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Supplementation with polyunsaturated fatty acids in pregnant rats with mild diabetes normalizes placental PPARγ and mTOR signaling in female offspring developing gestational diabetes

Evangelina Capobianco[a], Daiana Fornes[a], Sabrina Lorena Roberti[a], Theresa L. Powell[b,c], Thomas Jansson[c], Alicia Jawerbaum[a,*]

[a]Laboratory of Reproduction and Metabolism, CEFYBO, CONICET, School of Medicine, University of Buenos Aires, Argentina
[b]Section of Neonatology, Department of Pediatrics, University of Colorado Denver Anschutz Medical Campus, Aurora, CO 80045, USA
[c]Division of Reproductive Sciences, Department of OB/GYN, University of Colorado Denver Anschutz Medical Campus, Aurora, CO 80045, USA

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Abstract

Maternal diabetes impairs fetoplacental development and programs metabolic diseases in the offspring. We have previously reported that female offspring of pregnant rats with mild diabetes develop gestational diabetes mellitus (GDM) when they become pregnant. Here, we studied the effects of supplementation with polyunsaturated fatty acids (PUFAs) in pregnant mild diabetic rats (F0) by feeding a 6% safflower-oil-enriched diet from day 1 to 14 followed by a 6% chia-oil-enriched diet from day 14 of pregnancy to term. We analyzed maternal metabolic parameters and placental signaling at term in pregnant offspring (F1). The offspring of both PUFAs-treated and untreated mild diabetic rats developed GDM. Although gestational hyperglycemia was not prevented by dietary PUFAs treatment in F0, triglyceridemia and cholesterolemia in F1 mothers were normalized by F0 PUFAs dietary treatment. In the placenta of F1 GDM rats, PPARγ levels were reduced and lipoperoxidation was increased, changes that were prevented by the maternal diets enriched in PUFAs in the F0 generation. Moreover, fetal overgrowth and placental activation of mTOR signaling pathways were reduced in F1 GDM rats whose mothers were treated with PUFAs diets. These results suggest that F0 PUFAs dietary treatment in pregnancies with mild diabetes improves maternal dyslipidemia, fetal overgrowth and placental signaling in female offspring when they become pregnant. We speculate that an increased PUFAs intake in pregnancies complicated by diabetes may prove effective to ameliorate metabolic programming in the offspring, thereby improving the health of future generations.
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Keywords: Maternal–fetal exchange; Fetal programming; Polyunsaturated fatty acids; Oxidative stress; Pregnancy

1. Introduction

Gestational diabetes mellitus (GDM), a common metabolic disorder in pregnancy, causes maternal, placental and fetal complications and is associated with adverse outcomes such as maternal hypertensive disorders, cesarean section, macrosomia, birth trauma, neonatal respiratory distress, hypoglycemia, polycythemia hyperbilirubinemia and increased infant adiposity [1,2]. In addition, the offspring of GDM mothers are susceptible to develop metabolic and cardiovascular disease later in life, which is believed to reflect intrauterine programming in response to an adverse intrauterine environment [3,4]. Fetal overgrowth, resulting in the delivery of a large-for-gestational-age infant, is common in GDM and constitutes a phenotypic marker of intrauterine programming [5].

We recently developed a novel animal model of GDM involving intrauterine programming in the offspring of neonatal streptozotocin-induced diabetic female rats with mild diabetes (maternal glycemia in F0 generation, 150–250 mg/dl) [6]. The offspring (F1 generation) of mild diabetic rats (F0) have normal fasting blood glucose before pregnancy but develop hyperglycemia and hyperinsulinemia during late gestation, leading to GDM [6]. The phenotype of this rat model of GDM includes increased fetal growth and activation of placental mechanistic target of rapamycin (mTOR) signaling [6].

mTOR is a serine/threonine kinase regulated by cellular nutrient and metabolic status which regulates gene transcription and protein translation to control cellular growth [7]. mTOR is a positive regulator of system A and system L amino acid transport in the placenta which controls transfer of amino acids to the developing fetus [8]. mTOR forms two functionally distinct protein complexes, mTORC1 and mTORC2, in association with raptor and rictor, respectively [7]. P70 S6

Abbreviations: GDM, gestational diabetes mellitus; PPARγ, peroxisome proliferator activated receptor γ; mTOR, mechanistic target of rapamycin; PUFAs, polyunsaturated fatty acids.

* Corresponding author.
E-mail address: a.jawerbaum@gmail.com (A. Jawerbaum).

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kinase 1 (S6K1), ribosomal protein S6 (rpS6) and binding protein 4E-binding protein 1 (4EBP1) are downstream effectors of mTORC1 that positively regulate protein synthesis in association with mTORC1 activation [7]. Phosphorylation of 4EBP1 induces the release of the initiation factor elF4E and activation of protein synthesis [9]. Activation of mTORC2 stimulates amino acid transport mediated by activation of protein kinase Cα (PKCα) and serum and glucocorticoid-inducible kinase 1 (SGK1), direct targets of this signaling pathway [7,10]. Placental mTOR signaling is inhibited in fetal growth restriction both in animal models and in humans [8]. On the other extreme, increased placental mTOR signaling has been reported in obese women delivering large babies and in experimental models of maternal obesity associated with fetal overgrowth [11,12].

An intrauterine pro-oxidant and proinflammatory environment has been proposed to contribute to adverse outcomes and intrauterine programming in pregnancies affected by metabolic diseases [13]. Oxidative stress is a hallmark in pregnancies complicated by diabetes [14], and activation of pro-oxidant/proinflammatory pathways in combination with a reduction in antioxidant/anti-inflammatory signaling has been reported in diabetes in pregnancy both in women and in animal models [14,15].

Peroxisome proliferator activated receptor γ (PPARγ), one of the three known PPARs, is essential for placental development and plays an important role as a regulator of lipid metabolism and anti-inflammatory pathways [16,17]. PPARs are ligand activated transcription factors that heterodimerize with RXR receptors for retinoic acid and, after ligand binding, lead to the release of co-repressors and the recruitment of co-activators that will allow the transcription of multiple target genes, including antioxidant enzymes [16]. In addition, PPARγ activation inhibits the expression of multiple proinflammatory genes [18,19]. Placental PPARγ expression has been reported to be reduced in women with diabetes and in experimental models of both mild maternal diabetes and GDM [6,20–22].

Endogenous ligands for PPARγ are of lipid nature and include 15deoxy$\Delta^{12,14}$prostaglandin J_2, an arachidonic acid derivative, and various monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). PUFAs capable of activating PPARs include those from the n-6 series such as arachidonic acid and linoleic acid and those from the n-3 series such as α-linolenic acid and its derivatives eicosapentaenoic acid and docosahexaenoic acid [19,23]. Both n-3 and n-6 PUFAs are required during pregnancy, n-6 PUFAs are particularly needed during implantation and organogenesis, and n-3 PUFAs are specifically required to sustain the growth and development of the neural system during the third trimester of pregnancy [24,25]. Previous studies have shown that n-3 PUFAs inhibit mTOR signaling in various tissues and cell types [26,27]. Furthermore, n-3 PUFAs inhibit proinflammatory pathways in dams and fetuses in experimental models of diabetes and in adipose tissue and placenta of obese women [28–30].

Given their important antioxidant and anti-inflammatory properties and the essential nature of PUFAs in pregnancy, we hypothesized that PUFA supplementation in mild diabetic pregnant rats (F0) ameliorates metabolic parameters and fetoplacental development in the female offspring that develop GDM. PUFAs of the n-6 series are essential to sustain embryo organogenesis, and PUFAs of the n-3 series are highly needed in the second half of gestation to meet the increased fetal requirements during neural system development and growth at late gestation [24,31]. Therefore, the aim of this work was to treat mild diabetic rats during the F0 pregnancy with a standard diet or a diet enriched in PUFAs (6% safflower oil from day 1 to 14 of pregnancy and 6% chia oil from day 14 to term) and to determine metabolic parameters, fetal and placental weight, as well as placental PPARγ levels, lipoperoxidation and expression of total and phosphorylated proteins of the mTORC1 and mTORC2 signaling pathways in the F1 female in late pregnancy.

2. Materials and methods

2.1. Animals

Albino Wistar rats bred in our animal facility were fed *ad libitum* with commercial rat chow (Asociación Cooperativa Argentina, Buenos Aires, Argentina). A mild diabetes was induced by injecting 2-day-old neonates with streptozotocin (90 mg/kg, s.c, Sigma-Aldrich, St. Louis, MO, USA), diluted in citrate buffer (0.05 M pH 4.5, Sigma-Aldrich), as previously [32]. Controls were injected with citrate buffer alone. The diabetic state was confirmed in 2-month-old rats prior to mating. Rats were considered diabetic when their fasting glycemia values were higher than 130 mg/dl. Glycemia values in the mild diabetic female rats (F0) were 150–250 mg/dl and below 120 mg/dl in controls. This model allows the study of the pregnancy until term and the evaluation of offspring [33]. In this study, 3-month-old adult female offspring were studied on day 21 of pregnancy. The animal protocol was approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAL, Resolution CD No. 1497/2013), School of Medicine, University of Buenos Aires, and conducted according to the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (NIH Publication, Eighth Edition, 2011) http://www.ncbi.nlm.nih.gov/books/NBK54050/?report=reader.

2.2. Treatments and sample collection

F0 control and mild diabetic female adult rats were mated with control adult males. The presence of sperm cells in vaginal smears confirmed the first day of pregnancy. During pregnancy, the control rats were fed with a standard diet (commercial rat chow, Asociación Cooperativa Argentina, Buenos Aires, Argentina), whereas the mild diabetic rats were fed with a standard diet (commercial rat chow, Asociación Cooperativa Argentina) or a standard diet enriched in 6% safflower oil (Flora, Lynden, WA, USA) administered from days 1 to 14 of pregnancy, followed by a standard diet enriched in 6% chia oil (*Salvia hispanica* L seed oil, Sol Azteca, Buenos Aires, Argentina) administered from day 14 to term (PUFA group) (Fig. 1). The composition of the diet, determined by the National Institute of Food of Argentina (INAL, Buenos Aires, Argentina), and the

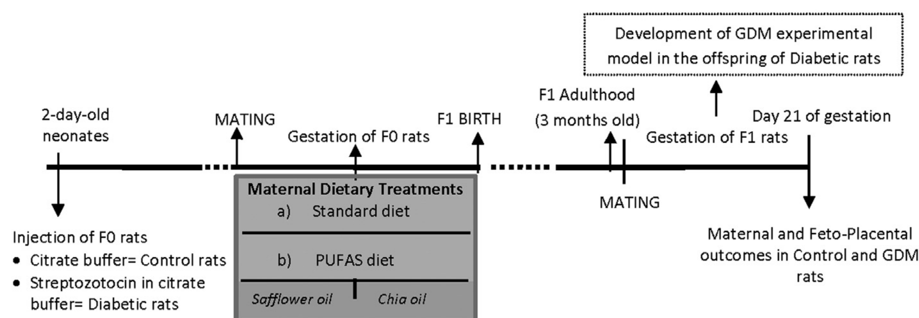


Fig. 1. Experimental design: Mild pregestational diabetes was induced in F0 Wistar rats by neonatal administration of streptozotocin. Control and diabetic rats were mated with control males. During F0 pregnancy, rats were dietary treated with a standard diet or a diet supplemented with 6% safflower oil (days 1 to 14 of gestation) or with 6% chia oil (days 14 of gestation to term). At 3 months of age and after glycemic control, the offspring from control and diabetic rats were mated and studied on day 21 of pregnancy.

Table 1
Diet composition

Diet composition	Standard diet	6% Safflower-oil-supplemented diet	6% Chia-oil-supplemented diet
Carbohydrates (g/100 g)	50	48	48
Proteins (g/100 g)	25	23	24
Lipids (g/100 g)	5	11	11
Calories (kcal/100 g)	324	345	342
Major PUFAs (g/100 g)			
Linoleic acid 18:2 (n-6)	1.99	**6.49**	2.93
Linolenic acid 18:3 (n-3)	0.73	0.55	**5.2**

major PUFAs content, determined by gas chromatography as previously [34], are shown in Table 1. After delivery, all dams received the standard diet. In 3-month-old female offspring (F1 generation), glycemia levels were determined by reactive strips. Subsequently, female F1 offspring were mated with control males and euthanized at day 21 of pregnancy (Fig. 1). Blood was collected and processed for determination of glucose, triglycerides and cholesterol in plasma by spectrophotometric enzymatic assays (Wiener Lab., Rosario, Argentina). Placentas from F1 females (n=8 in each experimental group, each dam from a different F0 mother) were removed and prepared for immunohistochemical analyses of PPARγ levels, frozen at −80°C for further determination of thiobarbituric acid reactive substances (TBARS, an index of lipoperoxidation and marker of oxidative stress) or homogenized in ice-cold buffer D (250 mM sucrose, 10 mM Hepes–Tris, pH 7.4, with protease and phosphatase inhibitors) and stored at −80°C for Western blot analysis of downstream effectors of the mTOR pathway.

2.3. Immunohistochemistry

To perform immunostaining of PPARγ, placentas (n=8 in each experimental group) were paraffinized and serially cut in 5-μm-thick sections. Subsequently, sections were deparaffinized and rehydrated through a graded series of ethanol, and the endogenous peroxidase activity was blocked. The sections were processed using anti-PPARγ primary antibody (rabbit polyclonal antibody, 1:100 dilution, Cayman Chemical Company, Ann Arbor, MI, USA) in a humidified chamber at room temperature overnight and incubated with the biotinylated anti-rabbit secondary antibody (anti-rabbit IgG, dilution 1:200, Vector Laboratories, Burlingame, CA, USA) in a humidified chamber at room temperature for 1 h. Sections were incubated with the Avidin–Biotin Complex (Vectastain, Vector Laboratories) for 1 h, and the presence of PPARγ antibody was detected with 3,3′-diaminobenzidine, as previously described [32]. Control sections were generated by omitting the primary antibody. Two skilled blinded observers evaluated three serial sections per placenta. Immunoreactivity intensity was quantified using the ImageProPlus software. Data are shown as relative to a value of 1, arbitrarily assigned to the control. A semiquantitative score leading to similar results was also performed (data not shown).

2.4. Lipoperoxidation analysis

Lipoperoxidation was assessed by determining the concentration of TBARS, a method widely used to assess peroxidation of fatty acids, as previously described [35]. Briefly, each placenta (n=8 in each experimental group) was homogenized in Tris–HCl buffer (0.1 M, pH: 7.4). The homogenate was mixed with 40% trichloroacetic acid (Merck Darmstadt, Germany). After centrifugation, the supernatant was mixed with an equal volume of thiobarbituric acid (46 mM) (Sigma-Aldrich), and the solution was heated at 95 °C and, after cooling, quantified spectrophotometrically at 540 nm. A range of concentrations of malondialdehyde (Sigma-Aldrich), subjected to the same conditions as the tissue homogenates, was used as standards.

2.5. Western blot analysis

Proteins from placental homogenates (n=7 rats in each experimental group) were separated by SDS-PAGE and transferred to PVDF membranes (35 V constant, overnight at 4°C), as previously described [6]. The membranes were stained with Amido Black staining solution for total proteins (Sigma-Aldrich) to confirm equal loading and transfer. Blocking was carried out for 1 h at room temperature in 5% nonfat milk in TBS-Tween, and membranes were incubated with the primary antibody (diluted in 1% BSA in TBS-Tween) overnight at 4°C. The expression of the following proteins was determined using antibodies from Cell Signaling Technology: total and phosphorylated S6K1, total and phosphorylated rpS6, total and phosphorylated 4EBP-1, total and phosphorylated PKCα, and total and phosphorylated SGK1. After washing, the membranes were incubated with the appropriate peroxidase-conjugated secondary antibody, visualized using ECL detection solution (Thermo Scientific) and captured in a G:BOX gel imaging system (Syngene). Densitometry analysis was performed with ImageJ software. The expression of the target protein in each individual lane was normalized for total protein staining to adjust for unequal loading. The mean of all the samples was calculated, and the expression of the target protein in each sample was calculated as a percentage of that mean (target/total protein density).

2.6. Statistical analysis

Data are presented as the mean±S.E.M. Groups were compared by one-way ANOVA followed by Bonferroni's *post hoc* test to compare all groups to each other. A P value less than .05 was considered statistically significant.

3. Results

3.1. Maternal and fetal metabolic parameters and fetal growth

We have previously reported that the offspring of mild diabetic rats are normoglycemic at 3 months of age but develop GDM when mated with control males [6]. In this work, we found that the 21-day pregnant female offspring of mild diabetic rats were hyperglycemic, independent of whether their mother was treated with PUFAs, indicating that PUFA treatment in pregnancy (F0) did not prevent GDM in the F1 generation (Fig. 2A). GDM F1 rats whose mothers received no PUFA treatment (GDM group) were hypertriglyceridemic when compared to controls (P<.05), and these changes were prevented by the F0 PUFA diet (GDM-PUFA group) (GDM *vs.* GDM-PUFAs, P<.001) (Fig. 2A). Likewise, cholesterol levels were increased in GDM rats when compared to controls (P<.001), and PUFA treatment during pregnancy of the previous generation prevented this elevation in cholesterol (GDM *vs* GDM-PUFAs, P<.01) (Fig. 2A).

We found that fetal glycemia was increased in both the GDM and GDM-PUFA groups when compared to controls (P<.05), although a significant decrease was evident in the GDM-PUFA group compared to GDM (P<.01) (Fig. 2B). Fetal triglycerides did not change in the GDM group compared to controls but decreased in the fetuses from GDM-PUFA dams as compared to the GDM group (GDM *vs.* GDM-PUFAs, P<.05) (Fig. 2B). Fetal cholesterol levels showed no differences in the control, GDM and GDM-PUFA groups (Fig. 2B).

Fetal weights were increased in GDM rats compared to controls (P<.001), and fetal overgrowth was partially prevented in GDM rats whose mothers received the PUFA treatment (GDM *vs.* GDM-PUFAs, P<.05, Fig. 3A). Placental weight showed no differences between the control, GDM and GDM-PUFA groups (Fig. 3B).

3.2. Placental PPARγ and lipoperoxidation

PPARγ is a nuclear receptor essential for placental development and relevant as a placental regulator of antioxidant/anti-inflammatory pathways [17,20]. We found a decrease in placental PPARγ protein levels in GDM rats as compared to controls (P<.001), which was prevented when their diabetic mothers were treated with PUFAs during the F0 pregnancies (GDM *vs.* GDM-PUFAs, P<.05) (Fig. 4A). Lipoperoxidation, evaluated through TBARS concentrations, was increased in the placenta from GDM rats when compared to controls (P<.05), an increase which was prevented in the GDM group whose mothers received the PUFA diet in the F0 pregnancy (GDM *vs.* GDM-PUFAs, P<.05) (Fig. 4B).

3.3. Placental mTOR signaling

We found no differences in total or phosphorylated S6 K1 levels between the control, GDM and GDM-PUFA groups (Fig. 5A), while phosphorylated rpS6 levels were reduced with no changes in total rpS6 levels in the placentas from the GDM-PUFA group when compared to the control and the GDM groups (P<.05, Fig. 5B). Moreover, although no differences were found in phosphorylated 4EBP1 levels between the groups, total 4EBP1 levels were reduced in

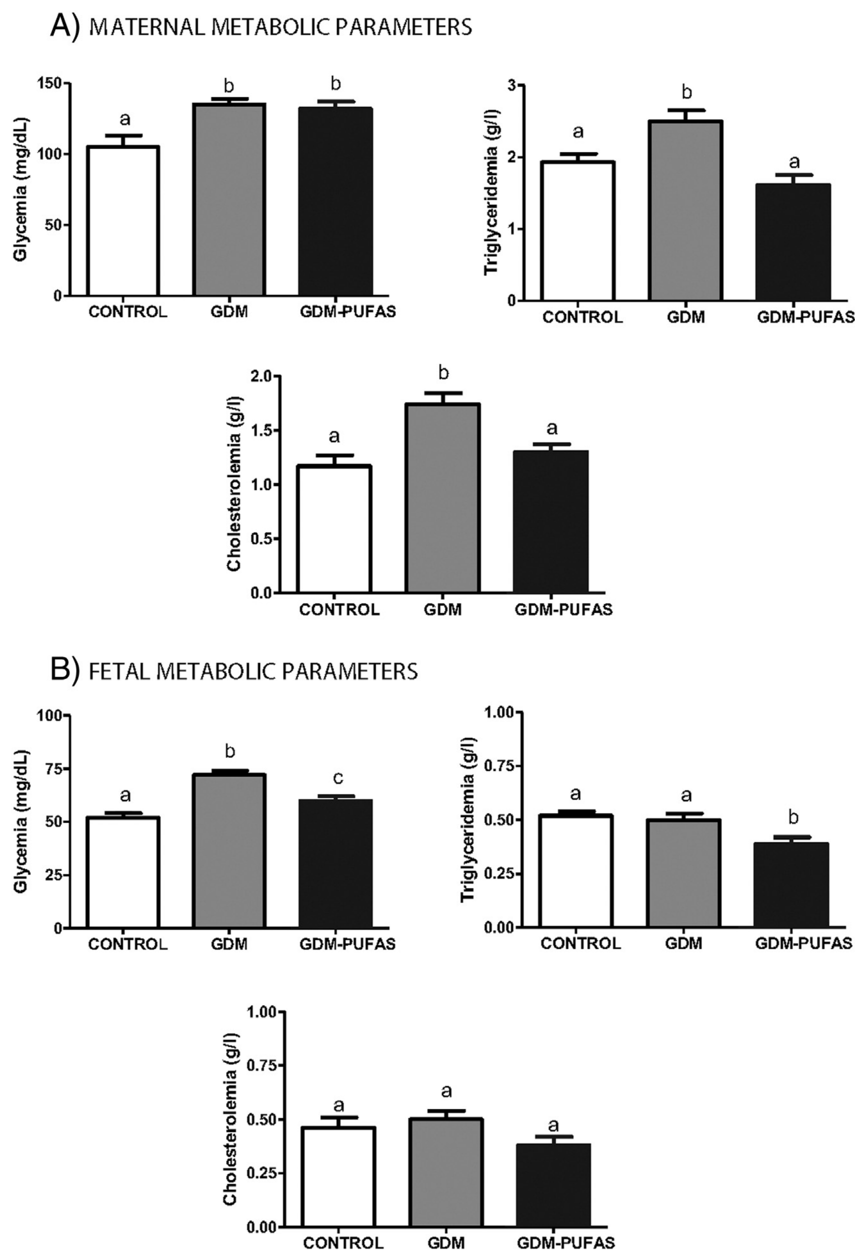


Fig. 2. Metabolic parameters in the offspring of control and mild diabetic rats that develop GDM on day 21 of pregnancy (F1). Mild diabetic rats (F0) received a standard diet or a 6% enriched PUFA diet during their pregnancies, and their pregnant female offspring were studied (GDM and GDM-PUFA groups, respectively). (A) Maternal glycemia, triglyceridemia and cholesterolemia. (B) Fetal glycemia, triglyceridemia and cholesterolemia. Values represent mean±S.E.M. obtained from eight F1 rats from different F0 mothers in each experimental group. Statistical analysis: one-way ANOVA in conjunction with Bonferroni's test. Different letters denote significant differences between groups. $P<.05$.

GDM placentas when compared to controls ($P<.05$) and increased to control values in the placentas from the GDM-PUFA group (GDM *vs.* GDM-PUFAs, $P<.05$) (Fig. 5C).

Downstream proteins in the mTORC2 pathway were also modified in placentas from GDM rats whose mothers received the PUFA-supplemented diets. Although no differences were found in phosphorylated PKCα levels, the levels of total PKCα were reduced in placentas from the GDM group ($P<.01$) but not in the placentas from the GDM-PUFA group when compared to the control group (Fig. 6A). Moreover, levels of phosphorylated SGK1 in placentas from the GDM group were markedly increased when compared to controls ($P<.001$) and reduced to control values when the mothers of GDM rats were treated with PUFAs during their pregnancies (GDM *vs.* GDM-PUFAs, $P<.001$) (Fig. 6B).

4. Discussion

Many previous studies have highlighted the importance of altered fetal nutrition in intrauterine programming of metabolic diseases [25], but little is known regarding the effects of a diet enriched in PUFAs during diabetic pregnancies on the pregnancies of the next generations. The main novel findings of this work are that PUFA treatment in mild diabetic pregnant rats (F0 generation) does not prevent GDM in the next generation but clearly improves lipid metabolic parameters,

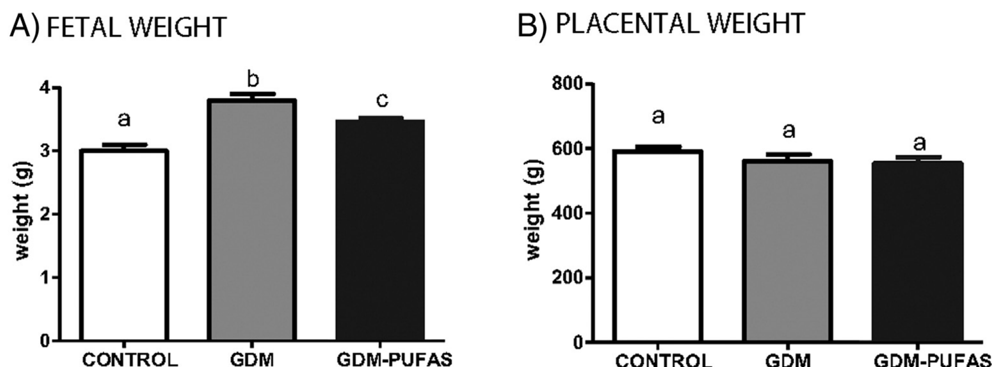


Fig. 3. Weight of fetuses and placentas of control and GDM rats on day 21 of pregnancy. GDM develops through intrauterine programming in the offspring (F1) of mild diabetic rats. Mild diabetic rats (F0) received a standard diet or a 6% enriched PUFA diet during their pregnancies, and their pregnant female offspring were studied (GDM and GDM-PUFA groups, respectively). (A) Fetal weight. (B) Placental weight. Values represent mean±S.E.M. obtained from eight F1 rats from different F0 mothers in each experimental group. Statistical analysis: one-way ANOVA in conjunction with Bonferroni's test. Different letters denote significant differences between groups. $P<.05$.

ameliorates fetal overgrowth and normalizes placental signaling by reducing lipoperoxidation, increasing PPARγ levels and reducing mTOR activation.

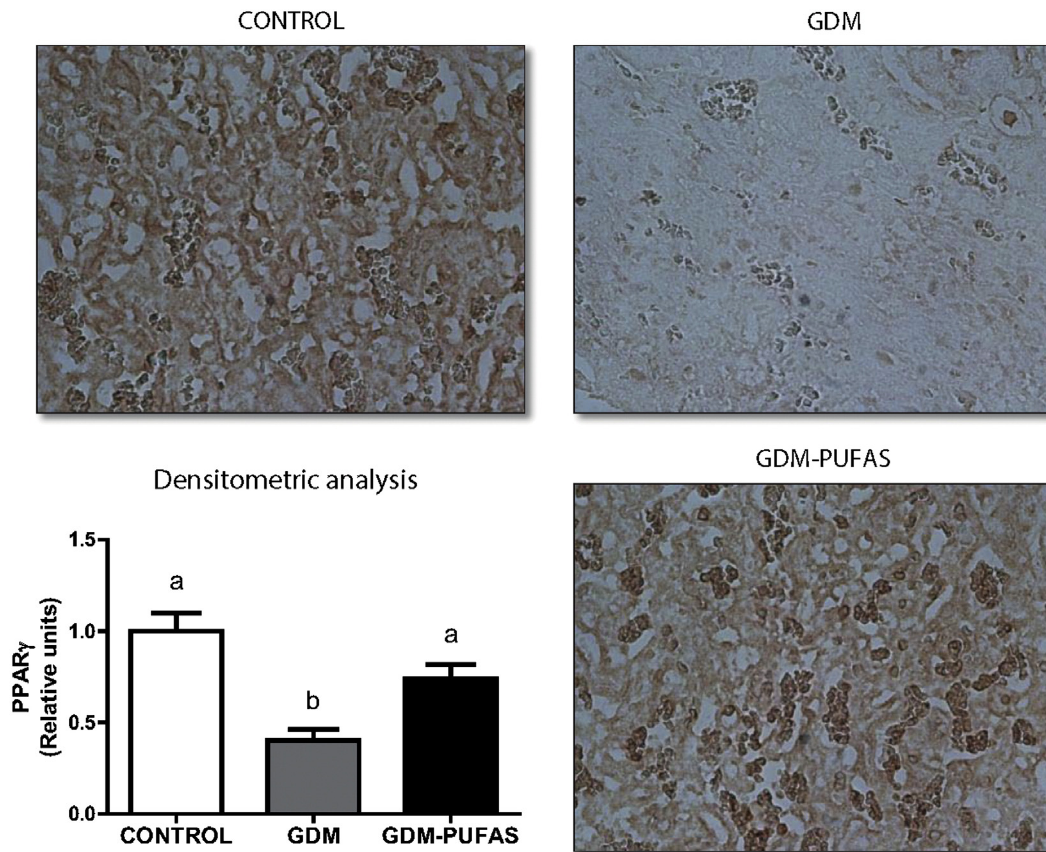
In many countries, more than 10% of pregnant women develop GDM, which increases the risk of adverse perinatal outcomes [1,2]. Fetal overgrowth and a pro-oxidant intrauterine environment are common in human GDM [5,14] and also characterize a recently developed rat model of GDM [6]. In this experimental model, female offspring from mild diabetic rats spontaneously develop GDM in late pregnancy after being exposed to intrauterine programming by maternal diabetes [6]. Our previous work in mild diabetic animals has shown that maternal diets enriched in olive oil and safflower oil lead to PPAR activation in the intrauterine compartment and prevent the pro-oxidant/proinflammatory intrauterine environment [20,32]. Moreover, fish oil and other n-3 PUFAs dietary supplements improve lipid metabolic and proinflammatory pathways in pregnant diabetic animals [28,29]. To address whether PUFAs diet in F0 pregnancy influences pregnancy in the F1 generation and taking into account the major requirements at different gestational ages [24,31], we provided diets enriched in n-6 PUFAs during the first half of pregnancy and in n-3 PUFAs during the second half of gestation to mild diabetic rats (F0) and analyzed the effect in the adult female offspring (F1) that develop GDM in pregnancy.

PUFA-enriched diet did not prevent the development of GDM in the female offspring of diabetic rats. Similarly, a diet enriched in olive oil in the F0 generation was previously found to be ineffective in preventing the development of type 2 diabetes in 5-month-old offspring of diabetic rats [35]. We did, however, find that PUFA supplementation in the F0 generation ameliorated the impaired lipid serum metabolites in GDM rats. This may be highly beneficial to the fetus, as lipids are metabolic substrates that can promote fetal overgrowth, be accumulated in fetal organs and are involved in the induction of a pro-oxidant environment, as evidenced in obese women and animal models of maternal obesity [36,37]. PUFAs of the n-3 series, and chia oil in particular, have been identified as important regulators of lipid metabolism [38,39]. The n-3 PUFAs of the F0 maternal diet may be critically involved in the regulation of lipid levels in the pregnant offspring that develop GDM; however, the reprogramming mechanisms involved will require further studies.

Both glycemia and triglyceridemia were decreased in the fetuses (F2) from the GDM-PUFA group when compared to the GDM group, metabolic changes that may result from the improvements in maternal lipid metabolic parameters and the improved placental signaling. Placental function is a key determinant of nutrient transfer to the developing fetus [14,40,41], and a decrease in placental PPARγ levels in GDM dams was prevented by F0 diet supplemented with PUFAs. This is important as PPARγ is a crucial ligand activated transcription factor involved in placental development and acts as regulator of placental antioxidant/anti-inflammatory pathways throughout gestation [20,21]. We have previously found that diets enriched in olive oil and safflower oil, respectively, enriched in MUFAS and PUFAs, regulate placental PPARγ signaling and reduce inflammatory markers in the placenta from pregestational diabetic rats [20,32,42]. Critically important is that PUFAs supplementation in the F0 generation has beneficial effects on placental PPARγ in the future generation. This suggests an involvement of epigenetic regulation, and PPAR genes are highly susceptible to epigenetic control [21,43]. In addition to up-regulated PPARγ levels, lipoperoxidation in the placenta was reduced to control values in the GDM-PUFAS group, pointing to possible antioxidant effects exerted by PPARγ signaling [14].

Only n-6 PUFAs lead to the formation of prostaglandins of the 2-series, which are required during early organogenesis [24], whereas n-3 PUFAs have been described to inhibit mTOR signaling pathways in various cell types [26,27]. This prompted us to determine if mTOR signaling, previously found to be activated in GDM placentas [6], was regulated by treatments with diets enriched in PUFAs in the F0 generation. mTOR signaling regulates the transfer of amino acids to the fetuses and is therefore a critical regulator of fetal growth [10]. Although placental S6K1 levels were not altered in the GDM or the GDM-PUFA groups, a reduction in the levels of phosphorylated rpS6 in the GDM-PUFA group indicates an effect on the mTORC1 pathway. Increased total 4EBP1 levels in the GDM-PUFA group prevents release of elf4E and activation of protein synthesis [7], suggesting a second pathway for modulating fetal growth.

The mTORC2 pathway, which was activated in placentas from GDM rats, was down-regulated by the treatments with PUFAs in the F0 generation, as evidenced by a reduction of phosphorylated SGK1 levels in the GDM-PUFA group compared to the GDM group and by the unchanged levels of total PKCα and SGK1 in the GDM-PUFA group compared to controls. Although placental weight was similar in the control, GDM and GDM-PUFA groups, fetal weight was reduced in the GDM-PUFA group when compared to the GDM group, suggesting that regulation of the mTORC1 and mTORC2 pathways in the placentas of the GDM rats as a result of the PUFAs treatment in their diabetic mothers contributes to establishing appropriate placental transfer of



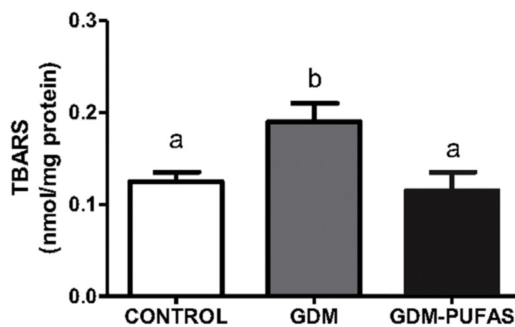


Fig. 4. PPARγ protein expression and lipoperoxidation in placentas of control and GDM rats on day 21 of pregnancy. GDM develops through intrauterine programming in the offspring (F1) of mild diabetic rats. Mild diabetic rats (F0) received a standard diet or a 6% enriched PUFAs diet during their pregnancies, and their pregnant female offspring were studied (GDM and GDM-PUFA groups, respectively). (A) PPARγ protein expression and (B) lipoperoxidation. Values represent mean±S.E.M. obtained from eight F1 rats from different F0 mothers in each experimental group. Statistical analysis: one-way ANOVA in conjunction with Bonferroni's test. Different letters denote significant differences between groups. $P < .05$.

nutrients and supports normal fetal growth [40]. The mechanisms that impact the placental mTOR pathway in the F1 generation may be epigenetic changes and could be due to an interaction between PPAR and mTOR pathways in response to F0 maternal PUFAs, although this speculation requires further studies.

Although the experimental results obtained cannot be extrapolated to the human condition, they constitute a base to perform further clinical studies. In summary, this study provides evidence that F0 PUFA dietary treatment in pregnancies with experimental mild diabetes improves maternal dyslipidemia, fetal overgrowth and placental signaling in female offspring when they become pregnant. We speculate that an increased PUFA intake in pregnancies complicated by diabetes may prove effective to ameliorate metabolic programming in the offspring, thereby improving the health of future generations.

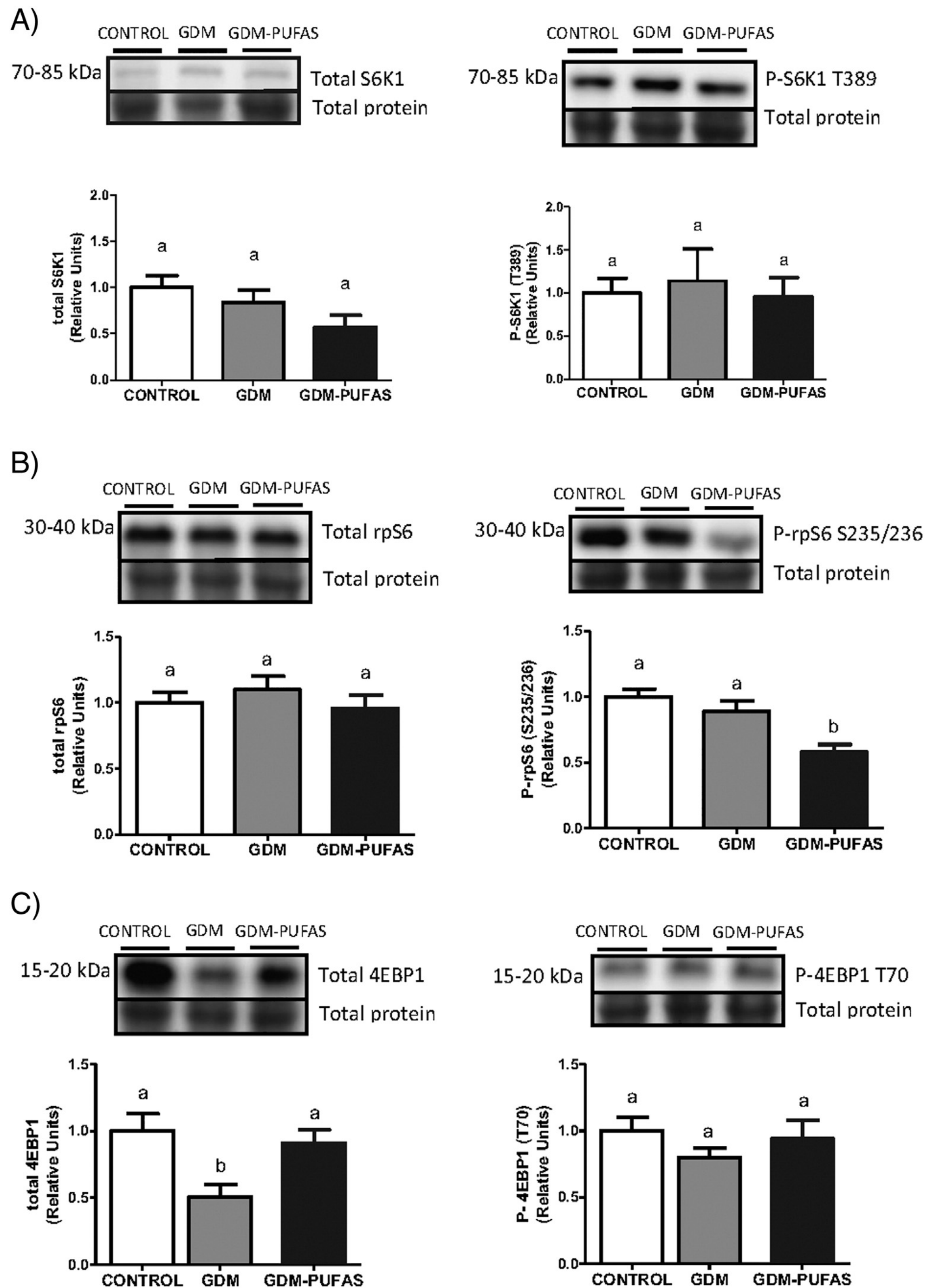


Fig. 5. Expression of proteins involved in mTORC1 pathway in the placenta of control and GDM rats on day 21 of pregnancy. GDM develops through intrauterine programming in the offspring (F1) of mild diabetic rats. Mild diabetic rats (F0) received a standard diet or a 6% enriched PUFAs diet during their pregnancies, and their pregnant female offspring were studied (GDM and GDM-PUFA groups, respectively). (A) Representative Western blots and data summary of phosphorylated and total S6K1. (B) Representative Western blots and data summary of phosphorylated and total rpS6. (C) Representative Western blots and data summary of phosphorylated and total 4EBP-1. Values represent mean±S.E.M. obtained from seven F1 rats from different F0 mothers in each experimental group. Statistical analysis: one-way ANOVA in conjunction with Bonferroni's test. Different letters denote significant differences between groups. *P*<.05.

Conflict of interest

The authors report no conflicts of interest.

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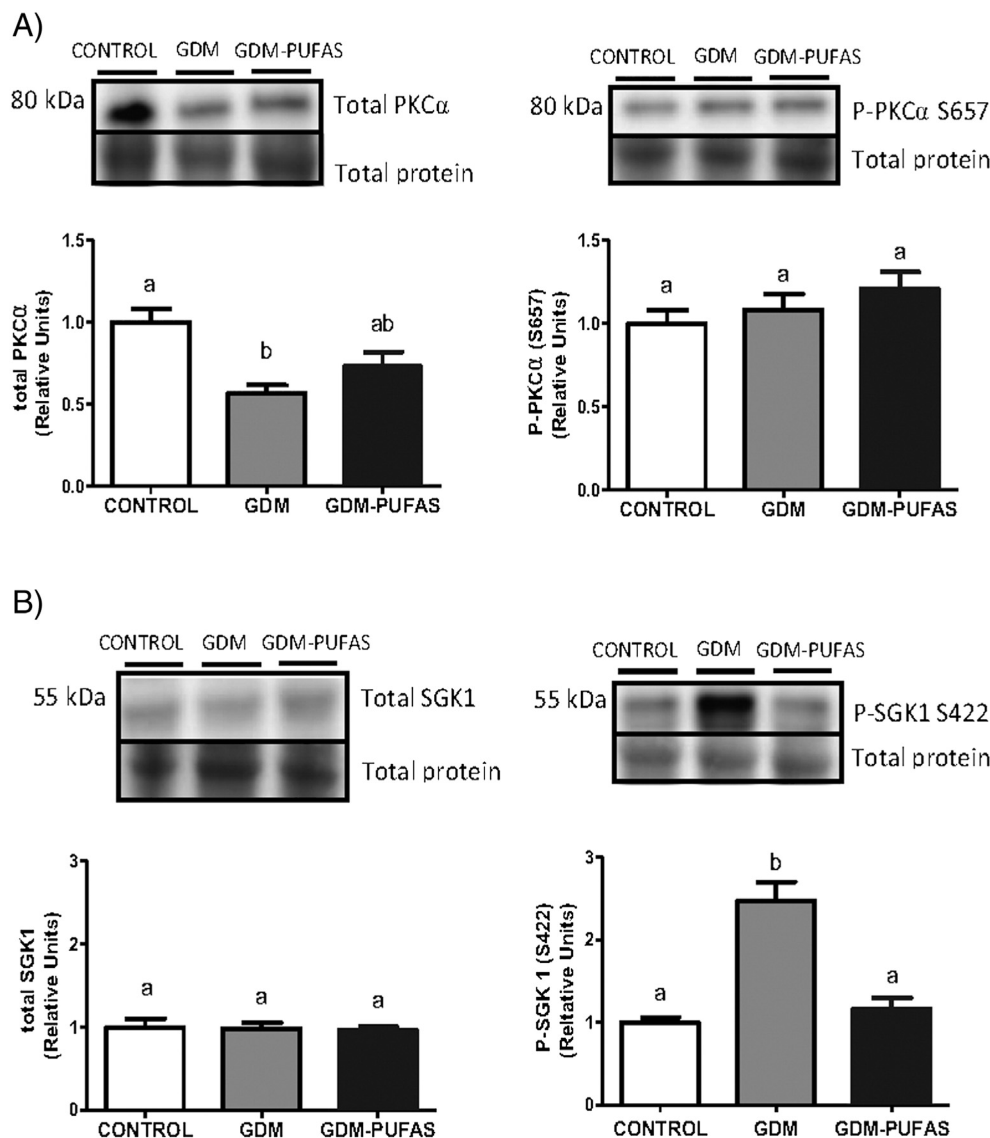

Fig. 6. Expression of proteins involved in mTORC2 pathway in the placenta of control and GDM rats on day 21 of pregnancy. GDM develops through intrauterine programming in the offspring of mild diabetic rats. Mild diabetic rats (F0) received a standard diet or a 6% enriched PUFAs diet during their pregnancies, and their pregnant female offspring were studied (GDM and GDM-PUFA groups, respectively). (A) Representative Western blots and data summary of phosphorylated and total PKCα. (B) Representative Western blots and data summary of phosphorylated and total SGK1. Values represent mean±S.E.M. obtained from seven F1 rats from different F0 mothers in each experimental group. Statistical analysis: one-way ANOVA in conjunction with Bonferroni's test. Different letters denote significant differences between groups. $P<.05$.

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