Original Article

Differences in Liver Injury and Trophoblastic Mitochondrial Damage in Different Preeclampsia-like Mouse Models

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Abstract

Background: Preeclampsia is a multifactorial disease during pregnancy. Dysregulated lipid metabolism may be related to some preeclampsia. We investigated the relationship between triglycerides (TGs) and liver injury in different preeclampsia-like mouse models and their potential common pathways.

Methods: Preeclampsia-like models (Nw-nitro-L-arginine-methyl ester [L-NAME], lipopolysaccharide [LPS], apolipoprotein C-III [Apo] transgnic mice + L-NAME, β2 glycoprotein I [βGPI]) were used in four experimental groups: L-NAME (LN), LPS, Apo-LN and βGPI, respectively, and controls received saline (LN-C, LPS-C, Apo-C, βGPI-C). The first three models were established in preimplantation (PI), early-, mid- and late-gestation (EG, MG and LG). βGPI and controls were injected before implantation. Mean arterial pressure (MAP), 24-hour urine protein, placental and fetal weight, serum TGs, total cholesterol (TC) and pathologic liver and trophocyte changes were assessed.

Results: MAP and proteinuria were significantly increased in the experimental groups. Placenta and fetal weight in PI, EP and MP subgroups were significantly lower than LP. Serum TGs significantly increased in most groups but controls. TC was not different between experimental and control groups. Spotty hepatic cell necrosis was observed in PI, EG, MG in LN, Apo-LN and βGPI, but no morphologic changes were observed in the LPS group. Similar trophoblastic mitochondrial damage was observed in every experimental group.

Conclusions: Earlier preeclampsia onset causes a higher MAP and urine protein level, and more severe placental and fetal damage. Preeclampsia-like models generated by varied means lead to different changes in lipid metabolism and associated with liver injury. Trophoblastic mitochondrial damage may be the common terminal pathway in different preeclampsia-like models.

Key words: Liver; Mitochondria; Preeclampsia; Triglyceride; Trophoblast

Introduction

Preeclampsia is defined as a new-onset hypertensive disorder with elevated urine protein, which occurs in pregnant women after 20 weeks gestation. Preeclampsia is a serious threat to maternal and fetal health. Severe preeclampsia, a liver disease that is unique to pregnancy, is often complicated by severe liver damage. Dani *et al*. detected microvesicular fat droplets in the liver of patients with preeclampsia.[1] This indicates that preeclampsia and acute fatty liver in pregnancy, which is another liver disease that is unique to pregnancy, belong in the same disease spectrum. Dysregulation of fatty acid oxidative metabolism may be common to all these liver diseases that are unique to pregnancy.[2]

Preeclampsia is a multifactorial disease. Plasma free fatty acid (FFA) and triglyceride (TG) levels increase in patients with preeclampsia.[3] This may be a result of a decrease in the FFA oxidation-related enzyme long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) mRNA expression and a reduced capacity for FFA oxidation in preeclampsia patients.[4] Other dysregulated lipid metabolism may be involved in preeclampsia, such as the increased serum apolipoprotein C-III (ApoC3) lipid transport protein.[5] In addition, Derzsy *et al*. found that complement activation and C-reactive protein levels in patients with preeclampsia were higher than those in normal pregnancy, suggesting that activation of the inflammatory response is associated with preeclampsia.[6] Antiphospholipid syndrome (APS) is also associated with early-onset preeclampsia, and abnormal lipid metabolism and inflammatory activation also exist in APS patients.[7,8] Therefore, maternal underlying diseases that are presented in prepregnancy, such as hyperlipidemia and APS, and adverse environmental factors during pregnancy, such as inflammation, are all related to preeclampsia.

Poon *et al*. detected different placental hemodynamics, pregnancy-associated plasma protein-A and abnormal

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placental growth factors between patients with early-onset and late-onset preeclampsia as well as in healthy pregnant women in the first trimester.[9] Our previous study found that enzyme protein expression related to placental FFA oxidation in severe preeclampsia patients with an onset time before 32 weeks gestation, especially in patients with liver injury, was dramatically decreased compared with normal pregnancy, while there were no significant differences in severe preeclampsia patients with an onset time after 32 weeks gestation.[10] In early- and mid-onset preeclampsia-like mouse models, LCHAD protein expression was significantly reduced in the placenta, but the late-onset model showed no significant difference from controls,[11] suggesting that different onset times of preeclampsia may result in different lipid metabolism.

The etiology of preeclampsia is unknown. According to different pathogenesis hypotheses, various animal models of preeclampsia have been established, such as the reduced uterine perfusion pressure model, tumor necrosis factor-α-injected model, nitric oxide synthase inhibitor-injected model, endotoxin-injected model and inflammatory factor-injected model.[12] Our previous study found that serum FFA increased in wild type or ApoC3 knock-in transgenic preeclampsia-like mouse models induced by Nw-nitro-L-arginine-methyl ester (L-NAME) or β2 glycoprotein I (βGPI), and hepatic and placental fatty infiltration were observed in these models. However, serum FFA, hepatic and placental fatty infiltration in the lipopolysaccharide (LPS) group were not significantly different from control. Carnitine palmitoyltransferase 1, carnitine palmitoyltransferase 2 and LCHAD protein expression were abnormal in L-NAME or βGPI preeclampsia-like models,[13-15] and all these enzymes are localized in mitochondria. Trophoblastic mitochondria are an important place for long-chain fatty acid β oxidation during pregnancy, and abnormal long-chain fatty acid β oxidation may induce mitochondrial damage in trophoblast cells. In addition, a previous study reported that LPS can inhibit Bcl-2 expression and promote Bax and caspase-8 expression to induce apoptosis in trophoblast cells via the mitochondrial pathway.[16] Different preeclampsia pathogenesis may have a similar mitochondrial injury in trophoblast cells. The liver and placenta are important organs for FFA β-oxidation during pregnancy, and complex links may exist between them in different preeclampsia-like models. The present study used L-NAME and LPS injections, knock-in mice with the human ApoC3 gene as an adverse genetic background, and a basic maternal disease model with APS to create different pathogenic preeclampsia-like mouse models. All models were created at the preimplantation (PI), early-, mid- and late-gestation (EG, MG, LG) stages to study the relationship between lipid metabolism and liver injury in different models that are created at different times. In addition, trophoblastic mitochondrial damage as a possible common pathway of different preeclampsia-like mouse models was investigated.

Methods

Animals

Animal experiments were approved by the Animal Care Committee and Medical Ethics Committee of Peking University, and all procedures were conducted in strict accordance with the guidelines of the Principles of Laboratory Animal Care, published by the NIH. Randomly-selected 8–10-week-old virgin female and 10–14-week-old male SPF C57BL/6J wild type mice and ApoC3 knock-in transgenic mice were purchased from the Department of Laboratory Animal Science of Peking University Health Science Center and mated. The mice were fed standard mouse chow and water *ad libitum* under controlled conditions. The day when vaginal plugs were found was designated as day 1 of pregnancy.

The C57BL/6J mice were randomly assigned to one of six groups: The L-NAME (LN) group (injected with l-NAME), LPS group, βGPI group, and their normal saline–injected control groups (the LN-C, LPS-C, and βGPI-C groups, respectively). The ApoC3 knock-in mice were randomly divided into the Apo-LN group and a normal saline–injected control group, the Apo-C group. Mice in the LN and Apo-LN groups received daily subcutaneous injections of l-NAME at a dose of 50 $mg \cdot kg^{-1} \cdot d^{-1}$.[17] Mice in the LPS group received a single intraperitoneal injection of ultra-low-dose LPS (1 μg/kg).[18] The LN, LPS and Apo-LN groups and their control groups were further subdivided depending on when the first injection was administered: PI subgroup, day 3 of gestation; EG subgroup, day 7 of gestation; MG subgroup, day 11 of gestation; and LG subgroup, day 16 of gestation. Each subgroup contained 10 pregnant mice. Two and 3 weeks before mating, we divided another 20 C57BL/6J mice into two groups, and subcutaneously injected them with human βGPI dissolved in incomplete Freund's adjuvant (Sigma, USA; 0.1 ml)[19] or physiological saline (0.1 ml) to create the βGPI group and its control group, respectively.

Sample collection and tests

The CODA noninvasive tail-cuff acquisition system (Kent Scientific Corporation, USA) was used to measure the mean arterial pressure (MAP) every 2 days, starting from day 2 after mating. Mice were placed in standard metabolic cages on the 17th day of gestation, and 24-h urine samples were collected from them for the measurement of the urine protein level, using a protein assay kit (Bio-Rad Protein Assay Kit I, USA). On the 18th day of pregnancy, the pregnant mice were sacrificed using an injection of 10% chloral hydrate (3 ml/kg). Blood samples were immediately collected from the retro-orbital plexus and centrifuged; the supernatant was stored at − 80°C. Serum TG concentration and total cholesterol (TC) were measured using a blood lipid chemical assay kit (Wako Chemicals, Japan). Cesarean section was performed, and placental and fetal wet weights were recorded. The maternal liver was embedded in paraffin sliced into 5-μm sections, which underwent routine Hematoxylin and Eosin staining, and observed and photographed under an optical microscope (Nikon, Japan). The placenta was

sliced into 1-mm thick sections and fixed with 3.0% glutaraldehyde at 4°C. After dehydration using a series of graded alcohol concentrations, the placenta was embedded in Epon812 Epoxy, and sliced ultrathin into 50–100 nm sections, double-stained with uranyl acetate and lead citrate, and observed and photographed using transmission electron microscopy (JEM1200EX, Japan).

Statistical analysis

SPSS 20.0 (SPSS Inc., USA) was used for data analysis. Quantitative data are expressed as the mean ± standard deviation. Multi-way analysis of variance (ANOVA) was used for comparing MAP. A Student's *t*-test was used for comparing two groups, and one-way ANOVA followed by the least-significant difference *post-hoc* test was used for differences between multiple groups. A $P < 0.05$ was considered statistically significant.

Results

Identification of models

Within each experimental group, MAP was significantly higher in every experimental subgroup than in the corresponding control subgroup ($P < 0.05$). Moreover, the earlier the injection began, the higher was the MAP ($P < 0.05$).

Among the experimental groups, MAP in the Apo-LN subgroups was higher than that of the corresponding subgroups in the other three experimental groups, but only the MAP in the Apo-LN-MP and Apo-LN-LP subgroups was significantly different ($P < 0.05$). MAP was also significantly higher in the Apo-C group than in other control groups that were established using wild type mice ($P < 0.05$). The MAP level in the βGPI group was similar to that in the LN-PI and LPS-PI subgroups. There was no significant difference in MAP between the βGPI-C, LN-C and LPS-C groups [Figure 1a].

Every experimental subgroup showed significantly higher 24-h urine protein concentrations than their corresponding control subgroups at 18 day of pregnancy ($P < 0.05$). Moreover, the earlier the injection began, the more the urine protein increased ($P < 0.05$).

In the LN, LPS and Apo-LN experimental groups, the PI, EP and MP subgroups showed significantly higher urine protein levels than those in LP subgroups ($P < 0.05$). The urine protein level in the Apo-LN group was higher than those in the other experimental groups, but only that in the Apo-LN-LP subgroup showed significance ($P < 0.05$). The urine protein level in the Apo-C group was not significantly different from that in the control groups of wild type mice. The urine protein level in the βGPI group was similar to that in the LN-PI and LPS-PI subgroups, and the difference between the βGPI-C, LN-C and LPS-C groups was not significant [Figure 1b].

Placental and fetal weight

Within each experimental group, PI, EP and MP subgroups' placental and fetal weight was less than that of the corresponding control subgroup for LN, LPS and Apo-LN ($P < 0.05$). LN-LP placental and fetal weight were not significantly different within the control subgroups. LPS-LP placental and fetal weight were significantly lower than that of the control subgroup ($P < 0.05$). Apo-LN-LP fetal weight was significantly less than the control subgroup ($P < 0.05$), but placental weight was not significantly different from the control subgroup. βGPI placental and fetal weight were significantly lower than the control group ($P < 0.05$), and there was no significant difference between LN-PI and LPS-PI. Within each experimental group, the earlier the preeclampsia onset, the more severe the placental and fetal damage ($P < 0.05$).

Lipopolysaccharide-LP placental weight was significantly lower than that of the other late subgroups. Fetal weight in all the LPS and Apo-LN subgroups was significantly less than the corresponding LN subgroups ($P < 0.05$). Fetal weight in the Apo-LN-PI, EP and MP groups was significantly lower than in the corresponding LPS subgroups ($P < 0.05$), but LPS-LP was significantly lower than Apo-LN-LP ($P < 0.05$). There were no significant differences between any other corresponding subgroups [Tables 1 and 2].

Serum triglyceride and total cholesterol concentrations

Within each experimental group, the serum TG concentration was significantly higher in all LN subgroups, except for the LN-LG subgroup, compared with those in the corresponding LN-C subgroups ($P < 0.05$), and the earlier the injection began, the higher the serum TG concentration. The serum TG concentration in the LPS subgroups did not significantly differ from those in the LPS-C subgroups. Except for the Apo-LN-LG subgroup, all Apo-LN subgroups showed a significantly higher TG concentration than those in the corresponding Apo-C subgroups ($P < 0.05$). TC concentrations did not significantly differ among all the groups and subgroups in this study.

Among the experimental groups, the TG concentrations in all the Apo-LN and Apo-C subgroups were significantly higher than those in any of the other subgroups ($P < 0.05$). The TG concentration in the βGPI group was similar to that in the LN-PI subgroup [Figure 2].

Liver morphologic changes

The liver showed visible morphological differences between the different models. Spotty necrosis was observed in PI, EG and MG for LN, Apo-LN and βGPI, but no obvious morphologic changes were observed in the LG subgroups for LN and Apo-LN. In the LN group, the earlier the injections began, the more severe were the changes in liver morphology. Cytoplasmic vacuolization was observed in Apo-LN and Apo-C. No obvious morphologic changes were observed in the LPS group [Figure 3].

Trophoblast cell ultrastructure

A decrease in microvilli, endoplasmic reticulum expansion, trophoblastic mitochondrial swelling and cristae disappearance were observed in all the experimental groups. There was no obvious difference in cell ultrastructure between the different models or between different onset times [Figure 4].

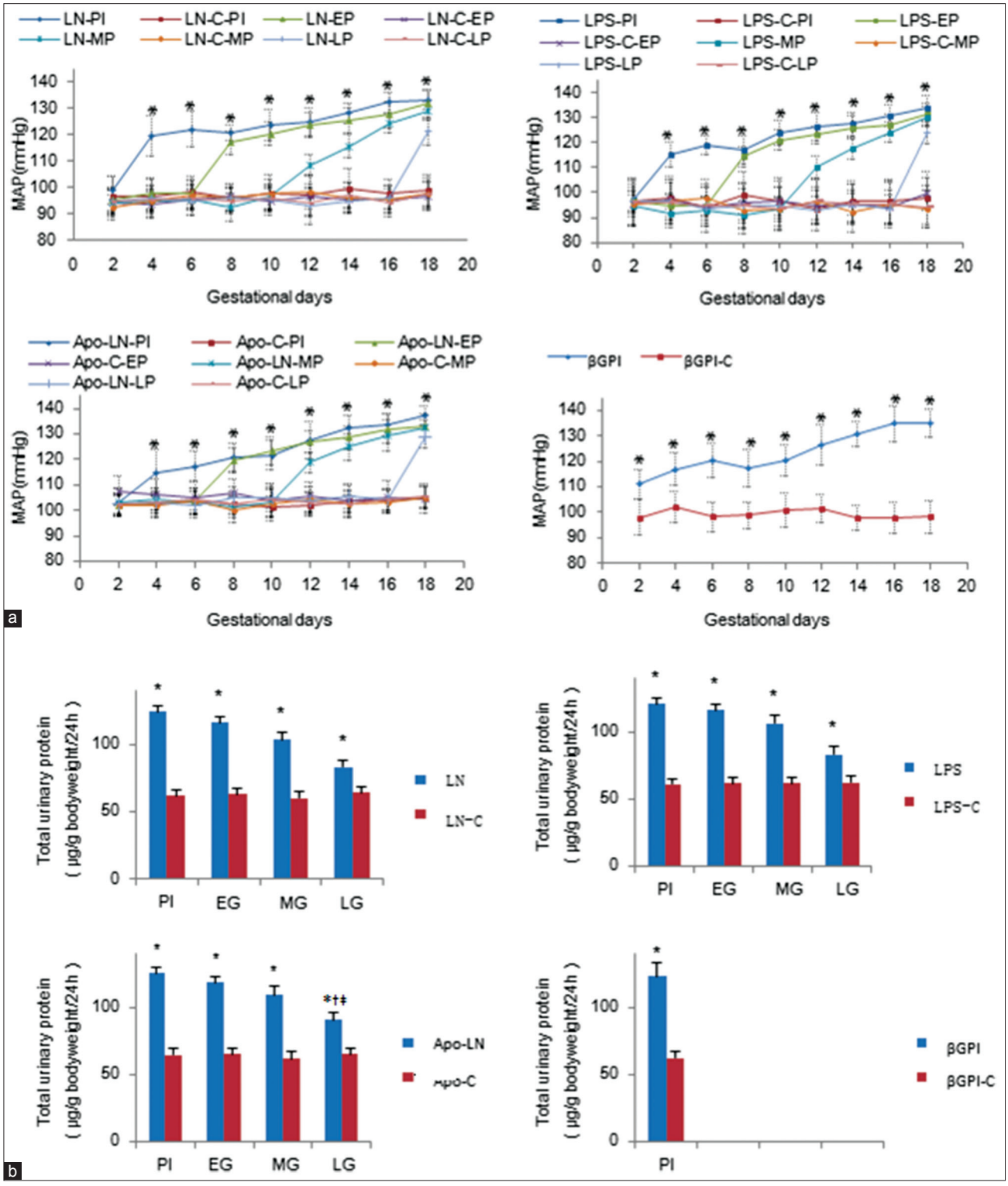

Figure 1: Mean arterial pressure and 24-h urine protein level during pregnancy. (a) Mean arterial pressure and (b) urine protein levels. *$P < 0.05$, compared with the corresponding control subgroup; †$P < 0.05$, compared with the corresponding L-NAME subgroup; ‡$P < 0.05$, compared with the corresponding lipopolysaccharide subgroup. PI: Preimplantation; EP: Early pregnancy; MP: Mid-pregnancy; LP: Late pregnancy.

Discussion

The present study used two typical models of preeclampsia: An endothelial injury model generated using L-NAME and an inflammation model generated using LPS. In addition, we used a transgenic model that overexpresses the human ApoC3 gene and has an abnormal lipid metabolism background, and the APS model that is generated by βGPI, which is injected before mating. MAP and 24-h urine protein were significantly elevated in pregnant mice after injection, and experimental groups showed preeclampsia-like symptoms. Four different models were used, and preeclampsia-like

Table 1: Placental weight on day 18 of pregnancy in each group (mg)

Groups	PI	EP	MP	LP
LN	70.0 ± 7.8*	78.9 ± 10.0*	80.7 ± 5.6*	91.2 ± 7.2†
LN-C	94.4 ± 6.0	90.3 ± 6.5	90.4 ± 5.9	92.4 ± 5.8
LPS	73.3 ± 3.6*	78.7 ± 5.6*	79.4 ± 4.5*	86.9 ± 6.1*,†,‡
LPS-C	95.9 ± 4.2	95.6 ± 3.6	93.4 ± 3.7	95.3 ± 3.6
Apo-LN	75.9 ± 5.2*	77.6 ± 7.7*	78.2 ± 4.9*	96.8 ± 4.6†
Apo-C	97.2 ± 5.9	91.3 ± 7.4	94.2 ± 4.0	94.8 ± 6.7
βGPI	77.1 ± 6.9*			
βGPI-C	97.5 ± 7.1			

*$P < 0.05$, compared with the corresponding control subgroup; †$P < 0.05$, the LP subgroups were significantly different from the other subgroups in the same group; ‡$P < 0.05$, the LPS-LP was significantly different than LN-LP and Apo-LN-LP. PI: Preimplantation; EP: Early pregnancy; MP: Mid-pregnancy; LP: Late pregnancy; LPS: Lipopolysaccharide; βGPI: β2 glycoprotein I; LN: L-NAME; Apo-LN: Apolipoprotein L-NAME.

Table 2: Fetal weight on day 18 of pregnancy in each group (g)

Groups	PI	EP	MP	LP
LN	0.71 ± 0.08*,‡	0.73 ± 0.09*,‡	0.73 ± 0.06*,‡	0.83 ± 0.07†,‡
LN-C	0.82 ± 0.08	0.83 ± 0.07	0.84 ± 0.06	0.85 ± 0.07
LPS	0.67 ± 0.07*	0.70 ± 0.06*	0.71 ± 0.06*	0.73 ± 0.10*,†
LPS-C	0.81 ± 0.04	0.82 ± 0.06	0.80 ± 0.09	0.80 ± 0.06
Apo-LN	0.63 ± 0.07*,§	0.66 ± 0.05*,§	0.67 ± 0.07*,§	0.76 ± 0.09*,†
Apo-C	0.80 ± 0.09	0.84 ± 0.07	0.81 ± 0.08	0.82 ± 0.06
βGPI	0.71 ± 0.06*,‡			
βGPI-C	0.81 ± 0.09			

*$P < 0.05$, compared with the corresponding control subgroup; †$P < 0.05$, the LP subgroups were significantly different from the other subgroups in the same group; ‡$P < 0.05$, subgroups in LPS and Apo-LN were significantly lower than the corresponding subgroup in LN and βGPI; §$P < 0.05$, compared with the corresponding LPS subgroup. PI: Preimplantation; EP: Early pregnancy; MP: Mid-pregnancy; LP: Late pregnancy; LPS: Lipopolysaccharide; βGPI: β2 glycoprotein I; LN: L-NAME; Apo-LN: Apolipoprotein L-NAME.

symptoms were induced at different onset times. The earlier the preeclampsia onset, the higher were the MAP and urine protein levels, indicating that successful preeclampsia-like model generation. In the LN (except for the late subgroup), βGPI, Apo-LN and Apo-C groups, but not in the LPS group, serum FFAs significantly increased. All experimental groups, except the LPS group, showed significant hepatic and placental lipid deposition.[13-15]

Roberts *et al*. proposed that maternal constitutional factors, including genetic, behavioral and environmental factors, affect the clinical manifestations of preeclampsia.[20] Different pregestational maternal situations and different pathogenic factors may induce different pathophysiological changes in lipid metabolism, resulting in different clinical outcomes for patients with preeclampsia. In this study, the earlier the preeclampsia onset, the higher were the MAP and urine protein levels, and the lower were the placental and fetal weights, indicating that early pregnancy is an important time for placental development; the earlier the adverse factors impact upon the mother, the more severely affected are placental and fetal development. Compared with other subgroups, serum TG concentration in LN-LG subgroup was significantly reduced, and there were no obvious morphologic changes in the liver. Liver injury in the Apo-LN group was also affected by ApoC3 knock-in, and there was no obvious tendency in this group, but TGs and liver injury in the Apo-LN-LG subgroup were significantly different from other subgroups. This is consistent with FFA oxidative metabolism and liver injury that often occurred in patients with early-onset preeclampsia, suggesting that the interaction between adverse factors and late-onset preeclampsia patients may be different from early-onset preeclampsia patients. In early-onset preeclampsia, maternal and placental influence is mutual. The adverse factors act on the mother's body and affect the developing placenta, and maternal-placental interactions may produce a vicious cycle by influencing the clinical manifestations of preeclampsia. In late-onset preeclampsia, the adverse factors affect the developed placenta, and this may lead to clinical manifestations that are mainly caused by the maternal stress reaction.

The classic preeclampsia-like model, the LN group, showed increased serum TGs, indicating that the L-NAME-generated model had dysregulated lipid metabolism. Because of a decrease in placental LCHAD protein expression,[21] accumulation of long-chain fatty acids may be the reason for the increase in TGs, and increased levels of long-chain fatty acids in the blood, which could lead to more absorption of long-chain fatty acids by the liver. When the anabolism of TGs exceeds the capacity of fatty acid oxidation, lipids in liver and other organs increase, resulting in maternal ectopic deposition of lipids and systemic metabolic disorder.[22] Another classic preeclampsia-like model, the LPS group, showed a similar MAP and urine protein level as that in the LN group, but serum TG levels in the LPS group differed from those in the other experimental groups. This finding might be a result of inflammatory cytokines stimulated by LPS that act on endothelial cells to activate NF-κB and other inflammatory pathways, resulting in endothelial cell damage and dysfunction, and leading to preeclampsia-like changes.[23,24] This indicates that the LPS-generated preeclampsia-like model may not have a long-chain fatty acid oxidative metabolism dysfunction.

Serum ApoC3 can cause an increase in hepatic TG anabolism, leading to elevation of serum TG levels.[25] Apo-C and ApoC3 transgenic mice without L-NAME injection had an elevated MAP, but their urine protein was not significantly different from the wild type control groups. ApoC3 transgenic mice with abnormal lipid metabolism showed gestational hypertension.[14] After injection of L-NAME in the Apo-LN group, MAP and urine protein increased significantly, and placental and fetal weight was significantly reduced. This suggests that the Apo-LN group showed a preeclampsia-like syndrome, and LCHAD mRNA and protein expression in the liver and placenta were significantly higher for the Apo-C, Apo-LN and βGPI groups and lower for LN group compared

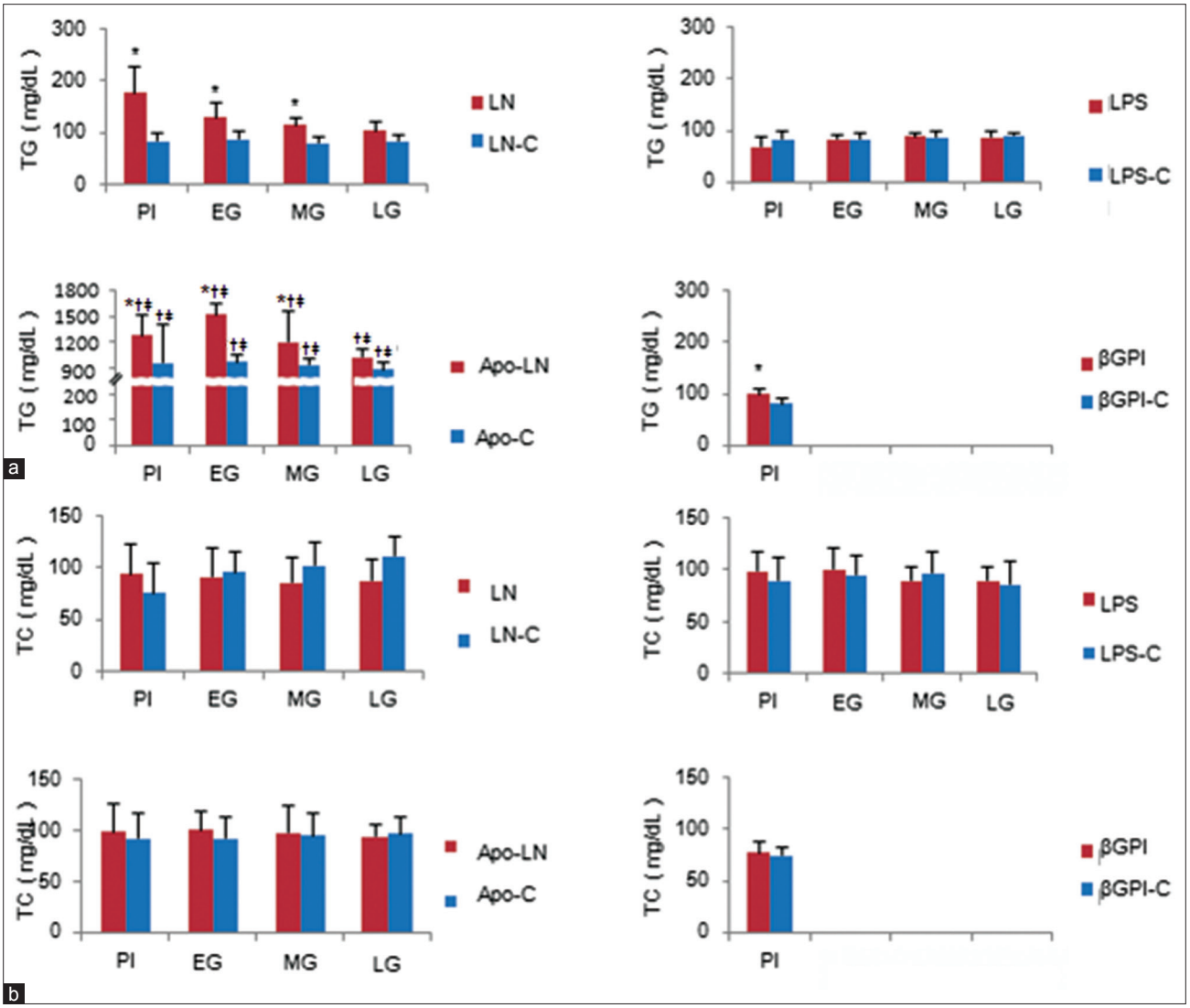

Figure 2: Serum triglyceride and total cholesterol concentrations. (a) Triglyceride and (b) total cholesterol concentrations. *$P < 0.05$, compared with the corresponding control subgroup; †$P < 0.05$, compared with the corresponding L-NAME (LN) or LN-C subgroup; ‡$P < 0.05$, compared with the corresponding lipopolysaccharide (LPS) or LPS-C subgroup. PI: Preimplantation; EG: Early-gestation; MG: Mid-gestation; LG: Late-gestation.

with controls. However, this did not differ between the LPS group and controls,[15] indicating that different maternal constitutional factors play different roles in pathogenesis of preeclampsia. Although some negative factors cannot induce preeclampsia-like syndrome alone, they can exacerbate the burden of pregnancy so that preeclampsia-like symptoms will be more serious with other conditions. In the LN group, the increase in TGs was caused by FFA accumulation. Unoxidized palmitoyl-CoA, a FFA metabolite, is the main cause of lipid-generated tissue injury,[26] suggesting that FFAs are more harmful than TGs. This difference in the source of serum TGs may account for the absence of liver injury in the Apo-C group. This phenomenon indicates that preeclampsia is the result of the interaction between genetic and environmental factors and that a lipid metabolism disorder acting on a different genetic background may result in different pathophysiological changes.

In the present study, βGPI-generated a preeclampsia-like mouse model that had significantly increased serum TG levels, which were similar to that in the LN-PI subgroup. Our previous study showed increased placental LCHAD mRNA and protein expression in the βGPI-generated preeclampsia-like model, and decreased placental LCHAD mRNA and protein expression in the L-NAME-generated preeclampsia-like mouse model, but both models had increased serum FFA.[13,21] This suggests that dysregulated long-chain fatty acid metabolism exists in the βGPI-generated preeclampsia-like mouse model, but fatty acid β-oxidation may be differently regulated in these two models.

In this study, spotty liver necrosis was observed in the LN and Apo-LN groups, which were generated using L-NAME, and in the βGPI group, which was generated using βGPI, but the LPS-generated model had no obvious hepatic necrosis. Fat mobilization increases during pregnancy, and adverse

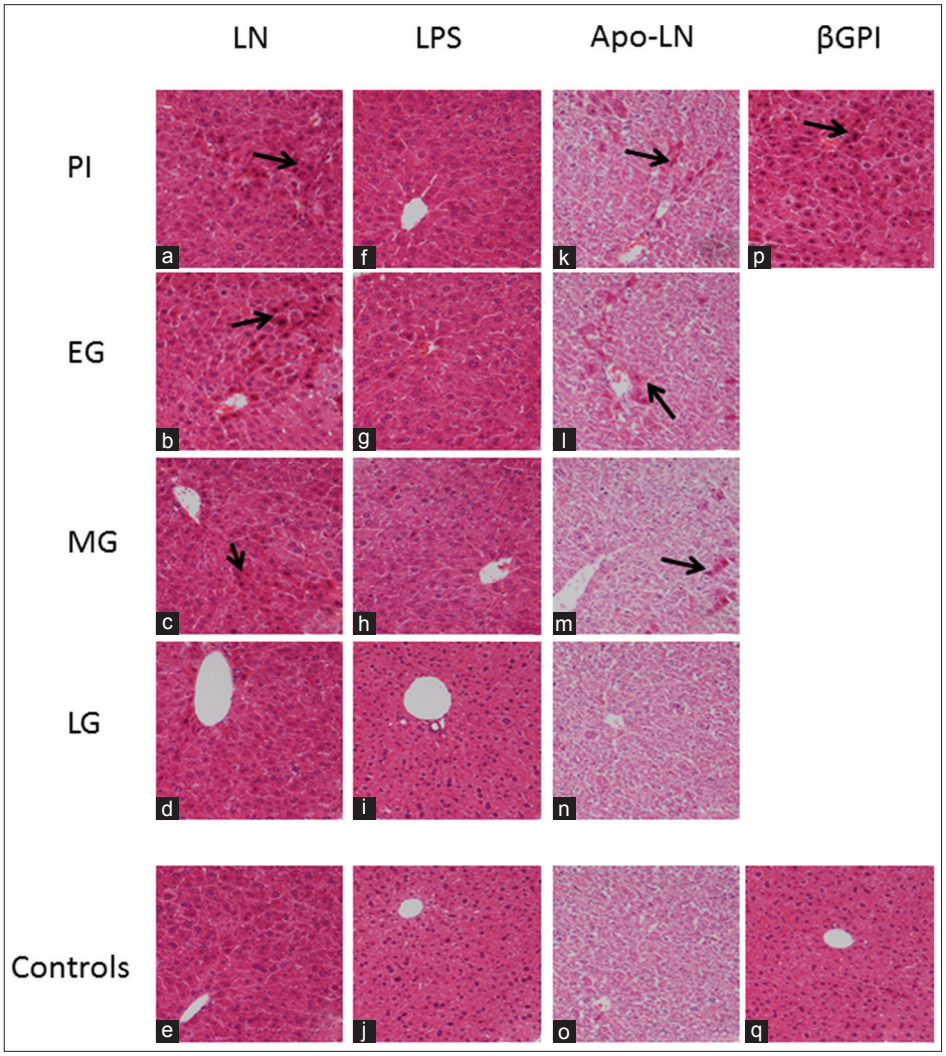

Figure 3: Morphologic changes in the liver at day 18 of pregnancy. Spotty necrosis was observed in PI, EG, MG of L-NAME (LN), Apo-LN and β2 glycoprotein I (βGPI) (black arrow). In the LN group, the earlier the injections began, the more severe were the liver morphologic changes. No morphologic changes were observed in the lipopolysaccharide (LPS) group. (a-d) LN; (e) LN-C; (f-i) LPS; (j) LPS-C; (k-n) Apo-LN; (o) Apo-C; (p) βGPI; (q) βGPI-C. PI: Preimplantation; EG: Early-gestation; MG: Mid-gestation; LG: Late-gestation (H and E; original magnification, ×200).

maternal constitutional factors may decrease placental long-chain fatty acid oxidation, resulting in increased serum FFA. The accumulation of long-chain fatty acids in the liver may be the reason for liver injury in preeclampsia.[27] LCHAD is an important enzyme for long-chain fatty acid oxidation, and the preeclampsia-like LN, Apo-LN and βGPI groups with abnormal serum TG showed abnormal LCHAD expression. The LPS group had no significant dyslipidemia, and there was also no obvious abnormal LCHAD expression, confirming that long-chain fatty acid metabolism may be a reason for liver injury in preeclampsia.[13-15] In addition, Apo-LN and Apo-C groups showed significant hepatic cytoplasmic vacuoles, which may be caused by congenital hyperlipidemia. The liver and placental protein LCHAD expression in Apo-C were significantly higher than those in the wild type saline control group, but placental LCHAD expression in the Apo-C group was significantly lower than that in Apo-LN. This suggests that highly expressed ApoC3 plays a role in the regulation of LCHAD in preeclampsia, and the difference in placental LCHAD expression may be the reason for different liver injuries between Apo-LN and Apo-C.[15] These phenomena suggest an association between placental long-chain fatty acid oxidative disorders and liver injury in preeclampsia.

Previous studies suggested that trophoblast cell dysfunction causes preeclampsia.[28] In this study, no matter what kind of model or induction time, preeclampsia-like symptoms always emerged and they were accompanied by abnormal mitochondrial structure in trophoblast cells. It is unlikely that cholesterol, which is mainly metabolized in the cytosol, is involved, but mitochondria are important places for FFA metabolism in trophoblast cells. Experimental groups with abnormal TG levels have abnormal LCHAD protein expression,[13-15] suggesting that the trophoblastic mitochondrial damage caused by long-chain fatty acid oxidative metabolic disorders is associated with some preeclampsia, which is also corroborated in the clinic by increased oxidative stress and mitochondrial damage in placental trophoblast cells

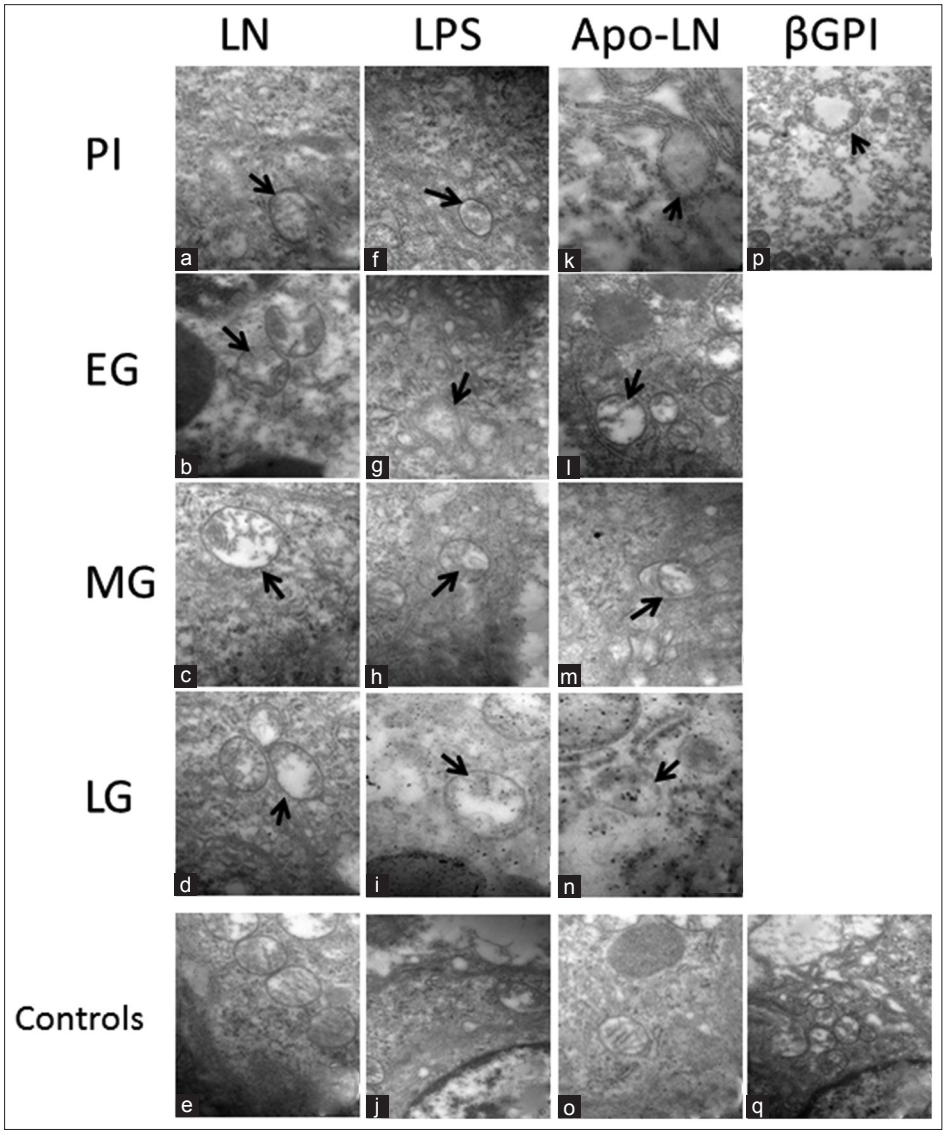

Figure 4: Trophoblast cell ultrastructure. Trophoblastic mitochondria swelling and cristae disappearance were observed in all the experimental groups (black arrow). There were no obvious differences between different preeclampsia models or different onset times. (a-d) L-NAME (LN); (e) LN-C; (f-i) lipopolysaccharide (LPS); (j) LPS-C; (k-n) Apo-LN; (o) Apo-C; (p) β2 glycoprotein I (βGPI); (q) βGPI-C. PI: Preimplantation; EG: Early-gestation; MG: Mid-gestation; LG: Late-gestation (Double stained with uranyl acetate and lead citrate, magnification × 8000).

from preeclampsia patients.[29,30] In addition, a previous study reported that LPS can promote caspase-8 expression in trophoblast cells, leading to apoptosis via the mitochondrial pathway.[31] Thus, there is also mitochondrial damage in the LPS group without a lipid metabolic disorder. All the preeclampsia models had trophoblast cell mitochondrial damage, suggesting that trophoblastic mitochondrial damage may be the common terminal pathway in different preeclampsia-like models.

Our results show that the earlier the preeclampsia onset, the higher were the MAP and urine protein levels, and the lower was the placental and fetal weight. Different preeclampsia-like mouse models may have different lipid metabolism, and a long-chain fatty acid oxidation metabolic disorder may be one of the reasons for liver injury in preeclampsia. Preeclampsia may be a similar clinical manifestation in a group of diseases, and the trophoblastic mitochondrial damage may be their common terminal pathway.

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