Effects of natural ligands of PPARγ on lipid metabolism in placental tissues from healthy and diabetic rats

Evangelina Capobianco, Verónica White, Romina Higa, Nora Martínez and Alicia Jawerbaum

Laboratory of Reproduction and Metabolism, CEFYBO-CONICET, School of Medicine, University of Buenos Aires, Paraguay 2155, 17th floor, Buenos Aires C1121ABG, Argentina

1Correspondence address. E-mail: a.jawerbaum@abaconet.com.ar

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent nuclear receptor that plays an important role in placental development and function metabolism in diabetic and control rats after midpregnancy, as well as the concentrations of the PPARγ endogenous agonist 15deoxyD12,14Prostaglandin J2 (15dPGJ2). In vitro experiments showed that 15dPGJ2 did not regulate placental concentrations of triglycerides, cholesterol esters, phospholipids and free fatty acids, but decreased the de novo synthesis of these lipid species. PPAR agonists were administered in vivo through dietary supplementation with either 6% olive oil or 6% safflower oil. These treatments led to increases in placental lipid mass in control tissues and more markedly in diabetic tissues. In addition, they led to reductions in the de novo lipid synthesis both in control and in diabetic placental tissues. In the placenta from diabetic rats fed with the standard diet, 15dPGJ2 concentrations were greatly reduced. Both dietary supplementations increased the concentrations of 15dPGJ2 in placentas from control and diabetic rats. These data indicate that, in the placenta, PPARγ natural ligands regulate the concentration of their own endogenous ligands. In addition, they increase the placental capacity to accumulate maternal-derived lipids, and reduce the de novo lipid synthesis, thus regulating metabolic pathways that are altered in the placenta from diabetic rats and involved in the lipid transfer to the developing fetus.

Keywords: diabetes in pregnancy; lipids; placenta; PPARs; prostaglandins

Introduction

Diabetes mellitus is a disorder that affects the normal development of pregnancy (Schwartz and Teramo, 2000; Jawerbaum and Gonzalez, 2006). In association with both hyperglycaemia and dyslipaemia, poorly controlled maternal diabetes can lead to increased risks of spontaneous miscarriage, congenital malformations and neonatal morbidity and mortality (Kitzmiller et al., 1978; Eriksson et al., 2003). In addition, it can increase the susceptibility to obesity, diabetes and cardiovascular diseases in the offspring later in life (Van Assche et al., 2001).

The placenta is critical in guiding fetal development, and can present several developmental, morphological and functional derangements in a diabetic environment (Desoye and Shafrir, 1994; Saldeen et al., 2002; Radaelli et al., 2003). Increased fat accretion in both developing fetuses and placentas from diabetic mothers is in part the result of the impairments in maternal lipid metabolism (Diamant, 1991; Herrera and Amusquiar, 2000; Catalano and Kirwan, 2001). The placenta is critical in guiding fetal development, and can present several developmental, morphological and functional derangements in a diabetic environment (Desoye and Shafrir, 1994; Saldeen et al., 2002; Radaelli et al., 2003). Increased fat accretion in both developing fetuses and placentas from diabetic mothers is in part the result of the impairments in maternal lipid metabolism (Diamant, 1991; Herrera and Amusquiar, 2000; Catalano and Kirwan, 2001). The placenta governs the transfer of lipids from mother to fetus by regulating their transport and metabolism (Herrera et al., 2006). The placenta is endowed with several transport proteins and enzymes needed to metabolize, utilize or accumulate lipids and to direct lipid release to the fetal circulation (Haggarty, 2002; Duttaroy, 2004). Although the mechanisms that regulate lipid metabolism in the placenta are largely unknown, recent studies suggest that peroxisome proliferator-activated receptors (PPARs) may be involved in these regulatory pathways (Xu et al., 2007).

PPARs are ligand-activated nuclear transcription factors which are key regulators of lipid homeostasis and anti-inflammatory processes (Desvergne et al., 2004; Beaven and Tontonoz, 2006). There are three PPAR subtypes named PPARα, PPARγ and PPARβ, which form a functional transcriptional unit upon heterodimerization with retinoid X receptors and activation by their ligands (Hihi et al., 2002). Each PPAR subtype has particular tissue distributions, physiological functions and ligands. 15DeoxyD12,14Prostaglandin J2 (15dPGJ2) is an endogenous ligand of PPARγ, but can also activate PPARβ and have PPARγ-independent effects. Indeed, anti-inflammatory effects of 15dPGJ2 frequently involve PPARγ-dependent and -independent inhibition of NFKappaB signalling pathway (Forman et al., 1997; Straus and Glass, 2001). In addition, various fatty acids, including oxidized lipids, monounsaturated fatty acids such as oleic acid and polyunsaturated fatty acids such as linoleic acid, are PPARγ ligands (Kersten and Wahli, 2000; Barlic and Murphy, 2007). Linoleic acid is a precursor of arachidonic acid, which is both an agonist of PPARγ and a substrate of cyclooxygenases (COX-1 and COX-2), the rate-limiting enzymes in the formation of prostaglandins (Smith et al., 1996). On the other hand, PPARβ pharmacological ligands are the thiazolidinediones, usually employed to ameliorate altered lipid profiles and insulin resistance in metabolic syndrome (Forman et al., 1997; Desvergne et al., 2004).

PPARγ controls the expression of multiple genes involved in lipid metabolism (Desvergne et al., 2004). It is highly expressed in adipose
tissue, and is an important determinant of adipocyte differentiation, lipid storage and metabolism (Forman et al., 1995; Lee et al., 2003). In addition, this nuclear receptor is crucial for the proper development of the labyrinth layer of the placenta and for the establishment of proper vascularity within the labyrinth. PPARγ null mice present alterations that lead to embryonic lethality at embryonic age 10.5 (Barak et al., 1999). At this developmental stage (equivalent to embryonic age 12 in rats), mother-to-fetus blood circulation is initiated, and an elevated placental PPARγ expression is observed (Asami-Miyagishi et al., 2004; Ain et al., 2006).

There is evidence for PPARγ function in placental development (Fournier et al., 2007b). PPARγ induces differentiation, modifies invasiveness and regulates endocrine function in human cytotrophoblasts (Schaiff et al., 2006; Fournier et al., 2007a). In addition, recent studies suggest that PPARγ may also regulate important functions related to the transfer of lipids through the placenta (Xu et al., 2007). Indeed, PPARγ up-regulates fatty acid uptake and the expression of the lipid droplet-associated protein adipophilin in cultured trophoblasts (Bildirici et al., 2003; Desmarais et al., 2007; Schaiff et al., 2007).

We have previously studied the modulation of lipid synthesis in rat term placenta and found that 15dPGJ2 regulates the de novo lipid synthesis through mechanisms that involve PPARγ activation (Capobianco et al., 2005). Besides, we have found reduced 15dPGJ2 concentrations in term placenta from diabetic patients and from rats with a diabetic condition induced through neonatal administration of streptozotocin (Jawerbaum et al., 2004; Capobianco et al., 2005).

The aim of this study was to improve the understanding of the capacity of endogenous PPARγ ligands to regulate lipid metabolism in the placenta after the initiation of mother-to-fetus circulation under both normal and diabetic environments. Therefore, we evaluated 15dPGJ2 capacity to modulate placental lipid metabolism after midgestation in healthy and diabetic rats. In addition, we analysed 15dPGJ2 concentrations and lipid metabolism in control and diabetic rats fed with a standard diet or either an olive oil- or a safflower oil-supplemented diet. The purpose of the use of these diets was to analyse the effects of dietary enrichment in unsaturated fatty acids capable of activating PPARs as well as in polyunsaturated fatty acids that are sources of arachidonic acid and thus of the synthesis of prostaglandins.

Materials and Methods

Animals and treatments

Albino Wistar rats were bred in the laboratory with free access to commercial rat chow (Asociación Cooperativa Argentina, Buenos Aires, Argentina) and water, in a lighting cycle of 14 h light:10 h dark. At 2 days of age, neonates were injected with either streptozotocin (90 mg/kg s.c.) (Sigma-Aldrich, St Louis, MO, USA) in citrate buffer (0.05 M, pH 4.5) (diabetic experimental model) or buffer alone (controls) (Portha et al., 1979). The reproductive characteristics of this diabetic experimental model have been reported previously (Jawerbaum and Gonzalez, 2005). In the evening of proestrus, control and diabetic females weighing between 200 and 300 g were caged overnight with control males. Mating was confirmed by the presence of sperm in vaginal smears. When a positive pregnancy was identified, this was designated as Day 0.5 of gestation. The guidelines for the care and use of animals approved by the local institution were followed, according to the ‘Principles of laboratory animal care’ (NIH publication No. 85-23, revised 1985, http://grants1.nih.gov/grants/olaw/references/olawpol.htm). On Day 0.5 of gestation, control and diabetic rats were randomized into three groups: (i) rats fed ad libitum with the commercial rat chow (standard diet), (ii) rats fed ad libitum with the standard diet supplemented with 6% olive oil and (iii) rats fed ad libitum with the standard diet supplemented with 6% safflower oil. Composition of the standard and oil-supplemented diets is reported in Table I. The three diets met the nutritional requirements of calories, fat, carbohydrates and proteins (Council SoLANNR, 1995). The olive oil-supplemented diet is enriched mostly in oleic acid (35.4%) as well as in palmitic acid (16.7%) and linoleic acid (22%), whereas the safflower oil-supplemented diet is enriched mostly in linoleic acid (22%) as well as in palmitic acid (67%) and oleic acid (42%), when compared with the standard diet (Table I). Animals were killed by cervical dislocation on Day 13.5 of pregnancy. Uteri were transferred to Petri dishes with Krebs Ringer bicarbonate (KRB) solution: 5.5 mM glucose, 145 mM Na+, 2.2 mM Ca2+, 1.2 mM Mg2+, 127 mM Cl−, 25 mM HCO3−, 1.2 mM SO42− and 1.2 mM PO43−. Whole placentas were dissected carefully by removing extraembryonic and fetal membranes and fetuses, and then washed three times in cold KRB and immediately weighed. Thereafter, placental explants were either frozen at −70°C or cultured as described below until evaluation of 15dPGJ2 concentrations and analysis of the synthesis and concentrations of free fatty acids and sterified lipids (triglycerides, phospholipids and cholesteryl esters). Rat glycaemia was measured with glucostix reagent strips and a glucometer in blood obtained from the tail vein (Bayer Diagnostics, Buenos Aires, Argentina). Rat triglyceridaemia was measured by a commercial enzymatic kit (Wiener Lab, Rosario, Argentina) in serum obtained through centrifugation of blood from the aorta artery.

Enzyme immunoasay of 15dPGJ2

Placental tissues (100 mg wet weight), including both the labyrinth and the junctional zones, were obtained at Day 13.5 of gestation from control and diabetic rats under the different dietary treatments and stored at −70°C until the determination of 15dPGJ2 concentrations. By employing a commercial enzyme immunoassy kit (Assay Design Co., Ann Arbor, MI, USA), 15dPGJ2 was measured, as previously (Capobianco et al., 2005). The minimum detectable concentration of 15dPGJ2 in this assay is 37 pg/ml. Cross-reactivity is detectable for 15dPGJ2 (100%), PGJ2 (49.2%), but <0.01% for other PGs according to the manufacturer’s information. Placental tissues (n = 7–8 rats in each experimental group) were homogenized in phosphate buffer saline, and an aliquot separated for protein determination by Bradford method using a protein assay reagent (Bio-Rad Laboratories Inc., CA, USA). Prostaglandins were extracted twice in absolute ethanol. The extracts were dried in a Savant (Hicksville, NY, USA) Speed-Vac concentrator and reconstituted with 50 μl ethanol and 200 μl of assay buffer provided by the commercial kit. Briefly, the kit uses a polyclonal antibody against 15dPGJ2 that binds in a competitive manner, either the prostaglandin in the sample or an alkaline phosphatase molecule, which has 15dPGJ2 covalently attached to it. After a simultaneous incubation, a p-nitrophenyl phosphate substrate was added, and the yellow colour generated was evaluated on a microplate reader at 405 nm.

Lipid concentrations assessment

Lipid concentrations were assessed in placental explants obtained from control and diabetic rats on Day 13.5 of gestation that were incubated in 1 ml KRB for 3 h in a metabolic shaker under an atmosphere of 5% CO2 and 95% O2 at 37°C, either with or without the addition of 15dPGJ2 2 μM (Cayman Chemical Co., Ann Arbor, MI, USA), as previously (Capobianco et al., 2005). No changes in lactate dehydrogenase concentrations were observed in the placental explant culture medium for the 3 h culture period. Lipid concentrations were also assessed in placentas explanted on Day 13.5 of gestation from control and diabetic rats fed with the different dietary treatments. Tissues were stored at −70°C until the lipid concentration analysis. In all cases, lipid concentrations were determined by thin layer chromatography (TLC), as previously described (Capobianco et al., 2005). Briefly, placental lipids were extracted in methanol–chloroform 2:1 (v/v) and then concentrated in a Savant (Hicksville) Speed-Vac concentrator. Total lipids were chromatographed with a solvent system consisting of hexane:ethyl ether:acetic acid 80:20:2 v/v. After development, the TLC plate was dried for 5 min and the lipids were stained with iodine vapours. Lipid species levels were quantified by comparison with known amounts of pure lipid standards run on the same plate. Intra-assay variations were <10%. The plates were scanned and analysed by densitometry using the Sigma Gel Program (Sigma-Aldrich).
De novo lipid synthesis analysis

Lipid synthesis was assessed in placentas explanted on Day 13.5 of gestation from control and diabetic rats fed with the different dietary treatments. Placentas (100 mg wet weight) were incubated in 1 ml KRB for 3 h added with 1 mCi 14C-acetate (53 mCi/mmol) (Amersham Biosciences, Arlington Height, IL, USA), and either with or without the addition of 15dPGJ2 2 μM, in a metabolic shaker under an atmosphere of 5% CO2 and 95% O2 at 37°C. After incubations, placental tissues were stored at -70°C until determination of the newly formed radioactive lipids as previously (Jawerbaum et al., 2002). Lipids in the samples, the standards and the internal control consisting of placental tissue incubated with no radioactivity were separated by TLC as described above. The radioactive spots corresponding to the different 14C-labelled lipid species were scrapped into vials and counted in a liquid scintillation counter.

Statistical analyses

All data are presented as mean ± SEM. Differences between the groups were compared using Student’s t-test, one- or two-way ANOVA with Tukey’s post hoc tests. A P-value of <0.05 was considered statistically significant.

Results

Effect of 15dPGJ2 on placental lipid metabolism

In order to analyse the influence of the PPARγ agonist 15dPGJ2 on the concentrations of esterified lipids (triglycerides, cholesteryl esters and phospholipids) and free fatty acids, placentas obtained from control and diabetic rats on Day 13.5 of gestation were cultured for 3 h in the presence or absence of 15dPGJ2 (2 μM). Placental lipid levels were then analysed. Placentas from diabetic rats showed increased levels of triglycerides (P < 0.01) and cholesteryl esters (P < 0.01) and no changes in the concentrations of the other lipid species analysed, when compared with controls (Fig. 1). No changes were observed in the levels of triglycerides, phospholipids, cholesteryl esters and free fatty acids when 15dPGJ2 was added to the culture media of placentas obtained from both control and diabetic rats.

![Figure 1: Effect of 15dPGJ2 additions on the levels of (A) triglycerides, (B) cholesteryl esters, (C) phospholipids and (D) free fatty acids in placental explants obtained from control and diabetic rats on Day 13.5 of gestation.](https://academic.oup.com/molehr/article-abstract/14/8/491/1017546)
To evaluate whether 15dPGJ2 modulates the de novo synthesis of sterified lipids (triglycerides, cholesteryl esters and phospholipids) and free fatty acids, placentas obtained from control and diabetic rats were cultured for 3 h in the presence of 14C-acetate as a tracer, either with or without 15dPGJ2 (2 μM) additions. Formation of radioactive lipids was then analysed. We found that 15dPGJ2 additions reduced the de novo synthesis of all lipid species analysed in the placentas from control rats (triglycerides, \( P < 0.01 \); cholesteryl esters, \( P < 0.01 \); phospholipids, \( P < 0.001 \) and free fatty acids, \( P < 0.001 \)) (Fig. 2). When control and diabetic tissues cultured with no additions were compared, we found a reduction in the de novo synthesis of triglycerides (\( P < 0.001 \), cholesteryl esters (\( P < 0.001 \), phospholipids (\( P < 0.01 \)) and free fatty acids (\( P < 0.001 \)) in placentas from diabetic rats. Despite these reduced basal levels, 15dPGJ2 was also able to reduce the de novo synthesis of triglycerides (\( P < 0.01 \), cholesteryl esters (\( P < 0.05 \) and phospholipids (\( P < 0.05 \) in placentas from diabetic rats (Fig. 2).

**Effects of the oil-supplemented diets on glycaemia, triglyceridaemia and placental and fetal weight**

Diet enriched in oleic acid and linoleic acid were created by supplementing commercial normal rat chow with 6% olive oil or 6% safflower oil (see details of fatty acid composition in Materials and Methods section, Table I). Control and diabetic rats were fed with the standard diet (commercial rat chow) or the oil-supplemented diets from Days 0.5 to 13.5 of gestation. Glycaemia, triglyceridaemia, energy intake, pregnancy weight gain, placental weight and fetal weight were similar in the oil-supplemented and non-supplemented control groups (Table II). Diabetic rats fed with the different diets showed hyperglycaemia (\( P < 0.001 \), hypertriglyceridaemia (\( P < 0.05 \), elevated energy intake (\( P < 0.05 \), similar weight gain during pregnancy (Days 0.5–13.5 of gestation) and similar placental and fetal weights when compared with their respective controls. No changes in glycaemia, triglyceridaemia, energy intake, pregnancy weight gain, placental weight and fetal weight were found when the oil-supplemented and non-supplemented diabetic groups were compared (Table II).

**Placental 15dPGJ2 concentrations under the dietary treatments**

The concentration of the endogenous PPARγ agonist 15dPGJ2 was analysed in placentas obtained on Day 13.5 of pregnancy from control and diabetic rats fed with the standard diet or the oil-supplemented diets. In placentas from diabetic rats fed with the standard diet, 15dPGJ2 was greatly reduced when compared with their controls fed with the same diet (\( P < 0.001 \)) (Fig. 3). Interestingly, both olive and safflower oil-supplemented diets greatly increased 15dPGJ2 concentrations in placentas from both control (\( P < 0.01 \) and diabetic rats (\( P < 0.001 \) when compared, respectively, with control and diabetic rats fed with the standard diet. Thus, there were no changes in 15dPGJ2 concentrations under the dietary treatments.

**Figure 2**: Effect of 15dPGJ2 additions on the de novo synthesis of (A) triglycerides, (B) cholesteryl esters, (C) phospholipids and (D) free fatty acids in placental explants obtained from control and diabetic rats on Day 13.5 of gestation. Placental explants were cultured either with or without the addition of 15dPGJ2 2 μM for 3 h in KRB in the presence of 1 μCi 14C-acetate (53 mCi/mmol) followed by evaluation of the incorporation of the tracer to lipids. Values are means ± SEM; \( n = 8 \) rats per group. One-way ANOVA with Tukey’s post hoc tests was performed. Different letters indicate significant differences between the groups (\( P < 0.05 \)).
concentrations when placentas from control and diabetic rats fed with the oil-supplemented diets were compared (Fig. 3).

Effects of the oil-supplemented diets on placental lipid concentrations

The concentrations of sterified lipids (triglycerides, phospholipids, and cholesteryl esters) and free fatty acids were analysed in placentas obtained on Day 13.5 of pregnancy from control and diabetic rats fed with the standard diet alone or the oil-supplemented diets. As observed in the studies performed in vitro, when placentas from control and diabetic rats fed with the standard diet were compared, higher triglyceride concentrations were found in placenta from diabetic rats (Table II).

When control rats were fed with the olive oil-supplemented diet, there were no changes in the concentrations of the lipids analysed when compared with placentas from control rats fed with the standard diet. When diabetic rats were fed with the olive oil-supplemented diet, placental triglyceride concentrations were increased when compared with placentas from diabetic rats fed with the standard diet (Fig. 4). On the other hand, when control rats were fed with the safflower oil-supplemented diet, an increase in the placental concentrations of triglycerides (P < 0.05) and cholesteryl esters (P < 0.01) was found when compared with placentas from control rats fed with the standard diet.

Table II. Characteristics of the animals fed from Days 0.5 to 13.5 of gestation with a standard diet alone or with a standard diet supplemented with 6% olive oil or 6% safflower oil.

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<th>Standard diet</th>
<th>Standard diet supplemented with 6% olive oil</th>
<th>Standard diet supplemented with 6% safflower oil</th>
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<tr>
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<td>Control</td>
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<td>Glycaemia (mg/dl)</td>
<td>100 ± 10a</td>
<td>230 ± 21b</td>
<td>91 ± 3a</td>
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<td></td>
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<td>204 ± 11b</td>
<td>86 ± 3a</td>
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<td>Triglyceridemia (g/l)</td>
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<td>2.0 ± 0.3b</td>
<td>0.7 ± 0.2a</td>
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<td>1.7 ± 0.2b</td>
<td>0.6 ± 0.1a</td>
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<tr>
<td>Energy intake (kcal/day)</td>
<td>55 ± 9a</td>
<td>103 ± 11b</td>
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<td>89 ± 8b</td>
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<td>Weight gain (Days 0.5–13.5 of pregnancy, g)</td>
<td>46 ± 4a</td>
<td>48 ± 6a</td>
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<td>43 ± 4a</td>
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<td>Placental weight (mg)</td>
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Values are means ± SEM. Students' t test, one- and two-way ANOVA with Tukey's post hoc tests were performed. Different letters indicate significant differences between the groups (P < 0.05).
(triglycerides, phospholipids and cholesteryl esters) and free fatty acids in placentas from control and diabetic rats, placental tissues explanted on Day 13.5 of gestation from control and diabetic rats fed with the different diets were cultured for 3 h in the presence of 14C-acetate as a tracer. Formation of radioactive lipids was then analysed. When placentas from control and diabetic rats fed with the standard diet were compared, there was a reduction in the synthesis of triglycerides \( (P < 0.001) \), cholesteryl esters \( (P < 0.001) \), phospholipids \( (P < 0.01) \) and free fatty acids \( (P < 0.001) \) in placentas from diabetic rats (Fig. 5).

When control rats were fed with the olive oil-supplemented diet, the synthesis of phospholipids \( (P < 0.01) \) and free fatty acids \( (P < 0.05) \) in the placenta was reduced when compared with placentas from control rats fed with the standard diet. Differently, when diabetic rats were fed with the olive oil-supplemented diet, no changes in the synthesis of lipids analysed were found when compared with placentas from diabetic rats fed with the standard diet (Fig. 5). On the other hand, when control rats were fed with the safflower oil-supplemented diet, the synthesis of cholesteryl esters \( (P < 0.01) \), phospholipids \( (P < 0.01) \) and free fatty acids \( (P < 0.001) \) was reduced in control placentas when compared with placentas from control rats fed with the standard diet. Moreover, all lipid species evaluated were reduced in placentas from diabetic rats treated with safflower oil when compared with placentas from diabetic rats fed with the standard diet (triglycerides, \( P < 0.001 \); cholesteryl esters, \( P < 0.05 \); phospholipids, \( P < 0.01 \) and free fatty acids, \( P < 0.01 \)) (Fig. 5).

**Discussion**

In the present work, we found reduced concentrations of the PPARγ endogenous agonist 15dPGJ2 in placentas from diabetic rats fed with a standard diet compared with control rats on a standard diet. We also found that olive oil- and safflower oil-supplemented diets were able to increase the concentrations of 15dPGJ2, and to modulate both the accumulation and the synthesis of different lipid species in placentas from both control and diabetic rats.

Several studies have shown that 15dPGJ2 has important properties as a regulator of lipid homeostasis and anti-inflammatory processes often exerted through the activation of PPARγ in different tissues (Straus and Glass, 2001; Jawerbaum and Gonzalez, 2005; Scher and Pillinger, 2005). Studies performed in human and rat gestational tissues have shown 15dPGJ2 capacity to control the formation of pro-inflammatory mediators (Lappas et al., 2002; Jawerbaum et al., 2004; Ackerman et al., 2005). Besides, in human...
placental trophoblasts, 15dPGJ2 regulates differentiation and endo-
crine function (Schaiff et al., 2006; Fournier et al., 2007b).

Maternal diabetes leads to impairments in the production of prosta-
glandins in several tissues (Reece and Eriksson, 1996; Jawerbaum and
Gonzalez, 2005). We have previously found reductions in 15dPGJ2
concentrations in embryos from diabetic rats during early organogen-
esis as well as in term placentas from diabetic rat models and diabetic
patients (Jawerbaum et al., 2002, 2004). Interestingly, in this work, we
found that 15dPGJ2 concentrations were reduced in placentas from
diabetic animals after midpregnancy, but that they were markedly
increased under both olive oil and safflower oil dietary treatments.
This increase may be the result of the increased linoleic acid concen-
trations both in the safflower oil-supplemented diet (226%) and in the
olive oil-supplemented diet (21%), as linoleic acid can lead to the gen-
eration of arachidonic acid, the substrate for the synthesis of 15dPGJ2.
Nevertheless, further research to confirm these changes by mass spec-
trometry would be needed. In agreement with our results, studies per-
formed in pregnant rats fed with diets supplemented with 10% olive
oil have shown increased serum levels of arachidonic acid (Amusqui-
var and Herrera, 2003). Moreover, studies in pregnant rats that
received oral treatments with safflower oil during early organogenesis
have shown increases in serum concentrations of arachidonic acid and
reductions in the malformation rate (Reece et al., 1996). Other PPAR
agonists have also been related to the regulation of prostaglandin gen-
eration. Indeed, PPARβ activation stimulates PGE2 production and
proliferation in human cholangiocarcinoma cells (Xu et al., 2006),
whereas carbaprostacyclin, a PPARβ agonist, increases embryonic
concentrations of PGE2, a prostaglandin required for the process of
closure of the neural tube (Higa et al., 2007).

In this work, we focused on PPARγ involvement in lipid metab-
olism and analysed the capacity of 15dPGJ2 and oil-supplemented
dietary treatments to regulate lipid concentrations and the
*de novo* lipid synthesis in the placenta. The oil-supplemented diets did not
modify glycaemia, triglyceridaemia or energy intake in controls and
diabetic rats. As expected, increases in glycaemia, triglyceridaemia
and energy intake were found in diabetic rats, well-documented
facts in the diabetic disease, related to the insulin deficiency and the
diminished capacity of carbohydrate utilization (Krishnamachar and
Canolty, 1986). In previous studies performed in rat term placentas
(Capobianco et al., 2005), and also in this study, 15dPGJ2 additions
did not modify placental lipid mass. Differently, olive and safflower
oil-supplemented diets led to placental accumulation of different
lipid species, an effect that was more marked in the safflower oil-
supplemented diet and in the diabetic condition. The fact that the
effects of PPAR agonists on placental lipid accumulation were
evident *in vivo* but not *in vitro* and greater in the diabetic placenta

![Figure 5](https://academic.oup.com/molehr/article-abstract/14/8/491/1017546/1017546)
suggestions that this increase is sustained from the lipids provided from maternal circulation, which are higher in maternal diabetes. As both olive and safflower oil-supplemented diets contain lipids that can activate the three PPAR isotypes, the involvement of each PPAR isotype should be established. Nevertheless, as suggested by recent evidence, placental lipid accumulation may be mediated by the activation of PPARγ. Indeed, recent studies have found that PPARγ agonists increase lipid content, expression of transport proteins involved in the uptake of lipids and expression of the lipid droplet-associated protein adipophilin in trophoblast cells (Bildirici et al., 2003; Schaff et al., 2005; Desmarais et al., 2007). Moreover, studies where rosiglitazone, a pharmacological PPARγ agonist, was administered in mice from Days 10.5 to 18.5 of pregnancy have also shown increased placental lipid concentrations (Schaiff et al., 2003; Schaiff et al., 1999). Also, the absence of lipid droplets is characteristic of placentas from PPARγ-null mice (Barak et al., 1999).

Concerning the placental de novo lipid synthesis, in previous studies, we have found that maternal diabetes induces alterations in this anabolic pathway and that 15dPGJ2 reduces this synthesis (White et al., 2004; Capobianco et al., 2005). In this work, the capacity of reducing the de novo placental lipid synthesis was observed not only in vivo in the presence of the PPARγ agonist 15dPGJ2, but also in the experimental groups treated in vitro with diets containing oils capable of stimulating PPARγ. These observed effects may occur both through PPARγ activation caused by the increased levels of mono and polyunsaturated fatty acids and through the formation of 15dPGJ2 that occurs under the two evaluated diets. Although 15dPGJ2 and unsaturated fatty acids can have PPARγ-independent effects (Straus and Glass, 2001; Schroeder et al., 2008), our previous studies have shown that 15dPGJ2 effects on the de novo lipid synthesis are not observed in the presence of a PPARγ antagonist in term placentas (Capobianco et al., 2005). Moreover, initial results performed in placentas obtained on Day 13.5 of gestation also showed that 15dPGJ2 effects on the de novo lipid synthesis were not observed in the presence of the PPARγ antagonist GW 9662 (data not shown).

In different tissues and cell types, both progestagenins and fatty acids have been found to control their own metabolism as a result of various positive loop mechanisms mediated by the activation of PPARs (Schoonjans et al., 1996; Xu et al., 2006; Schroeder et al., 2008). This positive feedback regulation results in the accumulation of lipids in adipocytes (Lee et al., 2003) and, as suggested by the results of this work, also in the accumulation of placental lipids provided by maternal circulation, which occur together with reductions in the endogenous synthesis of placental lipids. One of the protective functions of the placenta may rely on its capacity to control the endogenous lipid synthesis and to accumulate those lipids provided in excess by maternal serum, in order to prevent an increased transfer to the fetus. Therefore, studies are needed to address whether the changes observed under the diets enriched in lipid species that activate PPARs are beneficial for the developing fetus.

In conclusion, the results of this work provide novel evidence of the capacity of PPARγ agonists in the placenta to increase the concentrations of PPARγ endogenous ligand 15dPGJ2 to reduce the de novo lipid synthesis and to accumulate maternal-derived lipids, leading to a placenta with a greater capacity to accumulate lipids in adverse situations such as the diabetic environment.

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