

Serum lipoprotein composition, lecithin cholesterol acyltransferase and tissue lipase activities in pregnant diabetic rats and their offspring receiving enriched n-3 PUFA diet

N. A. Soulimane-Mokhtari¹, B. Guermouche¹, M. Saker¹, S. Merzouk², H. Merzouk¹, A. Hichami³, S. Madani³, N. A. Khan³ and J. Prost³

¹ *Laboratoire de Physiologie Animale et Biochimie, Département de Biologie, Faculté des Sciences, Université de Tlemcen, Algérie. E-mail: nassima_amel@yahoo.fr*

² *Département de Physique, Faculté des Sciences, Université de Tlemcen, Algérie*

³ *UPRES 2422, Lipides et Nutrition, UFR Sciences de la vie, 6 Boulevard Gabriel, Université de Bourgogne, Dijon 21000, France*

Abstract. The effects of dietary n-3 polyunsaturated fatty acids on lipoprotein concentrations and on lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL) and lecithin cholesterol acyltransferase (LCAT) activities were studied in streptozotocin-induced diabetic rats during pregnancy and in their macrosomic offspring from birth to adulthood. Pregnant diabetic and control rats were fed Isio-4 diet (vegetable oil) or EPAX diet (concentrated marine omega-3 EPA/DHA oil), the same diets were consumed by pups at weaning. Compared with control rats, diabetic rats showed, during pregnancy, a significant elevation in very low density lipoprotein (VLDL) and low and high density lipoprotein (LDL-HDL₁)-triglyceride, cholesterol and apoprotein B100 concentrations and a reduction in apoprotein A-I levels. HTGL activity was high while LPL and LCAT activities were low in these rats. The macrosomic pups of Isio-4-fed diabetic rats showed a significant enhancement in triglyceride and cholesterol levels at birth and during adulthood with a concomitant increase in lipase and LCAT activities. EPAX diet induces a significant diminution of VLDL and LDL-HDL₁ in mothers and in their macrosomic pups, accompanied by an increase in cholesterol and apoprotein A-I levels in HDL₂₋₃ fraction. It also restores LPL, HTGL and LCAT activities to normal range. EPAX diet ameliorates considerably lipoprotein disorders in diabetic mothers and in their macrosomic offspring.

Key words: Diabetes — Lipids — Lipoproteins — Macrosomia — Offspring — Polyunsaturated fatty acids — Pregnancy — Rats

Abbreviations: apo, apoprotein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPAX, concentrated marine omega-3 EPA/DHA oils; HDL, high density lipoprotein; HTGL, hepatic triglyceride lipase; Isio-4, vegetable oil; LCAT, lecithin cholesterol acyltransferase; LDL, low density lipoprotein; LPL, lipoprotein lipase; PUFA, polyunsaturated fatty acids; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein.

Introduction

Macrosomia or fetal obesity is a common complication associated to maternal diabetes during pregnancy. It results from

the combined effects of excessive transfer of maternal nutrients, and fetal hyperinsulinemia (Merzouk et al. 2000; Jones 2001; Fetita et al. 2006). Indeed, several studies showed that maternal diabetes is a major cause of lipid abnormalities in offspring and the development of obesity during childhood and adulthood (Merzouk et al. 2002; Huang et al. 2006).

Since diabetic pregnant rats showed similar metabolic perturbations compared to humans, this animal model was previously used to explore the association between

Correspondance to: Hafida Merzouk, Laboratoire de Physiologie Animale et Biochimie, Département de Biologie, Faculté des Sciences, Université de Tlemcen, Algérie
E-mail: hafidamerzouk_2@hotmail.com

birth weight and the predisposition of macrosomic pups of diabetic dams to obesity development and the onset of adult diabetes (Merzouk et al. 2002; Magaton et al. 2007). As expected, in rat, maternal diabetes implies an abnormal intrauterine environment for the fetal/maternal development, since the availability of fuels for the fetus is primarily dependent upon the maternal metabolic state. Diabetes-induced alterations in nutrient metabolism are then believed to result in perinatal complications in pups (Fetita et al. 2006).

On the other hand, increasing evidence is accumulating regarding the importance of n-3 polyunsaturated fatty acids (PUFA) specially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in exerting beneficial effects on serum lipids and lipoproteins (Merzouk and Khan 2003; Dimitrova-Sumkovska et al. 2006).

We have previously demonstrated the beneficial effects of n-3 PUFA in reducing the prevalence of macrosomia in diabetic pregnancy, on insulin sensitivity and on serum and liver lipids (Soulimane-Mokhtari et al. 2005). However, serum and liver lipids are not sufficient indicators of lipoprotein metabolism. In addition, serum lipoprotein concentrations and compositions are determined by many factors. Both secretion by liver and intestine and uptake and degradation by specific or non-specific pathways are involved. The combined action of several enzymes, such as lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL) and lecithin cholesterol acyltransferase (LCAT), on lipoproteins and movements of lipids and apoproteins (apo) in plasma also control the lipoprotein levels.

The purpose of this study was to evaluate the effects of n-3 PUFA-enriched diet on lipoprotein compositions as well as on LCAT, LPL and HTGL activities in diabetic rats and their offspring by determining the time course of changes in serum lipoprotein (VLDL, LDL-HDL₁, HDL₂₋₃) lipids and apo and enzyme activities. The following study should increase our understanding of the beneficial effects of n-3 PUFA in macrosomic offspring of diabetic mothers, with the aim of reducing the development of diabetes and its complications in these offspring.

Materials and Methods

Animals and experimental protocol

Adult Wistar rats were used. After mating, the first day of gestation was estimated by the presence of spermatozooids in vaginal smears. Sixty pregnant rats were placed in individual cages and were fed *ad libitum* two different diets, thirty for control Isio-4 diet (diet with vegetable oil) and thirty for EPAX diet (diet with concentrated marine omega-3 EPA/DHA oil). Control Isio-4 diet contains 58.7% starch,

20% casein, 5% saccharose, 5% cellulose, 4% minerals, 2% vitamins, 0.3% methionine, 5% vegetable oil Isio-4 which contains (mg/g): 18 : 2 n-6, 47.2; total n-3 1.7; and monounsaturated fatty acids 40.2 (largely 18 : 1). In EPAX diet, half of the vegetable oil Isio-4 was replaced by EPAX-7010 which contains 85% n-3 PUFA, i.e. EPA, 70; DHA 12% and tocopherol 2.1–3.2%.

Twenty Isio-4 and twenty EPAX pregnant rats were made diabetic by intraperitoneal injection of streptozotocin (40 mg/kg body weight) in 0.1 mol/l citrate buffer, pH 4.5, on the fifth day of gestation. Ten Isio-4 and ten EPAX pregnant rats were injected with citrate buffer alone as a control group. On the 12, 16, 18 and 20 days of gestation, maternal blood was collected for glucose concentration by cutting of the tip of the tail and squeezing it gently. Pregnant rats with plasma glucose levels between 5.55 and 16.65 mmol/l were designed as mildly hyperglycaemic (Merzouk et al. 2000) and were included in this study. The success rate in obtaining these mildly hyperglycemic dams in the current series was 70% for Isio-4 diet ($n = 14$) and 60% for EPAX diet ($n = 12$).

At delivery, pups from the streptozotocin-treated dams whose birth weights were 1.7 SD (above the 90th percentile) greater than the mean birth weight of the control pups were classified as obese pups and included in the study. The success rate of obtained obese pups was 62% (72 out of 116) for Isio-4-fed and 55% (44 out of 80) for EPAX diet. The protocol of selection of diabetic and obese animals has been described in detail, elsewhere (Merzouk et al. 2000, 2002; Soulimane-Mokhtari et al. 2005).

Twenty newborn rats of each group (control and experimental) and each diet (Isio-4 and EPAX) were killed by decapitation. Blood, liver and abdominal adipose tissue were collected. The remaining obese and control pups of each diet were left with their own mothers and were weighed weekly up to 12 weeks of age. Pups were weaned at 3 weeks of age, housed three rats *per* cage, and allowed Isio-4 or EPAX diet and water *ad libitum*. At different ages, six rats of each group were killed for blood and tissue samples.

Macrosomic rats from diabetic dams had significantly higher body weights than controls throughout the first 12 weeks of age in both the Isio-4 and EPAX diet-fed groups (Soulimane-Mokhtari et al. 2005).

Blood and tissue samples

At day 12 and 21 for dams, at day 60 and day 90 for pups, six rats of each group were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and then bled from abdominal aorta. Serum was obtained by low speed centrifugation (1000 g, 15 min). Liver and fat tissue surrounding the kidney and epididymal areas for the

male rats or ovary and fallopian tubes for female rats were removed, washed with cold saline, quickly blotted and prepared for lipolytic activities determination.

Isolation of lipoprotein fractions

Serum lipoproteins of density <1.21 kg/l were isolated by single ultracentrifugation flotation (model L8-55 ultracentrifuge, 50 Ti rotor, Beckman instruments, Palo Alto, CA, USA), according to Havel et al. (1955). The three lipoprotein fractions (VLDL, LDL-HDL₁, HDL₂₋₃) were isolated from total lipoproteins by a single-spin discontinuous gradient according to the method of Redgrave et al. (1975) as modified by Meghelli-Bouchenak et al. (1989). The three fractions were dialyzed against 0.15 mol/l NaCl and 1 mmol/l disodium EDTA (pH 7.4) at 4°C in spectra/por-2 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA).

Lipid determination

Lipoprotein triglyceride (TG) and total cholesterol (TC) contents were measured by means of a Boehringer kit (Mannheim, Germany), using enzymatic methods.

Apo determination

Apo A-I and B100 were measured on lipoproteins by Bioré kit, using turbidimetric methods.

Enzyme activities determination

Assay for LCAT activity

LCAT (EC 2.3.1.43) activity was assayed by conversion of unesterified [³H] cholesterol to esterified [³H] cholesterol, according to the method of Glomset and Wright (1964), as previously described Merzouk et al. (1997). Serum LCAT activity was expressed as nanomoles of esterified cholesterol per hour per milliliter of serum.

Assay for lipases activities

HTGL (EC 3.1.1.3) and adipose tissue LPL (EC 3.1.1.34) activities were assayed according to the method of Nilsson-Ehle and Ekman (1977), as previously described Merzouk et al. (2002). Tissue homogenates used as lipolytic sources (from liver and adipose tissue) were prepared according to the method of Tavangar et al. (1992).

Statistical analysis

Results are expressed as means \pm SD. The significance of differences among groups was analyzed statistically by

ANOVA, followed by Duncan's multiple range test (Duncan 1955) for parameter changes with age. The significance of differences between diabetic or macrosomic and control rats and between Isio-4 and EPAX diet at each age was assessed using Student's *t*-test. These calculations were performed using Statistica, version 4.1 (StatSoft, Tulsa, OK, USA). Differences were considered statistically significant at $p < 0.05$.

Results

Lipoprotein lipids and apo

Diabetic rats fed the Isio-4 diet showed a significant elevation ($p < 0.05$) of TG in VLDL and TC in both VLDL and LDL-HDL₁ compared to the control rats fed the same diet at days 12 and 21 of gestation. LDL-HDL₁-TG concentrations were also increased significantly in these rats but only at day 21 of gestation (Figure 1).

The macrosomic pups of Isio-4-fed mothers (at birth, days 60 and 90) also presented a significant increase in the level of TG and TC in VLDL and LDL-HDL₁ ($p < 0.05$) compared to control pups (Figure 2). HDL₂₋₃ presented a significant increase in TG at birth and day 90 and a decrease in TC at adulthood ($p < 0.05$).

Feeding the EPAX diet by diabetic rats and their macrosomic pups induced a significant decrease in VLDL and LDL-HDL₁-TG and -TC (Figures 1, 2). Comparing to macrosomic pups of Isio-4-fed mothers, macrosomic pups of EPAX diet presented a significant decrease in HDL₂₋₃-TG at days 0 and 90, and a significant increase in HDL₂₋₃-TC at days 60 and 90 ($p < 0.05$). The last increased significantly also in diabetic dams of EPAX diet than Isio-4 diet ($p < 0.05$).

Apo B100 levels were increased significantly ($p < 0.01$) in VLDL and LDL-HDL₁ of diabetic dams at days 12 and 21 and of their macrosomic offspring at days 0, 60 and 90 regardless to diets (Figure 3). apo A-I amounts were decreased significantly ($p < 0.01$) in HDL₂₋₃ of Isio-4-fed diabetic dams and of their adult macrosomic offspring. EPAX diet induced a significant decrease in apo B100 levels and an increase in apo A-I concentrations in diabetic dams and their macrosomic pups ($p < 0.01$) (Figure 3).

LCAT activity

Diabetic pregnant rats, Isio-4-fed, showed a significant reduction of serum LCAT activity compared to control rats ($p < 0.05$). In contrast, Isio-4-fed macrosomic pups (at birth, days 60 and 90) presented a significant increase in serum LCAT activity compared to their controls (Figure 4).

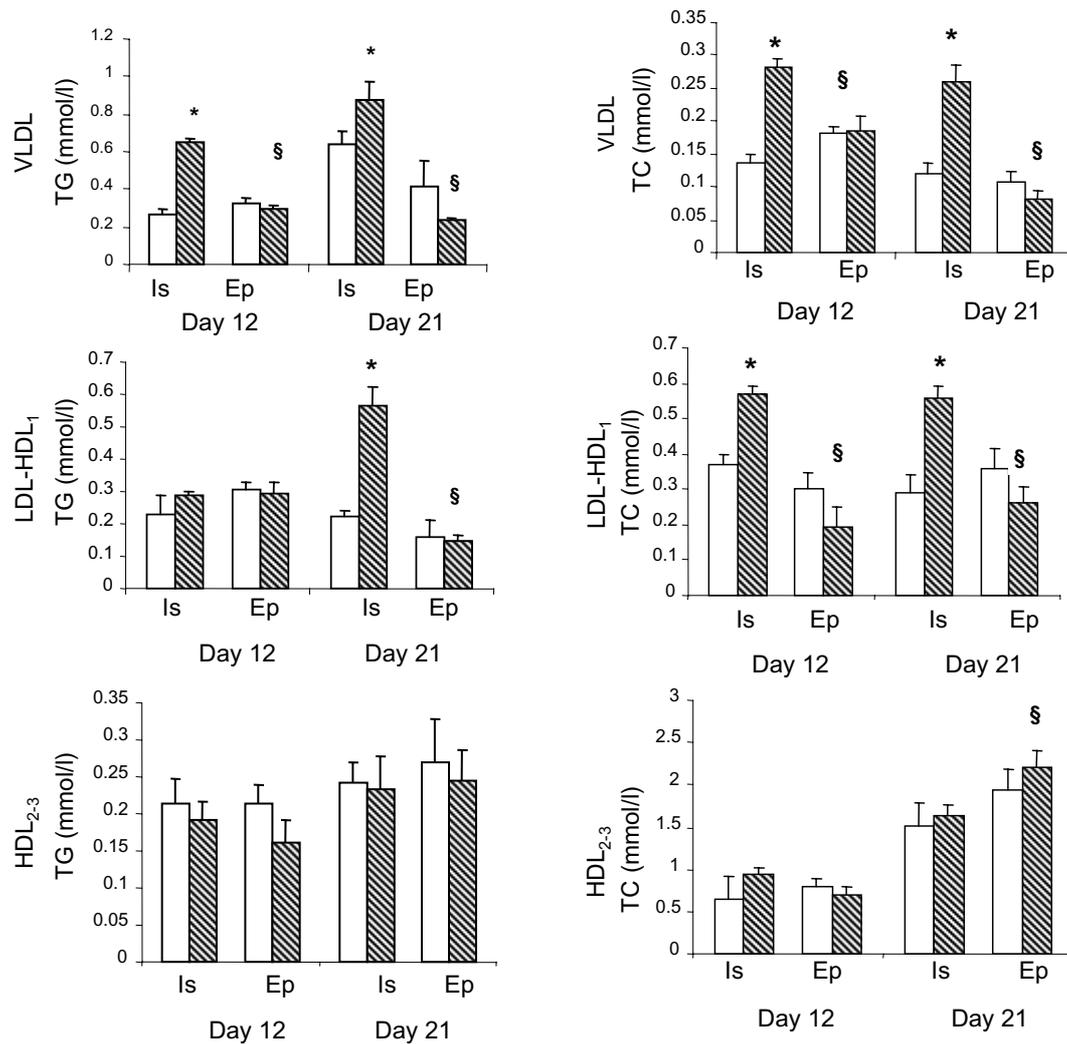


Figure 1. Lipoprotein triglyceride (TG) and total cholesterol (TC) levels during pregnancy in control (□) and diabetic (▨) rats. Is, Isio-4 diet; Ep, EPAX diet; * diabetics versus controls; § Ep versus Is; *, § $p < 0.05$.

EPAX diet significantly increased LCAT activity in diabetic dams, while it induced a decrease in this activity in macrosomic pups ($p < 0.05$) (Figure 4).

Lipases activities

HTGL activity was significantly high in diabetic rats of Isio-4 diet during gestation and in their macrosomic offspring at day 60 and 90. On the other hand, adipose tissue LPL activity was significantly decreased in Isio-4-fed diabetic dams, and increased in their macrosomic pups at day 60 and 90 ($p < 0.05$) (Figure 4).

EPAX diet reduced significantly HTGL activity in both diabetic dams and macrosomic pups compared to Isio-4 diet ($p < 0.05$) (Figure 4). In addition, EPAX modulated

adipose tissue LPL activity leading to a significant elevation in diabetic dams and to a significant decrease in this activity in macrosomic pups at day 60 and 90 (Figure 4).

Discussion

Our results showed that diabetes induced lipoprotein metabolism alterations in pregnant rats and their macrosomic offspring. Diabetic rats fed the Isio-4 diet showed a significant elevation of TG and TC in VLDL and LDL-HDL₁ lipoproteins with a concomitant increase in apo B100 levels. These findings were due probably to increased synthesis and secretion in lipoprotein observed during pregnancy as a consequence of hyperglycemia (King 2000). High VLDL concentrations could also be explained by decreased LPL

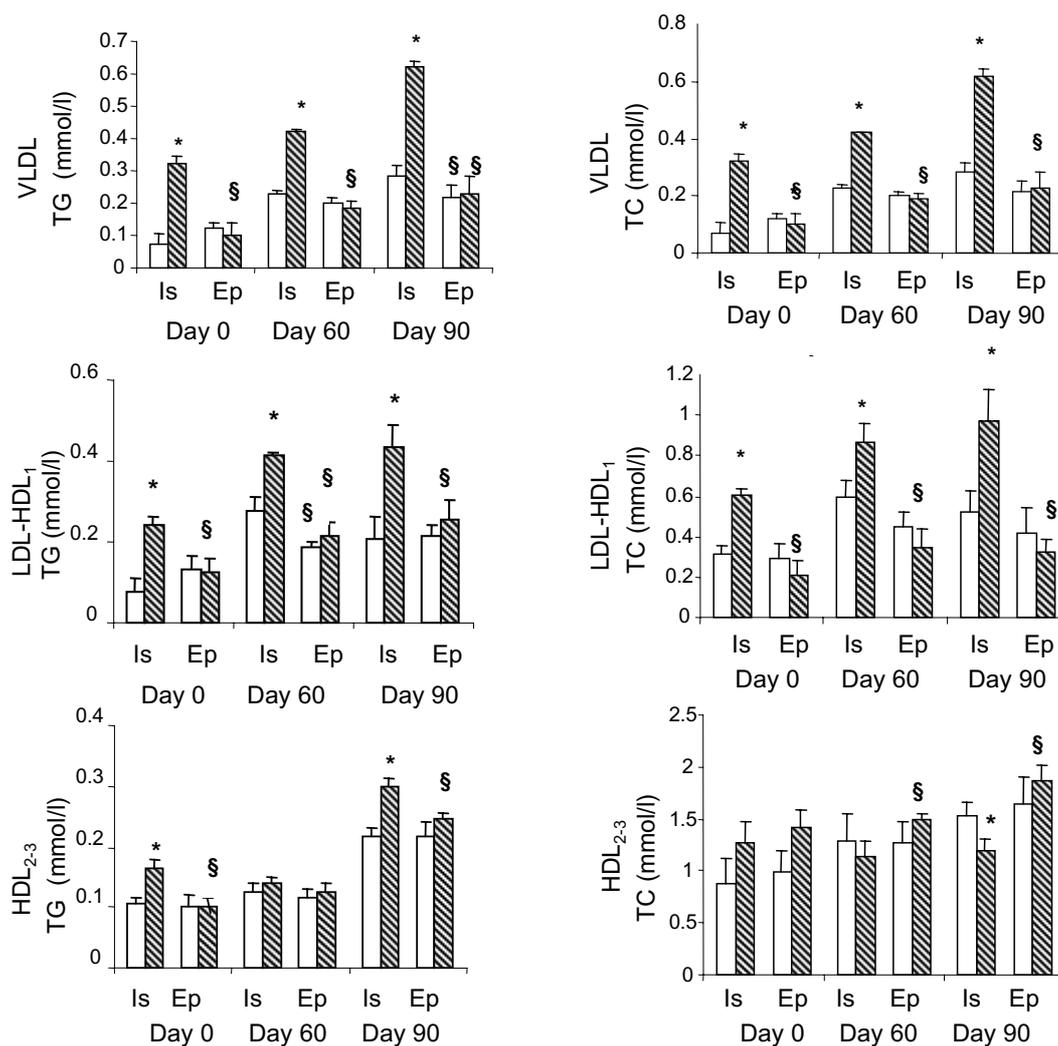


Figure 2. Postnatal changes in lipoprotein triglyceride (TG) and total cholesterol (TC) levels in macroscopic (▨) and control (□) offspring. Is, Isio-4 diet; Ep, EPAX diet; * macroscopic *versus* controls; § Ep *versus* Is; *, § $p < 0.05$.

activity secondary to insulin deficiency. In diabetic rats fed the Isio-4 diet, despite low LPL and high HTGL activities, LDL-HDL₁-TG levels were increased compared to controls. Decreased expression of apo B/E receptors due to insulin deficiency and resulting in diminished lipoprotein catabolism might contribute to maintain high LDL-HDL₁ levels in diabetic rats. These abnormalities are well known in diabetes (Stanely Mainzen Prince and Kannan 2006). Although HDL₂₋₃-TG and -TC were unchanged, HDL apo A-I levels were lower in diabetic Isio-4-fed rats than controls. These data suggested a reduction in HDL particle number with changes in their composition. In addition, there was a reduction in LCAT activity in diabetic rats, concomitantly to its cofactor apo A-I decrease. These results are in agreement with those found in experimental diabetes (Stanely Mainzen Prince and Kannan 2006).

At birth, the macroscopic offspring of diabetic dams were hyperglycemic and hyperinsulinemic and had accelerated growth compared to offspring of control rats, independently of maternal diet (Soulimane-Mokhtari et al. 2005). Indeed, the macroscopic pups of Isio-4-fed mothers presented an increase in the level of TG and TC in VLDL, LDL-HDL₁ and HDL₂₋₃ and of apo B100, compared to control pups. Previous studies reported raised fetal hepatic VLDL secretion and hypertriglyceridemia (Merzouk et al. 2000; Fetita et al. 2006). The hypercholesterolemia in the macroscopic pups at birth reflected an increase in lipoprotein particles, resulting probably from their enhanced synthesis. High LCAT activity in macroscopic pups was most likely due to an increase in enzyme mass, secondary to enhanced synthesis in these rats.

At days 60 and 90, macroscopic Isio-4-fed rats had high TG- and TC-VLDL and LDL-HDL₁ levels and apo B100 con-

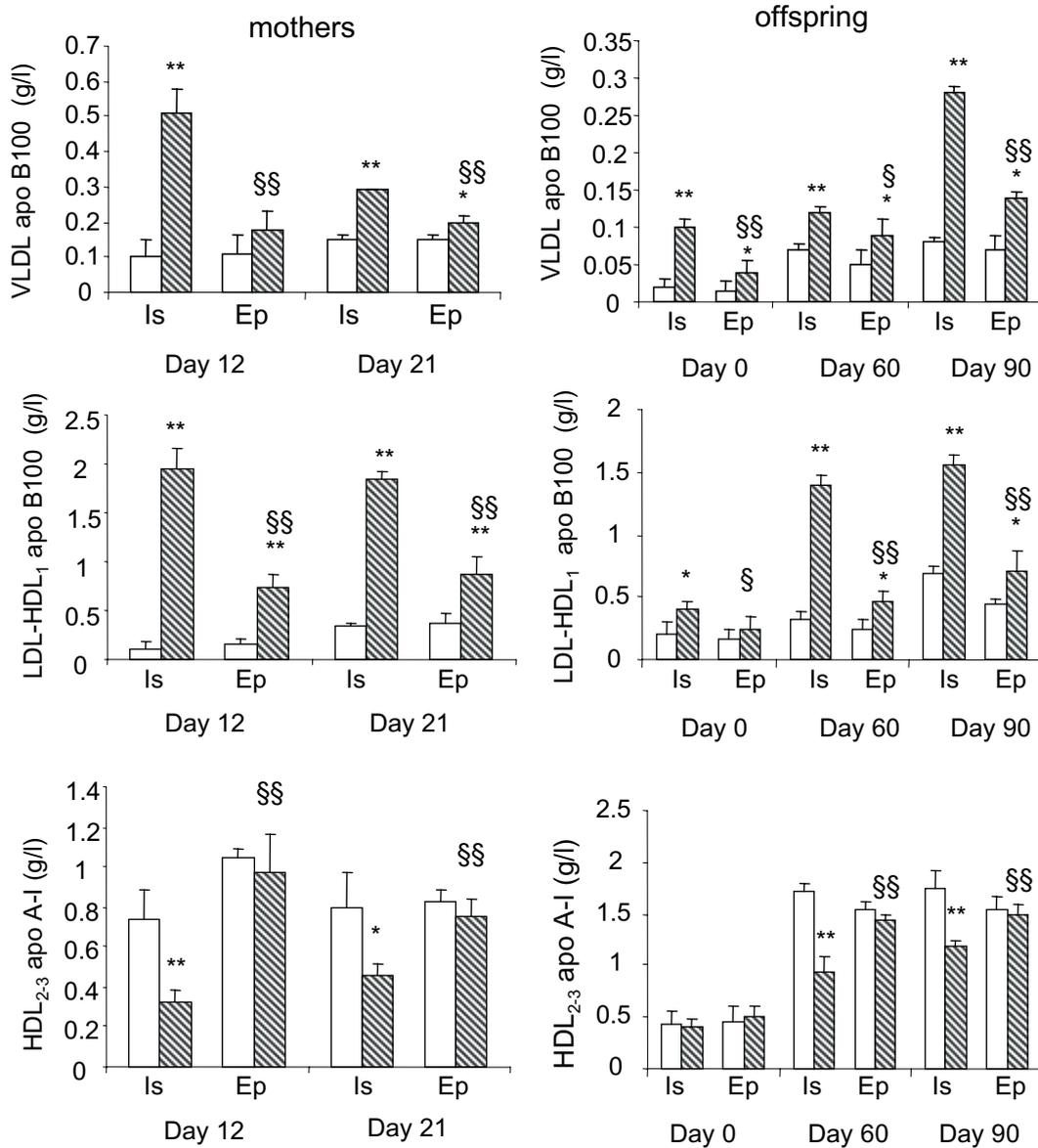


Figure 3. Apoprotein (apo) A-I and apo B100 levels in mothers and their offspring. Is, Isio-4 diet; Ep, EPAX diet; * diabetics or macrosomics *versus* controls; § Ep *versus* Is; *, § $p < 0.05$; **, §§ $p < 0.01$; □ control rats; ▨ diabetic or macrosomic rats.

centrations which could be explained by increased hepatic synthesis or reduced catabolism of lipoproteins containing apo B100. They also displayed significant increases in LPL and HTGL activities. In adulthood, these metabolic impairments are well known in obesity (Bioletto et al. 2000; Tian et al. 2006). We have previously showed that the macrosomic rats developed insulin resistance (Merzouk et al. 2000; Soulimane-Mokhtari et al. 2005). On the other hand, in these adult macrosomic rats, HDL₂₋₃-TC and apo A-I amounts were decreased significantly. HDL cholesterol levels are frequently reduced in obesity (Tian et al. 2006).

On the other hand, we have previously reported that macrosomic rats fed the EPAX diet had lower weight and had less hyperglycaemia and hyperinsulinaemia than macrosomic rats fed the Isio-4 diet, which is indicative of a beneficial effect of EPAX (Soulimane-Mokhtari et al. 2005). Several studies demonstrated that n-3 PUFA-enriched diets lead to changes in energy balance and body weight, and exert less obesogenic effects (Cunnane et al. 1986; Storlien et al. 1991) and improve glucose tolerance and insulin sensitivity in humans (Ebbesson et al. 2005).

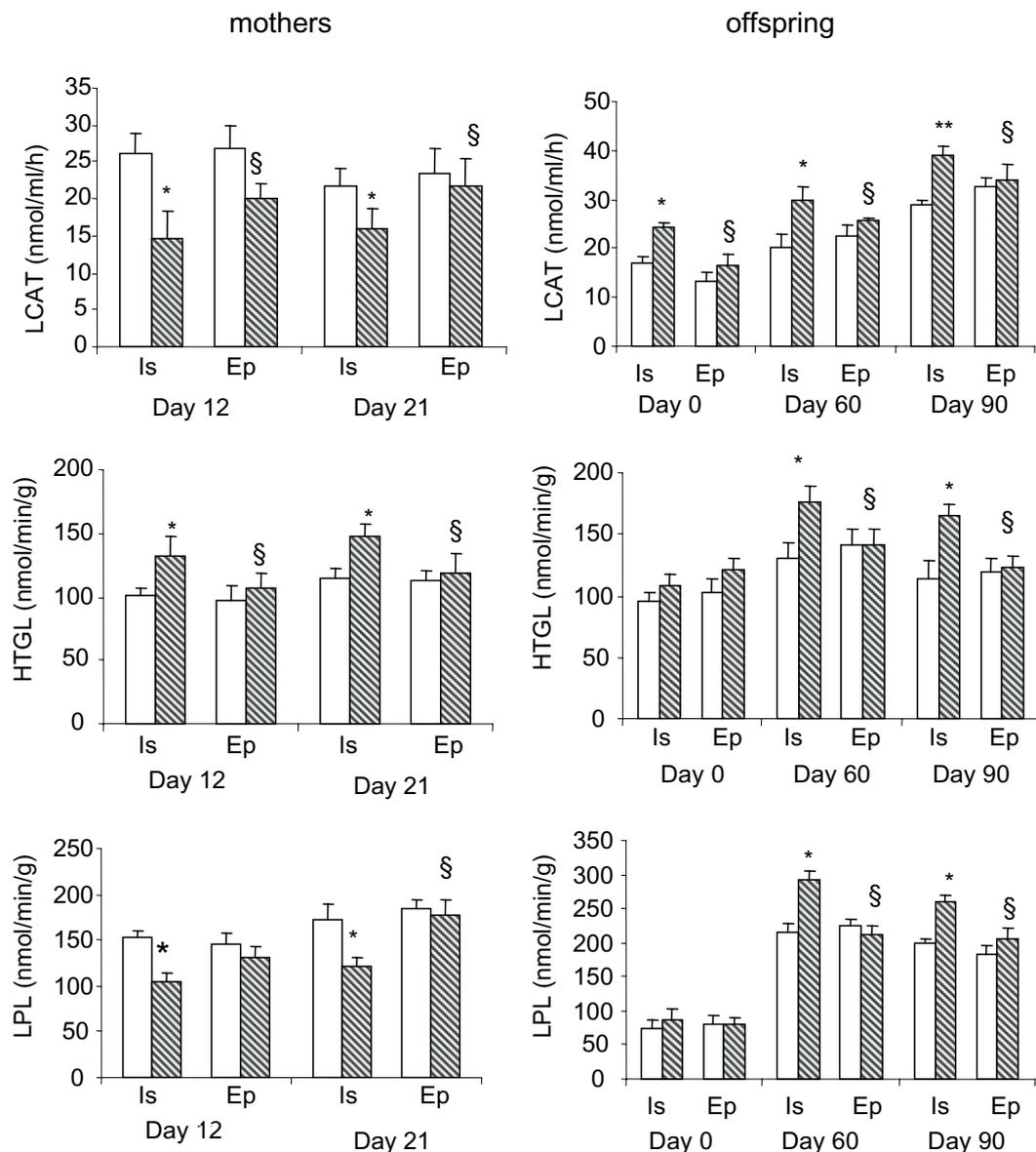


Figure 4. Enzyme activities (LCAT, HTGL and LPL) in mothers and their offspring. LCAT, lecithin cholesterol acyltransferase; HTGL, hepatic triglyceride lipase; LPL, adipose tissue lipoprotein lipase; Is, Isio-4 diet; Ep, EPAX diet; * diabetic or macrosomic versus control; § Ep versus Is; *, § $p < 0.05$; **, §§ $p < 0.01$; □ control rats; diabetic or ▨ macrosomic rats.

Our data showed that EPAX diet produced a correction of lipoprotein abnormalities observed in diabetic mothers and also in their macrosomic offspring. Feeding the EPAX diet by diabetic rats and their macrosomic pups during adulthood, induced a significant decrease in VLDL and LDL-HDL₁-TG with a concomitant decrease in apo B100 levels. Previous studies demonstrated a reduction in plasma TG levels in human as well as in experimental animals primarily by decreasing liver TG synthesis (Davidson 2006). We also demonstrate an hypocholesterolemic effect of n-3 PUFA in

diabetic dams and in their macrosomic pups at birth and during adulthood. The hypocholesterolemic effect of n-3 PUFA was mostly reflected in VLDL and in LDL-HDL₁, suggesting a decrease in cholesterol synthesis or increased cholesterol excretion into bile. EPAX diet also induced an increase in apo A-I and HDL-TC amounts in diabetic dams and their offspring. It modulated LPL, HTGL and LCAT activities to restore normal levels in experimental groups compared to controls. It has been reported that fish oil induced a reactivation of LPL and LCAT activities in hyperlipidemic

rats (Rizvi et al. 2003), which corresponded to our findings in diabetic dams. However, in other investigations, fish oil produced a reduction in LPL, HTGL and LCAT activities (Haug and Hostmark et al. 1987; Soria et al. 2002) which corresponded to our findings in macrosomic rats.

In conclusion, several lipoprotein metabolism abnormalities are noted in diabetic mothers and in their macrosomic offspring. n-3 PUFA diet had favourable effects on lipoproteins by counteracting maternal and also macrosomic hyperlipidemia and by restoring LPL, HTGL and LCAT activities to normal range. It ameliorated long-term prognostic of macrosomia. n-3 PUFA supplementation is then recommended during gestational diabetes and macrosomia.

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