Middle Iron-Enriched Fructose Diet on Gestational Diabetes Risk and on Oxidative Stress in Offspring Rats.

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Abstract Gestational diabetes mellitus (GDM) is associated with increased insulin resistance and a heightened level of oxidative stress (OS). Additionally, high iron consumption could also increase insulin resistance and OS, which could aggravate GDM risk. The aim of this study is to evaluate a high fructose diet (F) as an alternative experimental model of GDM on rats. We also have evaluated the worst effect of a fructose iron-enriched diet (FI) on glucose tolerance and OS status during pregnancy. Anthropometric parameters, plasma glucose levels, insulin, and lipid profile were assessed after delivery in rats fed an F diet. The effects observed in mothers (hyperglycemia, and hyperlipidemia) and on pups (macrosomia and hypoglycemia) are similar to those observed in women with GDM. Therefore, the fructose diet could be proposed as an experimental model of GDM. In this way, we can compare the effect of an iron-enriched diet on the metabolic and redox status of mother rats and their pups. The mothers' glycemic was similar in the F and FI groups, whereas the glycemic was significantly different in the newborn. In rat pups born to mothers fed on an FI diet, the activities of the antioxidant enzyme glutathione peroxidase (GPx) and glutathione-S-transferase in livers and GPx in brains were altered and the gender analysis showed significant differences. Thus, alterations in the glycemic and redox status in newborns suggest that fetuses are more sensitive than their mothers to the effect of an iron-enriched diet in the case of GDM pregnancy. This study proposed a novel experimental model for GDM and provided insights on the effect of a moderate iron intake in adding to the risk of glucose disorder and oxidative damage on newborns.

Keywords Gestational diabetes · Oxidative stress · High fructose diet · Iron

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Introduction

In the field of obstetrics, gestational diabetes mellitus (GDM) is one of most common complications of pregnancy affecting up to 14 % of all pregnancies, depending on the population studied and the diagnostic tests employed [1]. Since GDM is a cause of concern due to increased risks on both mother (e.g., hypertension, preeclampsia, cesarean delivery, and diabetes later in life) and fetus (macrosomia, neonatal hypoglycemia, shoulder dystocia), there is great interest in understanding the etiology and pathophysiological mechanisms of GDM. As the majority of cases return to normal glycemic levels postpartum, GDM has been considered a "transient condition." However, evidence suggests that GDM should be viewed more as a marker for chronic disease as mothers age [2], but it could also predict occurrence of diseases later in life for the newborn [3]. Despite the better diagnosis of GDM and recognition of its adverse consequences for mother and baby in many countries, there is still no consensus regarding the origin of GDM [4]. It is well known that this risk increases with advancing maternal

age, racial/ethnic disparities, and obesity, but other factors might also be involved [4, 5].

It is well documented that GDM is associated with oxidative stress (OS), owing to both overproduction of free radicals and/or a defect in the antioxidant defenses [6–9]. Multiple biochemical pathways and mechanisms of action for glucose toxicity have been suggested [10]; all these pathways have in common the formation of reactive oxygen species (ROS), and they relate to insulin resistance [11].

So far, streptozotocin, an agent of choice for experimental diabetes induction, leading to specific necrosis of the pancreatic β-cells [12], has been extensively [13, 14] used to clarify or to prevent [15] the biochemical mechanisms of GDM. However, this experimental model leads to type 1 diabetes, while the features of GDM are more like a type 2 diabetes (DT2). Therefore, streptozotocin is probably not a good model for GDM [13, 14]. A fructose-rich diet has been used as an experimental model for the study of insulin resistance [16, 17] but, so far, not in pregnancy.

Recent studies suggest that iron overload may impair the regulation of body glucose metabolism [18]. A meta-analysis concluded that high iron intake is significantly associated with a greater risk of type 2 diabetes [19]. It is still not clear whether iron leads to the development of GDM, and despite the association between iron intake and GDM risk being examined in several studies [20–24], so far no consensus has been reached [25]. Iron is an essential trace element required for crucial functions of the body, such as oxygen transport and energy production. However, a high iron level increases ROS production, which may cause pancreatic β-cell dysfunction [26], and insulin resistance and gestational diabetes have been associated with high plasma ferritin and biological evidence of oxidative stress [27].

This study had a dual purpose: to propose the fructose diet as an experimental model for studying GDM and to determine the effects of a moderate iron-enriched fructose diet on the metabolic and OS status of newborns.

Material and Methods

Animal Care

All experimental procedure was reviewed and approved by the Joseph Fourier University Institutional Ethic Committee for Animal Experiment. The rats were maintained and handled in agreement with the Guide for the Care and Use of Laboratory Animals. The female Wistar rats (Charles River, L'Arbresle, France), 12 weeks old, were housed in wire-bottomed cages in a temperature-controlled room (22 °C), 50 ± 10 % relative humidity, and a 12-h light/12-h dark cycle.

Diets

The diets were purchased from SAFE, 89290 Augis, France. The control group (C, $n = 6$) was fed by a standard Purina chow. The fructose group received the fructose-rich diet (F, $n = 6$) containing 65 % of fructose and 12 mg iron/100 g as indicated in Table 1. The fructose iron-enriched diet received the same fructose-rich diet but containing 22 mg iron/100 g diet (FI, $n = 6$) (Table 1). All rats were fed for 4 weeks before mating and during gestation for 3 weeks. The analytical measurement of the iron content of the pellets for the F and FI diet was determined using a quadrupole ICP-MS Thermo X-Series II equipped with collision/reaction cell technology (CCT), quartz impact bead spray chamber, and concentric nebulizer. The Xt interface option was used. The collision reaction gas was a mixture of He and H2 (97/7). The samples were mineralized in nitric acid and then diluted 100-fold in water prior to analysis. Fe 56 and Fe 54 were measured and Ga 71 was used as internal standard. An external standard calibration curve was generated using four calibration standards (0–200–1000 and 2000 nmol/l). Method accuracy was assessed by analyzing NIST standard reference material 1577b (bovine liver) and ARC/CL total diet reference material at the beginning and end of the analytical run. The between-run precision was 3.40 % and the bias was −1.67 %.

Table 1 Composition of the diets (g/100 g diet)

Composition	Purina chow (group C)	Fructose rich diet (group F)	Fructose iron diet (group FI)
Starch	62	0	0
Fructose	0	65	65
Casein	22.7	20	20
Vegetal oils	4.5	5	5
Mineral and vitamins	6,25	6,25	6,25
Iron mg/100 g diet	10	12	22
Cellulose	4,50	5	5
kCal/100 g diet	379	385	385

Experimental Procedures

Eighteen rats are weighed weekly and pups were weighed at delivery. One day after delivery and after overnight fasting, the mothers were anesthetized with sodium pentobarbital intraperitoneally. Some of the mothers were unfertilized which is common figure in animal facilities; therefore, the number of mother with pups was for each group: C ($n = 4$), F($n = 5$), FI ($n = 5$). Blood from mothers was collected by heart puncture in heparinized tubes protected from light and centrifuged at room temperature for 10 min at 3000*g*. Plasma was immediately isolated, aliquoted, and stored at −80 °C until analysis. The morning of sacrifice, males and females pups were weighed and decapitated without anesthesia. Blood glucose readings are taken via a drop of blood using a glucometer. Immediately after blood collection, the rats were sacrificed and visceral masses were weighed. Pups' livers and brains were removed, weighed, frozen in liquid nitrogen, and stored at −80 °C until analysis. Before analysis, tissue samples were homogeneized (10 %) in buffer (10 mM Tris-base, 1 mM diethylene triamine pentaacetic acid (DPTA)), 1 mM phenylmethanesulfonyl fluoride (PMSF), pH = 7.4) and centrifuged at 3000*g* and 4 °C for 10 min.

Biological Parameters

Fasting glucose, cholesterol, and triglyceride levels were evaluated by enzymatic and colorimetric methods on Roche/Hitachi modular 912 (Roche diagnosis, Meylan, France). Glycemia from pups were assessed using an Accu-Chek® glucometer (Roche Diabetes Care, Meylan, France). Insulin levels were assessed using commercial radioimmunoassay kit (Merck Millipore Corporation, Germany). Insulin sensitivity was calculated using the homeostatic model assessment-insulin resistance (HOMA-IR) (formula: fasting glucose (mg/dL) × fasting insulin μUI/mL/405). Plasma thiobarbituric acid reactive substance (TBARS) concentrations were assessed as described by Richard et al. [28]. Total plasma antioxidant status was estimated using ferric reducing antioxidant power (FRAP) assay as described by Benzie et al. [29]. Plasma thiol (SH) groups were assayed as described by Faure and Lafond [30]. The reduced (GSH) and oxidized (GSSG) form of glutathione was determined by a kinetic method as prescribed by Akerboom and Sies [31]. Glutathione peroxidase (GPx) activity was evaluated by the modified method of Gunzler et al. [32], using terbutyl hydroperoxide as a substrate instead of hydrogen peroxide. The glutathione-S-transferase (GST) activity was determined by the method of Habig et al. [33].

Statistical Analysis

Data statistical analyses were performed using the statistical software package (Statistica Program, Statistical Software, Paris, France). Values were expressed as mean ± standard error of the mean (SEM). Statistical analyses of the data were performed by analysis of variance, using the *t* test for comparison of the means. Statistical significance was set at $p < 0.05$.

Results

Evaluation of a Fructose Diet as a Model of Gestational Diabetes

The results of anthropometric and biochemical parameters of mother rats and their offspring are represented in Table 2. A high-fructose diet increased significantly the weight of their pups compared to the control fed group. The weight gain and the visceral fat mass were enhanced but not significantly different between the two groups of mothers. The livers of pups from the F group were significantly lower than those of the control group, but the brain weight was unaffected by the maternal diet. The results showed that a high-fructose diet increases significantly glycemia, insulin, cholesterol, and triglyceride of mothers, leading to a significant increase in insulin resistance followed by the HOMA test. On the contrary, glycemia of newborns were decreased.

Evaluation of Anthropometric and Oxidative Stress Parameters in Pups and Mothers Fed a Fructose Diet (F) or a Fructose Enriched with an Iron Diet (FI) during Pregnancy

Although a trend to an increase, the FI diet did not modify significantly the body weight gain during pregnancy, compared to the F fed group (Table 3). In addition, the FI diet had no effect on mothers'glycemia, insulinemia, cholesterol, and triglycerides. However, glycemia and body weight were increased in the pups born of the mothers fed the FI diet and the number of females in the FI group was significantly lower. There are gender differences since the glycemia was significantly increased only in male pups ($p < 0.03$) and the difference of body weight was significant for the female ($p < 0.003$) but almost significant ($p = 0.06$) in male pups.

The results of plasmatic OS parameters of mother rats are represented in Table 4. No significant difference was found on GPx activity, TBARS, and FRAP between rats fed with F and FI diet. The oxidative status was also assayed in livers and brains of offspring from the two groups, and results are

Table 2 Anthropometric and biochemical parameters of mother rats and pups after delivery

Parameters	Control	Fructose	*P* value
Weight gain (g)	17.2 ± 0.63	21.5 ± 1.95	*P* > 0.05
Total visceral fat (g/100 g body weight)	3.41 ± 0.53	4.11 ± 0.35	*P* > 0.05
Glycemia (mg/dL)	143 ± 4.14	163 ± 3.60*	0.008
Insulin (ng/mL)	0.58 ± 0.74	1.96 ± 0.80*	0.016
HOMA IR(U)	5.13 ± 0.75	19.46 ± 10.71*	0.014
Cholesterol (mg/dL)	54 ± 0.01	66 ± 0.03*	0.05
Triglyceride (mg/dL)	33 ± 0.05	88 ± 0.20*	0.02
Number of pups/dam	9.5 ± 2.18	10.0 ± 2.28	*P* > 0.05
Male pups/dam	4.0 ± 0.58	3.6 ± 0.81	*P* > 0.05
Female pups/dam	5.5 ± 1.66	6.4 ± 1.69	*P* > 0.05
Weight (g) all	8.5 ± 0.9	9.5 ± 1,7*	0.002
Male pups	8.94 ± 0.26	10.30 ± 0.45*	0.03
Female pups	8.17 ± 0.2	9.13 ± 0.26*	0.01
Liver weight all (g/100 g body weight)	3.54 ± 0.05	3.02 ± 0.04*	<0.001
Male pups	3.58 ± 0.09	2.94 ± 0.07*	<0.001
Female pups	3.51 ± 0.06	3.07 ± 0.05*	<0.001
Brain weight all (g/100 g body weight)	4.14 ± 0.06	4.12 ± 0.06	*P* > 0.05
Male pups	3.98 ± 0.07	3,93 ± 0.1	*P* > 0.05
Female pups	4.25 ± 0.07	4.21 ± 0.07	*P* > 0.05
Glycemia (mg/dL) all	106 ± 2	78 ± 2 *	<0.001
Male pups	112 ± 3	74 ± 3*	< 0.001
Female pups	100 ± 2	81 ± 2*	< 0.001

Results were expressed as mean ± SEM; the number of rats was in the control fed group ($n = 4$ mothers and $n = 38$ pups) and in the fructose fed group ($n = 5$ mothers and $n = 50$ pups). *$P < 0.05$

represented in Tables 5 and 6, respectively. In pups born to mothers in the FI diet group, brain and liver GPx activity was significantly decreased. The gender analysis showed that in the brain, GPx activity was related to a significant decrease in males, while the FI diet has no effect in females. Similarly, we found a gender effect on hepatic GPx, which is decreased in both groups, but the effect was also significant only in females.

Table 3 Anthropometric parameters of mothers and pups fed with an F diet or an FI diet

Parameters	F	FI	*P* value
Mothers weight gain (g)	21.5 ± 1.95	27.5 ± 1.6	*P* > 0.05
Mothers glycemia (mg/dL)	163 24 ± 3.6	161.44 ± 5.76	*P* > 0.05
Mothers insulinemia (ng/mL)	1.96 ± 0.78	1.05 ± 0.67	*P* > 0.05
HOMA IR(U)	19.46 ± 10.71	10.01 ± 6.15	*P* > 0.05
Mothers cholesterol (mg/dL)	66 ± 0.03	64 ± 0.66	*P* > 0.05
Mothers triglyceride (mg/dL)	88 ± 0.20	77 ± 0.17	*P* > 0.05
Number of pups/dam	10,0 ± 2,3	7,6 ± 2,2	*P* > 0.05
Male pups/dam	3.6 ± 0.8	3.8 ± 1.7	*P* > 0.05
Female pups/dam	6.4 ± 1.7	3.8 ± 0.9*	0.05
Pups glycemia (mg/dL) All	78 ± 1.8	85 ± 1.8 *	0.02
Male pups	74.4 ± 2.77	86.25 ± 2.52*	0.003
Female pups	81.13 ± 2.26	83.18 ± 2.64	*P* > 0.05
Pup weight (g) all	9.3 ± 0.10	11.0 ± 0.34*	<0.001
Male pups	10 ± 0.62	11.1 ± 0.16	0.06
Female pups	8.9 ± 0.37	10.9 ± 0.13*	0.003

Results were expressed as mean ± SEM; the number of rats was in fructose group (F: n = mothers and $n = 50$ pups) and in fructose iron (FI: $n = 5$ mothers and $n = 38$); *$P < 0.05$

Table 4 Plasmatic OS parameters in mother rats fed with the F or the FI diet

OS parameters of mothers	F (n = 5)	FI (n = 5)
TBARS (μM/L)	4.6 ± 0.13	4.03 ± 0.14
GPx activity (U/L)	6349 ± 304	6464 ± 110
FRAP (μM/L)	273 ± 20	284 ± 25
SH (μM/L)	327 ± 38	295 ± 35

Results were expressed as mean ± SEM

Even though there was no difference in total hepatic GSH between the two groups, the hepatic GSH concentration was significantly lower in male pups of the FI group but no difference was observed for female pups. The activity of GST was significantly increased in livers of the FI diet group, and the gender analysis showed a significant increase only in males' livers. In the brain, no difference for GSH and GST was observed between groups. There was no significant difference in total antioxidant capacity measured by FRAP assay between the two groups.

Discussion

Diabetes in pregnant women is associated with increased risk of maternal, fetal, and neonatal complications, which makes GDM a significant public health challenge. Aside from non-modulating factors of GDM as the mother ages, inadequate nutritional habits, such as high consumption of fructose and iron, might induce oxidative stress and insulin resistance. Therefore, we aimed to assess the fructose diet as a model of diet-induced GDM and then the effect of an iron-enriched fructose diet in inducing metabolic disorder and oxidative stress.

The Fructose Diet as a Model of GDM

So far, streptozotocin diabetes is the first choice for studying experimental GDM [12] and, to our knowledge, the only model. However, streptozotocin induces specific necrosis of the pancreatic 훽-cells, such as type 1 diabetes, rather than insulin-resistant diabetes, and it is still challenging for inducing GDM with streptozotocin [13, 34]. Thus, we evaluated a fructose-rich diet to feed pregnant rats as an experimental animal model for GDM by inducing insulin resistance. Here, the effect of an F diet to induce insulin resistance assessed with the HOMA score during pregnancy was consistent with that obtained with the same F diet in adult rats [35] and with diabetes induced by streptozotocin [36]. Furthermore, in this experimental model, the weight of newborns was significantly larger in the group of mothers fed with the F diet, and this effect is in agreement with the well-documented increased risk of macrosomia in the GDM women [37]. According to the recent guidelines of the Institutes of Medicine, weight gain associated with GDM increases the risk of miscarriages and other adverse outcomes [38], leading to a need for dietary counseling. While the pups were bigger, their blood glucose

Table 5 Liver OS parameters in newborn rats born to rats fed with F and with FI diet

OS parameters	Group F (n = 50) (18 males; 32 females)	Group FI (n = 38) (19 males;19 females)	P value
TBARS (μM/L)	1.05 ± 0.2	0.99 ± 0.2	$P > 0.05$
Male	1.12 ± 0.10	1.05 ± 0.10	$P > 0.05$
Female	0.95 ± 0.13	0.92 ± 0.19	$P > 0.05$
GPx activity (U/gP)	254 ± 7.0	225 ± 6.6*	0.03
Male	253 ± 15	232 ± 7.9	$P > 0.05$
Female	255 ± 10	217 ± 11*	0.005
GSH (μM/g P)	636 ± 57	569 ± 32	$P > 0.05$
Male	711 ± 73	522 ± 33*	0.03
Female	562 ± 82	625 ± 52	$P > 0.05$
GSH/GSSG	87.79 ± 6.34	88.87 ± 4.15	$P > 0.05$
Male	83.82 ± 30.9	87.63 ± 13	$P > 0.05$
Female	82.44 ± 21.36	91.79 ± 15.46	$P > 0.05$
FRAP (μM/g protein)	210 ± 5.8	197 ± 4.9	$P > 0.05$
Male	217 ± 7.8	201 ± 6.9	$P > 0.05$
Female	202 ± 8.0	193 ± 7.0	$P > 0.05$
GST (nM/mg protein)	232 ± 5.4	249 ± 4.3*	0.015
Male	228 ± 8.5	257 ± 6.9*	0.02
Female	236 ± 7.0	242 ± 4.0	$P > 0.05$

Results were expressed as mean ± SEM; *$P \leq 0.05$

Table 6 Brain OS parameters in newborn rats born to rats fed with F and with FI diet

OS parameters	F (n = 50) (18 males; 32 females)	FI (n = 38) (19 males;19 females)	P value
GPx activity (U/g protein)	104,1 ± 3.8	92,8 ± 3.5*	0.04
Male	113 ± 3.9	91 ± 2.4 *	0.001
Female	95 ± 4.0	95 ± 8.0	$P > 0.05$
GSH (μM/g protein)	273 ± 6.4	256 ± 6.7	$P > 0.05$
Male	259 ± 3.0	250 ± 6.0	$P > 0.05$
Female	301 ± 6.0	266 ± 1.4	$P > 0.05$
GSH/GSSG	76.08 ± 7.13	86.47 ± 11.16	$P > 0.05$
Male	79 56 ± 19.54	88.08 ± 40	$P > 0.05$
Female	70 55 ± 19.53	81.25 ± 24.2	$P > 0.05$
FRAP (μM/g protein)	254 ± 4.0	253 ± 4.1	$P > 0.05$
Male	258 ± 3.3	258 ± 6.5	$P > 0.05$
Female	250 ± 7.3	248 ± 4.8	$P > 0.05$
GST (nM/mg protein)	216 ± 8.3	228 ± 5.6	$P > 0.05$
Male	213 ± 36	230 ± 17	$P > 0.05$
Female	213 ± 27	226 ± 29	$P > 0.05$

Results were expressed as mean ± SEM; *$P \leq 0.05$

level was significantly lower than in the control group. This result is also consistent with the fact that the hypoglycemia associated with macrosomia is one of the most common metabolic disorders of the neonate of a GDM mother [37]. It occurs due to the hyperinsulinemia of the fetus in response to the maternal hyperglycemia in utero. The lower liver weight, observed both in male and female pups, could be explained by a decrease in glycogen storage level in response to a state of hepatic insulin resistance [39]. Taken as a whole, our results suggest that the fructose diet could be an alternative successful experimental model for studying GDM.

The Fructose Diet as a Model of GDM

In the second part of our study, we tested the hypothesis that an increased iron intake would induce metabolic and oxidative disorders during GDM. Iron requirements to meet the fetal pups' needs were estimated at 5–25 mg iron/100 g diet [40]. In our study, the dose of iron in the FI diet (22 mg/100 g diet) corresponded to a moderate, but not too heavy dose, because our goal was to assess the consequences of a high, but not an excessive iron intake, which is rarely the case during pregnancy. Furthermore, our goal was not to confirm the benefit of iron in cases of anemia, but we aimed to evaluate the effect of an iron-rich diet to rats of normal iron status in case of GDM, to increase insulin resistance and OS. Therefore, in the fructose diet, the level of iron was normal. Despite that the FI diet did not induce significant metabolic changes in mothers, the body weights of the newborns were increased and their glycemia was significantly increased. The increase in body weight induced by iron during GDM was more important in female. The number of female progeniture was also smaller in the group of pups born from mothers with a higher iron intake. In a previous study, iron supplementation to pregnant rats was associated to an increased number of placentas without a developing fetus, which could take in part to our result [41]. Since diet gender effects have been reported in offspring born from mothers fed with fructose liquid intake during pregnancy [42, 43], we can hypothesize that the FI diet could play a part in the small number of females in the FI diet group. Further experiments are necessary to confirm that this effect is caused by the FI diet.

The FI diet did not affect the oxidative status of the mothers. A previous study [44] in an experimental model of GDM induced by streptozotocin reported an increase in OS induced by intra-peritoneal iron. However, in this study, both the method and the high dose of iron, in contrast to ours, might explain the discrepancies in oxidative stress.

Interestingly, despite the apparently similar OS level between the mothers fed with the F or the FI diet, the redox status of the livers and brains of newborns was altered. This suggests that fetuses were more sensitive to the effect of iron during pregnancy than their mothers.

The GPx activity was decreased both in the liver and the brain of pups born from the FI-fed mothers. The inhibition of GPx activity has often been reported in cases of obesity and insulin resistance [45]. In line of our results, a difference by sex of hepatic GPx activity has also been reported in pups born to diabetic mothers [46]. The decrease in GPx is a common feature of the route toward DT2 [47] and aging [48] and in neurodegenerative diseases [49]. Interestingly, it has been shown that iron supplementation in neonate mice increases the

risk of Huntington's disease associated with an increased oxidative stress [50] while GPx was neuroprotective in a model of this neurodegenerative disease [51]. Therefore, the GPx decline observed in the brains of the FI males could have important implications for cognitive function. In future studies, it would be interesting to evaluate the consequences of this decrease on their behavior in adulthood.

In our study, males' livers seemed to be more sensitive than those of female to the effect of iron-rich diet. Indeed, the reduced GSH levels of males born to the FI diet-fed mothers served as an iron-induced OS indicator, while in the liver of females, the GSH levels were not modified. In line with this effect, Kim et al. [52] found that iron overload is associated with insulin resistance in men, but not in women. In addition, the GST activity was increased in the liver of pups from the FI group. This would appear to represent an adaptive response to cope with an increased ROS production induced by iron. Indeed, several lines of evidence suggest that GST plays a role in protecting cells from the consequences of such stress, since GST is regulated by ROS such as H_2O_2. The induction of GST by ROS has been described as an adaptive response to detoxify some oxidative metabolites produced by OS [53]. GST is a prototypical phase 2 antioxidant enzyme, which has a part in the detoxification of a broad range of toxic and potentially carcinogenic compounds [53]. In accordance with our results, an enhancement of GST was associated with a higher decrease in GPx in iron-treated mice [54]. Also in agreement with our data, suggesting that male might be more sensitive to female to the diet of mothers during pregnancy, a gender effect has been reported with a 10 % (*w*/*v*) fructose in drinking water during pregnancy, which increased oxidative stress in the liver of male progenitor but not in female [43]. In this study, in relation with an increased oxidative stress in livers of male offspring, a feature of a metabolic syndrome was observed in male while the females were more resistant. However, it is worth noting that the model of fructose in drinking water is not a model of GDM since it is not associated with glucose impairment [55] during pregnancy. Furthermore, in this study the analyses were performed later after delivery (90 days) while at delivery in our study. Therefore, we cannot exclude that postnatal nutrition could modify metabolic abnormalities induced by iron-fed fetal programming associated with GDM. This deserves further investigation to evaluate later in life the consequences of an oxidative stress in the liver of pups born from mothers with a GDM and with high iron intake.

In conclusion, the similarity in the metabolic and anthropometric effects observed in GDM in women and those of our experimental model allows us to propose the fructose diet as an experimental model of GDM. Clearly, these results indicate that a moderately high iron intake, in the case of GDM, induces adverse effects in the pups (macrosomia and impaired redox status both on the liver and the brain) without significant effects on their mothers. Future studies are warranted to clarify the link between these potential characteristics of a metabolic and oxidative altered state and a possible increased risk of chronic diseases later in life. Proper management of GDM, regarding iron intake in particular, would be of benefit for the newborn's health and the prevention of diseases in adulthood.

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Compliance with Ethical Standards All experimental procedure was reviewed and approved by the Joseph Fourier University Institutional Ethic Committee for Animal Experiment. The rats were maintained and handled in agreement with the Guide for the Care and Use of Laboratory Animals.

Conflict of Interest The authors declare that they have no conflict of interest.

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