Modulation of nitric oxide concentration and lipid metabolism by 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 in embryos from control and diabetic rats during early organogenesis

A. Jawerbaum, D. Sinner, V. White, C. Pustovrh,
E. Capobianco and E. Gonzalez

*Centro de Estudios Farmacologicos y Botánicos (CEFYBO),
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),
Serrano 669, (1414) Buenos Aires, Argentina*

The concentration of 15-deoxy $\Delta^{12,14}$ PGJ₂ (15dPGJ₂) and its effects on nitric oxide generation and neutral lipid in embryos from control and neonatal streptozotocin-induced (n-stz) diabetic rats during organogenesis were investigated. 15dPGJ₂ is produced in embryos during organogenesis, and its production is lower in embryos of n-stz diabetic rats than in embryos from control rats. Nitrate and nitrite concentrations were higher in embryos from n-stz diabetic rats and were reduced in the presence of 15dPGJ₂ both in embryos from control and diabetic rats. Thus, decreased 15dPGJ₂ concentrations in embryos from n-stz diabetic rats may be related to the high nitric oxide concentrations found in those embryos. Exogenous 15dPGJ₂ decreased cholesterol and cholesteryl ester concentrations

in embryos from control and n-stz diabetic rats, and reduced triacylglycerol concentrations in control embryos. Incorporation of [¹⁴C]acetate into lipids showed decreased *de novo* synthesis of cholesteryl ester and triacylglycerides in embryos from n-stz diabetic rats compared with controls. Exogenous 15dPGJ₂ reduced the incorporation of [¹⁴C]acetate into triacylglycerides, cholesterol and cholesteryl ester in embryos from both control and n-stz diabetic rats. 15dPGJ₂ is present in embryos during organogenesis, and reduces embryonic nitric oxide production and lipid synthesis. The lower 15dPGJ₂ concentration in embryos from n-stz diabetic rats may result in developmental alterations in this diabetic model.

Introduction

The cyclopentenone, 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 (15dPGJ₂), is a metabolite of prostaglandin D₂ (PGD₂). In contrast to classical prostaglandins, which bind to cell surface G protein-coupled receptors, 15dPGJ₂ is a natural ligand of a nuclear receptor, the peroxisome-proliferator activated receptor gamma (PPAR γ) (Forman *et al.*, 1995; Kliewer *et al.*, 1995). PPAR γ behaves as a ligand-activated transcription factor through its DNA binding domain, which recognizes response elements in the promoter of specific target genes linked to apoptosis, cell proliferation, differentiation, inflammation, lipid metabolism and glucose homeostasis (Kliewer and Wilson, 1998). PPAR γ was identified initially in differentiated adipocytes, and its role has been determined in relation to the pathogenesis of insulin resistance because members of the thiazolidinedione class of antidiabetic drugs such as troglitazone have been identified as a ligand of PPAR γ (Lehmann *et al.*, 1995).

In addition to hyperglycaemia, type 2 diabetic indi-

viduals almost invariably manifest a serious breakdown in lipid metabolism, often reflected by increased concentrations of circulating free fatty acids and triacylglycerides, together with excessive fat deposition in various tissues including muscle (McGarry, 2002). Such alterations are ameliorated by thiazolidinedione treatment (Mimura *et al.*, 1994; Cha *et al.*, 2001). Lipids play an essential role in embryonic growth and development as components of the newly formed cell membranes, oxidative fuels that support embryo growth and possibly as signalling molecules (Herz and Farese, 1999). Indeed, defects in endogenous cellular biosynthesis, receptor-mediated endocytosis and receptor-mediated transfer of neutral lipids, either through the effect of pharmacological agents or through the analysis of induced or naturally occurring genetic mutations, lead to disrupted development (Barbu *et al.*, 1984; Irons *et al.*, 1993; Farese *et al.*, 1996).

There is an increased incidence of congenital malformations in the offspring of diabetic patients and in experimental models of diabetes (Kitzmiller *et al.*, 1978; Eriksson *et al.*, 1991). Although it is clear that alterations in the synthesis of the lipid components of the pulmonary complexes in fetuses from diabetic mothers at term have been related to an increased incidence of respiratory

distress syndrome (Singh and Feigelson, 1983), little is known about the modulatory factors that influence lipid metabolism during early organogenesis, the period when most malformations are induced.

Administration of streptozotocin to 2-day-old neonates, a non-insulin-dependent diabetic model, results in reproductive abnormalities. Adult diabetic rats are mildly hyperglycaemic, mildly hypoinsulinaemic and glucose intolerant (Portha *et al.*, 1979). During pregnancy, uterine contractile activity and lipid metabolism are impaired, and there is an abnormal production of prostanoid in uterine, placental and amniotic tissues (Jawerbaum *et al.*, 1994). There are also increased resorption rates and enhanced PGE₂ production in embryos obtained from n-stz diabetic rats during organogenesis (Jawerbaum *et al.*, 1998). Indeed, these high concentrations of PGE₂ appear to be the result of an overproduction of nitric oxide (NO) that positively modulates PGE₂ generation (Jawerbaum *et al.*, 1998). The increased concentrations of NO in embryos from diabetic rats are probably related to the appearance of malformations. In fact, NO concentrations are tightly regulated, as NO mediates apoptosis in specific types of cell during embryo development, and is teratogenic both in deficit and in excess (Lee and Juchau, 1994).

15dPGJ₂ is a potent anti-inflammatory agent that represses the expression of a number of inflammatory response genes in activated macrophages, including the genes encoding pro-inflammatory cytokines, inducible NO synthase (iNOS) and cyclooxygenase 2 (COX-2) (Ricote *et al.*, 1998). The nuclear factor kappaB (NF-κB) and its activating kinase are key targets for the anti-inflammatory activity of 15dPGJ₂, which inhibits NF-κB-mediated transcriptional activation by PPARγ-dependent and -independent molecular mechanisms (Ricote *et al.*, 1998; Rossi *et al.*, 2000). There are reduced concentrations of 15dPGJ₂ in β cells isolated from diabetic rats, and addition of 15PGJ₂ inhibits NO and PGE₂ production, ameliorating the inflammatory response that leads to β-cell destruction (Gonzalez *et al.*, 2001). In the present study, 15dPGJ₂ concentration and the effect of exogenous 15dPGJ₂ on NO concentration, the lipid profile and the synthesis of labelled lipids from [¹⁴C]acetate in embryos obtained from control and neonatal streptozotocin-induced (n-stz) diabetic rats during early organogenesis were evaluated as part of an attempt to identify an endogenous inducer of NO overproduction and anomalies of lipid metabolism in embryos from diabetic rats.

Materials and Methods

Materials

Streptozotocin and citrate buffer were obtained from Sigma (St Louis, MO). Glucostix reagent strips were obtained from Bayer Diagnostics (Buenos Aires).

[1,2-¹⁴C]acetic acid, sodium salt was purchased from New England Nuclear Dupont (Boston, MA). 15-Deoxy

Δ^{12,14}prostaglandin J₂ Enzyme Immunoassay Kit was purchased from Assay Designs Inc. (Ann Arbor, MI). Nitrate-nitrite assay kit was purchased from Cayman Chemical Co. (Ann Arbor, MI). Phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, phosphatidyl inositol, triacylglycerol, fatty acids, cholesteryl ester and cholesterol standards were purchased from Doosan Serdary Research Laboratories (Yongin). Thin-layer chromatography (TLC) plates Silica gel 60 were obtained from Merck (Darmstadt).

Animals

Albino Wistar rats bred in the laboratory were fed Purina rat chow *ad libitum*. At 2 days of age the neonates from rats bred in the laboratory were injected i.p. with either 100 mg streptozotocin kg⁻¹ in 0.05 mol citrate buffer l⁻¹, pH 4.5 or with buffer alone (controls). Four days after birth, neonates exhibiting glycosuria (> 500 mg glucose dl⁻¹) (Diastix reagent strips; Bayer Diagnostics) were considered diabetic. Spontaneous response to this treatment led to a non-insulin-dependent diabetic state in the adult, which was stable and chronic, as described by Portha *et al.* (1979). The adult diabetic state was confirmed by means of glucose tolerance tests (Maloff and Boyd, 1986). Glycaemia was determined in blood from tail veins by Glucostix reagent strips (Bayer Diagnostics) and a glucometer. Basal blood glucose concentrations of diabetic rats were 215 ± 21 mg dl⁻¹, whereas blood glucose concentrations of control rats were 100 ± 10 mg dl⁻¹. Two hours after a glucose challenge (1.5 g glucose kg⁻¹ i. p.) blood glucose concentrations of diabetic rats were > 250 mg dl⁻¹, whereas blood glucose concentrations of control rats were < 110 mg dl⁻¹. Control rats were injected with buffer alone. Control and n-stz diabetic rats were caged overnight with normal male rats. Mating was confirmed by the presence of spermatozoa in vaginal smears. When a positive pregnancy was identified, this was considered day 0.5 of gestation. The guidelines for the care and use of animals approved by the local institution were followed, according to *Principles of Laboratory Animal Care* (NIH publication number 85-23, revised 1985).

Embryo preparations

Animals were killed by cervical dislocation on day 10.5 of pregnancy and the uterus of each animal was transferred to a Petri dish containing Krebs-Ringer bicarbonate (KRB) solution: 11.0 mmol glucose l⁻¹, 145 mmol Na⁺ l⁻¹, 2.2 mmol Ca²⁺ l⁻¹, 1.2 mmol Mg²⁺ l⁻¹, 127 mmol Cl⁻ l⁻¹, 25 mmol HCO₃⁻ l⁻¹, 1.2 mmol SO₄²⁻ l⁻¹ and 1.2 mmol PO₄³⁻ l⁻¹. A stereomicroscope and watchmaker forceps were used to remove the balls of decidual tissue from each uterus and to open them carefully to free the conceptuses. The embryos were dissected out of the yolk sacs and evaluated morphologically under a stereomicroscope. Viability was established by the presence of a beating heart. The embryos were categorized as morphologically normal or as showing neural tube defects or other malformations. Embryonic growth was quantified by direct measurement of

Table 1. 15-deoxy $\Delta^{12,14}$ PGJ₂ concentrations and morphological characteristics of embryos from control and n-stz diabetic rats on day 10.5 of gestation

	Embryos from control rats	Embryos from diabetic rats
Resorptions (embryos)	1% (1/127)	10% ^b (17/165)
Malformations (embryos)	1% (1/126)	10% ^b (15/148)
Number of somites (embryos)	14 ± 1 (64)	12 ± 0.5 (73)
Protein content (µg) (embryos)	66 ± 4 (21)	50 ± 2 ^a (21)
15-deoxy $\Delta^{12,14}$ PGJ ₂ (pg per µg embryonic protein) (number of rats)	22.6 ± 3.6 (8)	9.1 ± 2 ^a (8)

Values are percentage of embryos (chi-squared statistics) or means ± SEM. (Student's *t* test) for embryos obtained randomly from 8–10 rats: ^a*P* < 0.01; ^b*P* < 0.005 versus embryos from control rats.

the protein content (Bradford, 1976), and bovine serum albumin was used as a standard. Embryos in resorption stages were denoted as resorbed and received no further analysis. The viable embryos were stored at –70°C to determine 15dPGJ₂ concentration, or incubated as described below.

Four embryos obtained from control or diabetic rats were incubated in a metabolic shaker, under an atmosphere of 5% CO₂ in 95% O₂ at 37°C for 3 h, in 0.5 ml KRB with or without the addition of 2×10^{-6} mol 15dPGJ₂ l⁻¹. After incubation, the embryos were stored at –70°C until determination of nitrate–nitrite concentration or lipid extraction.

The *de novo* synthesis of labelled lipids from [¹⁴C]acetate was evaluated by incubating four embryos for 3 h in the same conditions as described above, with or without the addition of 2×10^{-6} mol 15dPGJ₂ l⁻¹ and 1.2 µCi [¹⁴C]acetate ml⁻¹ (55.2 mCi mmol⁻¹). After incubation, the embryos were washed three times in 10 ml KRB to remove adherent [¹⁴C]acetate and then stored at –70°C until lipid extraction.

15-deoxy $\Delta^{12,14}$ prostaglandin J₂ determinations

15dPGJ₂ was measured in the control and n-stz diabetic embryos using the 15-deoxy $\Delta^{12,14}$ prostaglandin J₂ Enzyme Immunoassay Kit (Assay Designs Inc.) Five embryos were sonicated in PBS; an aliquot was separated for protein determination as described by Bradford (1976); and embryonic prostaglandins were extracted twice in absolute ethanol. The extracts were dried in a Savant (Hicksville, NY) Speed-Vac concentrator and stored at –70°C until enzyme-immunoassay. When the assay was performed, the extracts were reconstituted with 50 µl ethanol and 200 µl assay buffer provided by the commercial kit. In brief, the kit provides a polyclonal antibody to 15dPGJ₂ to bind in a competitive manner the prostaglandin in the sample or an alkaline phosphatase molecule to which 15dPGJ₂ is covalently attached. After a simultaneous incubation, a *p*-nitrophenyl phosphate substrate is added, and the yellow colour generated is evaluated on a microplate reader at 405 nm. The results in the present study were expressed as pg per µg protein. Crossreactivities reported by Assay

Designs are: 100% for 15dPGJ₂, 49.2% for PGJ₂, 5.99% for $\Delta^{12,14}$ PGJ₂ and 4.92% for PGD₂.

Nitrate–nitrite assay

The embryonic nitrate and nitrite concentrations were quantified using an assay kit for nitrate and nitrite determinations (Cayman Chemical). The embryos were sonicated in Tris–HCl buffer solution, and an aliquot was separated for protein determination. Nitrates in the supernatant were reduced to nitrites using nitrate reductase, and total nitrites were measured by the Griess method (Green *et al.*, 1982). Absorbance was measured at 540 nm in a microtitre plate using NaNO₃ and NaNO₂ as standards. Results were expressed in nmol per µg protein.

Lipid analysis

Total lipids were extracted in methanol–chloroform 2:1 (v/v) (Blight and Dyer, 1959) and then concentrated in a Savant Speed-Vac concentrator. Neutral and total polar lipids were quantified by TLC and fluorescence emission of 1,6-diphenylhexatriene (DPH) as described by Igal *et al.* (2001). In brief, chromatography of the total embryo lipids was performed using a solvent system consisting of hexane–ethyl ether–acetic acid 80:20:2 plus 200 µmol DPH l⁻¹. After development, the TLC plate was dried for 5 min in the dark under a N₂ stream, and then the fluorescent lipid spots were photographed and analysed with a DS120 Kodak (Rochester, NY) Digital Science image system coupled to a UV transilluminator. Lipid species were quantified by comparison with known amounts of pure lipid standards run on the same plate. As a control for the fluorescence method, the lipids were also stained with iodine and with a ferric chloride solution and the spot images were processed in the same way as for the fluorescent spots; < 15% essential differences between the methods were apparent.

Neutral lipids were separated for radioactivity measurements by TLC as described above. Polar lipid species were resolved on TLC plates with chloroform–methanol–acetic acid–water 50:37.5:3.5:1.5 (v/v/v/v). Chromatography was carried out in all samples in parallel with pure lipid standards. [¹⁴C]-labelled lipids were detected with a

Berthold (Bad Wildbad) Scanner II. Radioactive spots were scraped into vials and counted in a liquid scintillation counter.

Statistical analyses

All values presented in this study are mean $\pm$ SEM. Comparisons among groups were performed using one-way ANOVA in conjunction with the Tukey's test. Differences between groups were considered significant when $P < 0.05$.

Results

15-deoxy $\Delta^{12,14}$ prostaglandin J_2 concentrations and morphological characteristics of embryos from control and n-stz diabetic rats

Embryos obtained from n-stz diabetic rats on day 10.5 of gestation showed a higher rate of resorption ($P < 0.01$), a higher rate of embryonic malformation ($P < 0.01$), reduced protein content (indicating growth delay, $P < 0.01$), and no alteration in the number of somites when compared with embryos from control rats (Table 1).

15dPGJ₂ concentrations were lower in the embryos from n-stz diabetic rats compared with controls ($P < 0.01$) (Table 1).

Effect of 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 on nitrate–nitrite concentrations in embryos from control and n-stz diabetic rats

Embryos obtained from control and n-stz diabetic rats on day 10.5 of gestation were incubated for 3 h in the presence or absence of 2×10^{-6} mol 15dPGJ₂ l⁻¹ and nitrate–nitrite concentrations were then evaluated. Nitrate–nitrite concentrations were higher in embryos from n-stz diabetic rats compared with controls (27%, $P < 0.05$). The presence of 2×10^{-6} mol 15dPGJ₂ l⁻¹ reduced the embryonic nitrate–nitrite concentrations in embryos from both control (21%, $P < 0.05$) and n-stz diabetic rats (42%, $P < 0.001$; Fig. 1).

Effect of 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 on lipid concentrations in embryos from control and n-stz diabetic rats on day 10.5 of gestation

Embryos obtained from control and n-stz diabetic rats on day 10.5 of gestation were incubated for 3 h in Krebs' medium, in the presence or absence of 2×10^{-6} mol 15dPGJ₂ l⁻¹, and concentrations of neutral lipids were then evaluated. Triacylglycerol, cholesterol and cholesteryl ester concentrations were similar in embryos from control and n-stz diabetic rats (Fig. 2). Addition of 15dPGJ₂ reduced the concentrations of triacylglycerol (35%, $P < 0.01$), cholesterol (63%, $P < 0.001$) and cholesteryl ester (34%, $P < 0.05$) in control embryos (Fig. 2). In embryos from n-stz diabetic rats, exogenous 15dPGJ₂ reduced the concentrations of cholesterol (39%, $P < 0.01$) and cholesteryl ester (34%, $P < 0.05$) but did not modify triacylglycerol content (0%; Fig. 2).

Fig. 1. Nitrate and nitrite concentrations in embryos from control (■) and neonatal streptozotocin-induced (n-stz) diabetic (□) rats on day 10.5 of gestation, incubated for 180 min in Krebs–Ringer bicarbonate solution with or without 2×10^{-6} mol 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 (15dPGJ₂) l⁻¹. ▨: Control plus 15dPGJ₂; ▩: diabetic plus 15dPGJ₂. Values are mean $\pm$ SEM for 36–40 embryos obtained randomly from 9–10 rats. Asterisks indicate significant difference from embryos from control rats without addition of 15dPGJ₂: * $P < 0.05$, ** $P < 0.02$; 'daggers' indicate significant difference from embryos from diabetic rats without addition of 15dPGJ₂: ††† $P < 0.001$ (ANOVA).

Effect of 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 on the incorporation of [¹⁴C]acetate into lipids in embryos from control and diabetic rats during organogenesis

Embryos obtained from control and n-stz diabetic rats on day 10.5 of gestation were incubated for 3 h in Krebs' medium added with tracer amounts of [¹⁴C]acetate to evaluate *de novo* lipid synthesis in the presence or absence of 2×10^{-6} mol 15dPGJ₂ l⁻¹. The rate of incorporation of [¹⁴C]acetate to triacylglycerol ($P < 0.01$) and cholesteryl ester ($P < 0.05$) was lower in stz-diabetic embryos than in controls, whereas no alterations in [¹⁴C]acetate incorporation to cholesterol were detected (Fig. 3).

The addition of 2×10^{-6} mol 15dPGJ₂ l⁻¹ induced a decrease in [¹⁴C]acetate incorporation to triacylglycerol (64%, $P < 0.001$), cholesterol (55%, $P < 0.01$) and cholesteryl ester (68%, $P < 0.001$) in embryos from control rats (Fig. 3). Similarly, in embryos from n-stz diabetic rats, exogenous 2×10^{-6} mol 15dPGJ₂ l⁻¹ reduced the incorporation of [¹⁴C]acetate to triacylglycerol (57%, $P < 0.01$), cholesterol (67%, $P < 0.01$) and cholesteryl ester (60%, $P < 0.01$) compared with diabetic embryos incubated without additions (Fig. 3).

Fig. 2. Neutral lipid concentrations in embryos from control (■) and neonatal streptozotocin-induced (n-stz) diabetic (□) rats on day 10.5 of gestation, incubated for 180 min in Krebs–Ringer bicarbonate solution with or without the addition of 2×10^{-6} mol 15-deoxy $\Delta^{12,14}$ prostaglandin J₂ (15dPGJ₂) l⁻¹. ▨: Control plus 15dPGJ₂; ▩: diabetic plus 15dPGJ₂. Values are mean + SEM for 36–44 embryos obtained randomly from 9–11 rats. Asterisks indicate significant differences from controls without addition of 15dPGJ₂: * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$; ‘daggers’ indicate significant differences from embryos from diabetic rats without addition of 15dPGJ₂: † $P < 0.05$, †† $P < 0.01$ (ANOVA).

Discussion

In the present study, rat embryos were shown to produce 15dPGJ₂ during organogenesis. Embryonic 15dPGJ₂ production was similar to that of other prostaglandins such as PGE₂ (Jawerbaum *et al.*, 1998). 15dPGJ₂, like other cyclopropanones, lacks cell surface receptors but is actively transported into cells, where it exerts a wide variety of biological actions, including modulation of lipid and glucose homeostasis, cell differentiation, cessation of cell growth, antitumoral activity and anti-inflammatory activity (Straus and Glass, 2001). However, the role of 15dPGJ₂ during embryo development is not known. 15dPGJ₂ is sequentially formed from PGD₂, PGJ₂ and Δ^{12} PGJ₂ *in vivo*, and has been identified in inflammatory fluids in increasing concentrations during the resolution phase of inflammation (Shibata *et al.*, 2002). Its precursor, Δ^{12} PGJ₂, which is increased by *in vivo* administration of PGD₂, is a natural component of human body fluids, and its generation is suppressed by treatment with cyclooxygenase (COX) inhibitors (Hirata *et al.*, 1988). In the n-stz diabetic rat model evaluated in the present study, in addition to morphological abnormalities and growth delay, embryonic 15dPGJ₂ concentrations were decreased. 15dPGJ₂ concentrations are lower in β cells obtained from diabetic rats (Gonzalez *et al.*, 2001) and

Fig. 3. Incorporation of [¹⁴C]acetate into neutral lipids in embryos from control (■) and neonatal streptozotocin-induced (n-stz) diabetic (□) rats on day 10.5 of gestation, incubated for 180 min in Krebs–Ringer bicarbonate solution with or without the addition of 2×10^{-6} mol 15-deoxy $\Delta^{12,14}$ prostaglandin J₂ (15dPGJ₂) l⁻¹. ▨: Control plus 15dPGJ₂; ▩: diabetic plus 15dPGJ₂. Values are mean + SEM for 36–44 embryos obtained randomly from 9–11 rats. Asterisks indicate significant differences from controls without addition of 15dPGJ₂: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ‘daggers’ indicate significant differences from embryos from diabetic rats without addition of 15dPGJ₂: † $P < 0.05$, †† $P < 0.01$ (ANOVA).

exogenous 15dPGJ₂ reduces PGE₂ and NO concentrations, an effect that involves inhibition of iNOS and COX-2 (Gonzalez *et al.*, 2001). Thus, a decreased concentration of 15dPGJ₂ in diabetic β cells appears to be related to the increase in NO and PGE₂ concentrations, resulting in cellular damage and β -cell death.

NO is clearly involved in embryo development. NO donors stimulate meiotic maturation and trophoblast outgrowth, whereas both NOS inhibitors and NO donors alter early embryo development (Sengoku *et al.*, 2001). During organogenesis, NO regulates cephalic morphogenesis, and both NO donors and NOS inhibitors have teratogenic effects (Lee and Juchau, 1994). In the last third of pregnancy, treatment with NO inhibitors results in significant intra-uterine growth retardation and haemorrhagic disruption of hind limbs (Diket *et al.*, 1994). Cellular growth and apoptotic events are required for normal embryonic development. As NO has been demonstrated to be a mediator of apoptotic events (Lee and Juchau, 1994), tight regulatory control of NO is required to ensure normal embryo development.

NO concentration is increased in the embryos from both insulin and non-insulin-dependent experimental models of diabetes, an alteration probably related to the increase in apoptosis, resorption and malformation rates induced by diabetes (Jawerbaum *et al.*, 1998, 2001; Moley, 2001). The potent anti-inflammatory activity of 15dPGJ₂ in different types of cell prompted the present authors to evaluate the relationship between 15dPGJ₂ and NO concentrations in normal and diabetic embryos during organogenesis. 15dPGJ₂ was found to inhibit NO generation in embryos from both control and n-stz diabetic rats. The lower 15dPGJ₂ concentration in embryos from n-stz diabetic rats is likely to be related to their increased NO concentration and may affect embryo development in diabetic mothers.

15dPGJ₂ and thiazolidinediones are pharmacological activators of PPAR γ , and modulate lipid and glucose metabolism, and promote adipocyte differentiation (Forman *et al.*, 1995; Lehmann *et al.*, 1995). PPAR α plays a major role in fatty acid catabolism and PPAR γ plays a major role in adipogenesis and lipid storage (Wahli *et al.*, 1995). However, thiazolidinediones induce enhancement of fatty acid oxidation in non-adipose tissues such as skeletal muscle (Cha *et al.*, 2001).

During development, lipids serve primarily as modifiers of the fluidity, structure and function of membranes. Although alterations in lipid metabolism affect development at term of the fetus of a diabetic mother (Singh and Feigelson, 1983), little is known regarding lipid metabolism in embryos from diabetic mothers during organogenesis. In the present study, no alterations in the content of triacylglycerol, cholesterol and cholesteryl esters in the embryos obtained from n-stz diabetic rats compared with controls were observed, but there was a reduced rate of incorporation of [¹⁴C]acetate to triacylglycerol and cholesteryl ester in the embryos from n-stz diabetic rats. A decreased embryonic lipid synthesis may be a metabolic response to increased maternal-embryo lipid transfer and may help to prevent an increase in the embryonic lipid concentrations. Indeed, triglyceride accumulation is present in pregnant uterine tissues and in the fetoplacental unit in n-stz diabetic animals (Jawerbaum *et al.*, 1994), and this is not reflected in the embryonic lipid profile. In contrast, in a more severe model of diabetes, there is a significant positive correlation between maternal plasma circulating lipids and fetal tissue lipid content (Shafrir and Barash, 1978).

In the present study, 15dPGJ₂ was demonstrated to decrease neutral lipid content in control and n-stz diabetic embryos during organogenesis. This decrease occurs via a mechanism that involves an inhibition of neutral lipid synthesis, as *de novo* incorporation of [¹⁴C]acetate to embryonic triacylglycerides, cholesterol and cholesteryl ester was decreased. Thiazolidinediones reduce hypertriglyceridaemia (Oakes *et al.*, 2001) via mechanisms that involve an enhanced ability of adipose tissue to take up and store triacylglycerides in newly differentiated adipocytes, and a reduction of triacylglyceride content in peripheral tissues

such as pancreatic islets (Shimabukuro *et al.*, 1997) and liver (Oakes *et al.*, 2001). In addition, troglitazone inhibits cholesterol biosynthesis in cultured Chinese hamster ovary cells (Wang *et al.*, 1999).

The inhibitory effect of 15dPGJ₂ on embryonic cholesterol content and synthesis is particularly interesting. Cholesterol is needed in sonic hedgehog (SHH) signalling pathways, and mutations in the SHH gene exhibit an indistinguishable phenotype from that seen in Smith-Lemli-Optiz syndrome (Farese and Herz, 1998), an autosomal recessive multiple congenital malformation syndrome associated with reduced cholesterol biosynthesis (Irons *et al.*, 1993). Indeed, inhibitors of delta 7-dehydrocholesterol reductase, an enzyme involved in the last step of the cholesterol biosynthesis pathway, are inducers of fetal malformations (Barbu *et al.*, 1984).

In the present study, reduced concentrations of 15dPGJ₂ were demonstrated in embryos from n-stz diabetic rats. Although much work is needed to understand the biological significance of this finding completely, the low concentration of 15dPGJ₂ may be related to NO overproduction that alters embryo development. In addition, the profound effects of 15dPGJ₂ on embryonic lipid content and synthesis during organogenesis indicated a significant role for 15dPGJ₂ in this important pathway providing developmental signals, energy storage and structural components for normal embryonic development.

This work has been supported by grants 'Ramón Carrillo-Arturo Oñativia' from Ministerio de Salud de la Nación Argentina (A. Jawerbaum), from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 0598/98) (E. Gonzalez), by Agencia de Promoción Científica y Tecnológica de Argentina (PICT 98 05-03375) (E. Gonzalez, A. Jaweraum) and by PROGRESAR program, ICIMER (D. Sinner). The authors thank Ariel Igal and Matias Caviglia for advice on the evaluation of lipid metabolism.

References

- Barbu V, Roux C, Dupuis J, Gardette J and Maziere JC (1984) Teratogenic effect of AY9944 in rats: importance of the day of administration and maternal plasma cholesterol levels *Proceedings of the Society for Experimental Biology and Medicine* **76** 54–59
- Blight EG and Dyer WJ (1959) A rapid method of total lipid extraction and purification *Canadian Journal of Biochemistry and Physiology* **37** 911–917
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding *Analytical Biochemistry* **72** 248–254
- Cha BS, Ciaraldi TP, Carter K, Nikoulina SE, Mudaliar S, Mukherjee R, Paterniti JR and Henry RR (2001) Peroxisome proliferator-activated receptor (PPAR) gamma and retinoid X receptor (RXR) agonists have complementary effects on glucose and lipid metabolism in human skeletal muscle *Diabetologia* **44** 444–452
- Diket AL, Pierce MR, Munshi UK, Voelker CA, Eloby-Childress S, Greenberg SS, Zhan XJ, Clark DA and Miller MJ (1994) Nitric oxide causes intrauterine growth retardation and hind-limb disruptions in rats *American Journal of Obstetrics and Gynecology* **171** 1243–1250
- Eriksson UJ, Borg H, Fordberg H and Styrd J (1991) Diabetic embryopathy. Studies with animal and *in vitro* models *Diabetes* **40** 94–97
- Farese R and Herz J (1998) Cholesterol metabolism and embryogenesis *Trends in Genetics* **14** 115–120

- Farese R, Cases S, Ruland S, Kayden H, Wong J, Young S and Hamilton R (1996) A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal–fetal lipid transport in mice *Journal of Lipid Research* **37** 347–360
- Forman BM, Tontoz P, Chen J, Brum RP, Spiegelman BM and Evans RM (1995) 15-deoxy-delta^{12,14} is a ligand for the adipocyte determination factor PPARgamma *Cell* **83** 803–812
- Gonzalez E, Jawerbaum A, Sinner D, Pustovrh C, White V, Capobianco E, Xaus C, Peralte C and Roselló-Catafau J (2001) Streptozotocin-pancreatic damage in the rat: modulatory effect of 15-deoxy delta^{12,14} prostaglandin J₂ on nitridergic and prostanoid pathway *Nitric oxide: Biology and Chemistry* **6** 214–220
- Green LC, Wagner, DA and Glogowski J (1982) Analysis of nitrate, nitrite and ¹⁵N-nitrate in biological fluids *Analytical Biochemistry* **126** 131–138
- Herz J and Farese R (1999) The LDL receptor gene family, apolipoprotein B and cholesterol in embryonic development *Journal of Nutrition* **129** 473–475
- Hirata Y, Hayashi H, Ito S *et al.* (1988) Occurrence of 9-deoxy-delta⁹, delta^{12,13,14}-dihydroprostaglandin D₂ in human urine *Journal of Biological Chemistry* **263** 16 617–16 625
- Igal RA, Caviglia JM, Gomez Dumm IN and Coleman RA (2001) Diacylglycerol generated in CHO cell plasma membrane by phospholipase C is used for triacylglycerol synthesis *Journal of Lipid Research* **42** 88–95
- Irons M, Elias E, Salen G, Tint GS and Batta AK (1993) Defective cholesterol biosynthesis in Smith–Lemli–Opitz syndrome *Lancet* **341** 1414
- Jawerbaum A, Roselló Catafau J, González ET, Novaro V, Gomez G, Gimeno AL and Gimeno MAF (1994) Glucose metabolism, triglyceride and glycogen levels, as well as eicosanoid production in isolated uterine strips and in embryos in a rat model of non-insulin-dependent diabetes mellitus during pregnancy *Prostaglandins* **47** 81–95
- Jawerbaum A, Gonzalez ET, Novaro V, Faletti A, Sinner D and Gimeno MAF (1998) Increased prostaglandin E generation and enhanced nitric oxide synthase activity in the non-insulin-dependent diabetic embryo during organogenesis *Reproduction Fertility and Development* **10** 191–196
- Jawerbaum A, Sinner D, White V, Pustovrh C, Capobianco E, Gimeno MAF and Gonzalez ET (2001) Modulation of PGE₂ generation in diabetic embryo: effect of nitric oxide and superoxide dismutase *Prostaglandins, Leukotrienes and Essential Fatty Acids* **64** 127–133
- Kitzmiller JL, Cloherty JP, Younger MD, Tabatabaai A, Rothchild SB, Sosenko I, Epstein MF, Singh S and Neff RK (1978) Diabetic pregnancy and perinatal morbidity *American Journal of Obstetrics and Gynecology* **131** 560–580
- Kliwer SA, Wilson TM (1988) The nuclear receptor PPARgamma – bigger than fat *Current Opinion in Genetics and Development* **8** 576–81
- Kliwer SA, Lenhard JM, Willson TM, Patel I, Morris DC and Lehmann JM (1995) A prostaglandin J₂ metabolite binds peroxisome proliferator-activated gamma and promotes adipocyte differentiation *Cell* **83** 813–819
- Lee Q and Juchau M (1994) Dysmorphogenic effects of nitric oxide (NO) synthase inhibition: studies with intra-amniotic injections of sodium nitroprusside and N^G-monomethyl-L-arginine *Teratology* **49** 452–464
- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Wilson TM and Kliwer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma *Journal of Biological Chemistry* **270** 12 953–12 956
- McGarry D (2002) Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes *Diabetes* **51** 7–18
- Maloff BL and Boyd BK (1986) Physiologic and cellular insulin action in a glucose-intolerant model of type 2 (non-insulin-dependent) diabetes in rats *Diabetologia* **29** 295–300
- Mimura K, Umeda F, Hiramatsu S *et al.* (1994) Effects of a new oral hypoglycaemic agent (CS-0.45) on metabolic abnormalities and insulin resistance in type 2 diabetes *Diabetic Medicine* **11** 685–691
- Moley KH (2001) Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women *Trends in Endocrinology and Metabolism* **12** 78–82
- Oakes ND, Thalen PG, Jacinto SM and Ljung B (2001) Thiazolidinediones increase plasma–adipose tissue FFA exchange capacity and enhance insulin-mediated control of systemic FFA availability *Diabetes* **50** 1158–1165
- Portha B, Picon L and Rosselin G (1979) Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes *Diabetologia* **17** 371–377
- Ricote M, Li AC, Willson TM, Kelly CJ and Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation *Nature* **391** 79–82
- Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M and Santoro MG (2000) Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase *Nature* **403** 103–108
- Sengoku K, Takuma N, Horikawa M, Tsuchiya K, Komori H, Sharifa D, Tamato K and Ishikawa M (2001) Requirement of nitric oxide for murine oocyte maturation, embryo development and trophoblast outgrowth *in vitro. Molecular Reproduction and Development* **58** 262–268
- Shafir E and Barash V (1978) Placental function in maternal–fetal fat transport in diabetes *Biology of the Neonate* **51** 101–112
- Shibata T, Kondo M, Osawa T, Shibata N, Kobayashi M and Uchida K (2002) 15-deoxy-delta^{12,14}-prostaglandin J₂: a prostaglandin D₂ metabolite generated during inflammatory processes *Journal of Biological Chemistry* **277** 10 459–10 466
- Shimabukuro M, Koyama K, Lee Y and Unger RH (1997) Leptin- or troglitazone-induced lipopenia protects islets from interleukin 1beta cytotoxicity *Journal of Clinical Investigation* **7** 1750–1754
- Singh M and Feigelson M (1983) Effects of maternal diabetes on the levels, synthetic rates and activities of synthetic enzymes of surface-active phospholipids in perinatal rat lung *Biochimica et Biophysica Acta* **753** 53–59
- Straus DS and Glass CK (2001) Cyclopentenone prostaglandins: new insights on biological activities and cellular targets *Medical Research Reviews* **21** 185–210
- Wahli W, Braissant O and Desvergne B (1995) Peroxisome proliferator-activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more *Chemistry and Biology* **2** 261–266
- Wang M, Wise SC, Leff T and Su T (1999) Troglitazone, an antidiabetic agent, inhibits cholesterol biosynthesis through a mechanism independent of peroxisome proliferator-activated receptor-gamma *Diabetes* **48** 254–260

Received 18 April 2002.

First decision 20 June 2002.

Revised manuscript received 18 July 2002.

Accepted 1 August 2002.