An extra virgin olive oil-enriched diet improves maternal, placental, and cord blood parameters in GDM pregnancies

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Abstract
Aims: To address the effect of a diet enriched in extra virgin olive oil (EVOO) on maternal metabolic parameters and placental proinflammatory markers in Gestational diabetes mellitus (GDM) patients.

Methods: Pregnant women at 24-28 weeks of gestation were enrolled: 33 GDM patients which were randomly assigned or not to the EVOO-enriched group and 17 healthy controls. Metabolic parameters were determined. Peroxisome proliferator activated receptor (PPAR) γ and PPARα protein expression, expression of microRNA (miR)-130a and miR-518d (which respectively target these PPAR isoforms) and levels of proinflammatory markers were evaluated in term placentas. Matrix metalloproteinases (MMPs) activity was evaluated in term placentas and umbilical cord blood.

Results: GDM patients that received the EVOO-enriched diet showed reduced pregnancy weight gain (GDM-EVOO:10.3 ± 0.9, GDM:14.2 ± 1.4, P = .03) and reduced triglyceridemia (GDM-EVOO:231 ± 14, GDM:292 ± 21, P = .02) compared to the non-EVOO-enriched GDM group. In GDM placentas, the EVOO-enriched diet did not regulate PPARγ protein expression or miR-130a expression, but prevented the reduced PPARα protein expression (P = .02 vs GDM) and the increased miR-518d expression (P = .009 vs GDM). Increased proinflammatory markers (interleukin-1β, tumour necrosis factor-α and nitric oxide overproduction) in GDM placentas were prevented by the EVOO-enriched diet (respectively P = .001, P = .001 and P = .001 vs GDM). MMPs overactivity was prevented in placenta and umbilical cord blood in the EVOO-enriched GDM group (MMP-9: respectively P = .01 and P = .001 vs GDM).

Conclusions: A diet enriched in EVOO in GDM patients reduced maternal triglyceridemia and weight gain and has antiinflammatory properties in placenta and umbilical cord blood, possibly mediated by the regulation of PPAR pathways.

KEYWORDS
anti-inflammatory effects, dietary treatment, foetal programming, maternal diabetes, monounsaturated fatty acids
**1 | INTRODUCTION**

Gestational diabetes mellitus (GDM) is a prevalent disease that increases the risks of maternal, placental, and perinatal adverse outcomes and induces long-term adverse effects on the offspring’s later life.1,2 Adverse outcomes in GDM have been related to an intrauterine proinflammatory environment.3 Indeed, proinflammation is a common alteration in gestational diseases and can influence the placental development and function, the foetal development and the offspring’s later life.4,5

Our previous studies performed in experimental models of diabetes and pregnancy allowed us to identify changes in pathways regulated by peroxisome proliferator activated receptors (PPARs) in embryos, foetuses, and placentas, related to a proinflammatory environment.6 PPARs are ligand-activated transcription factors capable of regulating metabolic and antiinflammatory pathways, as well as intrauterine development.7,8 PPARs are nutrigenomic agents, being monounsaturated fatty acids (MUFAs, the main components of olive oil), PPARs endogenous ligands.9,10 In experimental models of diabetes and pregnancy, our previous studies have shown that diets enriched in extra virgin olive oil (EVOO) lead to antiinflammatory effects, as shown by the ability of these diets to prevent increased levels of proinflammatory cytokines, nitric oxide overproduction, and matrix metalloproteinases (MMPs) overexpression in placentas, embryos and different foetal organs.11-16 Studies have also shown that although maternal EVOO-dietary treatments do not prevent programming of metabolic diseases in the offspring of diabetic animals, this treatment reduces the levels of proinflammatory markers in the offspring’s heart and prevents hypertriglyceridemia in the adult offspring of diabetic rats.11,16

In women with pregestational and gestational diabetes, placental levels and expression of PPARγ and PPARα are reduced.17,18 Besides, expression of microRNA-518d, a microRNA that targets PPARα, is increased in the placentas of GDM patients.19 Whether microRNAs that regulate PPARs are related to PPARγ changes and whether alterations in placental microRNAs that target PPARs can be prevented by diets enriched in PPAR ligands are unknown.

EVOO is the main vegetable oil that composes the Mediterranean diet, which is increasingly being considered as a medical treatment.20,21 The Mediterranean diet has been found associated with a lower incidence of GDM.22 Out of pregnancy, an EVOO-enriched diet has beneficial effects on metabolic and cardiovascular diseases, as demonstrated in the PREDIMED study and other clinical studies.23-25 In pregnant women, studies addressing a diet enriched in EVOO and pistachios reduced the incidence of GDM.26 No previous studies addressing a putative beneficial effect of an EVOO-enriched diet in GDM pregnancies have been conducted so far. In this work, we conducted a randomized clinical trial and tested the hypothesis that a maternal diet enriched in EVOO ameliorates triglyceridemia in the mothers, regulates placental PPAR protein levels, modulates the expression of microRNAs that regulate PPARs expression, and reduces proinflammatory markers in the placenta and the umbilical cord blood from GDM patients.

**2 | METHODS**

**2.1 | Study design**

Pregnant women with a singleton foetus at the time of GDM diagnosis (between 24 and 28 weeks) were randomly assigned in a 1:1 manner to receive a diet with additional three tablespoons of crude EVOO daily (36 g/day). GDM was diagnosed according to Latin American Diabetes Association (ALAD)/Argentine Society of Diabetes (SAD) diagnostic criteria, based on glycemia values either at fasting (>99 mg/dL in two measurements) or after the universal p75 g oral glucose tolerance test (>140 mg/dL at 2 hours).27 Control women were recruited at the same gestational age. Exclusion criteria included BMI over 30 kg/m² before pregnancy, multiple pregnancies and concurrent pathologies including: thrombophilia, preeclampsia, gestational diabetes, vascular or renal complications associated with chronic hypertension, anaemia with total haemoglobin below 8 g/dL and positive serology for HIV, VDRL, Hepatitis B or Chagas disease. Potential participants received written and oral information about the trial and had at least 24 hours to decide their participation. This clinical trial was approved on 14th January 2016 by the Ethics and Research Committee of the Pirovano Hospital (Review Board Project: DI-2016-29-HGAIP) and registered at the Ministry of Health of the City of Buenos Aires on October 2016, IF-2016-22 533 767. Participants were recruited at Hospital de Agudos Dr. Ignacio Pirovano, Buenos Aires, Argentina, from December 2016 to December 2018. The study was undertaken in accordance with the Declaration of Helsinki and followed the 2010 Consort guidelines. All women that agreed to participate provided their written informed consent.

All eligible women received standardized personal advice on healthy eating and appropriate medical care. The study was not blinded, as those women in the EVOO-enriched group received commercial bottles of EVOO at the nutrition visits to ensure adherence. In the three experimental groups (Control, GDM, GDM-EVOO), the women received dietary indications to follow a nutritional plan with the following composition: 2100-2400 Kcal/day; carbohydrates 48-50%, proteins 18-20%, and lipids 30-32%. In the intervention group, women were indicated to include three tablespoons of EVOO daily (36 g/day). The EVOO was indicated to be consumed uncooked and within the main meals. The group that did not receive the EVOO-enriched diet was indicated to include none to one tablespoon of EVOO daily (0 to 12 g per day). Follow-up appointments to the obstetrics and nutrition professionals in charge of the study were frequent (every 1 to 4 weeks according to the gestational age and requirements). To assess adherence to the EVOO dietary intervention, questionnaires were performed and diet composition was evaluated at each nutritional visit. Adherence was considered good if reports indicated a daily EVOO consumption over 26 g/day 5 to 7 times a week, regular if daily EVOO consumption was over 26 g/day 3 or 4 times a week and bad if daily EVOO consumption was lower than 26 g/day or lower than 3 times a week. Clinical data of the participants were recorded at each visit. At GDM diagnosis, women received the dietary treatment and the indications for glucose monitoring. In subsequent visits, insulin was added if target blood-glucose values were not achieved.27
2.2 Metabolic parameters and pregnancy outcomes

Eight-hour fasting blood was collected at enrolment (gestational weeks 24 to 28) and at gestational week 37. Plasma glucose, fructosamine, and glycated haemoglobin were evaluated using an Abbott autoanalyzer (Architect C8000). Triglycerides were evaluated by colorimetric methods (Wiener lab, Rosario, Argentina). Gestational weight gain (defined as the difference in maternal weight between the reported weight at term and prior to pregnancy) was determined. Maternal and foetal complications were reported. Neonatal and placental weight were obtained at birth.

2.3 Placenta and umbilical cord blood sampling

Umbilical cord blood was collected before placental delivery and citrate plasma immediately obtained and conserved at —80°C. Placental tissues were collected from centrally located cotyledons, avoiding decidua and membrane layers. Placental tissues were stored in 10% formalin for immunohistochemical studies, immersed in RNA-later solution and stored at —80°C for PCR studies, or immediately stored at —80°C for Western Blot, zymography and nitric oxide production evaluation.

2.4 Western blot analysis

Placental explants were homogenized, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes (35 V constant, overnight at 4°C), as previously described. The membranes were stained with Ponceau Red staining solution for total proteins (Sigma–Aldrich, St Louis, Missouri) to confirm proper transfer. After blocking for 1 hour, the membranes were incubated overnight at 4°C with the primary antibody. Two skilled blinded observers evaluated three sections per placenta. Immunoreactivity intensity was quantified with the primary antibody. Two skilled blinded observers evaluated three sections per placenta. Immunoreactivity intensity was quantified with the ImageProPlus software. Data are shown as relative to a value of 1 assigned to the mean values in the control group.

2.5 Total RNA and microRNA isolation, and qRT-PCR analysis

Total RNA and microRNA were isolated from placental explants (100 mg) using RNAzol (MCR Inc., Ohio) and their concentrations determined using the NanoDrop spectrophotometer.

For microRNA evaluation, cDNA was obtained using the TaqMan MicroRNA reverse transcription kit (Applied Biosystems, California). The relative expression of miR-130a and of miR-518d was determined using the TaqMan detection system (Applied Biosystems), the appropriate primers (assay ID 000454 and 002389 respectively), and the U6 spliceosomal RNA (assay ID 001973) as endogenous control (Applied Biosystems).

From total RNA, cDNA was synthesized incubating 1 μg of extracted RNA in a buffer containing 200 U MML-V enzyme (Promega, Wisconsin), 7.5 mM random primer hexamers and 0.5 mM of each dNTPs, as previously. The reaction mixture was incubated for 60 minutes at 37°C followed by 15 minutes at 70°C. Then, 2.5 μL of cDNA was used to perform the amplification in 10 μL reaction buffer containing dNTPs mix 20 mM, GoTaq Polymerase (Promega), Eva Green 20x, and gene specific primer (Cu/Zn SOD, forward: 5′-ACAAAGATGGTGTTGCAGAT-3′, reverse: 5′-AAGACTTCCA GCGTTTCCT-3′). The qPCR conditions started with a denaturation step at 95°C for 5 minutes and followed by up to 40 cycles of denaturation (95°C), annealing (62°C), and primer extension (72°C). mRNA levels were normalized to the 60s ribosomal protein L30 levels (L30 primer: forward: 5′-TGACAGACAGCAAAGGCG-3′, reverse: 5′-GCGACTGTAAGATGACACC-3′).

From total RNA and microRNA, the course of PCR amplification was followed in each cycle by the fluorescence measurement on Corbett Rotor-Gene 6000 (QIAGEN, Maryland). Gene expression was quantified using the 2-ΔΔCt method. Relative mRNA and microRNA levels are reported as fold value of the control.

2.6 Immunohistochemistry

Placentas were paraffinized and serially cut in 5-μm-thick sections for further evaluation of interleukin 1β (IL-1β) and tumour necrosis factor-α (TNF-α) immunostaining. Sections were deparaffinized, rehydrated through a graded series of ethanol, and the endogenous peroxidase activity was blocked. The sections were processed overnight using anti-TNF-α primary antibody (mouse monoclonal antibody, 1:100 dilution, Santa Cruz Biotechnology, California) and anti-IL-1β primary antibody (mouse monoclonal antibody, 1:100 dilution, Santa Cruz Biotechnology) in a humidified chamber at room temperature and then incubated with the biotinylated anti-mouse secondary antibody (anti-mouse IgG, dilution 1:200, Vector Laboratories, California) for 1 hour. Sections were incubated with the Avidin-Biotin-Complex (Vectastain, Vector Laboratories) for 1 hour and then the stain was developed with 3,3′-diaminobenzidine, as previously described. Control sections were generated by omitting the primary antibody. Two skilled blinded observers evaluated three sections per placenta. Immunoreactivity intensity was quantified with the ImageProPlus software. Data are shown as relative to a value of 1 assigned to the mean values in the control group.

2.7 Nitric oxide production

Nitric oxide production was determined by measuring the concentration of its stable metabolites nitrates/nitrites, as reported
previously. Briefly, placental explants were homogenized in 1 mL Tris-HCl buffer pH 7.6 and an aliquot was separated for protein analysis. After reducing nitrates to nitrites by using nitrate reductase enzyme, nitrites were measured by the Griess reaction using a commercial assay kit (Cayman Chemical Co.).

2.8 | Gelatinase activity of matrix metalloproteinases

Zymography was performed to evaluate the gelatinase activity of matrix metalloproteinases (MMP) 2 and 9, as previously described. Briefly, placental explants were homogenized in 50 mM Tris, 5 mM CaCl₂, 1 μM ZnCl₂, and 1% Triton X-100. Then, 30 μg of protein of the homogenates was mixed with loading buffer (2% SDS, 10% glycerol, 0.1% bromophenol blue, 50 mM Tris-HCl, pH 6.8) and subjected to a 7.5% SDS-PAGE containing 1 mg/mL gelatin (type A from porcine skin). Gels were rinsed, stained with Coomassie blue, and destained with 10% acetic acid and 30% methanol in water. The areas of proteolytic activity appeared as negatively stained bands in a dark background. MMPs were identified by their molecular weights and a positive internal control (conditioned medium of human fibrosarcoma HT-1080 cells). The enzymatic activity was quantified using ImageJ software and expressed as arbitrary densitometric units. Data are shown as relative to a value of 1 assigned to the mean value for MMP activity in the control group.

2.9 | Study endpoints

The primary endpoint was a change in PPARγ and PPARα levels and placental markers of a prooxidant/proinflammatory state (TNFα and IL-1β levels, nitric oxide production, SOD expression, and MMPs gelatinase activity). Second endpoints were changes in maternal

| TABLE 1 | Pregnancy and metabolic control data |
|--------------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|
|                                     | Control (n = 15) | GDM (n = 15) | GDM-EVOO (n = 15) | P-value |
| Adherence to dietary treatment      | -                | -              | Good: 80% (12/15) | -          |
|                                     |                  |                | Regular: 20% (3/15) |           |
|                                     |                  |                | Bad: None            |           |
| Age (years)                         | 27.4 ± 1.9       | 29.6 ± 1.6     | 31.1 ± 1.6         | >0.99 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.39 GDM-EVOO vs Control |
| Pre-pregnancy weight (kg)           | 57.5 ± 1.9       | 62.3 ± 2.0     | 61.3 ± 2.0         | 0.27 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.53 GDM-EVOO vs Control |
| Total weight gain (kg)              | 9.9 ± 0.7        | 14.2 ± 1.4     | 10.3 ± 0.9        | 0.02 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.03 GDM-EVOO vs GDM |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs Control |
| Fasting blood glucose at enrolment (mg/dL) | 81.4 ± 1.9       | 88.0 ± 2.7     | 86.0 ± 1.8        | 0.11 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.43 GDM-EVOO vs Control |
| Fasting blood glucose at term (mg/dL) | 81.3 ± 1.6       | 89.0 ± 3.6     | 87.6 ± 2.7        | 0.16 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.34 GDM-EVOO vs Control |
| Blood fructosamine at enrolment (mg/dL) | -                | 183.0 ± 6.1    | 181.1 ± 4.2       | 0.80 GDM-EVOO vs GDM |
| Blood fructosamine at term (mg/dL) | -                | 185.8 ± 5.8    | 189.6 ± 2.6       | 0.55 GDM-EVOO vs GDM |
| HbA1c at enrolment (%)              | -                | 5.0 ± 0.08     | 4.9 ± 0.10        | 0.44 GDM-EVOO vs GDM |
| HbA1c at term (%)                   | -                | 5.4 ± 0.10     | 5.5 ± 0.06        | 0.39 GDM-EVOO vs GDM |
| Blood triglycerides at enrolment (mg/dL) | 185 ± 8         | 209 ± 10       | 219 ± 15          | 0.43 GDM vs Control |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs GDM |
|                                     |                  |                |                   | 0.12 GDM-EVOO vs Control |
| Blood triglycerides at term (mg/dL) | 238 ± 6          | 292 ± 21       | 231 ± 14          | 0.04 GDM vs Control |
|                                     |                  |                |                   | 0.02 GDM-EVOO vs GDM |
|                                     |                  |                |                   | >0.99 GDM-EVOO vs Control |
| Treatment                           | -                | Diet only: 73% (11/15) | Diet+Insulin: 27% (4/15) | -          |
|                                     |                  | Diet only: 93% (14/15) | Diet+Insulin: 7% (1/15) |           |

Note: Data are presented as mean ± SEM. Statistical analysis: One-way ANOVA in conjunction with Bonferroni’s test or Student’s t test on continuous variables and chi-square test for categorical variables.
metabolic control, triglyceridemia, maternal weight gain, neonatal, and placental weight as well as microRNAs that target PPARγ and PPARα (miR-130a and miR-518d, respectively).

2.10 | Statistical analysis

Data are presented as the mean ± SEM. Groups were compared by one-way ANOVA followed by Bonferroni’s post-hoc test to compare all groups to each other. Student’s t-test or chi-square test as appropriate (Graphpad Prism 8 software). Normality of the variable distribution was corroborated with the Shapiro-Wilk test. Homogeneity of variance was evaluated with the Levene’s test, and in the cases where homogeneity of variance was not verified, the variance function Varldent was applied to the model (Infostat 2017 software). A P value lower than .05 was considered statistically significant.

3 | RESULTS

3.1 | Metabolic parameters and pregnancy outcomes

As shown in the study Flow chart (Supplementary Figure 1), we were able to obtain the complete samples at term of 45 women (15 of the control group, 15 of the GDM group and 15 of the GDM-EVOO-enriched group (GDM-EVOO)). Adherence to the EVOO dietary intervention was good in 80% of the GDM patients (Table 1). Age and pre-pregnancy weight were similar between the three groups (Table 1). Total weight gain was increased in the GDM group ($P = .02$ GDM vs Control), an alteration prevented by the maternal diet enriched in EVOO ($P = .03$ GDM-EVOO vs GDM) (Table 1). A very good metabolic control was achieved in both the GDM and the GDM-EVOO group, as shown by the fasting blood glucose, fructosamine and HbA1c levels at term (Table 1). GDM patients were treated with diet or diet and insulin, with no significant changes between the two groups ($P = .14$, Table 1). At enrolment, triglyceridemia values in the groups evaluated showed no changes (Table 1). Differently, at term, triglyceridemia was increased in the GDM group compared to Controls ($P = .04$), an alteration prevented in the GDM group that received the EVOO-enriched diet ($P = .02$ GDM-EVOO vs GDM) (Table 1). Regarding the pregnancy outcomes, gestational age at delivery, Cesarean delivery rate, maternal complications, neonatal complications, neonatal weight, and placental weight were similar in the three groups evaluated (Table 2).

3.2 | PPARs and microRNAs that regulate PPARs expression

We next addressed PPAR pathways and found reduced protein expression of PPARγ in the placentas from GDM patients that received or not the diet enriched in EVOO compared to controls ($P = .007$ GDM vs Control, $P = .03$ GDM-EVOO vs Control, Figure 1).

### TABLE 2 Pregnancy outcomes

| Parameter                        | Control (n = 15) | GDM (n = 15) | GDM-EVOO (n = 15) | P-value          |
|----------------------------------|-----------------|-------------|------------------|-----------------|
| Gestational age at delivery (weeks) | 38.1 ± 0.3     | 37.6 ± 0.3  | 37.8 ± 0.3       | 0.73 GDM vs Control |
|                                   |                 |             |                  | >0.99 GDM-EVOO vs GDM |
|                                   |                 |             |                  | >0.99 GDM-EVOO vs Control |
| Caesarean delivery                | 40% (6/15)      | 47% (7/15)  | 40% (6/15)       | 0.91            |
| Maternal complications            | None            | None        | None             | -               |
| Neonatal complications            | 0% (0/15)       | 26.7% (4/15)| 13.3% (2/15)     | 0.10            |
|                                   | Respiratory Distress Syndrome (1) | Hypoglycemia and Macrosomia (1) | Hyperbilirubinemia (1) |
|                                   | Hyperbilirubinemia (3) | | |
| Neonatal weight (g)               | 3312 ± 168      | 3205 ± 113  | 3332 ± 135       | >0.99 GDM vs Control |
|                                   |                 |             |                  | >0.99 GDM-EVOO vs GDM |
|                                   |                 |             |                  | >0.99 GDM-EVOO vs Control |
| Placental weight (g)              | 599 ± 27        | 613 ± 40    | 672 ± 32         | >0.99 GDM vs Control |
|                                   |                 |             |                  | >0.66 GDM-EVOO vs GDM |
|                                   |                 |             |                  | >0.39 GDM-EVOO vs Control |

Note: Data are presented as mean ± SEM. Statistical analysis: One-way ANOVA in conjunction with Bonferroni’s test on continuous variables and chi-square test for categorical variables. Maternal complications considered: pyelonephritis, cholestasis, preeclampsia, pregnancy-induced hypertension and threatened preterm labour. Neonatal complications considered: shoulder dystocia, brachial plexus injury, clavicle fracture, respiratory distress syndrome, intrauterine growth restriction, hypoglycemia, hyperbilirubinemia, birth defects and perinatal mortality.
Differently, the reduced protein expression of PPARα in the placentas from GDM patients was prevented by the diet enriched in EVOO \((P = .02 \text{ GDM vs Control, } P = .02 \text{ GDM- EVOO vs GDM, Figure 1})\). To determine whether microRNAs that target PPARs are involved in the changes observed, we evaluated the expression of miR-130a, which targets PPARγ in different tissues,\(^{29}\) and of miR-518d, which targets PPARα in the placenta.\(^{19}\) The expression of miR-130a showed no changes in the groups evaluated (Figure 1), but miR-518d expression, which was increased in the placenta of GDM patients \((P = .003 \text{ vs Control})\), was reduced in the placentas of the GDM patients that received the EVOO-enriched diet \((P = .009 \text{ vs GDM, Figure 1})\).

**3.3 Regulation of the proinflammatory environment**

As PPARs are largely involved in the regulation of the proinflammatory environment, and the EVOO diet is enriched in MUFAs, which are PPAR ligands, we next evaluated proinflammatory markers in term placentas from the GDM patients that received or not the EVOO-enriched diet. We found that TNF-α levels were increased in the placentas from GDM patients compared to controls \((P = .0002)\), an alteration prevented by the maternal diet enriched in EVOO \((P = .0002 \text{ vs GDM})\) (Figure 2). Similarly, IL-1β levels were increased in the GDM group compared to controls \((P = .0001)\), an alteration prevented by the maternal diet enriched in EVOO \((P = .0001 \text{ vs GDM})\) (Figure 2). The production of nitric oxide was increased in the GDM group compared to controls \((P = .03)\), an alteration prevented by the maternal diet enriched in EVOO \((P = .01 \text{ vs GDM})\) (Figure 2). Moreover, the gene expression of the antioxidant enzyme Cu-Zn superoxide dismutase was reduced in the placentas from GDM patients compared to controls \((P = .004)\), an alteration prevented by the maternal diet enriched in EVOO \((P = .01 \text{ vs GDM})\) (Figure 2). Finally, the activity of MMPs was measured both in the placenta and the umbilical cord blood. MMP-2 activity showed no changes, whereas MMP-9 activity was increased in the placentas from GDM patients compared to controls \((P = .003)\), an alteration prevented by the maternal diet enriched in EVOO \((P = .01 \text{ vs GDM})\) (Figure 2).
Moreover, MMP-2 and MMP-9 activities were increased in the umbilical cord blood from GDM patients compared to controls ($P = .03$ and $P = .009$, respectively), alterations prevented by the maternal diet enriched in EVOO ($P = .03$ and $P = .001$ vs GDM, respectively) (Figure 3). The study presented here is the first to address the effects of an EVOO-enriched diet in GDM pregnancies. In GDM mothers, the EVOO-enriched diet reduced triglyceridemia and weight gain. In the placenta of GDM patients, the maternal EVOO-enriched diet did not regulate miR-130a expression or PPARγ levels, but did regulate miR-518d expression and PPARα levels. Anti-inflammatory effects were observed in the placenta and the umbilical cord blood in the GDM patients that received the EVOO-enriched diet. If replicated in a larger number of patients, these results would have important clinical implications as a feasible dietary treatment that provides benefits to GDM mothers and their placentas, with possible beneficial effects on the offspring’s later life.

Maternal nutrition is crucial in GDM metabolic control. Modified diet interventions favourable influence outcomes related to maternal glycemia and birth weight\textsuperscript{30} and the follow-up of nutritional advices, such as consumption of low-glycemic index carbohydrates, has largely improved the metabolic control in this prevalent gestational disease.\textsuperscript{31,32} In the last years, the nutrigenomic concepts brought to a new dimension the interaction of nutrients and the genome, with implications in multiple diseases, including GDM.\textsuperscript{33} PPARs are ligand activated transcription factors that respond to nutrients to bring transcriptional regulation of metabolic, developmental, and proinflammatory pathways.\textsuperscript{7} Endogenous PPAR ligands have lipid nature and can be incorporated through the diet.\textsuperscript{9} Dietary unsaturated fatty acids are endogenous PPAR ligands that can efficiently be...
transferred through the placenta and the foetus to act as PPAR activators.\textsuperscript{6,34} Considering this, the low levels of PPAR\textsubscript{α} and PPAR\textsubscript{γ} previously observed in the placenta from GDM patients\textsuperscript{17} provide a rationale to provide PPAR agonists from the maternal diet to the placenta in GDM pregnancies.

Our previous studies performed in experimental models of diabetes and pregnancy have shown that a maternal EVOO dietary treatment regulates PPAR pathways and reduces proinflammatory markers in rat embryos, foetuses, placentas and in the offspring’s heart.\textsuperscript{6,11-13,15} The dose of olive oil that shows these beneficial effects in animal models of diabetes and pregnancy provides half of the lipid-derived calories (5\% in an 11\% lipid content diet). Based on this, in this translational clinical study, we provided a diet that brings about half of the lipid-derived calories by the olive oil (14\% in a 30\% lipid content diet). This amount of EVOO added to the diet was similar to that reported to be beneficial in different clinical studies, including those evaluating the ability of an EVOO-enriched diet to prevent GDM induction in a general population.\textsuperscript{21,26}

The PREDIMED and other clinical studies have addressed the ability of EVOO-enriched diets to ameliorate cardiovascular diseases and improve lipid metabolic profiles.\textsuperscript{21,23,25} Here, the fact that triglycerides and weight gain are reduced to control values in the GDM patients that received the EVOO-enriched diet is clinically relevant and deserves to be studied in larger populations. The mechanisms involved are likely to be related to effects on maternal target organs, including the liver, through mechanisms which are possibly related to the activation of PPAR\textsubscript{α}.\textsuperscript{35}

Other pregnancy outcomes, including gestational age, Caesarean delivery, maternal and neonatal complications, and neonatal and placental weight did not change between the groups evaluated, and a

FIGURE 3 Gelatinase activity of MMP-2 and MMP-9 in term placentas and umbilical cord blood in GDM patients that received or not a maternal diet enriched in three tablespoons of EVOO per day from weeks 24-28 of pregnancy until term and in controls. A, MMP-2 in term placentas. B, MMP-9 in term placentas. C, MMP-2 in umbilical cord blood. D, MMP-9 in umbilical cord blood. Values represent mean ± SEM. Statistical analysis: one-way ANOVA in conjunction with Bonferroni’s test. \textsuperscript{†}P < .05 and \textsuperscript{‡}P < .01 vs Control. \textsuperscript{§}P < .05 and \textsuperscript{‡‡}P < .01 vs GDM.
larger number of patients would be needed to address putative changes in these and other maternal/perinatal outcomes. Indeed, a limitation of this work was the limited number of patients included in this study. Another limitation is that we did not quantify overall caloric intake and macronutrient consumption, and thus we cannot rule out that differences in caloric intake could explain some of the benefits observed. A third limitation of this work is the lack of use of operator-independent biomarkers of fatty acid consumption such as circulating fatty acids.26

In this study, we focused in the placenta and the putative changes in PPARs expression. Similar to that previously found in a rat model of GDM,11 we here found that the maternal EVOO-enriched diet did not prevent the reduced PPARγ levels, but induced anti-inflammatory effects in the placenta. Indeed, PPAR ligands are provided by the EVOO-enriched diet, and thus, even without changing PPARγ levels, the activity of this nuclear receptor may be increased, in turn leading to the observed anti-inflammatory effects.37 Besides, the expression of miR-130, a microRNA that targets PPARγ and is related to PPARγ changes in the livers of GDM rats,29 was similar in the three groups evaluated, suggesting that other epigenetic regulators are those related to the observed PPARγ changes induced by GDM. Zhao et al. showed that the reduced PPARα levels in GDM placentas are related to increased levels of miR-518d.19 In this work, together with confirming this result, we found that the EVOO-enriched diet was able to prevent both increased miR-518d expression and reduced PPARα levels. This points to the miR-518d/PPARα pathway as a relevant component in the placental anti-inflammatory effects observed.

In non-pregnant subjects, EVOO-enriched diets have been found to exert potent anti-inflammatory effects in different tissues.21,24 In this work, the anti-inflammatory effects observed in the placentas from the GDM patients that received the EVOO-enriched diet included reductions in cytokine levels, regulation of antioxidant enzymes gene expression and reduction of nitric oxide overproduction. These proinflammatory markers have been previously found altered in GDM placentas and are difficult to modulate even with a good metabolic control.3,5 Also, the ability of the EVOO-enriched diet to prevent MMPs overactivity in the placenta and the umbilical cord blood observed in GDM patients is supported by previous studies addressing the effect of diets added with EVOO as negative regulators of MMPs in placentas from diabetic rats and in different human diseases15,38 and provides evidence of the capacity of the benefits of the EVOO-enriched diet to reach the foetal compartment.

5 | CONCLUSIONS

This randomized clinical study allowed identifying, for the first time, the ability of an EVOO-enriched diet in GDM pregnancies to reduce maternal triglyceridemia and weight gain, which are clinically relevant parameters, thus suggesting a putative benefit that should be addressed in larger populations. Also, this clinical study provides evidence of the capacity of an EVOO-enriched diet to induce placental anti-inflammatory effects, at least partly mediated by the activation/ regulation of PPAR pathways. Anti-inflammatory effects were also evidenced in the umbilical cord blood, suggesting benefits in the perinatal period and the offspring’s later life. Further studies addressing long-term effects of this dietary treatment will be needed to establish its clinical significance in the offspring’s later life.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

DGR: Protocol and project development, Data acquisition, Data analysis, Interpretation of the data. ED: Protocol and project development, Interpretation of the data, Manuscript editing. MVF and HLG: Protocol and project development, Manuscript editing. DF: Data acquisition and Data analysis. SBM and CAG: Project development, Manuscript editing. EC: Data acquisition, Data analysis, Manuscript editing. AJ: Conceptualization, Project development, Manuscript writing, Funding acquisition. All authors have read and approved the final manuscript.

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REFERENCES

1. Hod M, Kapur A, Sacks DA, et al. The international federation of gynecology and obstetrics (figo) initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet. 2015;131(Suppl 3):S173.
2. Monteiro LJ, Norman JE, Rice GE, Illanes SE. Fetal programming and gestational diabetes mellitus. Placenta. 2016;48(Suppl 1):S54-S60.
3. Lappas M, Hiden U, Desoye G, Froehlich J, Hauguel-de Mouzon S, Jawerbaum A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. Antioxid Redox Signal. 2011;15(12):3061-3100.
4. Perrone S, Santacroce A, Picardi A, Buonocore G. Fetal programming and early identification of newborns at high risk of free radical-mediated diseases. World J Clin Pediatr. 2016;5(2):172-181.
5. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta. 2015;36(7):709-715.
6. Jawerbaum A, Capobianco E. Review: effects of PPAR activation in the placenta and the fetus: implications in maternal diabetes. Placenta. 2011;32(Suppl 2):S212-S217.
7. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab. 2012;23(7):351-363.
8. Zendvain A, Deutsch MJ, Plosch T, Ensenauer R. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. Am J Physiol Endocrinol Metab. 2016;310(10):E797-E810.
9. Hiihi AK, Michalik L, Wahli W. PPARs: transcriptional effectors of fatty acids and their derivatives. Cell Mol Life Sci. 2002;59(5):790-798.
10. Moreno M, Lombardi A, Silvestri E, et al. PPARs: nuclear receptors controlled by, and controlling, nutrient handling through nuclear and cytosolic signaling. PPAR Res. 2010;2010:1-10.
11. Capobianco E, Gomez Ribot D, Fornes D, et al. Diet enriched with olive oil attenuates placental dysfunction in rats with gestational
diabetes induced by intrauterine programming. Mol Nutr Food Res. 2018;62(19):e1800263.
12. Higa R, Roberti SL, Musikan D, Mazzucco MB, White V, Jawerbaum A. Effects of maternal dietary olive oil on pathways involved in diabetic embryopathy. Reprod Toxicol. 2014;49C:185-195.
13. Kurtz M, Capobianco E, Careaga V, et al. Peroxisome proliferator-activated receptor ligands regulate lipid content, metabolism, and composition in fetal lungs of diabetic rats. J Endocrinol. 2014;220(3):345-359.
14. Kurtz M, Capobianco E, Martínez N, Roberti SL, Arany E, Jawerbaum A. PPAR ligands improve impaired metabolic pathways in fetal hearts of diabetic rats. J Mol Endocrinol. 2014;53(2):237-246.
15. Martínez N, Sosa M, Higa R, Fornes D, Capobianco E, Jawerbaum A. Dietary treatments enriched in olive and safflower oils regulate seric and placental matrix metalloproteinases in maternal diabetes. Placenta. 2012;33(1):8-16.
16. Capobianco E, Pelesson M, Careaga V, et al. Intrauterine programming of lipid metabolic alterations in the heart of the offspring of diabetic rats is prevented by maternal diets enriched in olive oil. Mol Nutr Food Res. 2015;59(10):1997-2007.
17. Holdsworth-Carson SJ, Lim R, Mitton A, et al. Peroxisome proliferator-activated receptors are altered in pathologies of the human placenta: gestational diabetes mellitus, intrauterine growth restriction and preeclampsia. Placenta. 2010;31(3):222-229.
18. Capobianco E, Martínez N, Fornes D, et al. PPAR activation as a regulator of lipid metabolism, nitric oxide production and lipid peroxidation in the placenta from type 2 diabetic patients. Mol Cell Endocrinol. 2013;377(1–2):7-15.
19. Zhao C, Zhang T, Shi Z, Ding H, Ling X. MicroRNA-518d regulates PPARalpha protein expression in the placenta of females with gestational diabetes mellitus. Mol Med Rep. 2014;9(6):2085-2090.
20. Lacatusu CM, Grigorescu ED, Floria M, Onofriescu A, Mihai BM. The Mediterranean diet: from an environment-driven food culture to an emerging medical prescription. Int J Environ Res Public Health. 2019;16(6):942. https://doi.org/10.3390/ijerph16060942.
21. Pirotti M, Albini A, Fabiani R, et al. Nutrigenomics of extra-virgin olive oil: a review. Biofactors. 2017;43(1):17-41.
22. Karamanos B, Thanopoulos A, Anastasiou E, et al. Relation of the Mediterranean diet with the incidence of gestational diabetes. Eur J Clin Nutr. 2014;68(1):8-13.
23. Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med. 2018;378(25):e334.
24. Wongwarawipat T, Papageorgiou N, Bertsias D, Siasos G, Tousoulis D. Olive oil-related anti-inflammatory effects on atherosclerosis: potential clinical implications. Endocr Metab Immune Disord Drug Targets. 2018;18(1):51-62.
25. Billingsley HE, Carbene S, Lavie CJ. Dietary fats and chronic non-communicable diseases. Nutrients. 2018;10(10):1385. https://doi.org/10.3390/nu10101385.
26. Assaf-Balut C, García de la Torre N, Duran A, et al. A Mediterranean diet with additional extra virgin olive oil and pistachios reduces the incidence of gestational diabetes mellitus (GDM): a randomized controlled trial: The St. Carlos GDM prevention study. PLoS One. 2017;12(10):e0185873.
27. Salzberg S, Alvaríñas J, López G, al e. Guías de diagnóstico y tratamiento de diabetes gestacional. ALAD 2016. Revista de la ALAD. 2016;6:155-169.
28. Capobianco E, Fornes D, Linenber I, Powell TL, Jansson T, Jawerbaum A. A novel rat model of gestational diabetes induced by intrauterine programming is associated with alterations in placental signaling and fetal overgrowth. Mol Cell Endocrinol. 2016;422:221-232.
29. Fornes D, White V, Higa R, Heinecke F, Capobianco E, Jawerbaum A. Sex-dependent changes in lipid metabolism, PPAR pathways and microRNAs that target PPARs in the fetal liver of rats with gestational diabetes. Mol Cell Endocrinol. 2018;461:12-21.
30. Yamamoto JM, Kellett JE, Balsells M, et al. Gestational diabetes mellitus and diet: a systematic review and meta-analysis of randomized controlled trials examining the impact of modified dietary interventions on maternal glucose control and neonatal birth weight. Diabetes Care. 2018;41(7):1346-1351.
31. Hernandez TL, Mande A, Barbou LA. Nutrition therapy within and beyond gestational diabetes. Diabetes Res Clin Pract. 2018;145:39-50.
32. Chester B, Babu JR, Greene MW, Geetha T. The effects of popular diets on type 2 diabetes management. Diabetes Metab Res Rev. 2019;35(8):e3188.
33. Franzago M, Fraticelli F, Stroppa L, Vitacolonna E. Nutrigenetics, epigenetics and gestational diabetes: consequences in mother and child. Epigenetics. 2019;14(3):215-235.
34. Lopez-Luna P, Ortega-Senovilla H, Lopez-Soldado I, Herrera E. Fate of orally administered radioactive fatty acids in the late-pregnant rat. Am J Physiol Endocrinol Metab. 2016;310(5):E367-E377.
35. Soto-Alarcon SA, Valenzuela R, Valenzuela A, Videlia A. Liver protective effects of extra virgin olive oil: interaction between its chemical composition and the cell-signaling pathways involved in protection. Endocr Metab Immune Disord Drug Targets. 2018;18(1):75-84.
36. Carbone S, Billingsley HE, Canada JM, et al. Unsaturated fatty acids to improve cardiopulmonary fitness in patients with obesity and HFpEF: the UFA-preserved pilot study. JACC Basic Translational Sci. 2019;4(4):563-565.
37. Croadell A, Duffney PF, Kim N, Lacy SH, Sime PJ, Phipps RP. PPARgamma and the innate immune system mediate the resolution of inflammation. PPAR Res. 2015;2015:549691.
38. Silva S, Combet E, Figueira ME, Koeck T, Mullen W, Bronze MR. New perspectives on bioactivity of olive oil: evidence from animal models, human interventions and the use of urinary proteomic biomarkers. Proc Nutr Soc. 2015;74(3):268-281.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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