed mothers (Table 4), in accordance with the

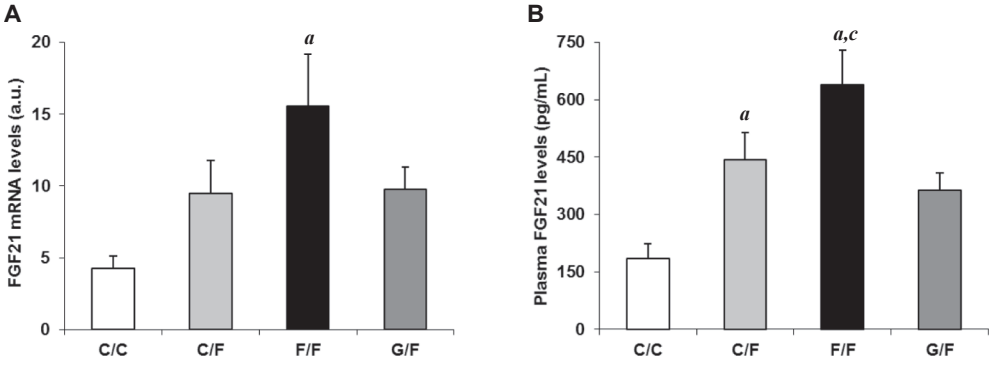

Fig. 1. Liquid fructose in gestation exacerbates fructose-induced FGF21 production in adult male progeny. (A) Hepatic gene expression of FGF21 and (B) plasma FGF21 of fructose-fed male adult progeny from control (C/F, light grey bar), fructose- (F/F, black bar), and glucose-supplemented (G/F, dark grey bar) mothers. Relative FGF21 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.). C/C: Control male 261-day-old offspring from control pregnant rats (empty bar, C/C). Data are means ± S.E. from 5 to 6 litters. *a*: at least $P < 0.05$ versus C/C; *b*: at least $P < 0.05$ versus C/F; *c*: at least $P < 0.05$ versus G/F.

Table 3
Liver weight and hepatic analytes of 261-day-old fructose-fed male progeny from fructose-fed (F/F) or glucose-supplemented (G/F) and control mothers (C/F).

	C/C	C/F	F/F	G/F
Liver weight (g)	17.5 ± 0.6	20.2 ± 0.9	18.0 ± 0.8	17.5 ± 1.7
Liver weight (g/kg BW)	30.2 ± 0.6	33.4 ± 0.7[a,c]	31.3 ± 1.0	30.1 ± 0.8
Total lipids (mg/g of tissue)	26.6 ± 1.4	25.2 ± 1.0[c]	24.4 ± 1.0[c]	30.1 ± 1.1
Triglycerides (mg/mg of protein)	0.107 ± 0.015	0.112 ± 0.002	0.102 ± 0.007	0.136 ± 0.016
Total cholesterol (mg/g of protein)	8.7 ± 0.6	9.1 ± 0.2	8.6 ± 0.3[c]	10.1 ± 0.3
Glucose (mg/mg of protein)	0.42 ± 0.04	0.24 ± 0.02[a]	0.24 ± 0.01[a]	0.30 ± 0.01
Lactate (mg/mg of protein)	0.49 ± 0.04	0.80 ± 0.08[a]	0.76 ± 0.06[a]	0.63 ± 0.03
Glycogen (mg/g of protein)	198.7 ± 32.0	292.8 ± 26.8[a]	280.9 ± 17.6	242.9 ± 7.5

Data are M ± SE, from five to six litters. C/C: Control male offspring of control dams. BW: Body weight.
[b]: at least $P < 0.05$ versus C/F.
[a] : at least $P < 0.05$ versus C/C.
[c] : at least $P < 0.05$ versus G/F.

findings observed in oxidative stress markers (Fig. 2).

Adipose tissue has been proposed as an important FGF21-target tissue (Kim & Lee, 2014; Luo & McKeehan, 2013). Therefore, we analysed lipid content in two locations of adipose tissue (lumbar and epididymal) and oxidative stress markers and antioxidant enzymes activity in lumbar adipose tissue of fructose-fed male rats.

As shown in Table 5, white adipose tissue (WAT) weight tended to be higher in F/F group both in lumbar and epididymal locations. However, there was no difference in brown adipose tissue (BAT) weight neither between the three groups of fructose-fed males nor in comparison to control rats (C/C) (Table 5). Since total lipids content in these two adipose tissues did not show significant differences between the four groups (Table 5), the total amount of adipose lipids turned out to be higher in F/F group both in lumbar (5.12 ± 0.56; 5.04 ± 0.71; 7.76 ± 1.77; 6.80 ± 1.24 g per organ, for C/C, C/F, F/F, and G/F, respectively) and epididymal (4.06 ± 0.38; 5.00 ± 0.25; 6.03 ± 0.73; 5.41 ± 0.79 g per organ, for C/C, C/F, F/F, and G/F, respectively) pads, without becoming significantly different.

The effects of FGF21 on adipose tissue lipolysis are complex. In fact, a role of FGF21 in controlling the futile cycle called triglyceride/fatty acid cycle has been proposed to explain why FGF21 has alternately been reported to stimulate or to repress lipolysis in white adipose tissue (Dutchak et al., 2012). Whereas FGF21 induces triglyceride hydrolysis and mobilization, it also induces genes that are involved in NEFA reuptake (mediated by fatty acid transporters such as CD36) (Schlein et al., 2016) and re-esterification with glycerol (mediated by the key gene of glyceroneogenesis, PEPCK) (Dutchak et al., 2012). Moreover, FGF21 has been shown to reduce the expression of lipogenesis-related genes (such as ATP citrate lyase) (Geller et al., 2019). Curiously, in the present study, although FGF21 was augmented in fructose-fed male rats, both lumbar adipose tissue lipolysis (measured as plasma NEFA levels, Table 2), and NEFA reuptake and re-esterification (Table 5) were augmented in G/F and C/F groups, being more pronounced in C/F males. However, lipogenesis was significantly increased only in F/F group in comparison to the control (C/C) and C/F groups (Table 5).

Regarding oxidative stress markers, adipose MDA content tended to be diminished by fructose intake in C/F and G/F groups and the opposite in F/F group. As shown in Fig. 3A, MDA values were significantly higher in F/F in comparison to the other two groups that received fructose when adults. However, protein carbonyls content was similarly induced by fructose intake in the three groups, becoming significantly different versus control rats (C/C) for F/F and G/F groups (Fig. 3B). To further investigate the mechanism behind this pro-oxidant effect of fructose, mainly observed in males from fructose-fed mothers, we determined several adipose components of the antioxidant system. Thus, as shown in Fig. 3C, whereas catalase activity was decreased by fructose intake in C/F and F/F groups, Gpx1 activity was significantly decreased in the three groups that ingested fructose in comparison to control animals (C/C) (Fig. 3D). Interestingly, GR and SOD2 activities showed similar profiles in response to fructose intake and in accordance to the values found for peroxidation of lipids (MDA, Fig. 3A). Thus, whereas liquid fructose promoted GR and SOD2 activities in males from control and glucose-fed mothers, these enzyme activities were not modified by fructose intake in males from fructose-fed mothers (F/F), maintaining the values observed in the control rats (C/C) (Fig. 3E and F). These findings reinforced the idea that maternal fructose-intake does affect fructose-induced effects on lipid accumulation and stress oxidative state both in liver and adipose tissue and is possibly a FGF21-mediated effect.

4. Discussion

In a previous study (Rodríguez et al., 2013), we reported an impaired hepatic transduction of the leptin signal and hepatic steatosis in fetuses from fructose-fed pregnant rats. Later, we demonstrated that these metabolic disturbances were responsible for a developmental programming of the progeny. We found that the maternal intake of a fructose-, but not glucose-, containing diet provoked hyperinsulinemia and impaired insulin signaling in male progeny (Rodríguez et al., 2015; Rodríguez et al., 2016a). Thus, in the 240-day-old male progeny used in the present study and before being subjected to the fructose-feeding

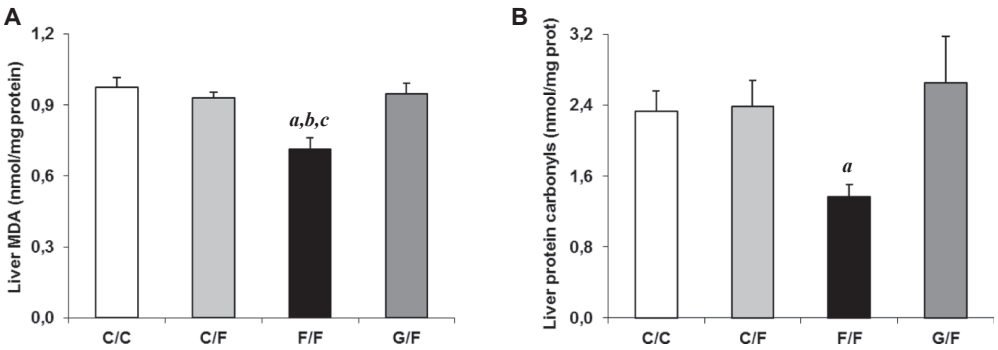

Fig. 2. Fructose-induced plasma FGF21 avoids hepatic oxidative stress in fructose-fed male adult progeny. (A) Hepatic MDA values and (B) liver protein carbonylation of fructose-fed male adult progeny from control (C/F, light grey bar), fructose- (F/F, black bar), and glucose-supplemented (G/F, dark grey bar) mothers. C/C: Control 261-day-old male offspring from control pregnant rats (empty bar, C/C). Data are means ± S.E. from 5 to 6 litters. *a*: at least $P < 0.05$ versus C/C; *b*: at least $P < 0.05$ versus C/F; *c*: at least $P < 0.05$ versus G/F.

Table 4
Liver antioxidant system in 261-day-old fructose-fed male progeny from fructose-fed (F/F) or glucose-supplemented (G/F) and control mothers (C/F).

	C/C	C/F	F/F	G/F
Catalase (mU/mg protein)	914.4 ± 59.7	862.0 ± 37.5	956.6 ± 43.0	974.1 ± 51.0
Gpx1 activity (U/mg protein)	3.97 ± 0.25	2.63 ± 0.19[a]	2.54 ± 0.08[a]	3.02 ± 0.16[a]
GR activity (mU/mg protein)	83.1 ± 4.1	73.6 ± 5.5	68.1 ± 2.1	69.9 ± 3.7
SOD1 activity (U/mg protein)	1.93 ± 0.42	1.87 ± 0.19	2.06 ± 0.20	1.83 ± 0.16
SOD2 activity (U/mg protein)	8.30 ± 0.18	11.74 ± 0.23[a]	11.05 ± 0.16[a]	11.65 ± 0.28[a]
GSH (μM/g tissue)	8.28 ± 0.33	9.17 ± 0.21	7.46 ± 0.21[b,c]	8.94 ± 0.25

Data are M ± SE, from five to six litters. C/C: Control male offspring of control dams.
[a] : at least $P < 0.05$ versus C/C.
[b] : at least $P < 0.05$ versus C/F.
[c] : at least $P < 0.05$ versus G/F.

period, those from fructose-fed mothers showed higher plasma insulin values (Table 1), although glycemia was similar independently of whether they were born from control, fructose- or glucose-mothers.

Therefore, bearing these metabolic disturbances in male progeny born from fructose-supplemented mothers in mind and in order to discover if this phenotype was conserved or aggravated by a short liquid fructose-feeding period (3 weeks) in these animals, we subjected male progeny from control, fructose- and glucose-fed mothers to a fructose liquid solution and determined if the maternal fructose intake influences the adult offspring's response to fructose.

Experimental and clinical studies have shown various adverse metabolic effects of high fructose consumption (Vilà et al., 2011; Rodríguez et al., 2013; Tappy & Lê, 2010; Johnson et al., 2007; de Koning et al., 2012; Brinton, 2016). The major effects of elevated fructose intake are, among others, obesity, insulin resistance, induced lipogenesis, increased levels of plasma triglycerides, fatty liver and, even, hepatic inflammation (Brinton, 2016). In the present study, male adult rats consumed around 25% of the amount of total calories from fructose, in accordance with a previous study (Vilà et al., 2011), and it is similar to the daily energy intake observed in heavy consumers of sweetened beverages in human populations (20–25%) (Rodrigo et al., 2016). Due to this augmented calorie consumption from simple sugar and, although total energy intake was similar between fructose-fed groups and the control (C/C), a clear body weight increase was observed in males consuming fructose in comparison to the control group, independently of which diet had been consumed by their mothers during pregnancy. Interestingly, although insulinemia was augmented in adult males from fructose-fed mothers after receiving fructose, the insulin sensitivity was not affected in comparison to the other groups. In fact, hepatic lipogenic genes tended to be augmented in fructose-supplemented males coming from fructose-fed mothers. Curiously, however, a hyperlipidemic effect of fructose intake was observed in males from control and glucose-fed mothers, but not in males from fructose-fed mothers.

Coincident with previous experimental and clinical studies (Fisher et al., 2016; Dushay et al., 2015), fructose consumption stimulated FGF21 hepatic expression increasing circulating FGF21 levels in adult males of the three groups. Surprisingly, the higher value was found in males from fructose-supplemented mothers, followed by males from control mothers. Thus, it has been demonstrated for the first time that fructose-induced FGF21 production is influenced by maternal intake. Increasing evidence indicates that FGF21 has beneficial effects on glucose and fat metabolism and thus, this higher FGF21 plasma level observed in F/F group could be related to the proposed protective role of FGF21 in situations where disturbances in lipids and carbohydrates are present (Chukijrungroat et al., 2017; Desai et al., 2017; Xu et al., 2009; Chavez et al., 2009; Gómez-Sámano et al., 2017; Tanaka et al., 2015). In fact, a fructose-induced accretion of hepatic lipids was observed only in males from glucose-fed mothers. Moreover, serum alanine aminotransferase (ALT), a liver function parameter that has been demonstrated to be reduced by FGF21 (Wang et al., 2015), was clearly augmented in G/F males in comparison to the other three groups (80.8 ± 9.1; 72.3 ± 8.4; 68.4 ± 4.4; 126.4 ± 20.0 U/L, for C/C, C/F, F/F, and G/F, respectively; $P < 0.05$, G/F versus C/F and F/F groups). Unexpectedly, fructose consumption in males from control and fructose-fed mothers provoked an accumulation of glycogen along with a diminution in hepatic glucose that parallel glycemia, and these effects could be mediated by FGF21. It has been demonstrated that FGF21 reduces hepatic glucose output by increasing glycogen levels and inhibiting gluconeogenesis (Liu et al., 2019), and this would be in

Table 5
Adipose tissues weight and analytes and lumbar adipose tissue expression of 261-day-old fructose-fed male progeny from fructose-fed (F/F) or glucose-supplemented (G/F) and control mothers (C/F).

	C/C	C/F	F/F	G/F
Lumbar adipose tissue weight (g)	6.22 ± 0.60	6.06 ± 0.68	9.10 ± 2.13	7.62 ± 1.19
Lumbar adipose tissue weight (g/kg BW)	10.7 ± 0.9	9.9 ± 0.9	15.0 ± 2.8	12.8 ± 1.1
Total lipids (mg/g of tissue)	820.4 ± 24.1	842.2 ± 24.8	855.5 ± 22.2	872.5 ± 38.2
Epididymal adipose tissue weight (g)	7.11 ± 1.01	7.26 ± 0.97	8.90 ± 1.12	7.40 ± 0.60
Epididymal adipose tissue weight (g/kg BW)	12.1 ± 1.4	11.8 ± 1.2	15.1 ± 1.3	12.9 ± 0.9
Total lipids (mg/g of tissue)	662.7 ± 17.2	721.1 ± 24.0	693.7 ± 50.0	726.9 ± 73.1
Brown adipose tissue weight (g)	1.17 ± 0.06	0.98 ± 0.03	1.04 ± 0.12	0.93 ± 0.07
Brown adipose tissue weight (g/kg BW)	2.0 ± 0,1	1.6 ± 0.0	1.8 ± 0.2	1.6 ± 0.0
Lumbar adipose tissue mRNA expression (a.u.)				
PEPCK	0.12 ± 0.05	0.28 ± 0.07	0.07 ± 0.01	0.14 ± 0.08
CD36	0.20 ± 0.04	0.44 ± 0.13	0.06 ± 0.02[b]	0.11 ± 0.04
ATP citrate lyase	0.23 ± 0.03	0.27 ± 0.09	0.66 ± 0.14[a,b]	0.41 ± 0.09

Data are M ± SE, from five to six litters. C/C: Control male offspring of control dams. BW: Body weight.
[c]: at least $P < 0.05$ versus G/F.
[a] : at least $P < 0.05$ versus C/C;
[b] : at least $P < 0.05$ versus C/F;

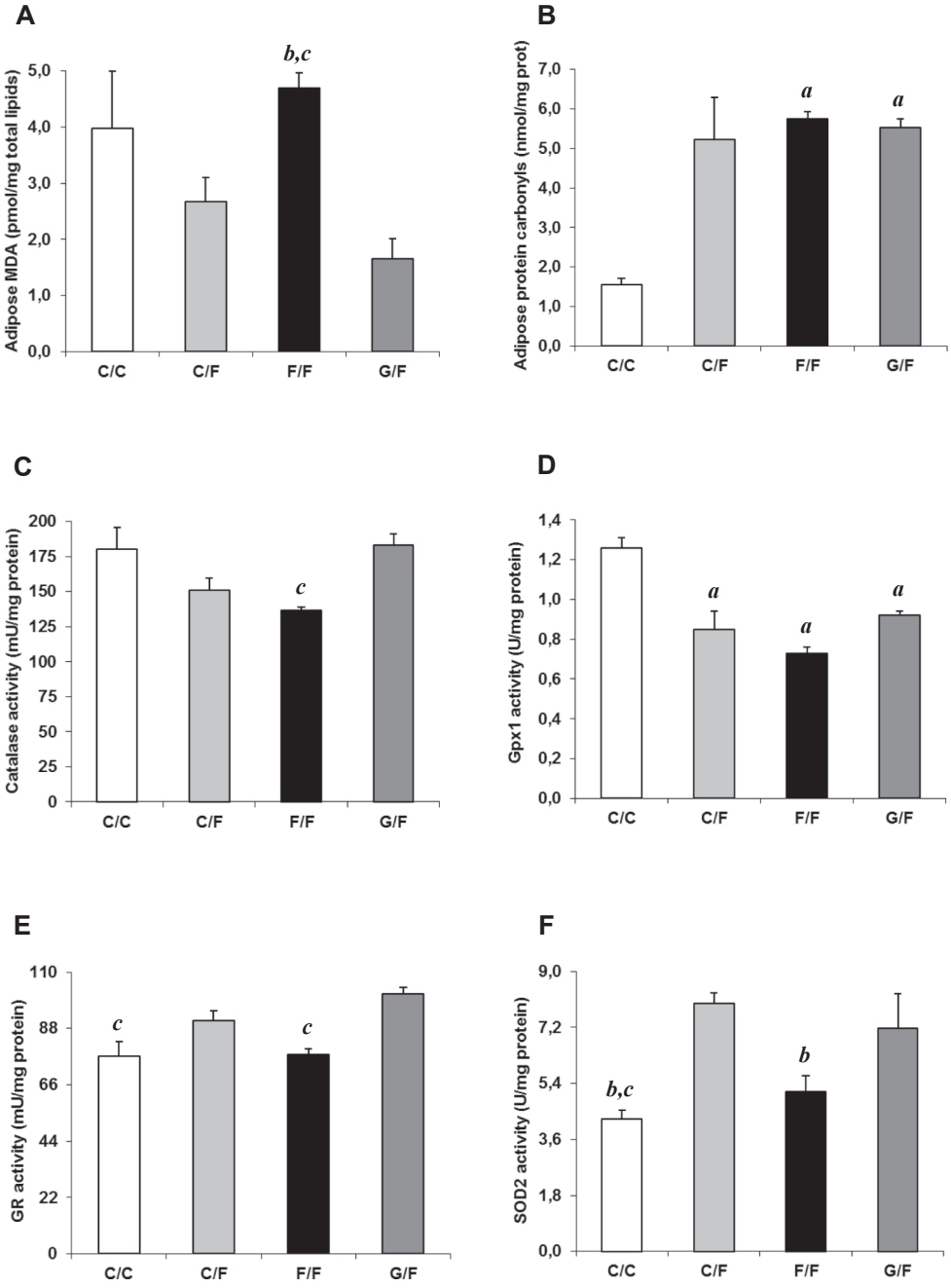

Fig. 3. Fructose-induced plasma FGF21 ameliorates oxidative stress in white adipose tissue in fructose-fed male adult progeny from control and glucose-fed, but not fructose-supplemented, pregnant rats. (A) Lumbar adipose MDA values, (B) protein carbonylation, and antioxidant enzyme activities of (C) catalase, (D) Gpx1, (E) GR, and (F) SOD2 of fructose-fed male 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 male offspring from control pregnant rats (empty bar, C/C). Data are means ± S.E. from 5 to 6 litters. *a*: at least $P < 0.05$ versus C/C; *b*: at least $P < 0.05$ versus C/F; *c*: at least $P < 0.05$ versus G/F. SOD: superoxide dismutase; Gpx1: glutathione peroxidase; GR: glutathione reductase.

consonance to the findings here observed in C/F and F/F males for PEPCK gene expression and hepatic lactate content.

Curiously, although fructose intake has been demonstrated to promote oxidative stress (Rodrigo et al., 2016; Castro et al., 2011; Francini et al., 2010; Rebolledo et al., 2008), fructose-fed males showed neither increase in hepatic lipid peroxidation (measured as MDA levels) nor in protein oxidation (determined as protein carbonylation) and, moreover, a significant decrease was observed in males from fructose-fed mothers. Recent findings suggest that increments in FGF21 protect tissues from an increased oxidative stress environment (Gómez-Sámano et al., 2017; Planavila et al., 2015; Yu et al., 2015), therefore, a role for FGF21 in fructose-fed males should not be discarded. Furthermore, SOD2 has been proposed as a FGF21 target gene (Planavila et al., 2015; Gómez-Sámano et al., 2017) and, although other enzyme activities of the antioxidant system were not changed (catalase, SOD1, GR) or even reduced (Gpx1) in fructose-fed males, SOD2 was clearly induced in the three groups. Interestingly, whereas GSSG content was diminished in the three groups, in accordance with the lack of regeneration of glutathione due to a decreased Gpx1 activity in fructose-fed males, reduced glutathione content (GSH) was specifically lower in F/F group, reflecting a higher utilization of this important antioxidant molecule and possibly explaining the findings observed in MDA and carbonyls levels for this group.

Adipose tissue seems to be another important FGF21-target tissue (Kim & Lee, 2014; Luo & McKeehan, 2013; Kliewer & Mangelsdorf, 2019; Gómez-Sámano et al., 2017) and acting on this tissue, FGF21 contributes to correct plasma triglyceride and glucose levels, insulin sensitivity and adiposity. Fructose ingestion, in contrast, has been related to excess intra-peritoneal and visceral adipose tissue (Brinton, 2016). In the present study, fructose-induced adiposity was observed in lumbar adipose tissue (for F/F and, to a less extent, G/F groups) and epididymal fat (for F/F group). Since F/F group showed the highest value of FGF21, this higher adiposity possibly indicates a lack of response of white adipose tissue to this hormone. In accordance with this,

lipid accumulation and oxidative stress markers in adipose tissue of F/F group also suggest this possible FGF21 resistance. Thus, lipolysis, CD36 and PEPCK (pathway/genes positively-regulated by FGF21) (Dutchak et al., 2012; Schlein et al., 2016) were augmented in C/F and G/F groups, but not in F/F males, and the opposite effect was observed for ATP citrate lyase (a negatively-regulated gene by FGF21) (Geller et al., 2019). Moreover, lipid peroxidation was diminished in fructose-fed males from control and glucose-fed mothers, but not in males from fructose-fed mothers, and this situation could be related to the enzyme activities measured for GR and, principally, SOD2 (a known FGF21 target gene), which were elevated in fructose-fed males, except for those from fructose-fed mothers (F/F).

Interestingly, we and others (Rodrigo et al., 2016; Furukawa et al., 2004) have demonstrated that plasma levels of lipid peroxidation resemble the lipid peroxidation levels in white adipose tissue but not in the liver. And, this is the situation in the present study, the low content of lipid peroxidation products found in lumbar adipose tissue of C/F and G/F groups (Fig. 3A) mirrored that found in plasma (65.5 ± 3.9; 46.9 ± 9.2; 75.5 ± 21.9; 36.7 ± 4.3 µmol MDA/mmol NEFA, for C/C, C/F, F/F, and G/F, respectively; $P < 0.05$, G/F versus C/C groups).

Altogether, these findings indicate that maternal intake does affect the function of fructose-induced FGF21 on glucose and lipid metabolism in the progeny. Thus, in males from control mothers (C/F), FGF21 seems to protect liver and adipose tissue against effects induced by fructose ingestion in terms of lipid accumulation and oxidative stress. However, in males from glucose-mothers (G/F), FGF21 is unable to avoid fructose-induced hepatic steatosis and, in males from fructose-fed mothers (F/F), FGF21 was unable to block adiposity and adipose oxidative stress promoted by fructose intake.

FGF21 expression and serum levels are increased by a variety of metabolic stressors including exercise, cold, fasting and nutrient overload. In some cases, this increase in FGF21 is beneficial and represents an adaptive metabolic response (Gimeno & Moller, 2014; Kim & Lee, 2014). And possibly, this would be the situation found here in males from control mothers (C/F) when exposed to a fructose overload. However, elevations of FGF21 levels have been observed in various human metabolic disorders (Gómez-Sámano et al., 2017; Chavez et al., 2009) as well as in obese and diabetic mice where FGF21 signaling is impaired in the liver and white adipose tissue (Fisher et al., 2010), suggesting that some kind of FGF21 resistance exists (Kim & Lee, 2014). Thus, the fundamental role of the endogenous FGF21 induced by obesity and other disturbances remains controversial. This could be the situation observed in the present study in males from glucose- and fructose-fed mothers (G/F and F/F) when subjected to a fructose overload.

FGF21 has recently emerged as a promising therapeutic agent for the treatment of obesity and insulin resistance. The administration of exogenous FGF21 leads to improvements in obesity-related metabolic deterioration and thus, the metabolically beneficial effects of exogenous FGF21 seem to be irrefutable (Kim & Lee, 2014). Nevertheless, some studies have demonstrated reduced responses to acute FGF21 treatments under obese diabetic conditions in various tissues, including liver, adipose tissue and pancreatic islets (So & Leung, 2016). Therefore, the findings in the present study suggest caution in using FGF21 and analogs as therapeutic agents and ensure the need for further studies to better define the role and mechanistic action of FGF21 in different physiological and pathological situations.

5. Conclusions

Finally, though the implication of FGF21 in the effects here reported needs to be confirmed by more direct studies (for example, by using FGF21 knockout mice from fructose-fed mothers), the most prominent result found here is that maternal carbohydrate intake determines production and function of fructose-induced FGF21 in adult male offspring. The common and worldwide frequent ingestion of fructose containing beverages by young adults, including reproductively active individuals, reinforces the notable clinical relevance of the observations obtained in the present study. Therefore, a reduction in the consumption of fructose-sweetened beverages should be recommended.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Jose M. Garrido and his team for their help in handling the rats, and Brian Crilly for his editorial help. This work was supported by a grant from Ministerio de Ciencia, Innovación y Universidades (MICINN) (SAF2017-89537-R), and the European Community FEDER funds. Silvia Rodrigo was supported with a FUSP-CEU fellowship. Elena Fauste is supported with a FPU fellowship from MICINN.

C.B. conceived and designed the study. S.R., E.F., C.D., P.O. and M.I.P. contributed reagents/materials/analysis tools for gene expression studies and parameter analysis. A.G., C.Ba., M.I.P., P.O. and S.R carried out the analysis for oxidative stress studies L.R. handled the animals. M.I.P. and J.J.A-M analyzed the data. C.B. wrote the paper. None of the authors have any conflicts of interest to report.

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